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Special Issue Reprint

Advances in Wildlife Conservation and Habitat Management in the Anthropocene

Edited by
Yiannis G. Zevgolis and Panayiotis G. Dimitrakopoulos

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Advances in Wildlife Conservation and Habitat Management in the Anthropocene

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Guest Editors

Yiannis G. Zevgolis
Panayiotis G. Dimitrakopoulos

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About the Editors

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Preface

This Reprint brings together the contributions published in the Special Issue "Advances in Wildlife Conservation and Habitat Management in the Anthropocene". It was prepared to offer readers an integrated overview of current challenges, methods, and applied perspectives in wildlife conservation and habitat management under conditions of accelerating environmental change.

The need for such a synthesis is increasingly clear. Wildlife populations, habitats, and ecosystems are now shaped by interacting anthropogenic, biotic, and abiotic pressures that often act simultaneously and produce complex ecological outcomes. These pressures challenge traditional conservation approaches and underline the importance of evidence-based, adaptive, and practically relevant responses.

Reflecting this context, the Reprint highlights studies that advance ecological understanding while also supporting conservation planning and management. The included contributions address different systems, taxa, stressors, and methodological approaches, including habitat assessment, species conservation, ecological monitoring, modelling, protected area management, and adaptive decision making.

We hope that this Reprint will serve as a useful resource for researchers, conservation practitioners, land managers, policymakers, students, and all readers interested in biodiversity conservation in the Anthropocene.

Yiannis G. Zevgolis and Panayiotis G. Dimitrakopoulos
Guest Editors

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Editorial

Stressor Complexity, Ecological Response, and Conservation Decisions: Advances in Wildlife Conservation and Habitat Management in the Anthropocene

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1. Introduction

Wildlife conservation and habitat management have entered a phase of irreducible complexity, in which the ecosystems where species must persist, populations must recover, and management must intervene are increasingly structured by interacting constellations of anthropogenic, biotic, and abiotic change [1,2]. Land-use transformation, habitat fragmentation, altered disturbance regimes, agricultural intensification, biological invasions, infrastructure expansion, overexploitation, and climate change now operate simultaneously across biological, temporal and spatial scales [3,4], generating ecological outcomes that are nonlinear, taxon-specific, historically contingent, and spatially heterogeneous in ways that linear or single-driver frameworks are poorly equipped to anticipate [5–7]. The defining conservation challenge of the Anthropocene therefore lies in the capacity to organise ecological knowledge in ways that can navigate complexity: characterising how stressors interact, detecting the biological responses through which species and communities register those interactions, translating those responses into spatial or demographic diagnosis, and sustaining the adaptive feedback between management action and ecological outcome on which effective conservation ultimately depends.

The 16 contributions assembled in this Special Issue collectively address this challenge, expressed through the full analytical passage from stressor characterisation to implemented, monitored, and revisable management action. The collection extends across terrestrial, insular, freshwater, agroecological, protected-area, and human-modified systems, addressing conservation problems that range from the continental-scale connectivity crisis facing the clouded leopard (*Neofelis nebulosa*) [8], to the intensely localised convergence of seasonal water scarcity, rising summer temperatures, and intensified predation threatening *Pelophylax cerigensis*—Europe's most threatened frog—within the restricted freshwater systems of an Aegean island [9]. These seemingly distant cases are joined by a shared evidentiary demand: to characterise interacting stressors with ecological precision, to measure the biological responses through which species and communities register those pressures, and to convert that evidence into decisions that are spatially explicit, adaptive, and accountable through future monitoring.

Together, the contributions advance the central argument of this editorial: wildlife conservation becomes most consequential when stressor complexity, ecological response, and conservation decision are organised within a single chain of evidence, capable of guiding action while remaining open to monitoring, challenges, and revision. This chain provides the basis for the Stressor–Response–Decision (SRD) framework (Figure 1) developed below, through which the 16 contributions are read as connected points along the passage from

interacting pressures to biological response, from response to spatial or demographic diagnosis, and from diagnosis to adaptive conservation action. Following this introductory section, this editorial develops this passage through four analytical arcs— the instability of conservation assumptions under interacting pressures; the biological responses through which those pressures become measurable; the spatial and quantitative diagnoses that convert ecological evidence into prioritisation; and the translation of diagnosis into adaptive, accountable action—before returning to the SRD framework as a synthetic argument for conservation science under Anthropocene complexity.

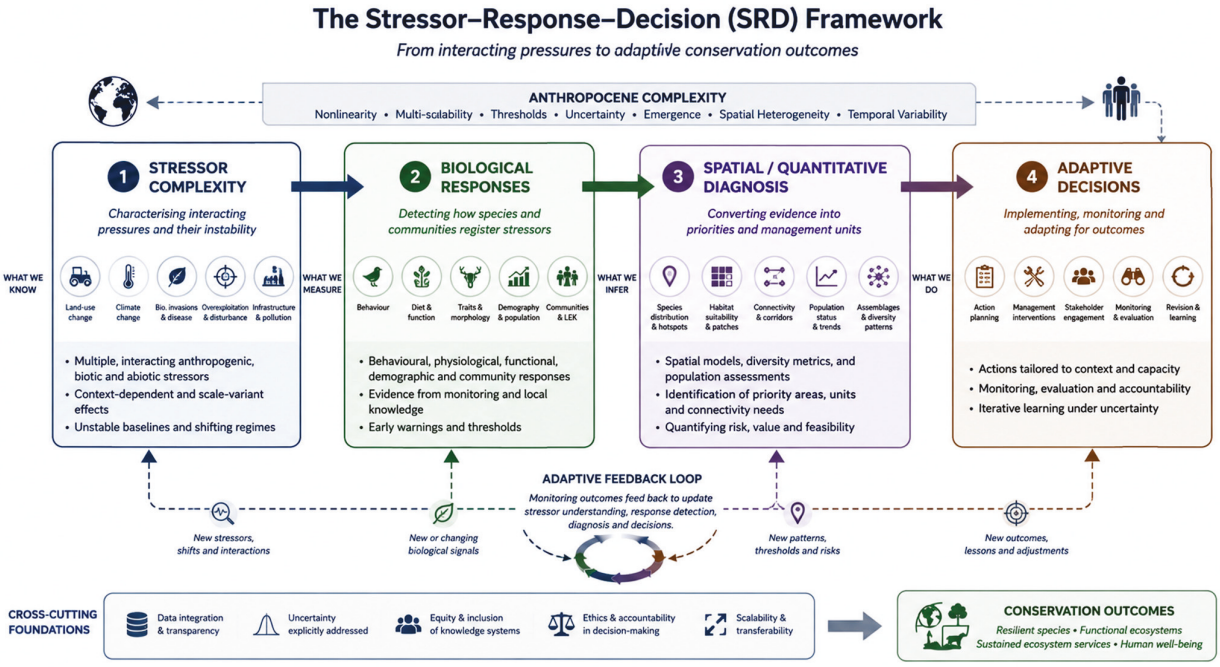

Figure 1. The Stressor–Response–Decision (SRD) framework for conservation science under Anthropocene complexity. The framework conceptualises conservation evidence as a connected circuit linking interacting anthropogenic, biotic, and abiotic pressures to biological responses, spatial or quantitative diagnosis, and adaptive conservation decisions. Stressor characterisation identifies the pressures and interactions structuring ecological systems; response measurement detects how species, populations, communities, and knowledge systems register those pressures; diagnosis translates evidence into hotspots, habitat patches, corridors, population status, assemblage patterns, and management units; and adaptive decisions implement, monitor, evaluate, and revise conservation action. The feedback loop emphasises that monitoring outcomes should update stressor understanding, response detection, diagnostic priorities, and management decisions, maintaining the integrity of the full evidence-to-decision chain.

2. Stressor Complexity and the Fragility of Conservation Assumptions

The first thematic arc of the SRD framework begins with the recognition that Anthropocene pressures do not merely intensify conservation problems; they also destabilise the conceptual categories through which those problems are identified, compared, and managed. Disturbance, recovery, mitigation, management, and prediction remain central to conservation thought, yet each carries an assumed ecological direction that interacting pressures can unsettle: disturbance should explain damage, recovery should mark improvement, mitigation should reduce risk, management should restore control, and prediction should make the future more legible. The contributions considered here show that these expectations acquire conservation meaning only when they are tested against biological response, landscape configuration, temporal contingency, and the historical conditions under which ecological processes unfold. Under Anthropocene complexity, a pressure

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becomes conservation-relevant through the measurable consequences it produces within a particular system, and the analytical task is therefore to identify when a process becomes a threat, a resource, a misleading signal, or a management opportunity.

This problem is especially visible in landscapes where disturbance and land use operate as structuring forces rather than discrete external events. In Mediterranean rangelands, livestock grazing generated divergent vertebrate responses across abundance, species richness, vegetation structure, and resource availability, showing that grazing intensity acquires ecological meaning only through the multiple response axes along which communities reorganise [10]; a prescription calibrated to richness alone may therefore misread abundance, vegetation structure, or resource supply. Fire poses a parallel challenge in post-disturbance wetlands, where burned and unburned patches function less as fixed habitat categories than as a spatially and temporally dynamic resource mosaic in which predator and scavenger activity is reorganised by season, prey exposure, carrion availability, and trophic opportunity [11]. In both cases, disturbance is transformed from a management label into an ecological question: its conservation meaning emerges from the biological response it elicits, the timing of that response, and the spatial configuration through which disturbance becomes constraint, release, or reorganisation.

Recovery introduces an even sharper test, because it is among the most intuitively powerful assumptions in applied conservation and therefore among the most consequential when it is treated as self-evident. The study of South China sika deer (*Cervus nippon kopschi*) over a decade of active forest restoration demonstrates that vegetation regeneration may advance structural and spectral indicators of recovery while simultaneously failing to improve functional habitat use by the target herbivore [12]. This distinction is central to conservation in recovering landscapes: greenness, canopy development, and vegetation density may describe the trajectory of the habitat while obscuring the ecological experience of the organism moving through it. A restored landscape can therefore be treated as biologically recovered only when structural change is confirmed through species use and functional habitat suitability. Otherwise, restoration remains a structural success whose conservation value is assumed rather than empirically demonstrated.

The decoupling between apparent and functional success is equally visible in conservation intervention and predictive modelling. Sustainable management of the olive fly (*Bactrocera oleae*) cannot be reduced to pest suppression alone, because host phenology, climatic sensitivity, natural enemies, pesticide resistance, and landscape structure all shape whether control is durable or ecologically costly [13]. Insecticide applications may reduce pest pressure while disrupting beneficial organisms and weakening the biological regulation they replace; climate change further widens this uncertainty by altering the timing, intensity, and predictability of olive fly pressure. The review's call for ecologically informed management is therefore also a conservation argument: in the Anthropocene, where agricultural landscapes occupy vast areas and interact continuously with surrounding biodiversity, pest management policy is biodiversity policy.

Predictive modelling faces a parallel demand for accountability. The retrospective evaluation of Egyptian vulture (*Neophron percnopterus*) projections under wind-farm mortality shows that the conservation authority of models depends on whether their assumptions are tested against the trajectories they claim to anticipate [14]. Short demographic baselines, weakly calibrated exposure estimates, and untested projections allow forecasts to influence regulation without being exposed to the consequences of error. Prediction, like disturbance, recovery, and management, becomes conservation-relevant only when it remains accountable to biological response. Identifying when and how that accountability is met, however, requires a different order of evidence: the biological responses through which species and

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communities make stressor effects visible, measurable, and interpretable at the resolution that management decisions demand.

3. Biological Responses and the Precision of Conservation Evidence

The second thematic arc of the SRD framework rests on the recognition that if interacting pressures destabilise the assumed meaning of disturbance, recovery, management, and prediction, then biological response becomes the point at which conservation interpretation regains precision. Species and communities register Anthropocene pressures through behavioural thresholds, dietary adjustments, trait variation, demographic structure, and the uneven ways vulnerability is expressed across biological scales. The response component of the SRD framework therefore shifts conservation evidence away from the question of whether a species is simply still present, and toward the more challenging question of how it is persisting, under what constraints, and with what hidden biological costs.

Behaviour is often the first place where pressure becomes measurable, because animals alter space use, escape decisions, and tolerance before changes in population size can be detected. Husby [15], through the reassessment of flight initiation distance in birds, shows that disturbance cannot be reduced to a generic buffer distance or average escape response; it must be interpreted through species identity, flock size, disturbance type, site context, and the probability structure of escape behaviour. The management value of behavioural metrics therefore lies in their capacity to preserve the variability through which animals actually experience risk, allowing disturbance thresholds to be designed from biological sensitivity instead of administrative convenience.

Dietary evidence carries the same logic into the functional dimension of habitat quality. In anthropogenic landscapes, the persistence of European ground squirrels cannot be inferred from occurrence alone, because feeding habits reveal the resource pathways through which animals negotiate modified habitats [16]. Diet becomes a diagnostic interface between habitat structure and biological requirement, showing whether a species is exploiting available resources, compensating for scarcity, narrowing its trophic options, or being pushed toward potentially suboptimal foods. In the populations studied, the use of plants with potentially adverse secondary compounds turns feeding ecology into evidence of functional constraint, converting the broad category of human-modified habitat into the more exact question of whether that habitat remains biologically sufficient.

Trait and demographic evidence deepen this response-based view by exposing forms of vulnerability that distributional data cannot resolve. The long-term morphometric assessment of sexual body size dimorphism in small mammals by Balčiauskas and Balčiauskienė [17] provides a baseline through which population structure, ecological variation, and future change can be interpreted with greater biological resolution. Tella et al. [18] extend this logic into demographic hiddenness, showing that conservation assessment of the Red-spectacled Amazon depends on distinguishing breeding and non-breeding fractions instead of treating visible abundance as demographic security. A population may remain numerically present while being structurally constrained; the conservation signal lies not only in how many individuals exist, but in how those individuals are organised, recruited, and able to sustain future persistence.

Local ecological knowledge expands this evidentiary architecture by capturing population visibility where conventional monitoring is logistically difficult, temporally sparse, or financially constrained. Wenborn et al. [19] show that structured knowledge from community game guards can support meaningful inference on elephant population size in remote landscapes, functioning as an organised observational system grounded in repeated encounter, spatial familiarity, and local continuity. Where survey capacity is absent, such

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systems extend the geographic reach of the evidentiary architecture that biological response monitoring requires.

4. Spatial Diagnosis and Conservation Prioritisation

The third thematic arc of the SRD framework begins at the point where biological response evidence must be converted into conservation choice. A behavioural threshold, a dietary constraint, a demographic imbalance, a locally observed population estimate, or a community-level shift becomes operational only when conservation science can locate it, compare it, and determine where action will matter most. Diagnosis therefore does more than map species or quantify their status; it identifies where vulnerability is concentrated, where ecological value and management conflict overlap, where future persistence depends on connectivity, and where limited conservation resources can be directed with the greatest justification.

This conversion of evidence into decision space is especially clear in the European rabbit study on Lemnos Island, where nearly 60% of high-density areas of *Oryctolagus cuniculus* coincide with formally designated conservation zones [20]. This overlap should be read as a diagnostic signal of how insular agroecosystems concentrate ecological value, population density, land-use context, and management conflict within the same landscape units. By combining 1534 occurrence records with environmental drivers, hotspot analysis, and spatial modelling, the study transforms distributional information into a map of management consequence, showing that the conservation relevance of a species is produced not by presence alone, but by where that presence intersects with institutional obligations, ecological function, and competing land-use demands.

Where the *Oryctolagus cuniculus* study diagnoses conservation conflict through concentration, studies of threatened and low-density species diagnose vulnerability through rarity, fragmentation, and spatial continuity. Khattak et al. [21] combine field evidence of Indian pangolin (*Manis crassicaudata*) presence with habitat suitability modelling to identify habitat patches of heightened conservation concern within a fragmented landscape, showing how sparse signs, local habitat structure, and predictive mapping integrate into a practical prioritisation framework. Abedin et al. [8] extend this diagnostic logic to a continental scale for the clouded leopard (*Neofelis nebulosa*), where habitat suitability forecasting, climate projections, and corridor identification reveal that conservation priority extends beyond occupied habitat to the spatial continuity required for persistence across fragmented Asian landscapes. One diagnosis works through localised patch concentration, the other through transboundary connectivity; both demonstrate that vulnerability produces conservation obligation only when its spatial structure is made explicit.

Freshwater systems complicate this diagnostic movement because what requires diagnosis is often the reorganisation of whole assemblages under cumulative pressure rather than the status of a single population. In the Pearl River Basin, He et al. [22] use zeta diversity and cumulative-effect analysis to show that human pressure reshapes fish communities through compositional turnover, uniqueness, and homogenisation that conventional richness metrics may not detect, shifting diagnosis to a comparative, community-level operation that identifies where ecological distinctiveness persists and where management should protect the spatial structure of biodiversity itself.

A parallel challenge emerges when the limiting factor is data scarcity rather than spatial complexity. The stock assessment of noncommercial fishes in Aras Dam Reservoir shows that sparse fisheries data can still be converted into management-relevant status, revealing depleted or unsustainable conditions in species that would otherwise remain outside conservation attention because they lack commercial prominence [23]. Diagnos-

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tic modelling here performs a corrective function: it prevents low visibility from being mistaken for low vulnerability.

Spatial diagnosis, in this sense, is the process by which conservation science decides where evidence becomes obligation. The SRD framework turns toward its decision component at this point: once vulnerability has been diagnosed, conservation must determine how that diagnosis becomes adaptive action, and how action remains accountable to the biological outcomes it was designed to secure.

5. From Diagnosis to Adaptive, Accountable Conservation Action

The fourth and final thematic arc of the SRD framework begins when diagnosed vulnerability becomes a demand for intervention. Spatial diagnosis identifies where conservation concern is concentrated, but its value is ultimately tested where evidence enters practice: when a hotspot becomes a management unit, a corridor becomes a planning priority, a depleted stock becomes a regulatory concern, or a threatened population becomes the focus of a coordinated recovery strategy. Decision-making is therefore the point at which conservation science becomes most exposed, because evidence is no longer judged only by analytical precision, but by its capacity to support actions that alter ecological trajectories.

The action plan for the Karpathos water frog, *Pelophylax cerigensis*, provides the clearest expression of this transition [9]. The species is confined to the restricted freshwater network of the island of Karpathos, where spatial limitation, seasonal water scarcity, rising summer temperatures, and predation pressure render generic prescriptions ecologically inadequate. Diagnosis must therefore become operational, with breeding sites protected, hydrological function secured, immediate pressures reduced, and monitoring sustained long enough to detect whether intervention is producing biological improvement. The action plan's value lies in this conversion of ecological understanding into a revisable conservation sequence organised around the persistence of a species whose margin for error is exceptionally small.

Adaptive management, in this sense, is the conservation form of accountability: intervention designed with enough ecological specificity to be implemented, enough monitoring structure to be evaluated, and enough institutional flexibility to be revised. The SRD framework reaches its practical conclusion here: conservation decisions become scientifically credible when they preserve the connection between the pressures that justify action, the responses that reveal ecological effect, and the monitored outcomes that determine whether management has altered the biological trajectories it was designed to change.

6. The Stressor–Response–Decision Framework: Synthesis and Future Directions

The four thematic arcs traced in the preceding sections are not sequential stages in a linear workflow, but interdependent analytical movements whose conservation value derives from their connection (Figure 1). Stressor characterisation without response measurement risks producing threat catalogues rather than management evidence; response measurement without spatial diagnosis produces biological knowledge without decision space; spatial diagnosis without adaptive action produces prioritisation without consequence; and adaptive action without monitoring produces intervention without accountability. The SRD framework is therefore best understood as a conservation circuit whose utility depends on maintaining the evidentiary links between interacting pressures, biological responses, spatial or demographic diagnosis, and revisable management action.

This circuit also clarifies what remains unfinished. Models require retrospective validation; restoration must be tested against organismal use rather than structural recovery alone; monitoring must move beyond abundance to behavioural, functional, demographic, and community-level responses; spatial prioritisation must be connected to institutions

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capable of acting on it; and action plans must remain open to ecological feedback. The contributions assembled in this Special Issue show progress across each of these fronts, but they also reveal that conservation effectiveness depends less on excellence within any single evidentiary domain than on integration across the whole chain.

The SRD framework makes no claim to resolve the complexity of Anthropocene conservation. Its value is more practical and more precise: it makes the structure of that complexity legible. By linking stressors to responses, responses to diagnosis, and diagnosis to adaptive action, it offers a way to organise conservation knowledge without dismissing the ecological uncertainty that management must confront.

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Vertebrate Community Responses to Livestock Grazing in an Ancient Mediterranean Rangeland System: Rethinking the Role of Grazing in a Biodiversity Hotspot

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Simple Summary: Livestock husbandry is a primary source of livelihood for more than a billion people globally and has been practiced in the Mediterranean since antiquity. Despite this long history, the impact of livestock grazing on wildlife populations and their habitats is poorly understood. The aim of this study was to understand how small mammals, reptiles, and bird species respond to differing degrees of livestock grazing pressure in the Mediterranean, a major biodiversity hotspot. We found that, as livestock grazing pressure increased, the number of small mammals and reptiles decreased. However, the number of these species peaked at moderate grazing levels in alignment with the Intermediate Disturbance Hypothesis. Bird populations, on the other hand, showed no direct response to livestock grazing levels but were influenced by changes in vegetation structure. Our findings suggest that there is no ideal level of livestock grazing that will benefit all wildlife species. Instead, a mosaic of light-to-moderate grazing across the entire region may best support wildlife conservation. This research has important implications for agricultural and environmental policy concerning the role of livestock grazing in wildlife conservation planning.

Abstract: Mediterranean ecosystems have been grazed by livestock for thousands of years. While considered both a major anthropogenic stressor and a potential habitat conservation tool, the effects of livestock grazing on vertebrate populations remain poorly understood. Our study focused on goat and sheep grazing on a large island off the coast of Greece in order to shed light on (1) the nature of the relationship between livestock grazing and vertebrate assemblages, and (2) the mediating mechanisms. Sampling small mammal, reptile, and passerine bird species across a range of livestock grazing intensities in a Mediterranean pastoral system, we used Generalized Linear Modeling to test for the presence of a unimodal relationship between grazing disturbance and vertebrate diversity in line with the Intermediate Disturbance Hypothesis (IDH). An information-theoretic approach helped elucidate which habitat characteristics best predicted vertebrate-grazing responses. Terrestrial species abundance decreased steadily with increasing grazing, while species richness exhibited a unimodal response, peaking at intermediate livestock stocking rates and offering support for the IDH. This response was best predicted by invertebrate food availability. Both passerine bird species' richness and abundance showed no clear relationship with grazing yet were significantly correlated with changes in vegetation structure. Our findings suggest that there is no ideal grazing level for broadly optimizing

both vertebrate abundance and richness. However, only light-to-intermediate livestock stocking rates are associated with healthy wildlife populations while also promoting terrestrial species richness. Agricultural policy that avoids overgrazing while encouraging a mosaic of different grazing intensities at the regional level is needed to best support diverse vertebrate assemblages.

Keywords: disturbance–diversity relationships; livestock grazing; reptiles; small mammals; passerine birds; Greece; habitat management; vertebrate diversity; intermediate disturbance hypothesis

1. Introduction

Livestock husbandry constitutes a globally important livelihood source, with 1.3 billion people relying on livestock as their principal means of subsistence [1]. However, livestock production is often viewed as a significant threat to ecosystem function [2,3]. The growing demand for livestock products and the amount of land designated for animal feed production [4,5] have brought the negative impacts of livestock production into sharp focus. Deforestation, water pollution, erosion, biodiversity loss, and greenhouse gas emissions are all associated with the intensification of domestic grazing systems [3,4,6].

Nevertheless, numerous studies also document the utility of grazing disturbance in promoting biodiversity [7,8]. Livestock grazing can enhance species richness [9], typically at intermediate intensities [10,11]. Commonly referred to as the Intermediate Disturbance Hypothesis (IDH) [12], the observed unimodal relationship between diversity and disturbance is attributed to the role of disturbance in preventing competitive exclusion, allowing for the coexistence of a greater number of species [10].

Although unimodal disturbance–diversity relationships are evident among a variety of ecosystems, taxonomic groups, and disturbance types [13], the IDH has received criticism on empirical and theoretical grounds [14]. Meta-analyses indicate that unimodal disturbance–diversity relationships are rare, with neutral, positive, or negative relationships between disturbance and species richness more commonly observed [15,16]. Variations in methodology may account for the range of disturbance–diversity relationships. For example, the IDH may not be evident if a study only analyzes a narrow range of habitat types or does not consider both disturbance frequency and intensity [17]. However, despite the lack of empirical support, Fox [14] suggests that the theoretical underpinnings of why diversity responds unimodally to disturbance regimes are flawed. He suggests nature's response to disturbance is more complex than the proposed mechanisms of species coexistence in the presence of disturbance, as outlined under the IDH. Yet, Pierce [18] warns that abandoning the IDH altogether may signal to policymakers that anthropogenic management of natural resources—such as grazing, burning, and mowing—is not important for conservation.

The majority of the grazing disturbance literature concentrates on vegetative responses to livestock grazing [9,19]. Few studies look at how grazing influences animal communities [20], most of which focus on invertebrates [21,22]. How vertebrate assemblages respond to grazing disturbance remains poorly understood. Several studies show grazing negatively impacts bird [23], reptile [24,25], and small mammal [26] abundance and richness, while others find species abundance increases with livestock grazing [27,28]. These studies are typically exclosure studies comparing vertebrate assemblages in grazed and ungrazed plots. Such exclosure studies are unable to detect how assemblages respond to intermediate grazing disturbance levels, nor do they account for the spatial and tem-

poral variations in grazing pressure under natural pastoral systems [7,29]. Additionally, these studies often focus on one particular species or group of animals, e.g., birds or small mammals, which is less informative for conservation managers interested in conserving multiple trophic guilds.

Studies on vertebrate responses to grazing generally fail to investigate the mechanisms driving grazing responses [13]. While the IDH provides a useful starting place for understanding why disturbance can promote species coexistence, a growing number of field studies suggest disturbance is a more complex phenomenon than the IDH acknowledges [14,30]. A more nuanced understanding of the mechanisms driving disturbance–diversity relationships is ultimately needed to inform land management strategies promoting wildlife diversity and ecosystem function.

We focus this study on the Greek island of Naxos, located in the Mediterranean Basin, a major biodiversity hotspot featuring many rare and endemic species [31–33]. In this region, livestock grazing has been a linchpin of human society since antiquity, with archeological evidence of domesticated animals dating back to at least 6500 BC [34]. Mediterranean scrublands have adapted to this long history of grazing [35], with many plant species exhibiting physical and chemical defenses (e.g., spiny leaves, thorns, phytotoxins) that make them resistant to herbivory [36,37].

On Naxos, like many other Aegean Sea islands, livestock husbandry of small ruminants (e.g., sheep and goats) provides an important source of subsistence and income from milk, cheese, and meat export [2,34,38]. Mixed flocks of sheep and goats are common throughout the region given their different, and thus complementary, foraging behaviors and plant species preferences, with sheep grazing and goats browsing opportunistically on the native shrubland species [39,40]. However, demographic shifts, global market fluctuations, and agricultural policies have simultaneously driven both the regional intensification and the abandonment of livestock farming practices across the Mediterranean [34,41,42]. Subsidies determined by animal numbers, in particular, have led livestock husbandry practices to shift from predominantly extensive grazing systems, where small, nomadic flocks graze primarily on natural vegetation, to intensive livestock farming systems with larger, more sedentary flocks that require high feed inputs. This has resulted in the abandonment of less-favored areas (LFAs) and heavy overgrazing of more favorable areas (e.g., accessible, fertile lowlands) [34,41]. Abandonment of pastoral lands can lead to woody plant encroachment, reducing biodiversity and increasing wildfire risk [22,42]. Alternatively, intensification of livestock production, where livestock rely primarily on artificial feed, can cause biodiversity losses, desertification, and water pollution [3,4]. Although the Mediterranean landscape has been shaped by anthropogenic stressors over millennia, the vegetation remains dynamic, often responding rapidly to changes in grazing regimes [6,35]. Understanding the responses of livestock-adapted communities to grazing, especially in an island ecosystem, provides an important contrast to other studies investigating these issues in less human-dominated systems and is imperative given the high biodiversity value of the region.

The objectives of this study were to (1) determine the nature of the relationship between small ruminant livestock grazing and vertebrate assemblages and (2) elucidate the mediating mechanisms. In contrast to previous studies focusing on a single species or animal group, we utilize a multitaxon approach, examining the response of multiple vertebrate groups simultaneously to a broad spectrum of grazing intensities. We hypothesized that vertebrate abundance would decrease with increasing livestock grazing pressure, while vertebrate species richness would peak at intermediate livestock stocking rates (Figure A1), in alignment with the IDH.

To understand the mechanisms underlying the observed disturbance–diversity responses, we investigated a number of a priori hypotheses informed by the disturbance literature. These hypotheses relate to changes in food availability [20,26], vegetation cover [43,44], and vegetation structural heterogeneity [45,46], which in turn drive vertebrate diversity responses. Using an information-theoretic approach and generalized linear modeling, we determined which habitat characteristics, modified through livestock grazing, best predict vertebrate abundance and richness.

2. Materials and Methods

2.1. Study Area

All fieldwork took place during May–July 2013 on the island of Naxos (446 km^2, 37°08′ N, 25°25′ E), located in the Cyclades archipelago (Aegean Sea, Greece) (Figure 1). A medium-sized island, Naxos was selected because it is representative of the hundreds of islands located in the region and because livestock grazing has been hypothesized to constitute an environmental challenge [38,47]. Climatic conditions are typical of the Mediterranean region, with mild wet winters and warm dry summers [48]. Phrygana, the dominant vegetation type on Naxos and in the Aegean region in general, consists of spiny, summer-deciduous aromatic shrubs and is the product of millennia of livestock grazing [49]. It is locally dominated by *Coridothymus capitatus*, *Genista acanthoclada*, and *Sarcopoterium spinosum*, interspersed with a few evergreen bush species such as *Pistacea lentiscus* and *Juniperus turbinata*. These woody plants are complemented by diverse herbaceous communities across numerous families (e.g., in the genera *Crespis* sp., *Centaurea* sp., *Bupleurum* sp., *Allium* sp., *Papaver* sp., *Plantago* sp., *Trifolium* sp., as well as numerous grasses, e.g., *Hordeum murinum*, *Avena sterilis*) [33,50]. This type of scrubland vegetation is widespread throughout the Mediterranean Basin and is home to a large number of endemic taxa [49].

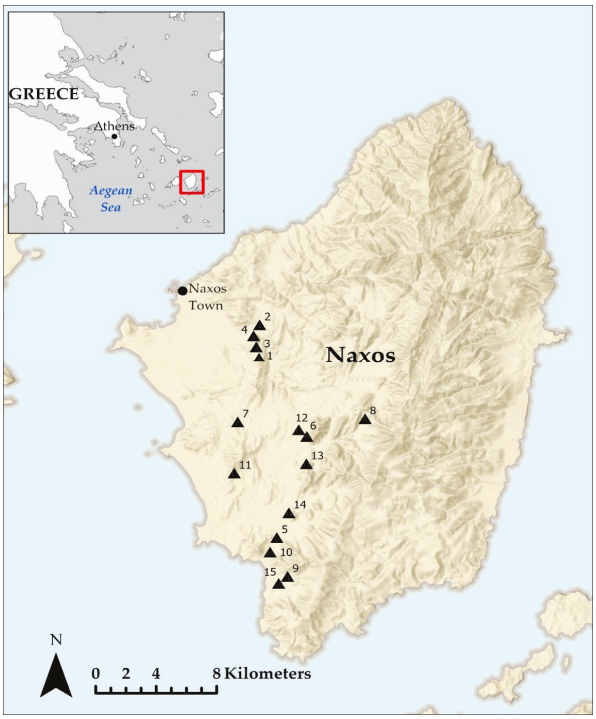

Figure 1. Map of Naxos Island. Study plots are indicated with triangles.

Naxos has a long history of livestock grazing, with remains of domesticated ungulates first documented from the Neolithic [40]. Pastoralists typically maintain mixed flocks of sheep and goats, and although livestock are often kept unfenced, they are grazed on specific land parcels [38]. Flocks of sheep and goats feed exclusively on natural pasture in the spring and summer, with supplemental feed used only during fall and the winter breeding season [51].

2.2. Study Plots

Fifteen plots were established in low elevation regions of Naxos (Figure 1) to represent a continuum of livestock grazing intensities (Figure 2). Plots were controlled for vegetation type (phrygana), substrate (limestone), and elevation (30–350 m), thus minimizing variation that may confound analyses (Table A1). Plots were 100 × 100 m (1 ha) in size such that they were large enough to reliably sample resident vertebrate taxa while still being homogeneous with regard to grazing pressure, vegetation cover, and species composition. Within each plot, four 50 m transects were established in the four cardinal directions from the plot center to quantify habitat and invertebrate diversity (Figure A2).

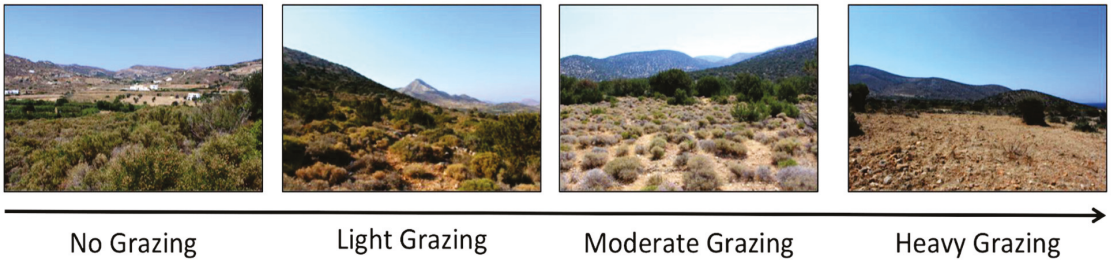

Figure 2. Images of study plots showing different vegetation types along the grazing intensity gradient.

2.3. Quantification of Grazing Conditions

We used stocking rate as a measurement of livestock (goat and sheep) grazing pressure on each study plot. Stocking rates, defined as the number of goats and sheep stocked per hectare, were based on interviews with pastoralists and validated through our own flock counts. Modifications were made to account for overinflation due to livestock passing through the plot without grazing (Appendix A). Stocking rates were further validated by quantifying livestock dung mass and general reconnaissance [52], which accounted for ground cover, vegetation height, and defoliation patterns in the area.

2.4. Vertebrate Population Measures

2.4.1. Terrestrial Vertebrates

Drift fences were used to survey ground-dwelling reptiles and mammals. Fences were constructed on each plot using a three-fence array design (modified from [53]), with three 5 m × 0.5 m lengths of plastic mesh and four 8 L buckets (Figure A2). Metal stakes were used to secure the fences at 1 m intervals. Buckets were sunk flush to the ground. Moist sponges and small rocks were placed in each bucket to prevent desiccation and overheating of animals. Each array was checked daily for a total of 20 trap nights during June and July. Traps were checked during the early morning hours prior to peak ambient temperatures (between 6:00 and 10:00 h UTC) to minimize animal stress. Animals were measured, marked using a permanent marker, and released at the point of capture. All animal handling methods were approved by the University of Michigan's Institutional Animal Care and Use Committee (IACUC) (Protocol No. 00004850).

2.4.2. Birds

We restricted our investigations to passerine bird species as they were the only birds regularly encountered on the study plots. Birds were surveyed twice at each plot in June and July 2013, overlapping with the breeding season, which lasts from late spring to early summer. Using a fixed radius point count method (modified from [54]), a researcher (E.V.) recorded birds detected (visually and aurally) within a 50 m radius from the center of the plot during a ten-minute monitoring period (Figure A2). Birds flying over the site were excluded from our analysis. Surveys were conducted between 6:00 and 10:00 h UTC under similar weather conditions (clear, low winds, and no precipitation).

2.5. Habitat Measurements

We collected habitat condition measurements to assess how livestock grazing influences habitat structure and quality. These included (1) vegetation biomass, (2) vegetation height, and (3) shrub cover.

2.5.1. Vegetation Biomass (Kg/m^2)

Eight, randomly selected, 80 × 80 cm quadrats (two quadrats along each of the four transects per plot) were sampled. All vegetation within a quadrat was clipped to ground level. All living and dead plant matter was collected, dried in the sun until brittle, and weighed (modified from [55]). Aboveground biomass values, expressed as kg/m^2 were averaged for all quadrats in each plot.

2.5.2. Vegetation Height (cm)

Vegetation height was sampled at 5 m intervals along 50 m transects radiating from the center of the study plot in the four cardinal directions. Vegetation height was later classified into six height classes: No plant, <10 cm, 10–50 cm, 50 cm–1 m, 1–2 m, and >2 m [56]. Height classifications were used to construct a vegetation structure heterogeneity index using the Shannon Index of Diversity [57] at each site, termed Foliage Height Diversity (*FHD*).

2.5.3. Shrub Cover

Shrub cover was calculated using a gap intercept method [58] to determine the amount of bare ground (gaps) greater than one meter between woody shrubs. Percent gap (% Gap) was calculated by dividing the number of recorded gaps by the total number of measurements on a plot. To obtain the % shrub cover, % Gap was subtracted from 100.

2.6. Invertebrate Population Measurements

We installed 12 invertebrate pitfall traps at each site along plot transects (three per transect at the 10, 30, and 50 m points). Pitfall traps were constructed using 320 mL plastic cups filled with 55 mL of ethylene glycol (Formula Flu Antifreeze) to preserve invertebrate samples. Cups were sunk flush to the ground under shrubs. Rocks were loosely placed over each cup to protect samples from livestock trampling. Traps were left out for ten consecutive trapping nights for a total of 120 trapping nights at each site. Invertebrates were identified to order, dried for five hours under a heat lamp, and weighed. Invertebrate biomass (g) was averaged per site. Invertebrate abundance and richness were calculated at the order level. Invertebrates were further classified according to the presence or absence of chemical and morphological defenses based on the presence of a heavily chitinized exoskeleton or noxious chemicals. This classification was then used to calculate the % undefended invertebrates at each site as a proxy for invertebrate food availability.

2.7. Statistical Analyses

We tested each variable for deviations from normality and used non-parametric statistics when transformations could not correct these violations. Relationships between livestock stocking rate and vegetation and invertebrate characteristics at each study plot were examined using Spearman's rank correlation. Livestock stocking rate was log-transformed to account for data overdispersion. Tests were two-tailed with a significance level set at $p \leq 0.05$. Generalized Linear Modeling [59] was used to determine the relationship between livestock grazing pressure and vertebrate assemblages and potential mediating mechanisms based on a priori models informed by the literature (Table 1). Vertebrate abundance, as is often the case with discrete count data, followed a Poisson error distribution, so when modeling abundance, we used Poisson errors and a log link function. Vertebrate richness, which had a normal distribution, was modeled with normal errors and an identity link function. For model selection we used an Akaike Information Criterion (AIC) approach corrected for small sample size [60,61]. The model with the lowest AICc score was considered to be the "best" model of those compared. To compare the various models, we calculated ΔAICc by subtracting the "best" model's AICc score from the AICc of the model in question. If ΔAICc < 2, a model was considered to be equally well supported by the data. Akaike weights (w_i) and evidence ratios were used to determine the degree of certainty in model selection. All statistical analyses were performed using the IBM SPSS v. 31 software package (IBM, New York, USA).

Table 1. A priori models corresponding to our hypotheses of potential mechanisms driving the grazing–vertebrate relationship.

Mechanism	Model	Reference
Food Availability	Vertebrate Richness/Abundance~Vegetation Biomass	[26]
Food Availability	Vertebrate Richness/Abundance~Invertebrate Biomass	[20]
Shrub Cover	Vertebrate Richness/Abundance~Shrub Cover	[43,44]
Vegetation Structural Heterogeneity	Vertebrate Richness/Abundance~FHD	[45,46]

2.7.1. Livestock Grazing-Environment Interactions

We used Spearman's rank correlations to elucidate the relationship between livestock stocking rate and habitat and invertebrate measurements. Habitat measurements included vegetation biomass, foliage height diversity (FHD), plant species richness, and shrub cover (Table A2). Invertebrate measures included total invertebrate biomass, overall invertebrate abundance, order-level richness, and % undefended invertebrates (Table A3). We additionally investigated the correlation between the abundance of each invertebrate order and stocking rate. Livestock stocking rate was log-transformed to account for data overdispersion.

2.7.2. Relationships Between Livestock Grazing and Vertebrate Populations

The influence of grazing on vertebrate abundance and richness was assessed using Generalized Linear Modeling. Abundance was calculated as the total number of individual birds or terrestrial vertebrates found at each study site, respectively. Species richness was the number of unique terrestrial vertebrates or bird species found at each site. In each case, we tested three models: a linear relationship between vertebrate populations and stocking rate, a unimodal relationship, and the intercept-only model. To test for unimodality, we compared a model using a second-order term against a linear model.

2.7.3. Mechanism

After determining the relationship between livestock grazing pressure and vertebrate assemblages, we ran a number of a priori models to explore potential mechanisms that could explain how grazing influences these vertebrate populations (Table 1).

3. Results

3.1. Effects of Livestock Grazing on Vegetation Traits and Invertebrate Numbers

Livestock grazing pressure correlated with several vegetation and invertebrate measures assessed at each study plot. As livestock stocking rates increased, vegetation biomass, shrub cover, and plant species richness all decreased significantly (see Table 2). No clear relationship with foliage height diversity (FHD) was observed (Table 2). Livestock grazing also had important quantitative and qualitative effects on invertebrates. While overall invertebrate abundance and order richness did not change significantly with stocking rate (Table 2), invertebrate biomass increased with stocking rate, suggesting an increase in average invertebrate body size. More importantly, there was a significant increase in the percentage of chemically and morphologically defended invertebrates as grazing pressure increased (Table 2). This correlation between livestock stocking rates and % undefended invertebrates is probably explained partially by changes in three specific invertebrate orders. Indeed, rising livestock pressure was negatively correlated with the abundance of Blattodea ($\rho = -0.615$, $p = 0.015$, n = 15, Spearman), an undefended order of invertebrates. Concomitantly, stocking rate was positively associated with numbers of Coleoptera ($\rho = 0.685$, $p = 0.005$, n = 15, Spearman) and Hemiptera ($\rho = 0.550$, $p = 0.033$, n = 15, Spearman) populations, which frequently possess morphological and chemical defenses.

Table 2. Results of Spearman's rank correlations (ρ) analyzing the relationship between stocking rate and vegetation and invertebrate measurements at each site. N = 15. Significant results ($p \leq 0.05$) are indicated with an asterisk.

Response Variable	Spearman's Rank Coefficient (ρ)	Significance (*p*-Value)
Vegetation measures:		
Vegetation biomass	−0.699	0.004 *
Shrub cover	−0.790	<0.001 *
Foliage height diversity (FHD)	−0.51	0.857
Plant species richness	−0.890	<0.001 *
Invertebrate measures:		
Invertebrate biomass	0.629	0.016 *
Invertebrate richness	0.325	0.237
Invertebrate abundance	0.396	0.144
% undefended invertebrates	−0.711	0.003 *

3.2. Vertebrate Populations Across Stocking Regimes and Proximate Drivers of Terrestrial Vertebrate and Avian Abundance and Species Richness

We recorded a total of 459 vertebrates (reptiles, mammals, and birds), representing 21 species (Table A4). Of these, 54% (n = 250, 9 species) were terrestrial vertebrates (reptiles and mammals) and 46% were passerine birds (n = 209, 12 species). Certain species were found on all sites, including the Aegean wall lizard (*Podarcis erhardii*) and the Sardinian warbler (*Curruca melanocephala*). No site harbored all study species. Furthermore, sites with the richest avian assemblages, such as Site 1, where eight different avian species were observed, had low terrestrial vertebrate richness, with only three species recorded. Several

rare terrestrial vertebrate species, for example, *Ablepharus kitaibelii* and *Sorex minutus*, were found only on grazed sites, whereas none of the less common species were found exclusively on ungrazed sites. In contrast, no such pattern was evident in birds, with several uncommon passerine species found exclusively on grazed (e.g., *Emberiza melanocephala*, *Saxicola torquata*) or ungrazed sites (e.g., *Emberiza calandra*, *Linaria cannabina*) (Table A5).

3.2.1. Terrestrial Vertebrates

Terrestrial vertebrate abundance exhibited a negative linear relationship with livestock stocking rate (Table 3 and Figure 3A). Terrestrial vertebrate abundance was best predicted by invertebrate biomass from pitfalls (Table 4). A unimodal relationship was observed between terrestrial vertebrate species richness and stocking rate (Figure 3B), with the unimodal model providing a better fit for the data than the linear or intercept-only models (Table 3). Vertebrate species richness was greatest at Site No. 11, which had a stocking rate of approximately 36 livestock/ha. However, the fitted curve in Figure 3B suggests that vertebrate species richness actually peaks at much lower stocking rates. Invertebrate biomass was the best predictor of this unimodal relationship between terrestrial vertebrate richness and livestock stocking rate; however, foliage height diversity and the intercept-only models also received considerable support with $\Delta AIC_c < 2$ (Table 4).

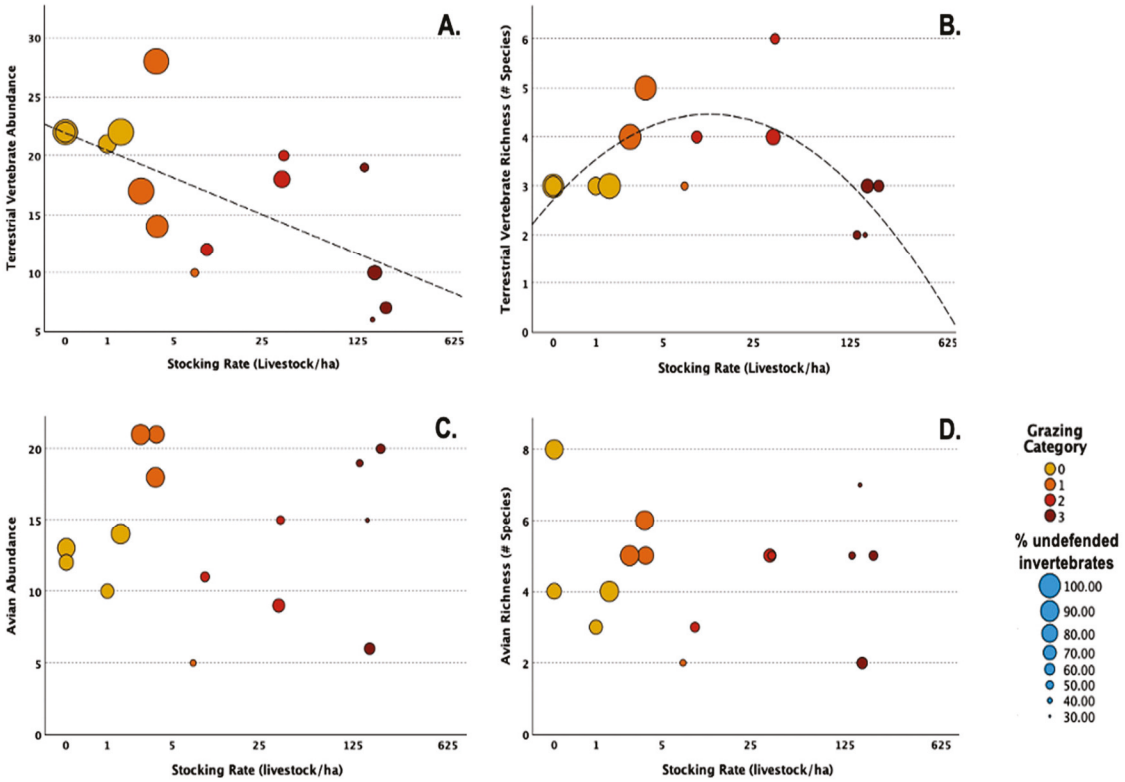

Figure 3. Relationship between livestock stocking rate (livestock/ha) and vertebrate wildlife population characteristics. (**A**) Terrestrial vertebrates: abundance (left) and (**B**) species richness (right). (**C**) Birds: abundance (left) and (**D**) species richness (right). Dashed lines show the best model using an information-theoretic approach to multimodal inference. The absence of a dashed line indicates the null model (intercept-only) provided the best fit (as indicated by the lowest AICc score). The color of each point represents the grazing category: no grazing (yellow); light grazing (orange); moderate grazing (dark orange); and heavy grazing (red). The size of the circle indicates the percent of undefended invertebrates found at each site (30–100%).

Table 3. GLM comparisons for the response of terrestrial vertebrates (small mammal and reptile) (A) and avian (B) abundance (1) and richness (2) to changes in livestock stocking rates. Models are ranked in ascending order by ΔAIC_c. The log likelihood, deviance (D), AIC_c score, AIC_c weights (w_i), and evidence ratios are given for each model. Model variables include linear and squared terms for livestock stocking rate. TVA designates terrestrial vertebrate abundance, TVR terrestrial vertebrate richness, AA avian abundance, and AR—avian richness.

	Log Likelihood	D	AIC_c	ΔAIC_c	w_i	Evidence Ratio
A1. Terrestrial Vertebrate Abundance						
TVA~stocking_rate	**−45.880**	**23.227**	**96.759**	**0.000**	**0.810**	**1.000**
TVA~stocking_rate + stocking_rate^2	−45.749	22.966	99.680	2.921	0.188	4.308
TVA~intercept	−53.017	37.502	108.342	11.583	0.002	327.504
A2. Terrestrial Vertebrate Richness						
TVR~stocking_rate + stocking_rate^2	**−18.028**	**9.717**	**48.055**	**0.000**	**0.647**	**1.000**
TVR~intercept	−22.540	17.733	50.079	2.024	0.235	2.751
TVR~stocking_rate	−21.635	15.719	51.452	3.397	0.118	5.466
B1. Avian Vertebrate Abundance						
AA~intercept	**−47.570**	**29.035**	**97.448**	**0.000**	**0.725**	**1.000**
AA~stocking_rate	−47.536	28.967	100.073	2.625	0.195	3.715
AA~stocking_rate + stocking_rate^2	−46.835	27.564	101.853	4.405	0.080	9.048
B2. Avian Vertebrate Richness						
AR ~intercept	**−28.565**	**39.600**	**62.130**	**0.000**	**0.805**	**1.000**
AR~stocking_rate	−28.560	39.572	65.301	3.171	0.165	4.882
AR~stocking_rate + stocking_rate^2	−28.372	38.594	68.744	6.614	0.030	27.303

Table 4. Model selection for predicting terrestrial (A) and avian (B) richness (1) and abundance (2) as mediated through grazing-induced changes in habitat characteristics. Models are ranked in ascending order by ΔAIC_c. The log likelihood, deviance (D), AIC_c score, AIC_c weights (w_i), and evidence ratios are given for each model. Model variables include invertebrate biomass, foliage height diversity (FHD), vegetation biomass, and shrub cover. TVA designates terrestrial vertebrate abundance, TVR terrestrial vertebrate richness, AA avian abundance, and AR—avian richness.

	Log Likelihood	D	AIC_c	ΔAIC_c	w_i	Evidence Ratio
A1. Terrestrial Vertebrate Abundance						
TVA~invertebrate biomass	**−37.376**	**10.378**	**79.843**	**0.000**	**1.000**	**1.000**
TVA~shrub cover	−48.291	28.049	101.581	21.738	0.000	52,522.661
TVA~vegetation biomass	−50.797	33.063	106.595	26.752	0.000	644,351.717
TVA~intercept	−53.017	37.502	108.342	28.499	0.000	154,3402.573
TVA~FHD	−52.495	36.459	109.991	30.148	0.000	3,520,100.154
A2. Terrestrial Vertebrate Richness						
TVR~invertebrate biomass	**−20.616**	**15.585**	**49.631**	**0.000**	**0.387**	**1.000**
TVR~intercept	−22.540	17.733	50.079	0.448	0.309	1.251
TVR~FHD	−21.576	15.586	51.325	1.694	0.166	2.333
TVR~vegetation biomass	−22.385	17.371	52.951	3.320	0.073	5.259
TVR~shrub cover	−22.504	17.649	53.189	3.558	0.065	5.924
B1. Avian Abundance						
AA~FHD	**−45.384**	**24.662**	**95.768**	**0.000**	**0.430**	**1.000**
AA~shrub cover	−46.190	26.274	97.380	1.612	0.192	2.239
AA~intercept	−47.570	29.035	97.448	1.680	0.186	2.316
AA~invertebrate biomass	−46.485	26.865	97.971	2.203	0.143	3.009
AA~vegetation biomass	−47.562	29.018	100.124	4.356	0.049	8.829
B2. Avian Richness						
AR~FHD	**−22.880**	**18.556**	**53.941**	**0.000**	**0.971**	**1.000**
AR~intercept	−28.565	39.600	62.130	8.189	0.016	60.009
AR~invertebrate biomass	−28.274	38.095	64.730	10.789	0.004	220.192
AR~vegetation biomass	−28.276	38.103	64.734	10.793	0.004	220.633
AR~shrub cover	−28.300	38.228	64.783	10.842	0.004	226.105

3.2.2. Avian Species

Avian abundance and richness showed no clear relationship with stocking rate (Figure 3B), with the intercept-only model providing the best model fit in both cases (Table 3). Avian abundance and richness were best predicted by foliage height diversity, with shrub cover also receiving support as an important driver of avian abundance (Table 4).

4. Discussion

4.1. Response of Vertebrate Assemblages to Grazing

4.1.1. Terrestrial Vertebrates

In this study, we found that while terrestrial vertebrate abundance decreased strongly with livestock stocking rate, species richness peaked at intermediate levels of livestock grazing. In both cases, invertebrate populations appear to be an important mediating mechanism, though more detailed investigations are needed.

Despite increases in reptile abundance among species preferring open habitats [28], most studies show decreased population sizes among reptiles [24,25] and small mammals [26,29] with grazing. We similarly found that the abundance of terrestrial vertebrates (reptiles and small mammals) decreased with increased stocking rates. Although previous studies speculate that food availability may drive the decrease in small mammal and reptile abundance on grazed sites (e.g., [26,62]), this has not been empirically demonstrated. Here, we find that invertebrate biomass was the best predictor of terrestrial vertebrate abundance, which is not surprising given that all the terrestrial vertebrates in this study are, to varying degrees, insectivores (Table A4). Interestingly, invertebrate biomass measured in pitfall traps increases with livestock stocking rate; however, these results likely do not reflect actual arthropod prey availability to wildlife. First, we found that the percentage of morphologically and chemically defended arthropods increases steadily with grazing intensity, suggesting that arthropod biomass alone does not capture the amount of invertebrates truly available for insectivores at a site. In addition, data from Naxos suggest that pitfall traps may provide inflated estimates of arthropod abundance in heavy-grazing contexts [63]. Grazing shifts arthropod communities away from smaller, flying, and non-defended taxa toward larger, armored, desiccation-resistant terrestrial taxa, a group that is preferentially captured in pitfall traps. As such, using different sampling methods (e.g., pan traps, sticky traps) would likely reveal that with intensified livestock grazing, the observed increase in ground arthropods is accompanied by a larger decline in undefended invertebrate numbers [63]. This study suggests terrestrial vertebrates are dependent on the invertebrate populations present. Nonetheless, a finer taxonomic resolution of arthropods, more diversified sampling methods, and a nuanced understanding of vertebrate species' dietary preferences will help further elucidate the relationship between terrestrial vertebrate abundance and invertebrate populations.

Despite reductions in overall terrestrial population abundance, we found terrestrial vertebrate richness peaked at intermediate stocking rates in accordance with the IDH. This unimodal relationship was best predicted by invertebrate biomass, implying the importance of grazing-mediated changes in invertebrate populations. However, how the increase in invertebrate biomass drives this unimodal response in terrestrial vertebrate richness is unclear. We hypothesize that (1) invertebrate biomass is one of multiple mechanisms driving terrestrial species richness [30] or that (2) invertebrate biomass is, in part, reflecting inverse changes in invertebrate richness.

Our GLM analysis indicates foliage height diversity (FHD), a vegetation structural heterogeneity measure, is also an important predictor of terrestrial vertebrate richness, giving some support to our first hypothesis. Sites with variation in vegetation structure

presumably provide greater foraging and nesting opportunities, therefore supporting more diverse terrestrial vertebrate assemblages [44]. As for the second hypothesis, although we found no significant relationship between stocking rate and invertebrate richness, previous studies show increases in invertebrate richness at intermediate grazing disturbance levels [64]. A finer taxonomic resolution [65] and a variety of invertebrate sampling methods may reveal a significant unimodal relationship between grazing and invertebrate species richness in this system. An unanswered question is as follows: Is it the quantity of food available and changes in vegetation structure driving terrestrial species richness responses? Or, is invertebrate biomass acting as a surrogate for the heterogeneity of the food source available under different grazing regimes?

4.1.2. Avian Species

Avian species richness and population abundance, contrary to our expectations, did not show any significant relationship with stocking rate. We hypothesized that avian species richness, similar to terrestrial species richness, would peak at intermediate grazing intensities, mediated through changes in vegetation structure [46]. Additionally, we expected an overall decrease in total avian populations with increased grazing, as seen in previous avian studies [23]. However, we found that neither abundance nor richness was correlated with livestock stocking rates. Instead, both avian abundance and richness were best predicted by vegetation structure complexity. Interestingly, the changes in vegetation structural heterogeneity were not related to livestock stocking rate and were likely the result of management history. The lack of a significant relationship between avian assemblages and livestock grazing is presumably because birds, by virtue of their greater mobility, select and use the landscape on a coarser scale than terrestrial vertebrates.

Although birds have shown both positive (e.g., [46]) and negative (e.g., [23]) responses to livestock grazing, several studies suggest that livestock grazing does not affect bird communities. Reynolds and Trost [66] found similar avian richness on grazed and ungrazed sagebrush sites in Idaho and attributed this finding to the mobility of birds. Similarly, in a pastureland in Australia, avian assemblages were best predicted by tree presence [28]. Since grazing intensification did not impact tree presence, birds showed no response to changes in sheep and cattle grazing pressure [28]. The bird species observed in this study tend to prefer open agricultural habitats (Table A4). Therefore, these species are most likely already adapted to a grazed landscape, and small-scale changes in livestock intensification are unlikely to impact these avian species. While outside of the scope of this study, a more robust sampling of bird populations may be able to reveal differential species responses to grazing presence and should be considered for future studies. Utilizing a naturally occurring livestock grazing continuum, our study scale (1 ha plots) was chosen to reflect current grazing practices in the region. Comparing larger study plots with more homogenous grazing pressure may result in an observable relationship between grazing intensity and avian populations. The current grazing regime in the Mediterranean does not appear to influence passerine richness or abundance, presumably because grazing does not significantly alter the food or habitat availability for avian species in the system at a regional level.

This study focused on vertebrate responses to current small ruminant livestock grazing practices on a large Mediterranean island ecosystem. However, disturbance is a multifaceted phenomenon characterized by its intensity, frequency, extent, duration, and timing [67]. Although we detected no relationship between current stocking rates (disturbance intensity) and avian richness and abundance, other aspects of grazing disturbance, such as frequency or timing, may be correlated with avian assemblages. For example, historically,

Naxian shepherds rotated grazing sites, avoiding grazing the same parcels in successive seasons to allow vegetation time to recover. However, with changing animal husbandry practices on the island, many sites are now continuously grazed. Livestock grazing can alter vegetation structure, creating heterogeneous vegetation structures [68], which our study shows is important in determining bird species richness and abundance. Therefore, we assume vegetation structural changes are correlated with the timing, duration, and/or frequency of livestock grazing rather than grazing intensity alone.

Future studies looking at multiple scales and disturbance characteristics are necessary to better understand if and how avian populations respond to livestock grazing regimes. Meanwhile, our study adds to the growing body of literature indicating that vertical vegetation structural heterogeneity plays a critical role in supporting diverse avian populations [44,45], suggesting rangeland managers and conservationists should strive to create and maintain vertical structural diversity, especially in areas where avian populations are threatened.

5. Conclusions

In this study, we found that while insectivorous terrestrial vertebrates with more limited dispersal abilities showed evidence of a unimodal disturbance–diversity response, avian richness did not show an apparent disturbance–diversity response. The mechanisms believed to drive unimodal disturbance responses (e.g., resource partitioning, competition–colonization trade-offs, temporal and spatial relative nonlinearity, and storage effects) [30,67], all focus on competitive interactions. While moderate grazing disturbance may benefit vertebrate assemblages, finding evidence of unimodal diversity–disturbance relationships among terrestrial vertebrates depends upon the methods of analysis, such as how species are grouped. Unimodal responses may only be evident when grouping species with similar dietary or habitat preferences, which are therefore in competition with one another. For example, if disturbance enables coexistence through suppression of competitive dominants, the reduction in Sardinian warbler (*Curruca melanocephala*) numbers, a primarily insectivorous bird, is unlikely to benefit the predominantly seed-eating goldfinch (*Carduelis carduelis*).

In demonstrating distinct disturbance–diversity relationships for terrestrial and avian vertebrate groups, as well as unique mechanisms mediating these disturbance responses, our findings support the need for more nuanced disturbance models than provided by the IDH. This is in line with Rivera-Núñez and colleagues' argument that the IDH lacks "clarity in defining the intensity, duration, and spatial scale of disturbances" [17]. Our results are useful in informing models that are better able to predict the impact of various disturbance regimes. Future studies should investigate a wider range of potential mechanisms, as well as how different grazing regime characteristics such as livestock type(s) (e.g., sheep, goat, pig, cow, bison, etc.) [3,41], frequency, duration, and timing of livestock grazing [38,67], or the concurrence of multiple disturbances [19] influence vertebrate populations.

Conservation Implications

These results underscore the importance of investigating the effects of disturbance across multiple groups of species. Grazing, particularly by feral goats, has long been recognized as a threat to the conservation of endemic plants and animals on islands [69,70]. Here, focusing solely on avian population responses might suggest that livestock grazing is irrelevant for wildlife conservation. However, by examining both terrestrial and avian vertebrate assemblages simultaneously, this study not only highlights their divergent responses to livestock grazing but also identifies two different mediating habitat characteristics (food availability and vegetation structural heterogeneity) that conservation managers should consider. In addition, we highlight the utility of limited livestock grazing as an

important conservation tool in Mediterranean scrublands. That said, caution is warranted when applying these findings to other insular communities. Naxos is a large island with a long history of livestock grazing; the authors expect that vertebrate responses to livestock grazing practices will depend both on island size and grazing history. In general, species communities on smaller and drier islands are less likely to tolerate intense livestock grazing, and stocking rates need to be correspondingly adjusted.

The differential disturbance responses of terrestrial and avian vertebrate species suggest no single livestock stocking rate will maximize all vertebrate groups. Our results indicate that while small mammal and reptile populations decline with increasing livestock herbivory, their species richness peaks at intermediate stocking rates (perhaps as high as 30–40 animals/ha). At the same time, avian assemblages do not appear to show a significant response to livestock grazing in this system. Taken together, this evidence suggests that while overstocking islands can profoundly impact terrestrial wildlife populations, light-to-moderate grazing is not antithetical to, and may even promote, vertebrate conservation efforts. Nonetheless, these results need to be balanced with parallel considerations of how grazing impacts plant diversity and ecosystem functioning.

Overall, livestock grazing's long history in the region supports the sustainability of this practice. This finding dovetails with previous claims that grazing can be an important conservation practice. Other scholars have suggested that policy should encourage extensification of livestock practices (e.g., lower stocking rates where livestock feed primarily on natural vegetation) rather than intensification (e.g., higher stocking rates supported by artificial feed) and abandonment of pastoral lands [7,51]. A recent study focused on Mediterranean reptile species found that, while climate was the primary factor limiting species distributions, land abandonment demonstrated a strong homogenizing effect on reptile assemblages [71]. While this suggests low-intensity grazing is critical for preserving reptile habitat, we believe a mosaic of grazing intensities would best support maximum vertebrate species richness, considering certain less common species were found exclusively on ungrazed or heavily grazed sites. Such a grazing mosaic has been suggested for optimizing invertebrate [72] and bird richness [64]. A shifting mosaic provides habitat for multiple species, as well as conditions necessary for vertebrates relying on several habitat characteristics throughout different lifecycle stages. Although our study did not investigate the effects of fire disturbance on vertebrate populations, previous work has shown the importance of fire–grazing interactions [8], and conservation managers should consider the combination of prescribed burns and livestock grazing as a way of fostering heterogeneous habitats.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board at the University of Michigan, Institutional Animal Care and Use Committee (IACUC) (Protocol No. 00004850, date of approval 30 May 2013).

Data Availability Statement: The original contributions presented in this study are included in the article and the Appendix A. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: Author Scott Brenton was employed by the company Foxfire Mushrooms, LLC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

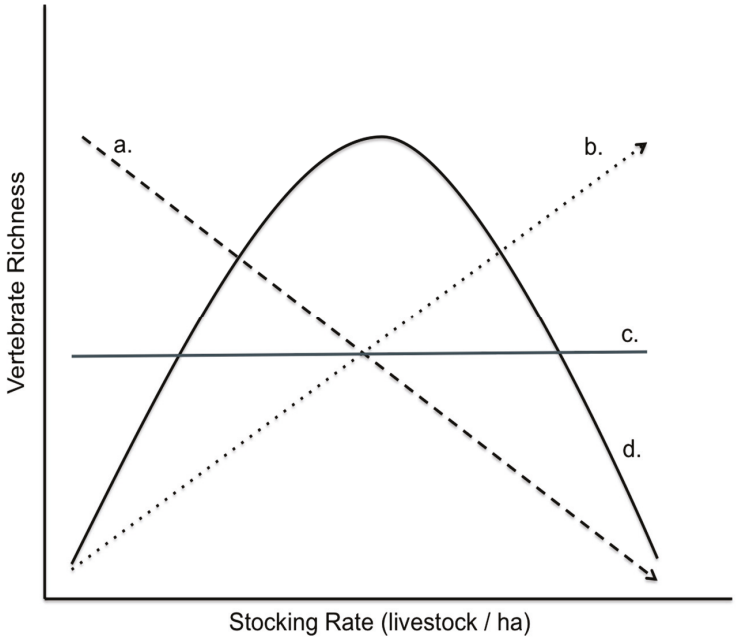

Figure A1. Hypothesized diversity–disturbance relationships for vertebrate species responses to livestock grazing: (a) negative linear, (b) positive linear, (c) neutral, and (d) unimodal.

Table A1. Site characteristics. Location, elevation, aspect, vegetation characteristics, stocking rate, and dung biomass for each study plot.

Site No.	Latitude	Longitude	Elevation (m)	Aspect	Primary Vegetation	Secondary Vegetation	Stocking Rate*	Livestock Dung Biomass (g/m^2)
1	N37°04.463′	E025°25.348′	179.53	N	*Genista*/*Calicotome*/*Cistus* Phrygana	Kermes Oak	0	0.00
2	N37°04.758′	E025°25.378′	66.49	E	*Cistus*/*Calicotome* Phrygana	Pistacea	0	0.00
3	N37°04.682′	E025°25.256′	150.87	S	*Cistus*/*Calicotome* Phrygana	Kermes Oak	1	0.33
4	N37°05.023′	E025°25.161′	54.86	W	*Genista* Phrygana	Pistacea	1.5	0.00
5	N36°58.819′	E025°25.999′	32.87	S	*Coridothymus* Phrygana	Juniper	2.5	3.11
6	N37°02.026′	E025°26.963′	197.39	W	*Coridothymus*/*Genista* Phrygana	Kermes Oak/Juniper	3.5	1.33
7	N37°02.360′	E025°24.593′	170.38	E	*Cistus*/*Calicotome* Phrygana	Pistacea	3.57	1.00
8	N37°02.457′	E025°29.137′	326.25	E	*Coridothymus*/*Genista* Phrygana	Kermes Oak	7.5	2.94
9	N36°57.625′	E025°26.374′	54.86	S	*Coridothymus* Phrygana	Juniper	9.38	5.83
10	N36.58.375′	E025.25.751′	55.42	W	*Coridothymus* Phrygana	Juniper	35	6.22
11	N37°00.794′	E025°24.470′	140.20	E	*Coridothymus*/*Genista* Phrygana	Juniper	36.1	2.39
12	N37°02.067′	E025°26.875′	206.65	W	*Coridothymus* Phrygana	Juniper	140	14.67
13	N37°01.079′	E025°27.044′	206.95	N	*Coridothymus*/*Genista* Phrygana	None	160	16.72
14	N36°59.578′	E025°26.421′	124.05	W	*Coridothymus* Phrygana	Juniper	166	59.33
15	N36°57.554′	E025°25.949′	61.63	N	Sparse *Coridothymus*	None	200	39.50

* Note: Stocking rates are defined as the number of goats and sheep per hectare and modified based on interviews with local pastoralists. If livestock merely passed through a plot, the stocking rate was divided by a factor of ten to represent the fraction of time animals spent on the site (estimated at a tenth of a day); if animals were only present on a given site for half of the year, we corrected by dividing by a factor of two.

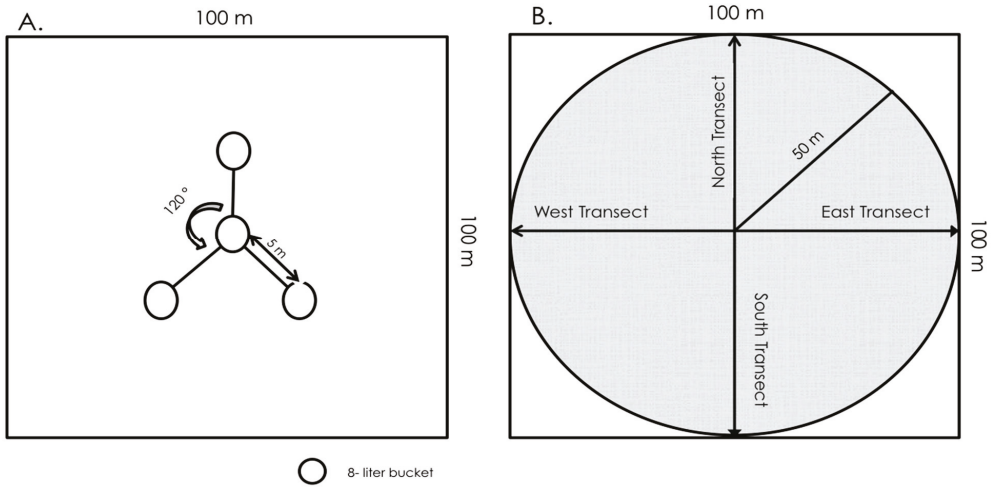

Figure A2. Implementation of vertebrate sampling methods. (**A**) Schematic of drift fence trap array with the locations of pitfall traps. (**B**) Avian point count survey design. Avian point counts were made from the center of the plot, recording birds detected within a 50 m radius (indicated in gray) during a ten-minute observation period.

Table A2. Vegetation response variables. Vegetation biomass (kg/m^2), shrub cover (%), Foliage Height Diversity (FHD), and plant species richness at each study plot.

Site No.	Vegetation Biomass (kg/m^2)	Shrub Cover (%)	FHD	Plant Species Richness
1	4.85	100	1.16	14
2	4.42	100	0.88	13
3	1.96	98	0.81	12
4	2.17	100	0.84	10
5	0.71	71	0.89	8
6	1.53	78	1.27	12
7	4.86	100	1.06	8
8	1.48	88	0.88	10
9	0.77	95	0.59	4
10	0.68	71	0.91	6
11	1.55	68	1.15	8
12	0.61	76	0.73	7
13	0.44	60	1.16	5
14	1.53	76	0.76	4
15	0.60	12	1.07	2

Table A3. Invertebrate response variables. Invertebrate biomass (g), invertebrate abundance, invertebrate order richness, and percentage undefended invertebrates at each study plot.

Site No.	Invertebrate Biomass (g)	Invertebrate Abundance	Invertebrate Richness	Undefended Invertebrates (%)
1	0.42	292	7	91.13
2	0.46	265	9	78.57
3	0.36	169	6	73.26
4	0.17	291	6	94.88
5	0.44	395	10	94.49
6	0.42	344	9	92.77
7	1.3	667	10	84.6
8	3.27	735	10	46.4
9	0.42	270	7	57.56
10	0.57	212	9	69.12
11	0.38	87	5	52.75
12	0.64	387	7	47.95
13	2.26	1376	8	37.17
14	1.29	612	10	63.17
15	1.42	401	8	56.47

Table A4. List of species observed during the study and associated foraging guilds.

Latin Name	Common Name	Foraging Guild
Curruca melanocephala	Sardinian warbler	Insectivore
Parus major	Great tit	Insectivore/Granivore
Carduelis carduelis	Goldfinch	Granivore/Omnivore
Galerida cristata	Crested lark	Granivore/Omnivore
Lanius senator	Woodchat shrike	Insectivore
Linaria cannabina	Linnet	Granivore/Insectivore
Emberiza calandra	Corn bunting	Granivore/Omnivore
Passer domesticus	House sparrow	Granivore
Emberiza cirlus	Cirl bunting	Granivore/Omnivore
Saxicola torquata	Stonechat	Insectivore
Chloris chloris	Greenfinch	Granivore/Omnivore
Emberiza melanocephala	Black-headed bunting	Granivore/Omnivore
Crocidura suaveolens	Lesser white-toothed shrew	Insectivore
Sorex minutus	Eurasian pygmy shrew	Insectivore
Lacerta trilineata	Balkan green lizard	Insectivore
Eryx jaculus turcicus	Javelin sand boa	Carnivore/Insectivore
Podarcis erhardii	Aegean wall lizard	Insectivore
Mediodactylus kotschyi	Kotschy's gecko	Insectivore
Hemidactylus turcicus	Mediterranean house gecko	Insectivore
Ablepharus kitaibelii	European snake-eyed skink	Insectivore
Vipera ammodytes meridionalis	Long-nosed viper	Carnivore/Insectivore

Table A5. Terrestrial vertebrate and avian species richness (R), abundance (A), and species assemblages by site. Species presence is indicated by an x. Sites are ordered based on livestock stocking rate (StR) from low to high. Terrestrial species abbreviations: Pe—*Podarcis erhardii*; Mk—*Mediodactylus kotschyi*; Cs—*Crocidura suaveolens*; Ak—*Ablepharus kitaibelii*; Lt—*Lacerta trilineata*; Ejt—*Eryx jaculus turcicus*; Vam—*Vipera ammodytes meridionalis*; Sm—*Sorex minutus*; Ht—*Hemidactylus turcicus*. Avian species abbreviations: Cm—*Curruca melanocephala*; Gc—*Galerida cristata*; Cch—*Chloris chloris*; Pd—*Passer domesticus*; Cca—*Carduelis carduelis*; Em—*Emberiza melanocephala*; Eci—*Emberiza cirlus*; St –*Saxicola torquata*; Eca—*Emberiza calandra*; Ls—*Lanius senato*r; Lc—*Linaria cannabina*; Pm—*Parus major*.

		Terrestrial Species											Avian Species											
Site No.	**StR**	**R**	**A**	***Pe***	***Mk***	***Cs***	***Ak***	***Lt***	***Ejt***	***Vam***	***Sm***	***Ht***	**R**	**A**	***Cm***	***Gc***	***Cch***	***Pd***	***Cca***	***Em***	***Eci***	***St***	***Eca***	***Ls***	***Lc***	***Pm***
1	0	3	22	x	x				x				8	13	x	x	x	x	x		x		x		x	
2	0	3	22	x		x		x					4	12	x			x	x				x			
3	1	3	21	x	x			x					3	10	x		x	x								
4	1.5	3	22	x	x				x				4	14	x	x	x	x								
5	2.5	4	17	x	x			x	x				5	21	x	x	x	x		x						
6	3.5	5	28	x	x	x	x				x		6	18	x	x	x	x		x	x					
7	3.57	5	14	x	x	x		x		x			5	21	x	x	x	x	x							
8	7.5	3	10	x	x	x							2	5	x							x				
9	9.38	4	12	x	x	x	x						3	11	x	x	x									
10	35	4	18	x	x	x				x			5	9	x	x			x	x						x
11	36.1	6	20	x	x	x			x		x	x	5	15	x	x	x		x	x						
12	140	2	19	x	x								5	19	x	x	x	x		x						
13	160	2	6	x	x								7	15	x	x	x	x	x		x	x				
14	166	3	10	x	x		x						2	6	x	x										
15	200	3	7	x		x	x						5	20	x	x	x	x						x		

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Article

Wildfire-Driven Shifts in Bird and Red Fox Activity: A Case Study from Biebrza National Park

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Simple Summary: Climate change is likely to cause more frequent and intense wetland fires, which can affect wildlife. This study looked at how birds that eat rodents or scavenge reacted to a large wildfire in Biebrza National Park, Poland, in 2020. We observed bird activity (expressed by the number of observed birds during a 1-h period) in both burned and unburned areas over different seasons. We found that bird activity depended on the combination of two factors, i.e., the fire and the season. Rodent-eating raptors and owls were more active in burned areas during summer, likely because it was easier to hunt when plant cover was removed by fire. Scavenging corvids were most active in burned areas right after the fire, probably due to the presence of dead animals and good feeding opportunities. Activity of wading birds (herons, cranes, and storks) feeding on a variety of food items (both plant and animal food) increased slightly in burned areas, but their activity mostly followed seasonal patterns. Overall, some bird species may benefit temporarily from fire-damaged areas, but their responses vary depending on the species and conditions of the fire.

Abstract: Climate change is expected to increase the frequency and severity of wetland fires, posing risks to biodiversity. We investigated changes in the foraging activity of predators and scavengers in response to a large wildfire (over 5500 ha of marshland) in Biebrza National Park, NE Poland, in the spring of 2020. Using GLMMs on bird records from 10 points in burned and unburned areas across seasons, we assessed the responses of rodent-eating raptors, corvids, and wading birds (herons, cranes, and storks). Bird numbers varied significantly with the interaction between fire and season, although fire alone was not a significant factor. Rodent-eating owls and raptors were recorded most frequently in burned areas during summer 2020 (3.5 in burned vs. 1.7 records/hour in control areas), likely due to improved hunting conditions in reduced vegetation. Corvids peaked in burned areas just after the fire (3.1 vs. 1.4 records/hour), presumably in response to carrion availability. Wading birds showed a slight post-fire increase in burned habitats (2.4 vs. 1.7 records/hour), yet their abundance was mostly season-driven. These results suggest that fire-altered habitats may provide short-term foraging advantages for certain bird groups. However, responses are species-specific and depend on fire timing, intensity, and habitat preferences.

Keywords: avian predators; rodent-eating raptors; scavengers; corvids; red fox; post-fire ecosystem recovery; post-fire foraging dynamics; seasonal predator activity

1. Introduction

Fires of natural or anthropogenic origin shape some ecosystems on Earth; this disturbance can maintain the landscape and influence many processes like vegetation structure, carbon, and hydrological cycle, climate, and others [1–3]. Because of climate change, wildfires occur more frequently and can have a devastating impact on wildlife, humans, and the economy [3–6]. Wetlands are recognized as biodiversity hotspots for both plants and animals [7,8], and Central European wetlands seem to be the most threatened by changes to the hydrological regime as predicted by climate projections [9]. Although burning can be among conservation measures within management strategies of marshlands [10], the predicted droughts affecting wetland water regimes are likely to result in more frequent fires [11]. Their effect, especially on more sensitive species, is hard to predict as the impact of wildfires on birds and mammals is multiform and insufficiently known. It may depend on fire characteristics, species' traits [12], and the time scale considered [13]. First, wildfire can directly influence animals by killing or injuring them [14–17]. Also, reduced protective cover may result in higher predation rates of survivors and newcomers in post-fire areas [18–20]. An increased predation risk can also be due to reduced prey body condition after fire and/or an increased need to forage in risky areas due to being energetically stressed [21,22]. All these may create good feeding opportunities for predators and scavengers. Indeed, corvids and some predatory birds travel to fire-affected areas to take advantage of easier hunting in burnt landscapes and increased scavenging opportunities [22–24]. They may even exploit fire fronts to capture prey flushed out by flames [23]. Adversely, fire may affect the rodent community [25], thus reducing prey for rodent-preying predators, which may result in significant declines in predatory species [13].

The Biebrza Valley is recognized as one of the most extensive wetlands in central and western Europe. In this study, we evaluated the impact of the large wildfire of spring 2020 (the largest open landscape fire in Poland after World War II, [26]) on the foraging activity of different groups of predators and scavengers. Following the expected increase in fire frequency, more knowledge of the impact on freshwater wetlands is crucial for future risk assessment. We focused on birds that belong to different trophic groups (and can be classified as predators, scavengers, or omnivores) by comparing the intensity of their penetration of open habitats of a vast freshwater wetland area shortly after the fire and during the following seasons. We may assume that in the case of rodent-eating avian predators, fire must have sharply reduced the abundance of available prey, especially in the short term [13,25]. Therefore, their reduced activity in the burned areas was expected, with an increased foraging activity when the area starts to be recolonized [19–21]. In turn, corvids, taking advantage of the increased availability of carrion of fire victims [24], were expected to be more active in the fire-affected areas shortly after the fire event. We also focused on wetland-associated long-legged wading birds that prey on variable vertebrate and invertebrate prey. We assumed that areas heavily disturbed by fire, with a limited vegetation cover, can be attractive foraging sites for this diverse bird group [13,27]. Finally, we focused on the red fox *Vulpes vulpes*, the most common and widely distributed medium-sized carnivore [28–31] for which small rodents are crucial prey [32,33]. Small rodent availability shapes space use by the red fox [30]. Although the species richness and abundance of small mammals are often lowest immediately after a fire, they recover (or even surpass pre-fire levels) as vegetation progresses [34]. We, thus, predicted that in winter (approximately eight months after the fire), small mammal availability would

be similar in the fire-disturbed and the control areas, reflected by the similar penetration intensity of this medium-sized carnivore.

2. Methods

2.1. Study Area

The study was conducted in Biebrza National Park, located in NE Poland (53°28′48.3″ N 22°38′19.3″ E, Figure 1). It is the largest (nearly 60,000 ha) national park in Poland and one of the biggest in Europe. It is one of central and western Europe's biggest, still well-preserved wetland areas [35]. The most important element of this area is typically lowland, around 160 km long, and meandering the Biebrza River. Biebrza is one of central and western Europe's last big natural rivers. The national park's landscape is dominated by open meadows, pastures, and rushes. Over 40% of habitats are hydrogenic [36]. Forests constitute around 25% of the park and are dominated by birches *Betula* spp., black alder *Alnus glutinosa*, and Scots pine *Pinus sylvestris* [37]. Biebrza National Park is recognized as a Natura 2000 site and included in the List of Wetlands of International Importance [38]. Because of climate change, flooding in the Biebrza River has been highly limited in recent decades [39]. Historically, all areas were flooded in spring, but recently, permanent drought has been noted. As a result, wildfires are becoming more and more common [40]. Most of the fires in the study area occur due to illegal burning [30].

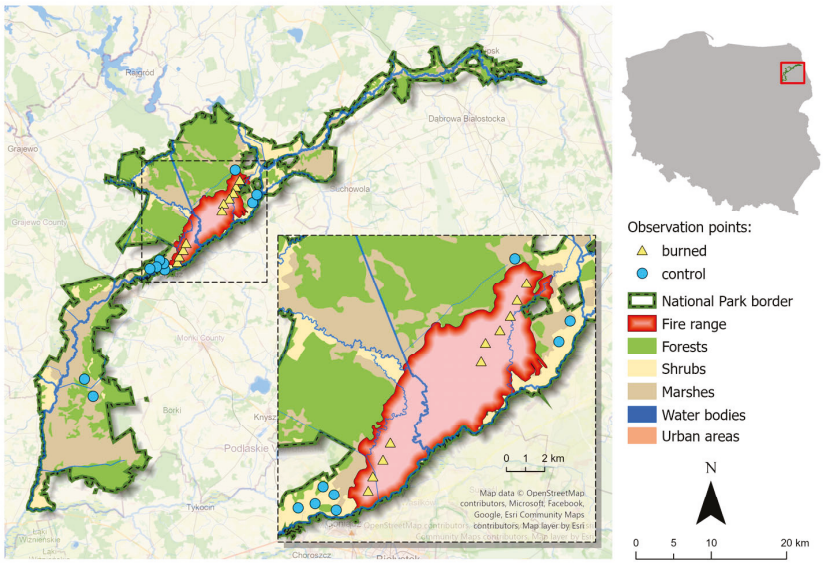

Figure 1. Location of Biebrza National Park in Poland, range of the wildfire in the Biebrza National Park (19–26 April 2020), and location of bird observation points within the burned and outside burned area (control).

2.2. The Fire in the Biebrza National Park

In April 2020, the water level in the river was the lowest (104 cm) compared to all measurements performed in this month since 1950, when the measurements started [40]. The wildfire that broke out on 19 April (and lasted until 26 April) was the biggest ever recorded in this area. Over 5526 ha of meadows and forests burned (Figure 1). The very rapid spread of the fire in the open space, with the blowing wind changing directions and the inaccessibility of the area for fire-fighting vehicles, made the fire one of the most extensive wildfires in Poland after the Second World War and the biggest outside forests [30]. Nevertheless, the fire did not affect the peat substrate. Detailed vegetation cover data were unavailable. Nevertheless, in spring 2020, burned and controlled areas differed very clearly;

in summer 2020, this difference was less vivid, yet the vegetation in the burned areas was less abundant [41] (Figure 2). Vegetation seemed to recover within a few months after the disturbance [42], and in spring 2021, the vegetation did not differ visibly (Figure 2).

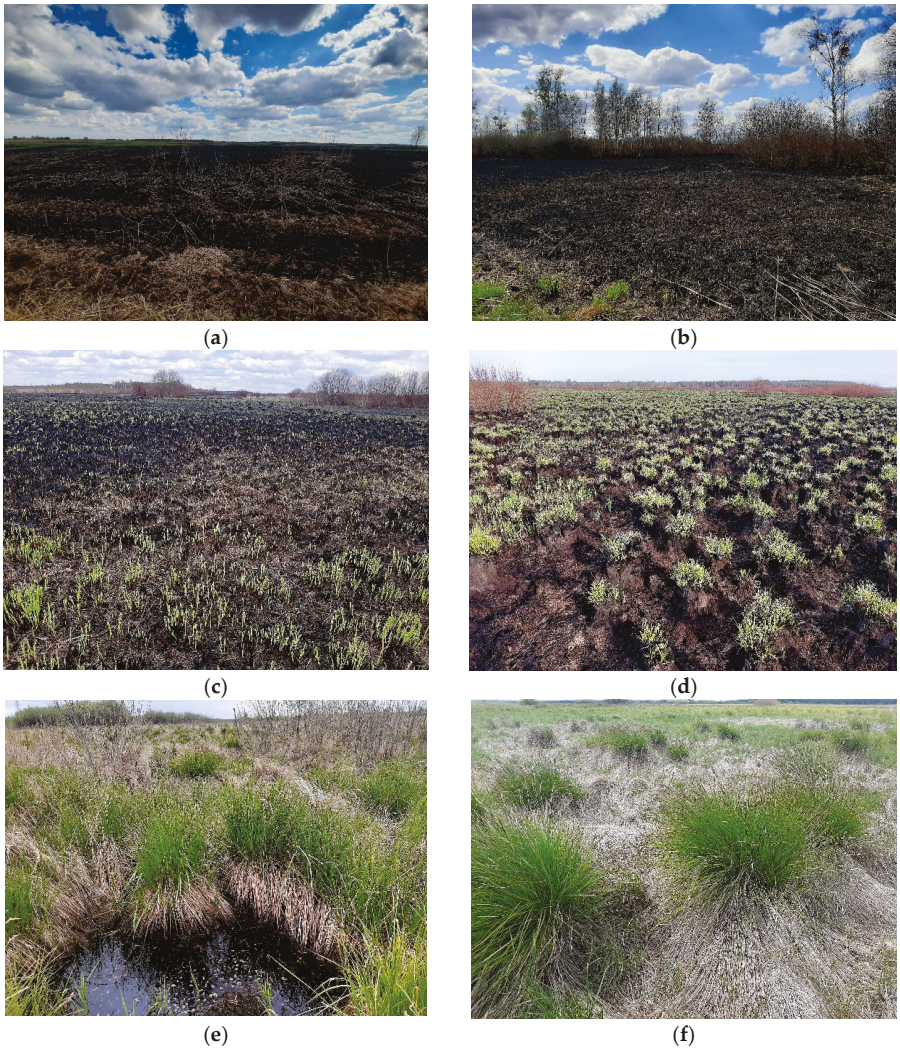

Figure 2. Landscape of study area in burned and control sites: (**a**,**b**) burned area just after the fire, (**c**) burned area—regenerating vegetation on 4 May 2020, (**d**) burned area—regenerating vegetation on 13 May 2020, and (**e**,**f**)—unburned area on 25 May 2020.

2.3. Fieldwork

2.3.1. Assessment of the Number of Fire Victims

Just after the end of the fire, we counted all dead vertebrates on a 23,438 m long and 4 m wide transect (93,752 m^2 in total, around 0.2% of the fire-destroyed area) to assess carrion availability for predators and scavengers and to roughly estimate the number of fire victims. We penetrated different parts of the burned area, moving along the selected observation points (see below). An observer walked slowly, looking for even the smallest vertebrates on the right and left sides of the transect. We used a GPS receiver and a 2 m long stick to assess the transect width properly. In the case of remnants of bird nests, we usually could not assess if they were occupied and how many chicks died/eggs were destroyed. Thus, to avoid overestimating the victims, we arbitrarily claimed that in one nest, one bird died. Finally, we extrapolated our results on the entire burned area to roughly estimate how many vertebrates were fire victims. This data gave us information about the potential scavenger food base and the scale of loss for

predators that hunt small mammals. During the fieldwork, we omitted remnants of animals that had clearly died before the fire, i.e., if we found only bones from big mammals without other tissues. In such cases, we assumed that they had probably been killed by wolves *Canis lupus*, or, in the case of wild boars *Sus scrofa*, were victims of African swine fever that broke out in this part of Poland in 2014 [43,44].

2.3.2. Assessment of Bird Activity in Burned and Unburned Areas

Simultaneously, we selected 10 bird observation points in the burned area. The distance between the nearest points was around 1 km. Similarly, 10 adequate observation points were selected in unburned (i.e., control) areas of the National Park around (Figure 1). The points were set as systematically as possible. Yet, their placement was adjusted so as to place them in similar surroundings (i.e., not in arable lands) and to avoid forest and willow bushes, which could hinder visibility and limit observational distance. Point placement was also affected by logistical reasons, as some parts of the park were inaccessible (i.e., there were no roads in the vicinity, and the interior of the marshes had very few paths and was hard to penetrate due to extensive peat bogs, marshes, and floodplains, being able to be reached only by boat when flooded). Each point was visited twice in spring (just after the fire, i.e., at the very beginning of May), in summer (July), and autumn (November) of 2020, and the next spring, in early May of 2021. One observation session at one point lasted 30 min. After this time, the observer moved to another point. The procedure was conducted for a few consecutive days. We pooled observations from one point from two 30-min sessions in the season. As a result, observation was conducted for 1 h each season at each point. Altogether, we conducted the observations for 80 h. Observations were conducted by binocular ZEISS (Oberkochen, Germany) 10 × 42 in the morning (mostly between 6 and 10 a.m.) during good weather conditions. We recorded the number of birds assigned to a certain species observed during one session. If one bird was observed for a longer period during a single session, it was counted just once. If a bird appeared more than twice during a session and we were not sure if it was the same individual or a new one, we adopted a 15-min time lapse until we recorded it once more. We pooled observations of various bird species into four groups: (i) raptors and owls preying on small rodents (the common buzzard *Buteo buteo* [45,46], rough-legged buzzard *Buteo lagopus,* the common kestrel *Falco tinnunculus* [47], long-eared and short-eared owl *Asio otus* and *A. flammeus* [48,49], lesser spotted eagle *Clanga pomarine* [50], greater spotted eagle *Clanga clanga* [51], the western marsh harrier *Circus aeruginosus*, or the Montagu's harrier *Circus pygargus* [52,53]), (ii) corvids (i.e., the common raven *Corvus corax*, hooded crow *Corvus cornix*, and magpie *Pica pica*), being opportunistic foragers often feeding on carrion, [54–59]), and (iii) long-legged wading birds (i.e., grey heron *Ardea cinerea*, great egret *A. alba*, white and black storks *Ciconia ciconia*, *C. nigra*, crane *Grus grus*), opportunistic predators, eating various prey [60–64]. From our observations, we excluded birds from the last group that flew at high altitudes, and their presence was clearly unrelated to foraging or prey search. The remaining raptors were other raptor species (i.e., white-tailed eagle *Haliaeetus albicilla*, goshawk *Astur gentilis*, sparrowhawk *Accipiter nissus*, hobby *Falco subbuteo*).

2.3.3. Penetration Intensity of Burned and Unburned Areas by the Red Fox

During winter 2020/2021 (approx. 10 months after the fire), we conducted snow tracking of the red fox in burned and unburned areas. The method of snow tracking is a well-established method for studying red fox habitat use, and it estimates activity levels of this medium-sized carnivore [29,31]. We carried out snow tracking 1–3 days after a snowfall, adjusting the number of tracks registered for 24 h of snow cover. The snow

tracking in the burned and control areas was conducted during the same days, so the snow conditions were similar. We calculated a relative index of fox abundance as N tracks/km/24 h of snow cover. Tracks were identified based on shape, size, and the placement of toepads. The length of the transects in the burned area was 22 km, and in the reference area, 23 km. The transects were placed in open areas, also in the vicinity of the birds' observation points.

2.4. Statistical Analysis

The difference in the total number of birds recorded in burned and control areas was checked with student's *t*-tests. To test if the number of recorded birds assigned to the four groups changed in response to the fire occurrence (and its interaction with season), we used two-way PERMANOVA (999 permutations) with repeated measures, using Bray–Curtis dissimilarity. The obtained *p*-values were adjusted for multiple comparisons using Bonferroni correction. Fire occurrence (burned vs. control areas), season (spring 2020—just after the fire, summer 2020, autumn 2020, or spring 2021), and the interaction between the two were included as independent variables. This analysis was conducted using the adonis2() function from the vegan package [65] in R [66]. Next, for each of the four groups of birds, we used a generalized linear mixed model (GLMM) to check if fire occurrence or the interaction between fire occurrence and the season affected the number of records of each defined bird group separately. The models assumed a Poisson distribution with a log link function. The response variable was the number of birds recorded per observation unit. Two fixed factors were included: fire (burned vs. control) and its interaction with season (spring_2020, summer_2020, autumn_2020, and spring_2021). A random effect was specified for the sampling location (observation points) to account for repeated measures. Next, post hoc pairwise comparisons using estimated marginal means (Bonferroni corrected *p* values) were used to test the difference between burned and control areas with seasons. Mann–Whitney U test was used to compare the number of red fox tracks in the burned and control areas. The analyses were completed with SPSS (PS IMAGO PRO 10) software [67].

3. Results

3.1. Number of Fire Victims

We found 47 fire victims on the transect in the burned area. The biggest share of them were amphibians (N = 19). The biggest animals found dead were red fox and white stork (Table 1). Extrapolating our results, on the whole, burned area, we can roughly assess the minimum number of vertebrates killed by fire on 27,630 individuals.

Table 1. List of fire victims detected on the sample transect (23,438 m long, 4 m wide) in the burned area of Biebrza National Park.

Fire Victim	N
Lepus europaeus (juv.)	1
Erinaceus sp.	1
Vulpes vulpes	1
Talpa europaea	2
Sorex sp.	1
Small rodents unidentified	5
Pica pica	1
Ciconia ciconia	1
Bird unidentified	3
Bufo spp.	9
Ranidae	6
Triturus/*Lissotriton*	1

Table 1. *Cont.*

Fire Victim	N
Amphibian unident.	3
Anguis fragilis	1
Natrix natrix	1
Lacerta/*Zootoca*	1
Fish	3
Unidentified vertebrates (excl. birds)	2
bird nests	4
Total	47

3.2. Bird Activity in the Burned and Unburned Areas

Overall, we recorded 531 bird observations, 272 in the burned and 259 in the reference areas; on average, there were 6.8 birds per hour of observations in the burned (SE = 0.57) and 6.48 (SE = 0.46) in the control sites (Appendix A). The number of observed birds assigned to the four groups (Table 2) depended on the interaction between the seasons and the fire occurrence (two-way PERMANOVA with repeated measures, fire*season F = 0.071, df = 3, $p < 0.05$).

Table 2. The number of birds observed from different trophic groups in the burned (F) and control (C) areas in the Biebrza National Park during four seasons. Spring 2020—just after the fire was extinguished. Wading birds—herons, cranes, and storks.

Season		Birds' Group (N Records)				
		Rodent-Eating Raptors and Owls	Corvids	Wading Birds	Other Raptors	Total
Spring 2020	F	13	31	25	5	74
	C	22	14	17	8	61
Summer 2020	F	35	16	23	6	80
	C	17	16	34	3	70
Autumn 2020	F	7	29	2	6	44
	C	10	33	2	2	47
Spring 2021	F	21	15	33	5	74
	C	23	21	31	6	81

Owls and raptors that prey on rodents were recorded 76 times in burned and 72 times in control areas (Table 2). The overall model (GLMM) for rodent-eating raptors and owls was statistically significant (F = 3.259, df = 7, $p = 0.005$). The interaction between fire and season was significant (F = 3.668, df = 6, $p < 0.005$), indicating that the effect of fire changed seasonally. Nevertheless, fire's main effect was insignificant (F = 0.111, df = 1, $p > 0.05$). Rodent-eating raptors and owls were recorded most frequently in burned areas in summer 2020 (a few months after the fire, 3.5 records/hour vs. 1.7 in control points at the same time, Figure 3). In turn, in spring 2020 (just after the fire) the abundance of birds was lower in burned than in control points (1.3 vs. 2.2 records/hour, Figure 3). Tested interactions pointed to a substantial increase (2.64-fold) in rodent-eating raptor abundance in burned plots in summer 2020 ($\beta = 0.972$, SE = 0.322, $p = 0.004$) (Table 3). Other interactions were not significant (Table 3). Post hoc pairwise comparisons of estimated marginal means revealed a significant difference between burned and control areas only in summer 2020 ($p = 0.017$) (Figure 3).

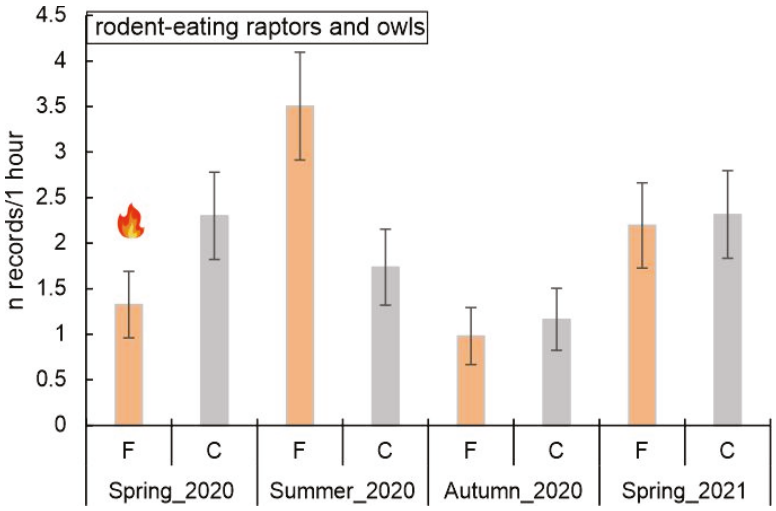

Figure 3. Predicted number of records of rodent-eating owls and raptors (marginal means, ±SE) across different seasons (spring_2020, i.e., just after the fire; marked with flame symbol) and fire conditions (F—burned, C—control areas). Post hoc pairwise comparisons (Bonferroni corrected) showed the difference between burned and control areas (p = 0.017) in summer 2020.

Table 3. The effect of fire occurrence, season (spring_2020, i.e., just after the fire) and the subsequent periods), and their interaction on the number of records of rodent-eating raptors (n records/1 h) observed in the 10 sampling locations (observation plots) located within the burned area and in the control areas shown by GLMM. 0 *—reference category. Significant effects marked in bold.

Model Term	β	SE	t	p	95% CI	
					Lower	Upper
Intercept	0.833	0.2085	3.997	<0.001	0.418	1.249
Fire = fire	−0.551	0.3448	−1.599	0.114	−1.239	0.136
Fire = control	0 *					
[Fire = fire] × [Season = Summer_2020]	**0.972**	**0.3224**	**3.015**	**0.004**	**0.329**	**1.615**
[Fire = fire] × [Season = Autumn_2020]	−0.301	0.4212	−0.715	0.477	−1.141	0.538
[Fire = fire] × [Season = Spring_2021]	0.504	0.3478	1.450	0.151	−0.189	1.198
[Fire = fire] × [Season = Spring_2020]	0 *					
[Fire = control] × [Season = Summer_2020]	−0.281	0.3179	−0.885	0.379	−0.915	0.352
[Fire = control] × [Season = Autumn_2020]	−0.681	0.3597	−1.894	0.062	−1.398	0.036
[Fire = control] × [Season = Spring_2021]	0.006	0.2944	0.021	0.983	−0.581	0.593
[Fire = control] × [Season = Spring_2020]	0 *					

Corvids were recorded 175 times, 91 times in burned areas and 84 times in the control areas. The number of observed corvids in the burned and control areas was similar during three out of four seasons. Only shortly after the fire (in spring 2020), the number of observations in burned areas was higher than in control points (3.1 vs. 1.4 records/hour). The model for corvids (GLMM) was statistically significant (F = 2.727, df = 7, p = 0.014). The effect of fire on corvid abundance depended on the season (Fire*Season, F = 3.159, df = 6, p = 0.008), while the fire occurrence itself was not significant (p > 0.05). In spring 2020 (just after the fire), corvid abundance was more than twofold higher in burned than in control observation points (β = 0.794, p = 0.026). In turn, negative interactions were shown for summer 2020 (β = −0.661, p = 0.035) and spring 2021 (β = −0.726, p = 0.024), pointing to a lower abundance of corvids in burned plots at those times than in spring 2020 (just after the fire). The abundance of corvids in the control areas was highest in autumn 2020 (β = 0.857, p = 0.009) (Table 4, Figure 4). Post hoc pairwise comparisons showed the effect of fire occurrence on corvids' abundance in spring 2020 (p = 0.025) (Figure 4).

Table 4. The effect of fire occurrence, season (spring_2020, i.e., just after the fire), and the subsequent periods, and their interaction on the number of records of corvids (n records/1 h) observed in the 10 sampling locations (observation plots) located within the burned area and in the control areas shown by GLMM. 0 *—reference category. Significant effects marked in bold.

Model Term	β	SE	t	*p*	95% CI	
					Lower	Upper
Intercept	0.320	0.2835	1.130	0.262	−0.245	0.885
Fire = fire	**0.794**	**0.3486**	**2.277**	**0.026**	**0.099**	**1.489**
Fire = control	0 *					
[Fire = fire] × [Season = Summer_2020]	**−0.661**	**0.3078**	**−2.149**	**0.035**	**−1.275**	**−0.048**
[Fire = fire] × [Season = Autumn_2020]	−0.067	0.2583	−0.258	0.797	−0.582	0.448
[Fire = fire] × [Season = Spring_2021]	**−0.726**	**0.3145**	**−2.308**	**0.024**	**−1.353**	**−0.099**
[Fire = fire] × [Season = Spring_2020]	0 *					
[Fire = control] × [Season = Summer_2020]	0.134	0.3660	0.365	0.716	−0.596	0.863
[Fire = control] × [Season = Autumn_2020]	**0.857**	**0.3190**	**2.688**	**0.009**	**0.222**	**1.493**
[Fire = control] × [Season = Spring_2021]	0.405	0.3450	1.175	0.244	−0.282	1.093
[Fire = control] × [Season = Spring_2020]	0 *					

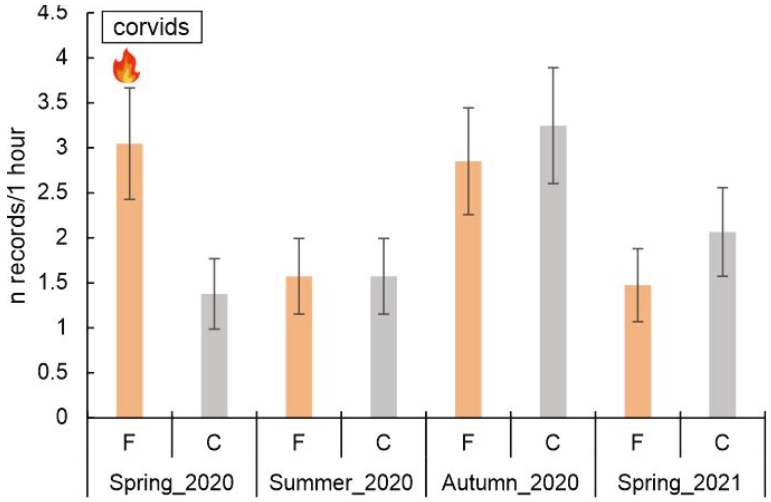

Figure 4. Predicted number of records of corvids (marginal means, ±SE) across different seasons (spring_2020, i.e., just after the fire; marked with flame symbol) and fire conditions (F—burned, C—control areas). Post hoc pairwise comparisons (Bonferroni corrected) showed differences ($p = 0.025$) between burned and control areas in spring 2020.

The long-legged wading birds (herons, cranes, and storks) were observed 167 times, 83 and 84 times in burned and control areas, respectively. The overall model for wading birds was highly significant (F = 5.010, df = 7, $p < 0.001$), indicating that the predictors (fire, season, and their interaction) explained a substantial amount of variance in wading bird abundance. The main effect of fire was not significant ($p = 0.980$). However, the interaction between fire and season was highly significant (F = 5.830, df = 6, $p < 0.001$). In burned plots, a substantial reduction in wading birds' abundance was observed in autumn 2020 (β = –2.526, $p < 0.001$). Within control plots, abundance varied seasonally. Wading birds' abundance was significantly higher in summer 2020 and marginally higher in spring 2021, while a significant decrease was observed in autumn 2020 (Table 5, Figure 5). Post hoc comparisons of estimated marginal means showed no statistical differences between burned and control areas in any season.

Table 5. The effect of fire occurrence, season (spring_2020, i.e., just after the fire) and the subsequent periods), and their interaction on the number of records of herons, cranes, and storks (n records/1 h) observed in the 10 sampling locations (observation plots) located within the burned area and in the control areas shown by GLMM. 0 *—reference category. Significant effects marked in bold.

Model Term	β	SE	t	*p*	95% CI	
					Lower	Upper
Intercept	0.526	0.2585	2.037	0.045	0.011	1.042
Fire = fire	0.364	0.3392	1.072	0.287	−0.313	1.040
Fire = control	0 *					
[Fire = fire] × [Season = Summer_2020]	−0.083	0.2889	−0.289	0.774	−0.659	0.493
[Fire = fire] × [Season = Autumn_2020]	**−2.526**	**0.7348**	**−3.437**	**<0.001**	**−3.991**	**−1.061**
[Fire = fire] × [Season = Spring_2021]	0.278	0.2651	1.047	0.299	−0.251	0.806
[Fire = fire] × [Season = Spring_2020]	0 *					
[Fire = control] × [Season = Summer_2020]	**0.693**	**0.2970**	**2.333**	**0.022**	**0.101**	**1.285**
[Fire = control] × [Season = Autumn_2020]	**−2.140**	**0.7475**	**−2.863**	**0.005**	**−3.630**	**−0.650**
[Fire = control] × [Season = Spring_2021]	0.601	0.3018	1.991	0.050	−0.001	1.202
[Fire = control] × [Season = Spring_2020]	0 *					

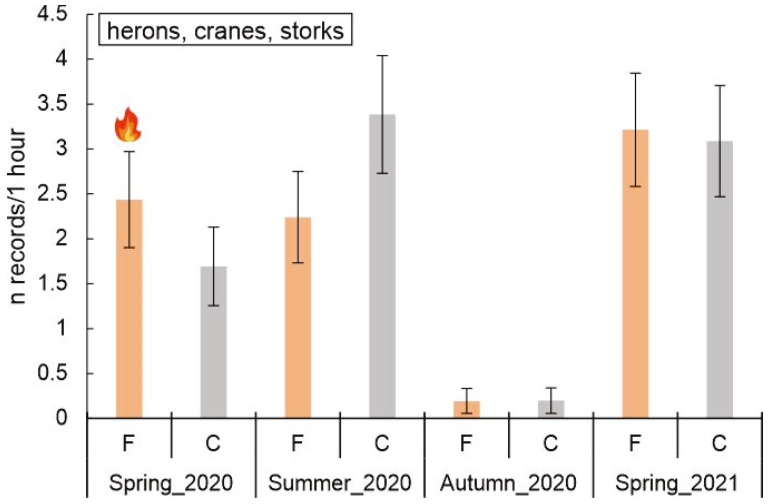

Figure 5. Predicted number of records of wading birds (herons, cranes, and storks) (marginal means, ±SE) across different seasons (spring_2020, i.e., just after the fire; marked with flame symbol) and fire conditions (F—burned, C—control areas). No significant differences between burned and control plots in any season were shown by post hoc pairwise comparisons.

The overall model (GLMM) for the remaining birds of prey pooled together was not significant (F = 0.784, df = 7, $p > 0.05$); their abundance was not affected by either the fire occurrence (F = 0.005, df = 1, $p > 0.05$) or the interaction between the two factors (F = 0.836, df = 6, $p > 0.05$).

3.3. Penetration Intensity of Burned and Unburned Areas by the Red Fox

The red fox density index during the winter of 2000/2021 was 6.86 tracks/km/24 h (SE = 0.411) in the burned area and 6.13 tracks/km/24 h (SE = 0.432) in the control area. These values did not differ significantly (Mann–Whitney test, U = 1851, $p > 0.05$).

4. Discussion

4.1. Fire-Induced Mortality

Our knowledge of fire-induced mortality of animals, especially via high-intensity wildfires as opposed to planned fires, is very low [21]. Our estimations of 30,000 fire victims are probably

highly understated, as spotting all small vertebrates in black ash is impossible, or their bodies may have been completely calcined [20]. Also, rodents, moles *Talpa europaea*, and some reptiles could have died in burrows because of high temperatures, lack of oxygen, or toxic smoke [20]. Some other individuals could have died even later due to, e.g., fire injuries [21]. It is unclear how many animals may have survived the fire [20,21], yet we can assume that a big part of the small mammals present in this area probably died during or after the fire. The most abundant rodent species in Biebrza Marshes is the root vole *Microtus oeconomus*. The spring density of this population fluctuates yearly, from just a few to 150 ind./ha [68]. Its abundance in the burned areas before the fire occurred is unknown, yet the population abundance in other Biebrza marshes areas was over 50 ind./ha [41]. Amphibian density on open marshland is challenging to estimate. However, on sedgeland in the Narew River Valley (located nearby, Biebrza being its tributary), the moor frog *Rana arvalis* density was between 300 and 1800 ind./ha. These values may be even higher in riparian habitats along the river [69]. Considering the annual life cycle of anurans inhabiting our study area, all the species of frogs and toads must have already emerged from the hibernation sites at the time of the fire, thus being at risk of fire. Even if just a fraction of amphibians were killed by the fire, the number of fire victims would be over 1.5 million. Overall, an estimated animal mortality would result in a very high short-term carrion availability for scavengers.

4.2. Bird Activity as a Response to Fire Occurrence

The two factors affecting bird occurrence in the burned areas are vegetation and food alterations [17]. The effect of fire is vivid primarily shortly after the event [70] and is species dependent [17]. In our case, a higher abundance of corvids in the burned area was observed shortly after the fire. This aligns with our data about available carrion assessment and the nature of the three observed corvid species, i.e., raven, hooded crow, and magpie, as opportunistic feeders [54–59]. During further observation sessions (i.e., seasons), the number of records of corvids in burned and control areas was similar, suggesting that trophic conditions were no longer affected by the fire event.

The fire probably reduced small rodent availability in the short term [17,29,41]. Indeed, in summer 2020, vole density was much lower in burned than in fire-unaffected areas [41]. However, at that time, the number of records of rodent-eating raptors peaked in fire-disturbed areas and was much higher than in control points. The difference in fresh vegetation matter between burned and unburned areas was not detectable on satellite images just 2 months after the fire [42], so small mammals possibly started recolonizing this site. Still, the vegetation cover was much reduced (like clumps of sedge or accumulated dry plant matter) compared to control areas, offering less cover for small mammals [41]. Therefore, rodents that colonized areas were probably easy prey for raptors [22].

The abundance of herons, cranes, and storks changed with the season and was very low in autumn when these birds had already flown away to their wintering sites [71–74] and highest in spring 2021 (the year after the fire, when the water level was high). They were more commonly observed in the burned areas than in the control areas shortly after the fire, and more often observed in control sites in summer. Yet these differences were insignificant. The burned area could have posed an attractive foraging site because of the presence of carrion and wounded animals, and its easy access to invertebrates with the scarce vegetation cover [17].

4.3. Response to Fire Occurrence by the Red Fox

Fire can influence predator–prey interactions by rapidly modifying the distribution of concealment cover and food resources [75]. Our results documented no differences in the

red fox density index in the burned and control areas in winter. Unfortunately, we have no data on food availability in the two areas at that time. Nevertheless, the most vivid changes (decrease in the number of rodents and increase in the carrion availability) were expected shortly after the fire (i.e., in spring and summer). Also, vegetation cover alteration, enabling easy hunting, was most significant at that time ([41,42], own observations). Probably, almost 10 months after the fire, prey availability for foxes was similar in burned and unburned habitats. Seemingly, the response to fire by medium-sized carnivores may vary and be context-dependent (review in [23]). In general, and according to the literature, the red fox was shown to respond positively to fire. However, some studies showed a neutral response [76]. For example, in Australia, fire history did not significantly affect red fox distribution at either landscape or site scales [77].

5. Conclusions

Large-scale wildfire in the Biebrza National Park locally sharply reduced populations of small vertebrates. This study highlights wildfire's immediate and short-term ecological consequences on bird activity and predator dynamics in a marshland ecosystem of Biebrza National Park. Although direct estimations of fire-induced animal mortality are limited, the available evidence suggests a substantial impact, particularly on small vertebrates. This supported elevated scavenger (i.e., corvids) activity due to increased carrion availability in burned areas shortly after the fire. Also, other predators (i.e., rodent-eating birds of prey and owls) responded positively to the fire event, taking advantage of the process of recolonization of burned areas by small mammals that occurred a few months after the fire, albeit with increased vulnerability to predation due to diminished vegetation cover. Conversely, red foxes exhibited no significant differences in winter activity between burned and unburned areas, suggesting that prey availability and habitat structure had equilibrated by that time. Overall, while the wildfire caused notable short-term ecological changes, these effects weakened within a year, underscoring the resilience of the studied marshland ecosystem. However, climate change results in increased wildfire frequency and intensity. While fire-adapted species may be less vulnerable to fire events, changes in the fire regime and their intensity may make many other species or areas fire-affected [21]. Future research should aim to quantify longer-term consequences and further explore the nuanced responses of different trophic groups to fire disturbances.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Number of records of birds assigned to each group per 1 h observation.

Point_ID	Fire Event	Season	Rodent-Eating Raptors and Owls	Wading Birds	Corvids	Other Raptors
1	fire	2020 spring	3	2	4	0
2	fire	2020 spring	1	3	2	1
3	fire	2020 spring	1	3	3	2
4	fire	2020 spring	1	1	3	0
5	fire	2020 spring	1	1	3	0
6	fire	2020 spring	0	2	3	0
7	fire	2020 spring	2	5	1	1
8	fire	2020 spring	1	2	3	1
9	fire	2020 spring	2	4	6	0
10	fire	2020 spring	1	2	3	0
11	control	2020 spring	1	2	2	0
12	control	2020 spring	3	2	2	0
13	control	2020 spring	1	1	0	1
14	control	2020 spring	6	0	1	0
15	control	2020 spring	3	1	1	4
16	control	2020 spring	4	2	0	0
17	control	2020 spring	2	2	1	0
18	control	2020 spring	0	2	1	2
19	control	2020 spring	1	4	1	0
20	control	2020 spring	1	1	5	1
1	fire	2020 summer	5	3	2	1
2	fire	2020 summer	5	2	1	1
3	fire	2020 summer	3	1	4	2
4	fire	2020 summer	3	2	0	1
5	fire	2020 summer	3	2	2	1
6	fire	2020 summer	2	3	1	0
7	fire	2020 summer	4	6	2	0
8	fire	2020 summer	2	4	2	0
9	fire	2020 summer	5	0	2	0
10	fire	2020 summer	3	0	0	0
11	control	2020 summer	3	4	3	1
12	control	2020 summer	2	2	1	0
13	control	2020 summer	1	5	1	1
14	control	2020 summer	0	1	1	0
15	control	2020 summer	1	3	1	0
16	control	2020 summer	2	4	2	0
17	control	2020 summer	3	5	1	1
18	control	2020 summer	1	3	3	0
19	control	2020 summer	3	3	2	0
20	control	2020 summer	1	4	1	0
1	fire	2020 autumn	2	1	4	1
2	fire	2020 autumn	0	0	1	0
3	fire	2020 autumn	2	0	2	0
4	fire	2020 autumn	0	0	0	1
5	fire	2020 autumn	0	1	1	0
6	fire	2020 autumn	2	0	2	1
7	fire	2020 autumn	0	0	3	1
8	fire	2020 autumn	0	0	9	1
9	fire	2020 autumn	1	0	6	1
10	fire	2020 autumn	0	0	1	0
11	control	2020 autumn	3	1	0	1

Table A1. *Cont.*

Point_ID	Fire Event	Season	Rodent-Eating Raptors and Owls	Wading Birds	Corvids	Other Raptors
12	control	2020 autumn	2	0	1	1
13	control	2020 autumn	0	0	2	0
14	control	2020 autumn	1	1	8	0
15	control	2020 autumn	0	0	9	0
16	control	2020 autumn	1	0	4	0
17	control	2020 autumn	1	0	0	0
18	control	2020 autumn	0	0	5	0
19	control	2020 autumn	0	0	2	0
20	control	2020 autumn	2	0	2	0
1	fire	2021 spring	5	3	2	1
2	fire	2021 spring	1	1	3	0
3	fire	2021 spring	2	2	2	1
4	fire	2021 spring	5	2	1	1
5	fire	2021 spring	1	2	1	0
6	fire	2021 spring	1	1	0	0
7	fire	2021 spring	3	7	5	1
8	fire	2021 spring	1	11	0	1
9	fire	2021 spring	2	3	1	0
10	fire	2021 spring	0	1	0	0
11	control	2021 spring	2	5	0	2
12	control	2021 spring	2	4	4	1
13	control	2021 spring	3	3	0	0
14	control	2021 spring	2	4	4	0
15	control	2021 spring	1	3	1	0
16	control	2021 spring	1	3	2	2
17	control	2021 spring	1	2	1	0
18	control	2021 spring	4	2	1	0
19	control	2021 spring	4	4	6	1
20	control	2021 spring	3	1	2	0

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Does Vegetation Recovery Limit the Habitat Use of Herbivore? Decadal Evidence of a Potential Ecological Mismatch

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Simple Summary

In the context of large-scale forest ecological restoration, we examined the relationship between decadal (2015–2024) activity intensity of sika deer and vegetation (leaf area index and normalized difference vegetation index), together with topographic and anthropogenic factors. We found that activity intensity of sika deer had a sustained increase over time. Vegetation indices had weak, periodical effects on sika deer activity, whereas topographic and anthropogenic variables exhibited significant effects. Our findings demonstrate that vegetation recovery within the reserve does not automatically improve habitats for forest-dependent herbivores, and could lead to a potential ecological mismatch.

Abstract

Large-scale forest ecological restoration is commonly expected to improve habitat quality and promote population growth of forest-dependent herbivores. Yet, whether vegetation recovery facilitates or constrains herbivore growth and habitat use at local scales within nature reserves remains unclear, as vegetation recovery and canopy closure might alter forage availability and lead to ecological mismatch between vegetation features and population dynamic. Here, we used the endangered species South China sika deer as the study species, and its dominant distribution region—Qingliangfeng Biosphere Reserve—as the study area. Using decadal camera-trapping data (2015–2024) and extracted vegetation and other environmental variables, we quantified decadal trends in sika deer activity intensity and interannual variation in vegetation (leaf area index, LAI, and normalized difference vegetation index, NDVI). We incorporated topographic and anthropogenic disturbance variables and applied generalized linear mixed models and generalized linear models to analyze its habitat use. We found that: (1) Numbers of independent photographs and the relative abundance index of sika deer increased significantly and consistently from 2015 to 2024. (2) LAI exhibited substantial interannual variability without a stable trend. In contrast, segmented regression identified a clear temporal breakpoint in NDVI, with a significant increasing trend before 2021 followed by a pronounced decline thereafter. (3) In all years, distance to settlement had a significant and negative effect on activity intensity, whereas distance to road, elevation, and year had significant positive effects. LAI and NDVI showed negative and weak effects on sika deer activity intensity. In specific years, LAI had a significantly negative effect in early periods whereas NDVI became significantly negative in mid and late periods. Other environmental variables exhibited interannual heterogeneity. Our findings demonstrate that vegetation recovery within the reserve does not automatically improve habitats for forest-dependent herbivores and could lead to a potential ecological mismatch.

Keywords: deer; ecological restoration; forest ecosystems; decadal camera-trapping

1. Introduction

Large-scale ecological restoration and conservation initiatives have accelerated forest recovery globally over recent decades [1]. In China, the Natural Forest Protection Program (NFPP) and the expansion of the protected-area network have markedly improved forest ecosystem conditions. By 2018, the NFPP had increased national forest cover from 12.7% in 1973 to 22.96%, while total forest growing stock rose from 8.7 billion m^3 to 17.6 billion m^3 [2]. As one of the world's largest restoration programs, the Natural Forest Protection Program has substantially reduced large-scale logging in natural forests [3]. These policy interventions have created favorable conditions for the recovery and expansion of large herbivore populations.

However, vegetation recovery does not necessarily lead to parallel improvements in local habitat quality for large herbivores [4]. Increases in ecological mismatches between vegetation recovery and large herbivores might occur. In the early stage of vegetation recovery, the increase in food resources promotes the growth of herbivore populations and allows them to reach a peak. In the middle and late stages of vegetation recovery, progressive canopy closure reduces understory light availability, thereby suppressing the growth and regeneration of herbaceous and shrub layers [5]. These changes may limit understory foraging and constrain herbivores that depend on understory vegetation [6]. In addition, as vegetation recovery and succession progress, plant communities in some shrub-dominated habitats may become excessively dense, forming closed shrub thickets that restrict the movement space of large herbivores [7]. Therefore, it is vital to identify the mismatch progress and conduct measures to moderate the paradox for regional sustainable development.

Gaining a deeper understanding of herbivore responses to vegetation recovery requires decadal empirical studies and the explicit evaluation of different vegetation metrics [6,8,9]. Previous research assessing vegetation effects on wildlife has largely relied on short-term monitoring data or single vegetation indices, most commonly the normalized difference vegetation index (NDVI) [10–13]. NDVI often saturates under closed-canopy and high-biomass conditions [14], limiting its ability to characterize forest canopy structure [15]. In contrast, the leaf area index (LAI) more directly characterizes canopy structural attributes and is widely regarded as an important indicator of forest structural succession [16]. Integrating NDVI and LAI with decadal wildlife activity data offers a more comprehensive approach for characterizing large herbivore responses to vegetation structural change.

Sika deer exhibit a fragmented distribution across East Asia [17]. The South China sika deer (*Cervus nippon kopschi*), an endemic and regionally endangered species in China, is listed as a Class I national protected species and is classified as endangered in the China Biodiversity Red List [18]. At present, this endangered species occurs only in fragmented forest habitats within hilly and mountainous areas of the Zhejiang, Jiangxi, and Anhui provinces [19]. Owing to agricultural, pastoral expansion, forest degradation, and historical poaching pressure, its population remained at extremely low levels for decades, with estimates of approximately 300 individuals by the late 20th century [19,20]. In recent years, strengthened conservation measures and reduced anthropogenic disturbance have facilitated a gradual population recovery. As a typical herbivore, the South China sika deer primarily feeds on shrubs or shrub-grass mosaics, and uses the forests for sheltering [21,22]. It is a kind of wildlife that favors landscapes with low canopy density forests and low-density shrubbery, and avoids the dense cover of forest and shrub [21,22].

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A stable population is currently found in the Qingliangfeng Biosphere Reserve. Within this typical subtropical montane forest ecosystem, under intensified conservation management and gradually denser forest and shrub, the South China sika deer provides an opportunity to examine activity intensity in relation to vegetation metrics, as well as topographic and anthropogenic disturbance variables under decadal forest recovery. Therefore, it is a favorable case to illustrate the responses of a large herbivore to vegetation across decadal spatiotemporal scales [23].

Based on camera-trapping monitoring data collected over a decade (2015–2024) and extracted vegetation and other environmental variables from the Qingliangfeng Biosphere Reserve, this study systematically examined the spatiotemporal dynamics of sika deer activity intensity in relation to vegetation succession. Specifically, we aimed to: (1) quantify decadal spatiotemporal trends in sika deer activity intensity; (2) characterize interannual spatiotemporal variation in key vegetation indices; and (3) evaluate the combined effects of vegetation indices and other environmental factors, including topography, hydrology, and anthropogenic disturbance on sika deer habitat use. We hypothesized that: (1) sika deer's activity intensity and vegetation feature would tend to be dynamic; (2) vegetation and other environmental factors contribute to its habitat use, and ecological mismatch between the activity of deer and vegetation recovery would appear. Our study provides new empirical evidence for understanding vegetation–habitat interactions and habitat use mechanisms in sika deer under ongoing forest succession.

2. Materials and Methods

2.1. Study Area

Globally, a total of 785 biosphere reserves have been designated, among which 14 biosphere reserves are distributed in subtropical regions. The Qingliangfeng Biosphere Reserve (30°01′–30°18′ N, 118°50′–119°12′ E) is one of the old reserves in this network and represents a typical subtropical montane forest ecosystem. It is situated in Northwest of Zhejiang, China, and spans an elevation range of 399–1787.4 m [24,25]. The reserve lies at the northern margin of the mid-subtropical zone and has a subtropical monsoon climate [24]. The Qingliangfeng Biosphere Reserve comprises three geographically isolated areas, including Longtangshan, Shunxiwu, and Qianqingtang, among which, the Qianqingtang area constitutes the primary distribution area of the South China sika deer (Figure 1). This area covers approximately 56.9 km^2 and is characterized by mountainous terrain at elevations of around 1000 m, with relatively gentle slopes and broad, shallow valleys. The mean annual precipitation is approximately 1862 mm, exhibiting strong seasonality, with rainfall concentrated in summer and autumn and comparatively lower precipitation during winter and spring. The mean annual temperature is 11.7 °C, and the accumulated annual temperature ranges from approximately 2200 to 4800 °C, with pronounced variation along elevational gradients and across seasons [26]. Vegetation is dominated by deciduous broad-leaved forests, coniferous forests, and mixed conifer–broad-leaved forests, with localized patches of evergreen broad-leaved forest and meadow habitats, forming a complex mosaic of forest structures and habitat types [27]. The Qingliangfeng Biosphere Reserve supports high biodiversity, with records of 2452 vascular plant species, 355 vertebrate species, and 2567 insect species [25]. This diversity of forest types and topographic conditions provides a highly heterogeneous habitat for forest-dependent herbivores.

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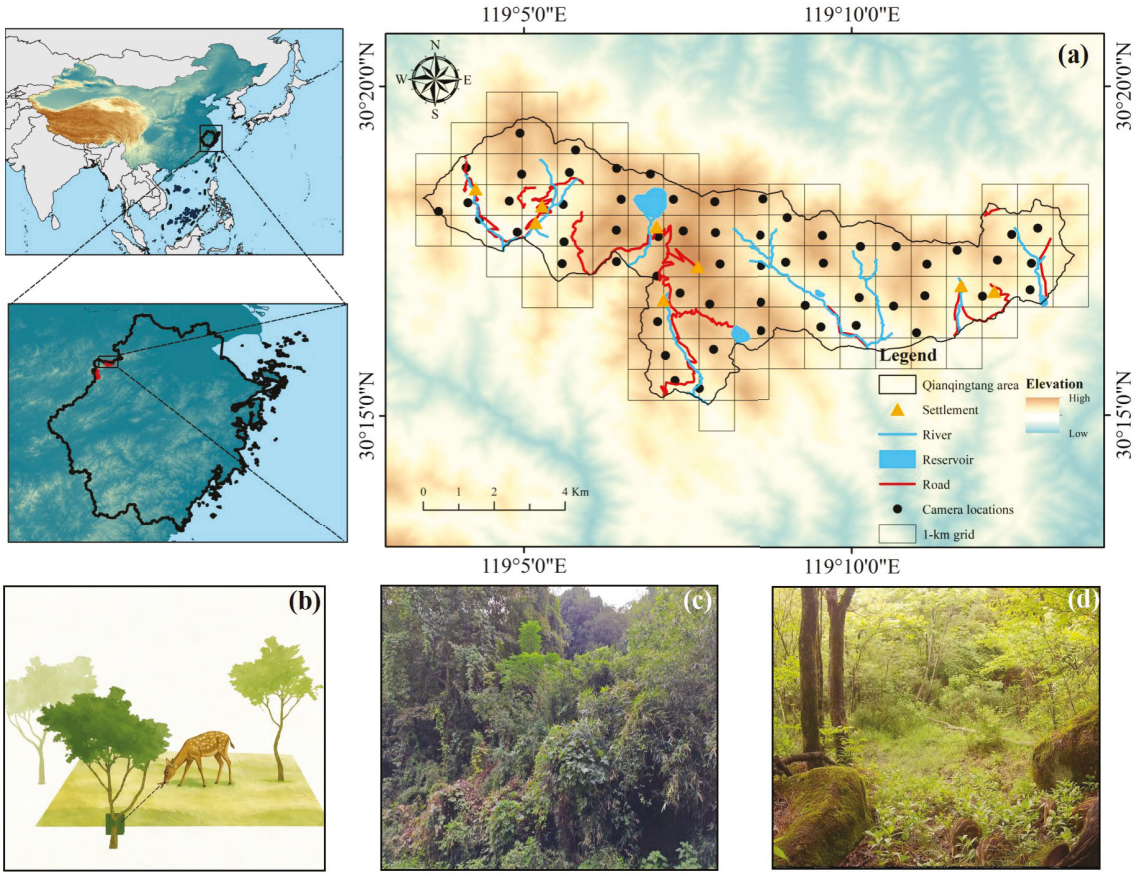

Figure 1. Distribution of the study area, camera-trapping locations, and forest structure in the Qingliangfeng Biosphere Reserve (**a**) Location of the study area and camera-trapping locations (N = 58) in the Qingliangfeng Biosphere Reserve from 2015 to 2024. (**b**) Schematic illustration of camera-trapping-based estimation of sika deer activity intensity. (**c**) Dense forest in the Qingliangfeng Biosphere Reserve. (**d**) Open forest in the Qingliangfeng Biosphere Reserve.

2.2. Camera-Trapping Survey

Firstly, we divided the study area into 107 square survey grid cells (1 km × 1 km) based on ArcGIS 10.8 (ESRI, Redlands, CA, USA) (Figure 1) [28]. We deployed infrared cameras (Ltl Acorn 6210, Zhuhai Ltl Acorn Electronics Co., Ltd., Zhuhai, China) across the grid system. Cameras were not installed in a small number of grid cells where the protected area was extremely limited. Some camera locations and identification numbers were adjusted during the survey period due to equipment malfunction or inadequate image quality (further details are provided in Table S1). For each grid cell, the geographic center was located in the field using a handheld GPS unit and used as the predefined camera placement point. Cameras were installed within a 50 m × 50 m area centered on this point, preferentially at locations with sparse understory vegetation, along animal trails, or near water sources [29]. Camera placement took local terrain conditions into account, including slope, aspect, and topography. To minimize interference from direct sunlight during sunrise and sunset, cameras were oriented toward the northeast or southwest and mounted on tree trunks at heights of 40–90 cm above ground level. Surrounding environmental conditions at each camera location were also recorded [30]. Prior to deployment, all cameras were configured using standardized settings, including time synchronization, two consecutive photographs per trigger, a minimum trigger interval of 5 s, and medium sensitivity. Cameras were inspected every 4–6 months, during which SD memory cards (32 GB) and batteries were replaced to ensure continuous operation. A total of 58 infrared cameras were deployed in the Qingliangfeng Biosphere Reserve.

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We examined retrieved photographs for species identification and recorded associated metadata, including capture time and the number of consecutive images [29,31]. Over the 10-year monitoring period (2015–2024), we accumulated 170,962 camera-trapping days and recorded 15,614 independent photographs. We defined independent photographs as consecutive images of the same species at the same camera location in each 30 min [31]. And pictures of the same species within 30 min were recorded as 1 independent photograph.

2.3. Explanatory Variables

Previous studies have demonstrated that topographic features, vegetation indices, and distance to water sources significantly influence habitat use by sika deer [21,22,32]. Accordingly, we considered four categories of explanatory variables, including topography, vegetation, hydrology, and anthropogenic disturbance (Table 1). Elevation and slope were derived from a digital elevation model (GDEMV3, 30 m resolution) using spatial analysis in ArcGIS 10.8. Vector data for the road network, settlements, and hydrological features were initially obtained from the National Geomatics Center of China (www.webmap.cn). To improve spatial accuracy, we manually corrected and supplemented these vector datasets in ArcGIS 10.8 using high-resolution satellite imagery, allowing a more realistic representation of current patterns of human activity and hydrological distribution within the study area. We then used Euclidean distance tools to calculate the distance from each sampling unit to the nearest water body and settlement.

Table 1. Description of explanatory variables used in the analysis.

Variable	Data Type	Description and Source Source
Hydrological		
Distance to water	Continuous	Euclidean distance to the nearest water, derived from NGCC vector data in ArcGIS 10.8.
Topography		
Elevation	Continuous	Extracted from the 30 m digital elevation model (GDEMV3) in ArcGIS 10.8.
Slope	Continuous	Calculated from the 30 m digital elevation model (GDEMV3) in ArcGIS 10.8.
Vegetation		
Leaf Area Index	Continuous	Annual mean LAI from MODIS MCD15A3H, processed in Google Earth Engine.
Normalized difference vegetation index	Continuous	Annual mean NDVI from MODIS MOD13Q1, processed in Google Earth Engine.
Anthropogenic disturbance		
Distance to road	Continuous	Euclidean distance to the nearest road, derived from NGCC vector data in ArcGIS 10.8.
Distance to settlement	Continuous	Euclidean distance to the nearest settlement, derived from NGCC vector data in ArcGIS 10.8

NGCC, National Geomatics Center of China; GDEMV3, Global Digital Elevation Model version 3 (30 m spatial resolution); MODIS MCD15A3H, MODIS Leaf Area Index product (4-day composite, 500 m spatial resolution); MODIS MOD13Q1, MODIS vegetation indices product providing NDVI (16-day composite, 250 m spatial resolution).

We used the LAI to represent total leaf area per unit ground surface area as an indicator of canopy structural characteristics [33] and the NDVI to characterize vegetation greenness and reflect vegetation growth activity [34]. LAI data were obtained from the MODIS MCD15A3H product (4-day composite, 500 m spatial resolution), and annual mean values were calculated using Google Earth Engine. NDVI data were derived from the MODIS MOD13Q1 product (16-day composite, 250 m spatial resolution) and were similarly

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processed to obtain annual mean values [35]. We calculated annual averages of both indices for each year from 2015 to 2023.

2.4. Statistical Analyses

We analyzed spatiotemporal pattern dynamics of sika deer in the Qingliangfeng Biosphere Reserve. To examine temporal trends in sika deer activity intensity from 2015 to 2024, we fitted generalized linear mixed models (GLMMs) with year as a fixed effect, the number of independent photographs and the relative abundance index separately as response variables [36]. Camera ID was incorporated as a random effect to account for repeated measurements across sampling locations. Prior to model fitting, we evaluated the distributional properties of the response variables by examining the relationship between the mean and variance (Table S1). Because the variance exceeded the mean (Table S1), we used a negative binomial distribution to account for overdispersion. We compared the trend model (including year) with a null model (excluding year) using AICc, and likelihood ratio test (LRT) [36]. Spatially, we applied the kernel density estimation to visualize the spatial distribution of sika deer within the Qingliangfeng Biosphere Reserve for each year.

We analyzed spatiotemporal dynamics of vegetation indices in the Qingliangfeng Biosphere Reserve. Temporally, we used one-way ANOVA to analyze annual grid level data to test for interannual differences in the LAI and the NDVI. In this analysis, LAI and NDVI extractions were based on the 1 km $\times$ 1 km grid cells, and we have calculated annual LAI and NDVI for each of the 107 grid cells across the full study period (2015–2024). In addition, to further examine decadal temporal trends in vegetation indices overall in the study area, we applied segmented linear regression to analyze decadal changes in vegetation indices [37,38]. Spatially, we fitted ordinary least squares regressions to grid-level LAI and NDVI time series (2015–2024) and extracted the interannual trend slope for each of the 107 grid cells. We used these slopes to quantify spatial variation in both the direction and magnitude of vegetation change across the study area. We then applied kernel density estimation to visualize the annual spatial patterns of vegetation change within the Qingliangfeng Biosphere Reserve.

To examine the effects of vegetation and other environmental factors on habitat use by the South China sika deer, we employed GLMMs and generalized linear models (GLMs) [39,40]. We conducted the all-year analysis and specific-year analysis. The response variable was the number of independent photographs of sika deer. Because the data were count-based and exhibited overdispersion (Table S1), we fitted all models using a negative binomial distribution implemented via the glm.nb function in the MASS package (version 7.3-65) in R (version 4.5.2) [41]. During all-year analysis, explanatory variables included elevation, slope, NDVI (or LAI), and distances to settlements, water sources, roads, and year. Due to potential collinearity between NDVI and LAI [42,43], two suits of models involving NDVI or LAI were created. Camera ID was served as random effect [44]. During specific-year analysis, we excluded the year and conducted GLMs. We also applied the negative binomial distribution because the overdispersion parameters ($\hat{c}$) lied 0.950–1.642 based on the assumed models with negative binomial distribution (Table S2). In addition, we generated candidate models and performed model selection based on Akaike's information criterion in all-year and specific-year analysis. Models with ΔAICc < 4 were treated as the optimal models, and retained for model averaging because this threshold captured most of the cumulative Akaike weight [45,46]. Parameter estimates were then obtained using model-averaged coefficients across the optimal models [47]. In addition, nested models within optimal models were further compared with the best model using LRT to assess whether inclusion or exclusion environmental variables significantly could improve the model fit.

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3. Results

3.1. Spatiotemporal Patterns of Sika Deer

From 2015 to 2024, we accumulated 170,962 camera-trapping days and recorded 15,614 independent photographs. Time-series analyses based on camera-trapping monitoring data revealed a consistent and statistically significant increase over time in both the number of independent photographs and the relative activity index of sika deer (Figures 2 and S1, Tables S3 and S4). The LRT results showed that the model including the year significantly improved model fit (χ^2 = 176.28, $p < 0.001$) (Table S3). Year had a significant positive effect (Estimate = 0.18 ± 0.012 SE, z = 14.69, $p < 0.001$) on the number of independent photographs of sika deer (Figure S1, Table S3). A similar increasing trend was detected for the relative abundance index of South China sika deer (Table S4 and Figure S1).

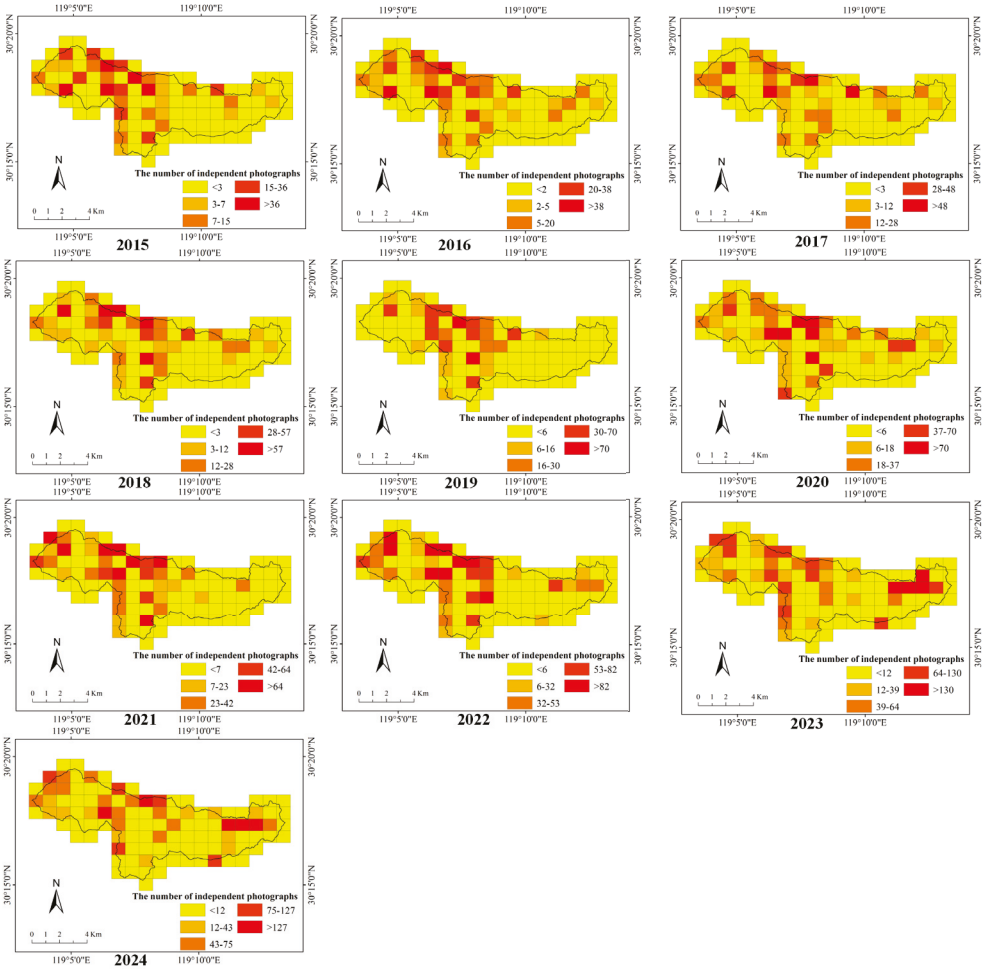

Figure 2. Spatial distribution of the number of independent photographs of sika deer in the Qingliangfeng Biosphere Reserve, 2015–2024.

3.2. Spatiotemporal Patterns of Vegetation Recovery

The interannual variations in LAI and NDVI at the grid scale within the study area are shown in Figures 3 and 4. One-way ANOVA showed that both LAI and NDVI differed significantly among years within the study area (Tables S5 and S6) ($p < 0.001$), indicating pronounced interannual variation in vegetation indices from 2015 to 2024. Interannual variation in NDVI was relatively small, whereas LAI exhibited greater variability. The temporal changes in the mean values of NDVI and LAI across the study area are shown in Figure S2 and Table S7. Segmented regression analysis indicated that the temporal trend of mean annual NDVI in the study area exhibited a significant breakpoint around

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2021.82 (SE = 0.430) (Table S8). Prior to this breakpoint, NDVI increased significantly with year (slope = 0.0067 ± 0.0023, t = 2.88, p = 0.028). In contrast, NDVI declined significantly after the breakpoint (slope = −0.0301 ± 0.009, t = −3.510, p < 0.010). The segmented model showed a good fit, explaining a substantial proportion of the interannual variation in NDVI (adjusted R^2 = 0.679). By contrast, segmented regression analysis did not identify a significant temporal breakpoint in mean annual LAI over the study period (Table S9). Although the estimated breakpoint occurred in 2018.73, its associated standard error was large (SE = 3.710). The slopes before and after the breakpoint were both non-significant (before breakpoint: slope = 0.050 ± 0.097, t = 0.52, p = 0.624; after breakpoint: slope = −0.030 ± 0.052, t = −0.57, p = 0.097). Overall, the segmented model exhibited poor explanatory power (adjusted R^2 = −0.356).

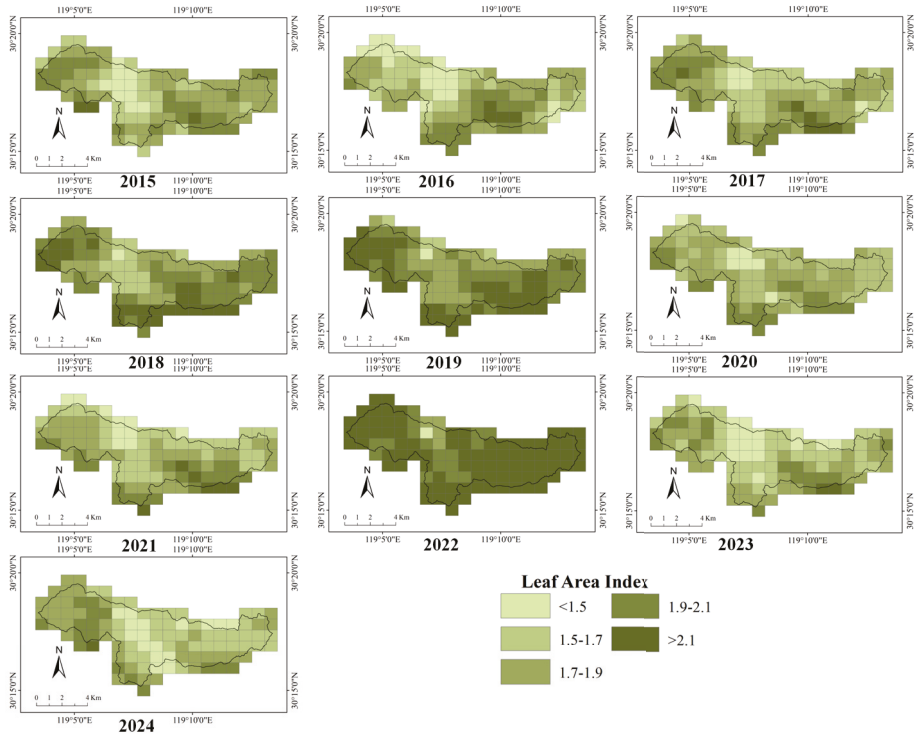

Figure 3. Heatmap of leaf area index in the Qingliangfeng Biosphere Reserve, 2015–2024.

Spatially, the multi-year distributions of LAI and NDVI at the decadal scale across the study area are shown in Figure S3. Both vegetation indices exhibited clear spatial gradients, with higher LAI and NDVI values primarily distributed along the peripheral areas of the reserve. Overall, the spatial pattern was characterized by higher values in the south than in the north, and in the west than in the east, whereas no clear pattern was observed in the central part of the study area. With respect to interannual trends (Figure S3), the directions of change in LAI and NDVI were spatially inconsistent. Some areas exhibited persistent increases (slope > 0), whereas others showed weak changes or a mixture of positive and negative trends, resulting in an overall patchy spatial pattern.

3.3. Vegetation and Other Environmental Variables' Contribution

Model ranking showed that LAI (or NDVI) fell into the candidate optimal models (Tables S10–S12). LRT results showed that models including LAI or NDVI could not significantly improve model fit compared with the best model (LAI: χ^2 = 0.744, p = 0.388; NDVI: χ^2 = 0.151, p = 0.697) (Tables S11–S13). And model-averaged estimates indicated no significant effects of LAI (Estimate = −0.010 ± 0.027 SE, p = 0.721) or NDVI (Estimate = −0.004 ± 0.025 SE, p = 0.860) (Table 2). Distance to settlement had a significant

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and negative effect, whereas distance to road, elevation, and year had significant and positive effects (Table 2). Model including or excluding variables could not improve the model fit significantly (Tables S11–S13).

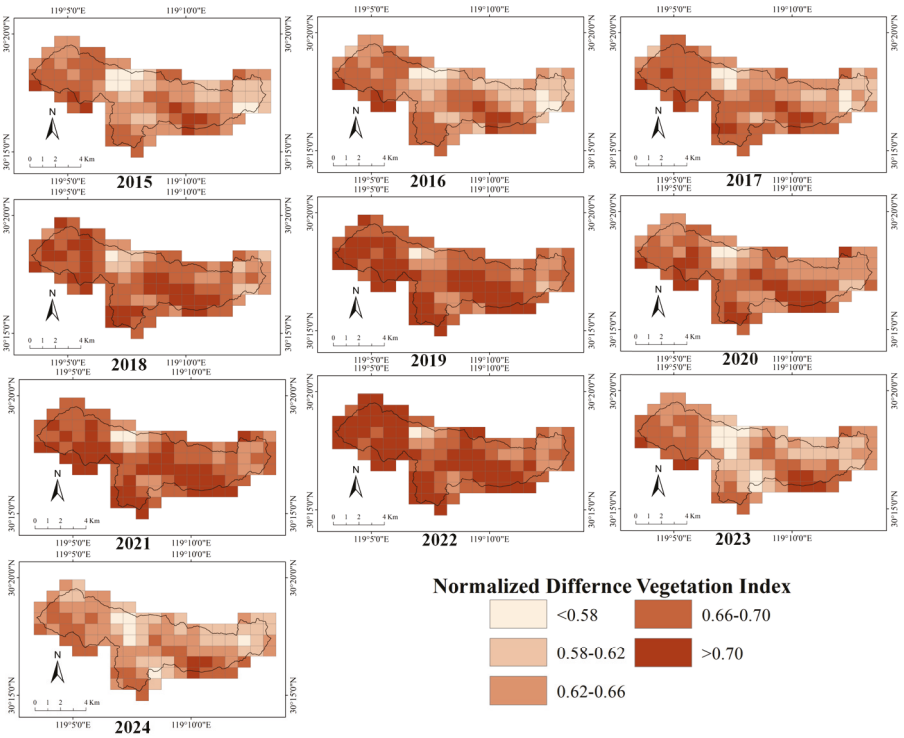

Figure 4. Heatmap of normalized difference vegetation index in the Qingliangfeng Biosphere Reserve, 2015–2024.

Table 2. Model-averaged results in examining the relationships between the number of independent photographs of sika deer and explanatory variables from 2015 to 2024. Separate models were constructed for leaf area index and normalized difference vegetation index. Explanatory variables included leaf area index, normalized difference vegetation index, distance to road, distance to settlement, distance to water, elevation, slope, and year.

Models	Variable	Estimate	SE	z Value	*p*
NDVI	Intercept	2.184	0.104	21.068	<0.001
	Distance to road	0.242	0.120	2.017	0.044
	Distance to settlement	−0.413	0.098	4.220	<0.001
	Distance to water	0.192	0.116	1.657	0.097
	Elevation	0.233	0.114	2.039	0.041
	Year	0.177	0.012	14.337	<0.001
	Slope	0.007	0.036	0.202	0.840
	NDVI	−0.004	0.025	0.176	0.860
LAI	Intercept	2.184	0.103	21.110	<0.001
	Distance to road	0.241	0.119	2.030	0.042
	Distance to settlement	−0.413	0.097	4.239	<0.001
	Distance to water	0.194	0.115	1.690	0.091
	Elevation	0.232	0.113	2.044	0.041
	Year	0.177	0.012	14.382	<0.001
	LAI	−0.010	0.027	0.357	0.721
	Slope	0.007	0.036	0.202	0.840

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LAI (or NDVI) also fell into the candidate optimal models in specific analysis (Tables S14 and S15). Model-averaged results showed that the effects of topographic, hydrological, and anthropogenic variables on sika deer activity intensity exhibited pronounced interannual heterogeneity (Figure 5; Table S16). LAI had a significant and negative effect on deer occurrence in 2015 and 2016 (Figure 5). NDVI showed negative associations with sika deer occurrence in most years except 2015, with a significant negative effect detected in 2019 and 2023.

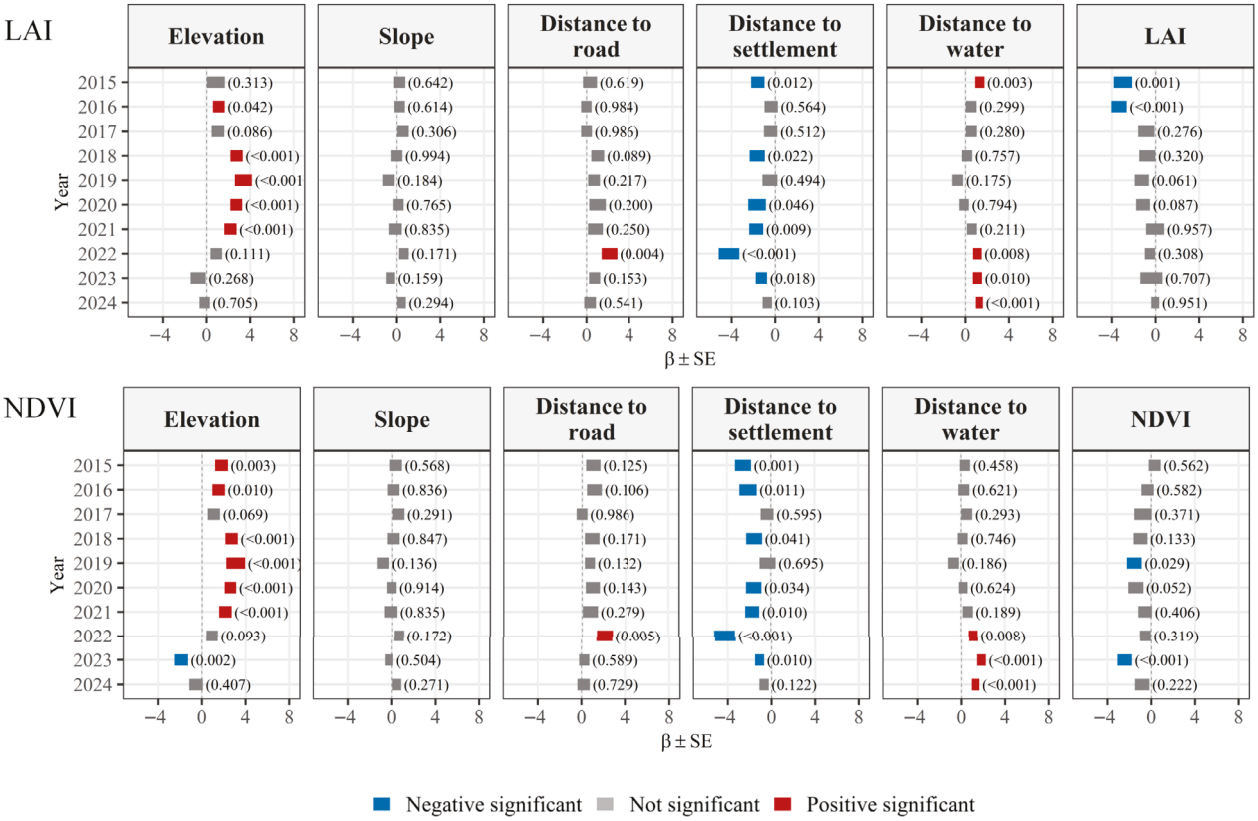

Figure 5. Visualization of generalized linear models regression coefficients describing the relationships between the number of independent photographs of sika deer and explanatory variables in the Qingliangfeng Biosphere Reserve from 2015 to 2024.

4. Discussion

In the context of vegetation recovery, clarifying the response patterns of herbivores to vegetation characteristics is essential for the conservation of endangered wildlife and for effective habitat management. This study was conducted in the Qingliangfeng Biosphere Reserve and focused on the sika deer as the focal species. By examining the effects of vegetation change on sika deer, this study provides a case example of herbivore responses to vegetation recovery. Vegetation recovery is a key process for alleviating habitat degradation and restoring ecosystem functions. For instance, natural succession and rewilding following farmland abandonment in Europe have facilitated the return and expansion of deer and other large herbivores [48]. In tropical secondary forests, landscapes with higher forest cover have been shown to support greater abundances of ungulates, such as white-tailed deer and collared peccaries [49]. In temperate secondary forests, structural restoration of forest stands can substantially increase deer foraging opportunities and habitat use [50]. These studies indicate that improved habitat conditions constitute a critical ecological basis for the persistence and potential growth of large herbivore populations [48–50].

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Sika deer activity intensity and vegetation features exhibited interannual heterogeneity, which supports our first hypothesis (both the vegetation and deer's activity intensity tend to be dynamic over time). At the grid level, vegetation trends within the reserve produced a highly heterogeneous, patchy spatial pattern. Meanwhile, the number of independent photographs of sika deer increased markedly from 2015 to 2024 in the reserve. These patterns contribute to decadal conservation management and reduced anthropogenic disturbance of the reserve. Under the broader framework of the Natural Forest Protection Program, the forests were restricted on logging, and conserved [3]. However, vegetation within the reserve showed pronounced interannual heterogeneity, with no clear overall temporal trend and spatially heterogeneous rates of change (Figure S3). This pattern might be due to the relatively short study period in relation to forest recovery processes, which tends to be insufficient for capturing long-term vegetation trends. Since the establishment of reserve in 1985 [20], the accessibility of residents was restricted, and farming and livestock grazing have decreased [51]. These rules reduced anthropogenic disturbance and contributed to the recovery of vegetation and sika deer's population.

Vegetation and other environmental factors contributed to deer habitat use in both the all-year and specific-year analyses, partly supporting our second hypothesis (that deer's habitat use was influenced by environmental factors and a potential ecological mismatch with vegetation recovery emerged). Neither LAI nor NDVI was significantly associated with sika deer activity intensity in all-year analysis. But in specific-year analysis, LAI had a significantly negative effect in early periods whereas NDVI became significantly negative in mid and late periods. The combined results showed an unclear pattern, which suggested obscure deer–forest relations. But negative effects of vegetation indices (NDVI/LAI), although not significant, appeared in most models, which might be the potential ecological mismatch and lie in the primary stage. In addition, due to the small population of sika deer, the abundance of this species is in a rapid growth stage in this region without large carnivorous wildlife. The limitation effects of vegetation have not yet appeared and may exhibit a lag effect [52]. A similar pattern has been reported in other deer studies [53], which proves that density dependence can obscure the relationships between remotely sensed environmental variables and demographic rates. Future research should incorporate the field microhabitat data and seasonal variation associated with vegetation structure to clarify its relationship. And it is necessary to conduct long-term monitoring, identifying the mismatch, and anticipating timely adaptive management.

Elevation, distance to settlement and distance to roads were the key factors influencing the habitat use of sika deer in our study. In all-year analysis and most specific-year analyses, deer tended to inhabit higher elevations in the reserve, and selected the lower elevation habitat in the recent year from 2023 and 2024. This pattern might be due to the density-dependent habitat expansion. In former years, the abundance of deer was small, and the deer prioritized the optimal habitat located in the higher elevation [32]. As the population grew, sika deer shifted to inhabit in the low elevation region [20,22]. Sika deer selected the near settlement regions, which could be explained by the special spatial distribution of settlements. The settlements in the reserve are protection stations [20,54], which could not produce threats for deer. Sika deer avoided roads, consistent with widespread evidence that roads function as high-risk landscape features that constrain ungulate movement and habitat use through persistent anthropogenic disturbance [55–57]. Overall, it is necessary to partition the conservation zone in relation to elevation, settlements and roads, and conduct specific measures for sustainable development.

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5. Limitations

Some limitations occurred in current study. We focused on the independent photographs of sika deer and explored its relationships with the vegetation. However, other aspects, such as group size, the use and dynamics of trails, interspecific relationships among herbivores, and browsing impacts of herbivores on the forest, were not included, which were directly related to vegetation structure and should be investigated in the future.

6. Conclusions

Using camera-trapping data collected over a decade (2015–2024) and associated with vegetation and environmental factors extracted for the Qingliangfeng Biosphere Reserve, this study systematically examined the spatiotemporal dynamics of sika deer activity intensity in relation to vegetation succession. We found that both the number of independent photographs and the relative abundance index of sika deer show a sustained increase over time. Vegetation indices showed weak, period-dependent effects on sika deer activity, whereas anthropogenic variables exhibited clearer effects. We recommend that long-term monitoring of sika deer and investigation of its habitat and microhabitat should be enhanced. Our findings provide new empirical evidence for understanding the habitat use of large herbivores under forest recovery and offer a scientific basis for forest structural regulation, habitat management, and adaptive management of population dispersal.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology15060491/s1, Figure S1: Predicted temporal trends in the number of independent photographs and relative abundance index of South China sika deer from 2015 to 2024. Solid lines represent model predictions and shaded areas indicate 95% confidence intervals; Table S1: The number of independent photographs and the relative abundance index of sika deer across camera-trappings in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Table S2: Overdispersion parameter (ĉ) for year-specific generalized linear models with a negative binomial distribution examining relationships between the number of independent photograph of sika deer and explanatory variables (distance to road, distance to settlement, distance to water, elevation, slope, and Leaf Area Index (Normalized Difference Vegetation Index) from 2015 to 2024; Table S3: Results of the Generalized Linear Mixed Model testing temporal trends in number of independent photographs of sika deer from 2015 to 2024; Table S4: Results of the Generalized Linear Mixed Model testing temporal trends in relative abundance index of South China sika deer from 2015 to 2024; Table S5: Results of one-way ANOVA testing interannual variation in Leaf Area Index in relation to South China sika deer in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Table S6: Results of one-way ANOVA testing interannual variation in Normalized Difference Vegetation Index in relation to South China sika deer in Qingliangfeng Biosphere Reserve from 2015 to 2024; Figure S2: Interannual variation in annual mean Leaf Area Index and Normalized Difference Vegetation Index in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Table S7: Annual mean Normalized Difference Vegetation Index and Leaf Area Index in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Table S8: Result of segmented regression analysis of temporal changes in annual mean Normalized Difference Vegetation Index in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Table S9: Result of segmented regression analysis of temporal changes in annual mean Leaf Area Index in the Qingliangfeng Biosphere Reserve from 2015 to 2024; Figure S3: Spatial patterns of multi-year mean Leaf Area Index and Normalized Difference Vegetation Index, and spatial distribution of their interannual trends in the Qingliangfeng Biosphere Reserve. (a) Spatial distribution of multi-year Leaf Area Index; (b) Spatial distribution of multi-year Normalized Difference Vegetation Index; (c) Spatial distribution of interannual trends in Leaf Area Index, represented by linear regression slopes; (d) Spatial distribution of interannual trends in Normalized Difference Vegetation Index, represented by linear regression slopes; Table S10: AICc-based model ranking results in examining the relationships between the number of independent photographs of

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sika deer and explanatory variables from 2015 to 2024. Candidate models were derived from the global model including distance to road, distance to settlement, distance to water, elevation, slope, year, and Normalized Difference Vegetation Index; Table S11: Likelihood ratio tests comparing nested candidate models with the best model (Independent Photographs ~ Distance to road + Distance to settlement + Distance to water + Elevation + Year); Table S12: AICc-based model ranking results in examining the relationships between the number of independent photographs of sika deer and explanatory variables from 2015 to 2024. Candidate models were derived from the global model including distance to road, distance to settlement, distance to water, elevation, slope, year, and Leaf Area Index; Table S13: Likelihood ratio tests for comparing nested candidate models with the best model (Independent Photographs ~ Distance to road + Distance to settlement + Distance to water + Elevation + Year); Table S14: Results of year-specific candidate models with ΔAICc < 4 assessing the relationships between independent photograph counts of sika deer and explanatory variables (distance to road, distance to settlement, distance to water, elevation, slope, and Leaf Area Index) from 2015 to 2024; Table S15: Results of year-specific candidate models with ΔAICc < 4 assessing the relationships between independent photograph counts of sika deer and explanatory variables (distance to road, distance to settlement, distance to water, elevation, slope, and Normalized Difference Vegetation Index) from 2015 to 2024; Table S16: Model-averaged results for exploring the relationships between numbers of independent photographs of sika deer and explanatory variables (distance to road, distance to settlement, distance to water, elevation, slope, year, Normalized Difference Vegetation Index, and Leaf Area Index) from 2015 to 2024. Leaf Area Index and Normalized Difference Vegetation Index were modeled separately in optimal models. Models with ΔAICc < 4 were selected and averaged.

Author Contributions: Z.L.: conceptualization; methodology; software; validation; formal analysis; investigation; data curation; writing—original draft; writing—review and editing; visualization. Z.C.: investigation; resources. R.G.: investigation; resources. Q.L.: investigation; resources. L.G.: investigation. X.S.: investigation. S.Z.: conceptualization; methodology; software; validation; formal analysis; investigation; resources; data curation; writing—original draft; writing—review and editing; visualization; supervision; project administration; funding acquisition. A.X.: formal analysis; resources; writing—original draft; writing—review and editing; visualization; supervision; project administration; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Wildlife monitoring is under the permission of Zhejiang Qingliangfeng National Nature Reserve administration (No. H141206, 20 September 2014). We also adhered to the ABS/ASAB "Guidelines for the treatment of animals in behavioral research and teaching" and Wildlife Protection Law of the People's Republic of China. All camera-trapping deployment was conducted in areas of natural animal activity, with a rapid and non-intrusive installation process, without the use of any food or sound lures. No wildlife individuals were captured, handled, marked, or experimentally manipulated.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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Review

Utilizing Olive Fly Ecology Towards Sustainable Pest Management

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Simple Summary: The olive fly is a major pest that threatens olive trees worldwide. It attacks olives, reducing both the quantity and quality of the harvest. This can disrupt supply chains and lead to economic losses for farmers and businesses. To combat this pest, farmers often rely on chemical insecticides, which can harm the environment and beneficial insects. Understanding the olive fly's life cycle and its relationship with olive trees is crucial for developing effective control strategies. Factors like temperature and humidity influence the fly's population dynamics. By studying natural enemies and sustainable agricultural practices, we can explore alternative control methods that minimize environmental harm. Climate change and intensive cultivation have made olive fly a more serious threat. The development of insecticide resistance further complicates the problem. To address these challenges, we need to shift towards sustainable approaches. This includes using biological control agents like natural enemies and employing attract-and-kill strategies. Additionally, a deeper understanding of olive fly's ecology, including its response to temperature and its ability to find refuge in different landscapes, is essential for predicting outbreaks and implementing effective protection measures. By combining ecological knowledge with sustainable control methods, we can protect olive trees, preserve the environment, and ensure the continued production of high-quality olive oil.

Abstract: The olive fly (*Bactrocera oleae*, OLF) is a major pest of global significance that occurs in places where olive cultivation thrives. This paper highlights the economic and environmental damage caused by OLF infestations, including reduced olive oil yield and quality, disrupted supply chains, and ecosystem imbalances due to heavy insecticide use. Understanding olive fly ecology is crucial for developing effective control strategies. The review explores the fly's life cycle, its relationship with olive trees, and how environmental factors like temperature and humidity influence population dynamics. Additionally, studying the role of natural enemies and agricultural practices can pave the way for sustainable control methods that minimize environmental harm. Climate change, intensive cultivation, and the development of resistance to insecticides necessitate a shift towards sustainable practices. This includes exploring alternative control methods like biological control with natural enemies and attract-and-kill strategies. Furthermore, a deeper understanding of OLF ecology, including its response to temperature and its ability to find refuge in diverse landscapes, is critical for predicting outbreaks and implementing effective protection strategies. By employing a holistic approach that integrates ecological knowledge with sustainable control methods, we can ensure the continued viability of olive cultivation, protect the environment, and produce high-quality olive oil.

Keywords: agroecology; olive fly; pest management; sustainability; *Bactrocera oleae*; climate change; sustainable agriculture; biological control

1. Introduction

The OLF, *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae), is one of the major pests of global significance to olive cultivation [1]. It is found almost anywhere cultivated olives are grown or wild olives naturally occur. The OLF has been reported in Africa, Europe, Canary Islands, Middle East, Chine, California, Mexico, and Central America [2]. The female lays its eggs directly within developing olive fruit, and the subsequent larval development causes significant economic losses. The economic losses caused by *B. oleae* worldwide are estimated to be around 800 million US dollars per year [3]. This makes *B. oleae* one of the most harmful olive pests globally [4]. As the larvae feed, they damage the fruit's flesh, leading to premature ripening, oil quality degradation, and reduced fruit weight. Additionally, OLF infestations create suitable conditions for fungal pathogens, further compromising fruit quality and yield [5,6].

The economic impact of OLF infestations is substantial. Damaged olive fruits have lower market prices or become entirely unmarketable in the case of edible olives, while if they are intended for oil, its production, quality, composition and therefore price are affected. Large-scale outbreaks can cripple olive oil production in entire regions, impacting the livelihoods of farmers and disrupting global olive oil supply chains [7]. Beyond direct economic losses, OLF infestations can have significant ecological consequences. The extensive use of insecticides aimed at controlling OLF populations can disrupt natural ecosystems and harm beneficial insects like pollinators, predators, and parasitoids [8].

Estimates suggest that the OLF causes losses of 5% to 15% in the total olive production annually [2,9]. For example, in 2013, more than 3.6 million tons of olives and 3.27 million tons of olive oil were lost [6]. This damage manifests in several ways: the larvae consume the fruit pulp, leading to premature fruit drop, reduced oil yield, and compromised oil quality due to the increased acidity from microbial growth [10]. In severe infestations, the losses can reach up to 80% of the oil's value and cause complete destruction of certain table cultivars [6,10]. The level of infestation is affected by many factors, such as the region, the climatic conditions, the tree varieties, and farming practices [11–13]. The pest's ability to thrive in diverse environments further complicates management efforts, as it can adapt to various climatic conditions [7,14].

This review aims to provide a comprehensive overview of OLF ecology. By understanding how temperature, humidity, and other environmental factors influence OLF behavior, development, and population dynamics, particularly in the face of climate change, we can develop more sustainable and effective OLF management strategies. By mitigating the economic and ecological impact of this persistent pest, we can ensure the continued viability and sustainability of olive cultivation.

2. Biogeographic Patterns and General Biology

An adult OLF is 4–5 mm long and ranges from light to dark brown. It has a whitish to yellowish scutellum, and the female has a prominent ovipositor (Figure 1). Its eggs are whitish and 0.7 mm long. The larva is whitish to yellowish when it grows in green fruits and purplish if found in dark, ripe fruits, reaching 7–8 mm. The pupa is ellipsoid and light brown [1].

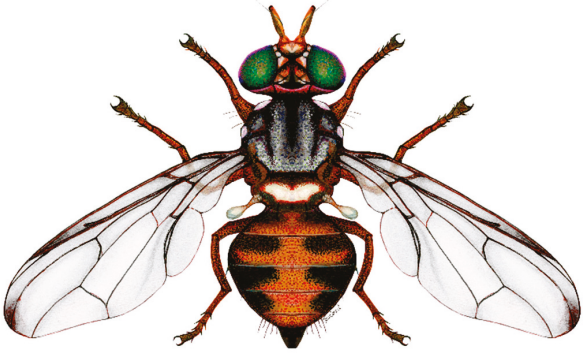

Figure 1. Olive fly, female adult.

During the OLF's life cycle, it undergoes complete metamorphosis, transitioning through distinct egg, larval, pupal, and adult stages. Each stage plays a vital role in ensuring the fly's survival and reproductive success. The OLF's reproductive behavior is intricately linked to olive fruit development [15]. The mating process involves several distinct phases, including the search for mates, where males are attracted to females; courtship; and successful copulation [16]. Male OLFs engage in wing fanning behavior during courtship, which has been shown to increase their mating success [17]. Once mated, females meticulously select oviposition sites, preferring developing olive fruits with the optimal moisture content and minimal physical damage. Fecundity varies depending on the size of the female and environmental conditions, with a single female capable of laying 12 eggs a day and between 200 and 500 eggs throughout its lifespan [18,19].

Molecular techniques, such as chromosome quotient (CQ) analysis and mitochondrial genome sequencing, have been used to elucidate the taxonomic classification and evolutionary relationships of *B. oleae* and other *Bactrocera* species [20]. A molecular clock analysis based on a combination of cytochrome oxidase I (COI) and NADH dehydrogenase subunit 1 (NADH) gene sequences was used to resolve the taxonomic status of several *Bactrocera* species. The analysis showed that *B. oleae* diverged from other *Bactrocera* species, such as *B. papayae* and *B. carambolae*, around 2.7 million years ago [21]. The OLF has a widespread distribution, primarily in the Mediterranean Basin, but also in other regions where olive cultivation is present. The native range of the insect is centered in the Afrotropics, including parts of Africa, Asia, and the Mediterranean region [22]. However, the pest has also been introduced and established in other areas, such as North and Central America, where it has become a significant problem for olive growers [23]. The distribution and spread of OLF are influenced by various factors, including climate, landscape structure, and the availability of its host plant, the olive tree (*Olea europaea* L.) [3,24].

Its development and survival rates differ across each life stage (Figure 2). Under the optimal conditions (10 °C−30 °C), its eggs hatch in 1 day, giving rise to larvae that are vulnerable to predators [6]. These larvae primarily feed on the olive fruit's pulp, with their development influenced by temperature and fruit quality. Warmer temperatures can accelerate larval development, while unfavorable temperatures (<23 °C, >29 °C) or damaged fruit can lead to increased mortality [25,26]. Following three instars (molting stages), the larvae open an exit hole in the olive epicarp and either escape from the fruit to pupate in the soil or pupates inside the fruit and open an exit hole for the adult [6]. Studies have found that larvae pupates in the top 3 cm [27], while other research studies have found that they can reach the depth of 7 cm in the soil [28]. The pupal stage lasts for several weeks, with the emergence timing depending on temperature [29]. Adult flies that emerge from pupae will seek out mates and suitable oviposition sites, perpetuating the cycle up to

6–7 generations per year. A comprehensive understanding of the factors influencing each life stage of the fly is paramount to developing targeted OLF management strategies that can disrupt specific phases of its development and reproduction.

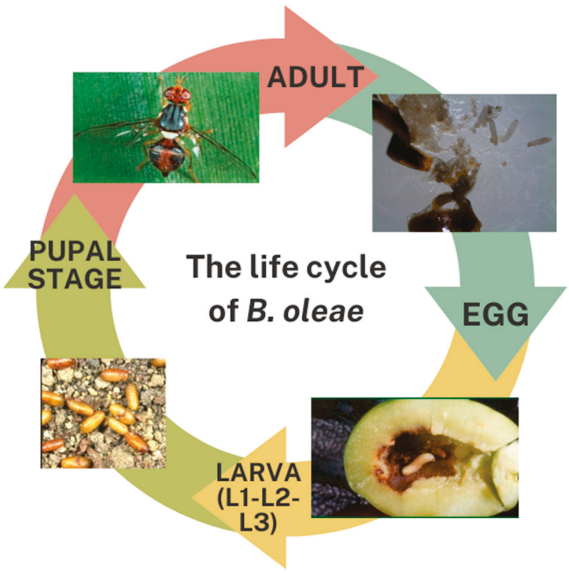

Figure 2. The life cycle of *B. oleae*.

Pupation occurs within olive fruits during the summer season, while during autumn, the majority of the third instar larvae exit the olive to pupate and undergo overwintering in the soil. Additionally, in regions characterized by mild winter climates, the OLF can survive the winter either as larvae or adults [30,31]. In the ensuing spring months, adults emerge between March and May, initiating a series of generations ranging from one to five, contingent upon environmental factors [31]. The OLF can complete a generation in as little as 30 to 35 days at optimum temperatures. Optimal temperatures ranging from 22 °C to 26 °C promote higher infestation rates [32,33].

The female introduces an egg into the pulp (mesocarp) of green or ripe olive fruits after having made an oviposition slit in the epicarp using her ovipositor and a minute chamber underneath in the pulp [1]. In mid-summer, females start to lay eggs (1 egg per olive—except in cases of very dense populations—until 12 eggs/day) and continue until late autumn, while each one can lay between 200 and 500 eggs [34,35].

3. Host Plants and Interactions

The OLF exhibits a strong association with its primary host plant, the *Olea* species (mostly the widespread *Olea europaea* L.), but its interactions with other plant species and microorganisms are also significant [10,36]. Olive fruits serve as the sole breeding ground for the fly, providing sustenance for developing larvae and ensuring the continuation of the OLF's lineage [2]. The OLF has developed a symbiotic relationship with the bacterium *Candidatus* Erwinia dacicola, which aids in overcoming the plant's chemical defenses, particularly oleuropein, a bitter compound found in olives [37,38]. This symbiosis enhances the fly's ability to exploit olive fruits, thereby increasing its reproductive success. Moreover, the OLF's attraction to specific volatile compounds produced by both the olive fruit and associated microorganisms has been documented [39,40]. These interactions underscore the complexity of the OLF's ecological niche and its reliance on both plant and microbial partners for survival and reproduction.

The larvae's ability to thrive in unripe fruits, which possess strong antimicrobial properties, is a unique adaptation that allows them to exploit a niche that is often less competitive [41,42]. The OLF's reproductive success is influenced by various factors, including the physical characteristics of the fruit. Research has shown that the fruit's texture can affect oviposition preferences, with softer fruits being more susceptible to infestation [43]. Furthermore, the presence of specific volatiles emitted by host plants plays a crucial role in attracting adult flies for mating and oviposition [44,45]. The olive tree's unique chemical profile, including the volatile organic compounds emitted during fruit development, acts as a powerful attractant for adult olive flies searching for oviposition sites. Additionally, the olive fruit's fleshy mesocarp offers a perfect nutritional source for larvae, facilitating their growth and development [46].

While the olive tree stands as the OLF's primary host, the potential role of alternative host plants in OLF population dynamics cannot be ignored. Certain wild olive relatives and other fruiting trees have been documented to harbor OLF populations [47]. Adult OLFs, during the search for their host plant, can use a variety of food sources, different pollens, honeydews, fruit and plant exudates, and bacteria [31]. They have been found on plant species such as lemon, cypress, fig, and orange trees [48–50]. While some studies suggest these alternative hosts may serve as reservoirs for OLF populations, particularly during periods of low olive fruit availability, their overall contribution to OLF population dynamics remains a subject of ongoing research [51].

The OLF's interaction with the olive tree is a complex interplay of attraction, exploitation, and potential harm [52]. As mentioned earlier, the olive's volatile compounds lure adult flies, guiding them towards suitable oviposition sites [44,53], specifically toluene, C6 compounds from the lipoxygenase pathway, hexanal, (E)-2-nonenal, and specific sesquiterpenes [44,54]. The subsequent egg-laying and larval development within the olive fruit translates into a significant investment of resources by the tree [23,55]. The OLF's feeding activity damages the fruit pulp, leading to premature ripening, oil quality degradation, and reduced yield [9,13,56–59]. Additionally, the fly may introduce pathogens with its feeding, further compromising fruit health [60–64]. Understanding the factors influencing host selection and the impact of OLF infestation on fruit quality is crucial for developing effective control strategies that protect olive trees and ensure sustainable olive production [52].

4. Environmental Factors

The OLF's population dynamics, development, and activity are intricately interwoven with climatic factors, and the presence of native aromatic species, such as *Cistus creticus* L. and *Lavandula stoechas* L., in understory plant communities can either promote or inhibit OLF infestations [32,65] (Figure 3). Temperature plays a pivotal role, influencing egg development, larval growth rates, and adult emergence times [66]. Warmer temperatures can accelerate OLF development, leading to faster population turnover and potentially higher infestation levels [67]. Conversely, cool temperatures can slow development and reduce overall population growth. The optimal temperature range, according to the research, is 23–29 °C [6]. Rainfall and humidity also have a significant influence, with the optimal level of relative humidity according to the research being around 60% (RH) [68]. While some level of humidity is necessary for egg development and adult survival, excessive rainfall can disrupt mating behavior and decrease egg viability. Humidity fluctuations can similarly impact development rates and adult activity levels. Understanding these climatic interactions is crucial for predicting OLF outbreaks and implementing targeted control measures. Furthermore, climate change poses a potential threat to olive cultivation and OLF manage-

ment strategies. Predictive models indicate that shifts in climate could alter the geographic distribution and abundance of OLF populations towards northern regions because of the changes in the suitability of different regions for olive cultivation [3,7,66], complicating existing management practices [7]. Understanding these environmental interactions is crucial for developing effective control strategies for more precise and low-input practices.

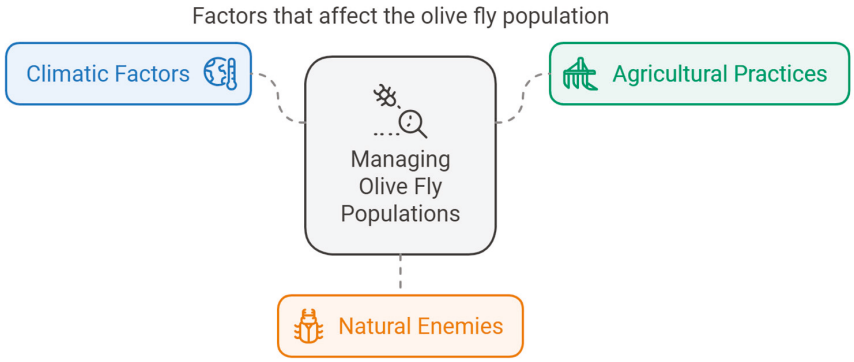

Figure 3. Factors that affect the olive fly population.

Agricultural practices also play a part in shaping OLF populations. Irrigation practices can influence fruit quality and susceptibility to attack. For instance, water stress in olive trees may lead to increased fruit sugar content, making them more attractive to egg-laying females. Conversely, fertilization practices can indirectly negatively affect OLF populations by influencing the abundance and diversity of natural enemies within the olive grove ecosystem [69,70].

Natural enemies, encompassing predators and parasitoids, play a vital role in regulating OLF populations. Predatory insects like ladybugs and ground beetles feed on OLF eggs and larvae, spiders, birds, bats, and lizards predate on OLF adults, while parasitoid wasps lay their eggs within OLF pupae, effectively reducing fly emergence rates [30,71]. Maintaining a healthy and diverse population of these natural enemies through the use of organic farming practices and the creation of a suitable habitat within olive groves offers a sustainable approach to OLF control [30,72]. By understanding the complex interplay between climatic factors, agricultural practices, and natural enemies, we can develop more sustainable and holistic strategies for managing OLF populations and ensuring healthy olive production.

5. Management Strategies

The OLF presents a persistent challenge for olive growers (Figure 4). Diverse methods and control strategies have been developed to combat this pest [73]. Farming practices represent the first line of defense [72,74,75]. Early harvesting, before peak OLF activity, can significantly reduce fruit damage [76,77]. Sanitation measures, such as removing fallen fruit and pruning to improve orchard ventilation, also play a role by minimizing potential breeding sites [57,78]. However, the effectiveness of farming practices alone is often limited, particularly in areas with high OLF pressure [2,79,80].

Chemical control, through the application of insecticides, has traditionally been the mainstay of OLF management [81]. Spraying applications are carried out using two methods: (i) bait sprays and (ii) cover sprays. Bite sprays use insecticides, such as organophosphate (at a concentration of 0.3% active ingredients), combined with a food attractant, such as 2% hydrolysate protein. This mixture is then applied from ground level to a limited section of the canopy, typically ranging from 1 to 2 cubic meters. The amount of spraying solution per tree does not exceed 300 milliliters. Cover sprays involve using insecticide at a concentration that is ten times lower (e.g., 0.03% organophosphate) compared to that in bait sprays. Moreover, cover

sprays require a significantly larger volume of spray solution (approximately 10 to 15 L per tree) to be applied across the entire tree canopy until runoff [82].

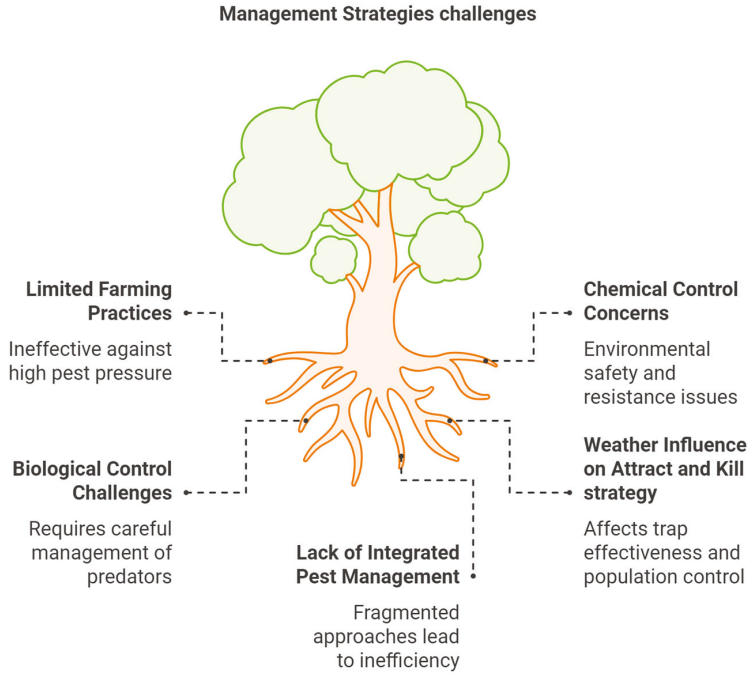

Figure 4. Management strategy challenges.

However, concerns over environmental safety, insecticide resistance development in OLF populations, and potential negative impacts on beneficial insects have spurred the exploration of alternative methods [83].

Biological control offers a more sustainable approach by harnessing the power of natural enemies. The release of predatory insects or the promotion of existing predator populations within an olive grove can help regulate OLF numbers [84,85]. Additionally, the use of parasitoid wasps that target OLF pupae is gaining traction. While promising, biological control often requires careful management to establish and maintain effective predator and parasitoid populations [86]. This can be achieved by maintaining native flora inside and at the edges of the olive groves [72]. Moreover, it has been proven that certain herbs, such as *Cistus creticus* L. and *Lavandula stoechas* L., can act as repellents for the OLF because of their olfactory cues, which can reduce fly attraction to odors from the olive host by 10% [65]. Additionally, the identification of beneficial soil arthropods that can naturally suppress OLF populations has opened new avenues for integrated pest management strategies [71]. Recent studies have also focused on the role of microbial antagonists in controlling OLF populations. The use of bacterial isolates, such as *Providencia entomophila*, has been shown to increase the mortality rates in olive flies, suggesting that these microorganisms could serve as biocontrol agents [87].

The development of biopesticides based on these natural enemies could provide a sustainable solution to OLF management, reducing the need for synthetic chemicals. Entomopathogenic fungi, such as *Metarhizium brunneum* and *Beauveria bassiana*, have shown efficacy in controlling the OLF and other Tephritid fruit fly pests [88–92]. These fungi can infect and kill the pest through direct contact, and some can also have sublethal effects on the pest's behavior and reproduction [93]. *Metarhizium brunneum* can be used for soil-based treatments targeting the pre-imaginal stages of the OLF [88,89]. This approach has been shown to reduce the emergence of adult flies by significant percentages in treated plots

compared to controls [89]. The persistence and efficacy of these fungal treatments can be influenced by factors such as the formulation type and environmental conditions [88].

Additionally, entomopathogenic fungi can be integrated with other management strategies, such as the use of pheromone-based attractants [45] and the development of molecular tools for pest detection and monitoring [56,94], to create a more comprehensive and effective integrated pest management approach against the OLF [2]. Interactions between entomopathogenic fungi, plants, and insects can be complex, and plant-mediated effects may influence the efficacy of these fungi as biocontrol agents [95]. Understanding these interactions could help optimize the use of entomopathogenic fungi in olive production systems.

Attract-and-kill (A&K) strategies utilize pheromone and food attractant traps that lure adult olive flies and trap them, effectively reducing mating success and egg-laying. These approaches are environmentally friendly and require less broad-spectrum insecticide application. However, their effectiveness can be influenced by weather conditions and OLF population densities [35].

One of the most promising technological advances in OLF management is the application of a sterile insect technique (SIT). This method involves the mass rearing of sterile male flies, which are then released into the wild to mate with wild females, resulting in no offspring and a gradual reduction in the population. Recent studies have demonstrated the feasibility of using genetically enhanced sterile insect techniques, such as the fsRIDL (female-specific Release of Insects carrying a Dominant Lethal) approach, which has shown promise in suppressing OLF populations in controlled environments [34]. This method not only reduces the reliance on chemical insecticides but also minimizes the ecological impact associated with traditional pest control methods. Furthermore, advancements in the understanding of the OLF's genetic makeup have facilitated the identification of specific traits that can be targeted for control. For instance, research into the gut microbiota of *Bactrocera oleae* has revealed potential avenues for manipulating these microbial communities to enhance pest management strategies [96,97]. By understanding the symbiotic relationships between the OLF and its gut bacteria, researchers can develop targeted interventions that disrupt these interactions, potentially leading to reduced pest viability.

Technological advancements in monitoring systems have significantly improved the ability to track OLF populations and inform management decisions. The development of decision support systems (DSSs) that integrate location-aware technologies allows farmers to receive real-time data on pest populations and environmental conditions [98–100]. These systems can provide tailored recommendations for pest management, optimizing the timing and application of control measures. Moreover, the use of attract-and-kill devices, which combine attractants with insecticides, has emerged as a promising strategy for managing OLF populations. These devices minimize the impact of full-cover spraying and target specific pest populations, thereby reducing the overall chemical load in the environment [85]. Research into new attractants derived from yeast associated with the olive fruit fly has further enhanced the effectiveness of these devices [45]. While the attract-and-kill approach is targeted towards OLF, there is a risk of non-target effects on other arthropod species in the olive grove ecosystem [31,101]. The use of insecticides, even in targeted bait stations, can still pose risks to other arthropods, including pollinators and natural enemies of OLF [31,102].

The future of OLF management lies in the development and implementation of integrated pest management (IPM) programs [103]. These programs combine various control strategies, including cultural practices, biological control, A&K techniques, and the judicious use of selective insecticides, to achieve long-term, sustainable OLF suppression [104].

This holistic approach minimizes the environmental impact while effectively managing OLF populations and ensuring high-quality olive production [103]. As part of an IPM program, farmers could opt to cultivate olive cultivars that are more resistant to OLF infestation. The susceptibility of olive cultivars to the OLF may be influenced by various factors, including the physical and chemical characteristics of the olive fruit, such as epicuticular waxes [105] and volatile organic compounds [44]. These cultivar-specific traits can affect the olive fly's ability to detect, access, and oviposit on the fruit. Additionally, there is genetic variation in olives' resistance to the OLF, so the identification and use of resistant cultivars could be an important tool for IPM programs [12,106]. However, "resistant" olive cultivars are not completely immune to OLF infestation [9]. The pest can still cause significant damage to these varieties, though potentially at lower levels compared to that in more susceptible cultivars. Moreover, we should not underestimate the importance of local varieties and the importance of their conservation.

6. Technological Advances

Recent technological advances have substantially enhanced OLF population monitoring and pest control strategies. Miranda et al. developed a decision support system (DSS) for managing the olive fruit fly in Mediterranean olive orchards, utilizing semi-automatic, location-aware, real-time monitoring that significantly enhanced the pest control efficiency [98]. They integrated electronic traps that monitored pest populations with georeferenced tree data, enabling precise, targeted insecticide applications. Based on such frameworks, further innovations have introduced electronic traps (e-traps). An example is the research by Diller et al., where deep learning algorithms are used to monitor fruit fly populations while enabling real-time data acquisition and analysis [107].

Further technological advances in OLF population monitoring and intervention have been achieved through machine learning techniques and deep learning techniques, aiming at the detection and counting of olive fruit flies. The study in Molina-Rotger et al. investigated the potential of machine learning (ML) techniques in electronic traps for remote monitoring of pests [108]. They used Random Forest (RF) and Support Vector Machine (SVM) algorithms to develop a tracking system, achieving a significant detection accuracy, enabling the remote tracking and detection of pests to facilitate precision agriculture. This approach reduces the need for on-site monitoring of olive groves and supports sustainable pest management by optimizing pesticide use and minimizing the ecological impact. In Mamdouh et al., an attempt is made to detect olive fruit flies captured in smart traps, proposing a specialized framework that exploits the strengths of the YOLO deep learning algorithm, adapted to olive fruit fly recognition and counting [109]. The algorithm is trained from images of smart pheromone traps. Yellow color normalization is applied, where the images are adjusted to the yellow color of the traps, making the framework illumination-invariant. For greater accuracy, the training dataset is enriched with negative samples, as well as data augmentation techniques being applied.

In addition to assessing the accuracy of OLF detection algorithms, Mira et al. also took the energy consumption into account [110]. They used three computer vision methods for object classification to detect the OLF in colored traps. The images used to train the algorithm consisted of a variety of realistic conditions to maximize the accuracy.

7. Discussion

The relentless quest for effective OLF management necessitates continuous research and exploration. Several key areas demand further investigation to ensure the sustainability of olive production in the face of evolving challenges. A crucial area of research concerns

the development of resistance in OLF populations to the existing control methods. The widespread use of insecticides has been selected for resistant fly strains, jeopardizing the effectiveness of chemical control programs [2,111,112]. For example, in Greece, there are reports of resistance to pyrethroid and alpha-cypermethrin insecticides [113,114]. Future research should focus on monitoring and understanding the evolution of their resistance mechanisms, while simultaneously exploring alternative insecticides with novel modes of action that can circumvent the existing resistance [113,115].

The looming threat of climate change necessitates a deeper understanding of its impact on OLF ecology [46,66]. Rising temperatures and altered precipitation patterns are predicted to influence OLF population dynamics, development rates, and geographical distribution. Research efforts should focus on simulating climate change scenarios and their potential consequences for OLF populations [106,116,117]. By understanding how climate change will reshape the OLF's behavior and development, we can develop proactive management strategies that remain effective under future climatic conditions [7,118,119].

The use of an integrated geoinformatics system for OLF monitoring and spraying could be an important factor for timely and effective control of this insect [120]. Exploiting the possibility of mapping the trap network and recording real-time data on the OLF population enable the direct identification of outbreaks and the correlation of insect density with various environmental parameters [98]. By tracking the spray vehicles, it is possible to monitor in real time the areas where spraying is carried out and to take corrective measures [121]. The use of the above applications leads to better management of insecticides and therefore has a positive environmental impact [122].

Finally, exploring novel control strategies holds immense promise for sustainable OLF management. Research efforts should investigate the potential of utilizing semiochemicals, such as kairomone lures that attract natural enemies, or the development of genetically modified olive trees with increased resistance to OLF infestation [44]. Additionally, advancements in biocontrol techniques, such as the mass rearing and release of effective parasitoid wasp species, need further exploration [123–125]. By investing in these innovative approaches, we can create a diverse toolbox for OLF control, ensuring the long-term viability and environmental sustainability of olive production.

The olive fly is one of the major threats to olive production, causing significant economic and environmental damage [3,80,126]. Infestations reduce olive oil yield and quality, impacting farmers' income and disrupting global supply chains [6]. Additionally, the heavy use of insecticides to control these pests harms beneficial insects and disrupts ecosystems [127,128].

To effectively manage this persistent pest, scientists need a deep understanding of OLF ecology. This review delves into the fly's life cycle, its relationship with its primary host (the olive tree), and how environmental factors like temperature and rainfall influence its population dynamics [24,129]. By understanding these aspects, researchers can develop targeted control strategies [130]. For instance, studying each life stage's vulnerabilities allows for interventions that disrupt specific parts of the fly's development and reproduction. Additionally, exploring the role of natural enemies and the impact of agricultural practices can lead to the development of sustainable control methods that minimize environmental harm [80].

Research shows that to effectively control the OLF, all of the parameters related to its ecology must be taken into account. It is clear that climate change, intensive cultivation, environmental degradation, and the development of resistance to agrochemicals, combined with the existence of alternative hosts and the insect's ability to cover considerable distances during the day, are creating new data for understanding and effectively managing it [100]. Research shows that the insect can take refuge in nearby areas when conditions are unfavorable for it [30]. In many cases, we cannot talk about population reductions under

extreme weather conditions but about the population finding shelter in a more diverse landscape so that it can return when these conditions are favorable. An understanding of these parameters, further study of the ecology of the OLF, and a more environmentally friendly approach to the subject could lead to the prediction of population outbreaks and more effective protection of olive groves [100].

Compared to other Tephritid fruit flies, such as *Ceratitis capitata*, while the management strategies share some similarities, such as the use of biological control and IPM approaches, the specific challenges and solutions may vary due to the unique biology and ecology of each pest species. The development of insecticide resistance is a common challenge across Tephritid fruit fly pests, highlighting the need for alternative, sustainable control methods [2, 50]. The role of the gut microbiome in the biology and management of Tephritid fruit flies, including *B. oleae*, is an emerging area of research that may lead to novel control strategies [50,80]. Meanwhile, molecular techniques, such as DNA barcoding and PCR-based diagnostics, have proven valuable for the identification and monitoring of Tephritid fruit fly species, which can support the implementation of effective management programs [94,131].

8. Conclusions

Despite the significant economic impact of the OLF on olive production worldwide, our understanding of its ecology remains incomplete. This gap in the knowledge presents a critical barrier to developing truly effective, precise, and sustainable management strategies. Further research is needed to elucidate key aspects of the OLF's life cycle, behavior, and interactions with the environment. This includes investigations into the factors influencing population dynamics, host–parasite interactions, and the impact of climate change on the OLF's distribution and abundance. We need to treat the OLF not only as a pest but firstly as an insect as a part of the agroecosystem. By filling these knowledge gaps, we can refine the existing management practices, develop novel control methods, and ultimately minimize the negative impacts of the OLF on olive production while preserving environmental health.

Successful OLF control offers significant economic and environmental benefits. Reduced crop losses translate into higher olive oil production and economic gains for farmers. Moreover, sustainable control methods minimize the reliance on harmful insecticides, promoting biodiversity and healthy ecosystems within olive groves. In conclusion, a comprehensive understanding of OLF ecology is the key to developing effective and sustainable management strategies, ensuring the continued viability of olive cultivation and the production of high-quality olive oil.

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How Accurate Are Population Predictions? Wind Farms and Egyptian Vultures as a Case Study

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Simple Summary: Population viability analysis is a modeling tool estimating the future size and risk of extinction for populations. Population viability analyses are widely used in setting conservation policies. There is disagreement about the precision of this method. To gain a clearer picture of the usefulness of population viability analysis packages, many more predicted population trajectories need to be evaluated. This will provide vital feedback information that can be used to refine the models, which should in turn produce increasingly accurate predictions of population viability. The objective of this work was to analyze discrepancies between predictions and reality of a population viability analysis for the Egyptian vulture population. Real and additional data have shown that the population viability analysis predictions, i.e., the complete extinction of the Egyptian vulture by those dates, did not come true, fortunately; in fact, the conservation status of the species in question has improved. We discuss reasons for that discrepancy and how to avoid these mistakes in the future.

Abstract: It is clear that scientists' predictions must be rigorous and based on scientific evidence, but, even more, it is crucial to review scientific predictions after a reasonable time. However, predictions of published PVAs have rarely been contrasted with real populations' trends over time. This is worrisome because this is the only way to keep learning and improve our ability to make more accurate predictions. In addition, conservation efforts can shift the initial predictions for the viability of threatened populations; thus, the evaluation of initial predictions becomes required over time. This is the case of the Egyptian vulture in Spain, where trajectories of real populations over the years differ from large-scale predictions. Its extinction in the Iberian Peninsula—due to mortality in wind farms, among other causes—was predicted by 2020, according to published viability analyses; yet, 14 years after this publication, not only did it not happen, but its national (and European) population remains stable and is even slightly increasing (+2.6%). These differences between predicted and observed trajectories of populations show the limitations of the simulations as a conservation tool and offer the opportunity to evaluate the used PVAs and the shortcomings that affected the assessment of the real trajectory of the species. With only four years of data available to simulate and generate 100-year predictions, along with the distribution and variance of mortality rates by collision in wind farms and an overestimation the number of pairs in risk areas, a clear relationship was assumed between predicted risk according to distances and the actual recorded mortality at wind farms, even though it is known that these are not closely related.

Keywords: PVA; viability of population; wind farm; bird mortality; *Neophron percnopterus*; prediction of population trajectories; observed versus predicted population trajectories

1. Introduction

The need to constantly review our understanding of endangered species' threats cannot be underestimated. In fact, conservationists should be encouraged to undertake such regular work to ensure that, firstly, when threats have been removed and/or mitigated, such work is appropriately acknowledged. Secondly, to celebrate the successes when a species' conservation status has improved, and thirdly, when additional data are available, to recognize that perhaps the initial concerns were incorrect.

Population viability analysis (PVA) is a modeling tool that estimates the future size and risk of extinction for the populations of organisms [1–3]. PVA is a way to predict the probability of population extinction by inputting actual life-history information and projecting it forward using stochastic computer simulation [4,5]. Although population viability analysis (PVA) is widely used and can be extremely useful in setting conservation policies, there is disagreement about the precision of this method. Objections have been raised concerning the precision of predictions, sometimes in view of the short time series of data available and the effects on the estimated parameters [6–9]. A review of the use of demographic models for endangered species management [5] pointed out that poor data cause difficulties in parameter estimation, which in turn lead to unreliable estimates of extinction risk. To gain a clearer picture of the usefulness of PVA packages, many more predicted population trajectories need to be evaluated. This feedback process is fundamental to evaluate the real utility of this approach as a useful tool for species conservation, especially in setting conservation policies. We need much more information about the real accuracy of past PVAs by confronting predicted versus observed trajectories in different species and populations. Only this process will provide us with vital feedback information that can be used to refine the models, which should in turn produce increasingly accurate predictions of population viability. This is the objective of this work.

In 2009, a paper dealing with the long-term effect of wind farm mortality on the viability of the Egyptian vulture population (*Neophron percnopterus*) in Spain was published [10]. Wind farms receive public and governmental support as an alternative energy source that mitigates air pollution and facilitates the transition to the decarbonization of society. With 61,460 MW of installed power as of 12 November 2020 and 56.04% of total installed power, Spain is one of the leading countries in the world [11] in the production of energy from renewable sources per capita. Developing renewable energy has become a common strategy and effort in countries all over the world, since it became fundamental to reverse global climate change [12]. Wind power is one of the oldest and most developed sources of renewable energy, with an increase in production exceeding 20% annually over the past decade [13]. In accordance with the current environmental issue, wind facilities generate a low environmental impact by reducing environmental pollution and water consumption [14].

However, it is known that wind farms can have adverse effects on wildlife, particularly through bird and bat collision with rotating turbine rotor blades. Bats and birds are the most impacted taxa because, as flying animals, they are more prone to collide with the turbine rotor [15–19]. Low collision rates have been registered in some studies, such as 0.001 birds/turbine/year [20], but also high collision rates have been reported in others (1.33/turbine/year), with the griffon vulture (*Gyps fulvus*) being the most frequently killed raptor species (0.41 deaths/turbine/year) [17,21]. These findings indicate that mortality rates per turbine are quite variable because the probability of collisions depends on a

range of factors such as species, species-specific flight behavior, weather, and topography around wind turbines [15,16,21–23]. There is a consensus that raptors may be more prone to colliding with blades than other birds [24] due to their morphology, foraging behavior, or flight behavior [25,26]. Wind farms are assumed to have an important impact on raptor populations, because raptors have longer life spans and lower reproductive rates, resulting in population growth rates that are highly sensitive to adult and subadult mortality [27–29].

2. Materials and Methods

To compare predicted and observed trajectories of populations of Egyptian vultures, we used published information on real historical trajectories according to national censuses [30] and other online information [31]. Using all the available data, we present in Figures 1 and 2, the real trajectories of both Spanish and the Southern Egyptian vulture populations.

In addition, we calculated the real values of some demographic parameters during the 2009–2022 period, now available from the same sources as above, and compared them with the ones available during the 2004–2008 period, which were used to construct the PVA used in [10]. We recorded and analyzed the results reported by the plan against poison, developed by the Andalusia Environmental Ministry, looking for some trend in this source of non-natural mortality, which was the most important for the species at the beginning of this century [32].

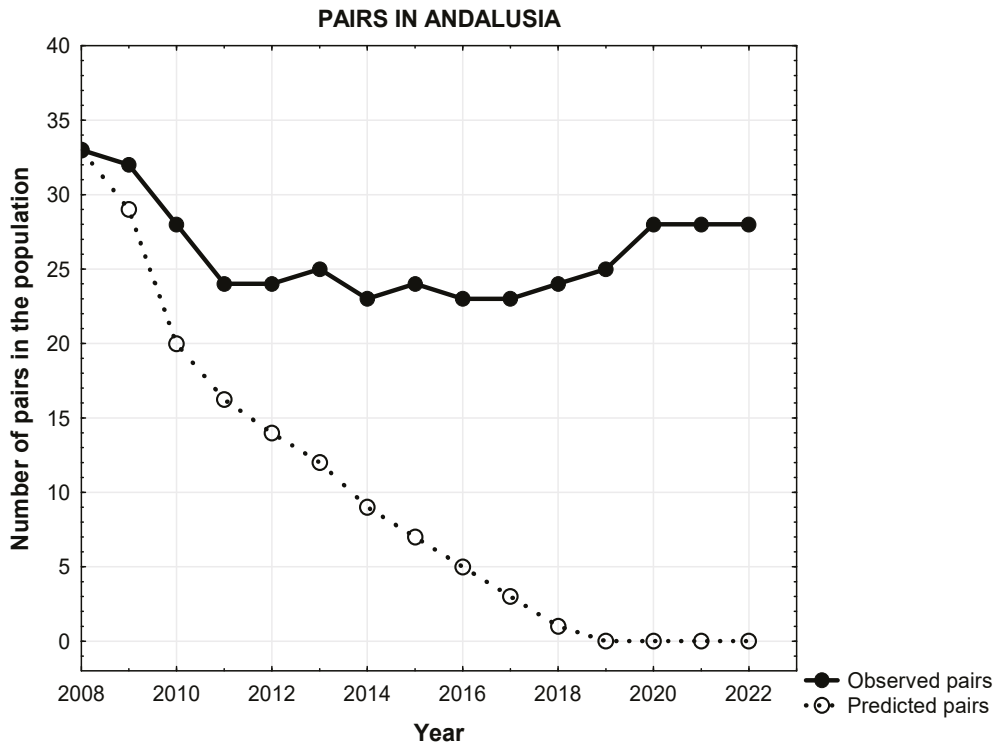

Figure 1. Predicted (according to [10]) versus observed trajectories for the Southern population of Egyptian vultures. As we can see, trajectories differ dramatically. In fact, ref. [10] predicted complete extinction of the species from the Southern population (Andalusia) by 2018. Breeding pairs and years are presented in the axis.

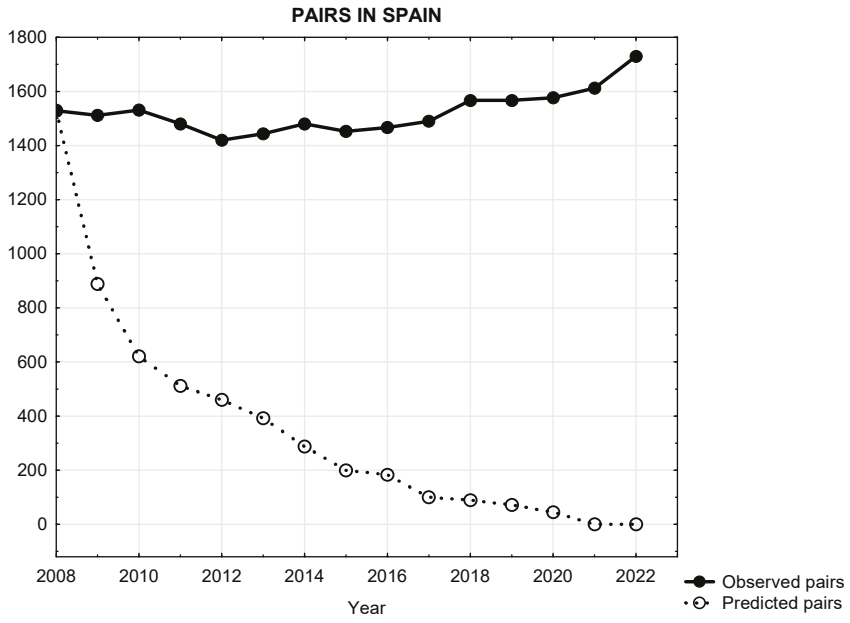

Figure 2. Predicted (according [10]) versus observed trajectories for the Iberian Peninsula population of Egyptian vultures. As we can see, trajectories differ dramatically. In fact, ref. [10] predicted complete extinction of the species from the Iberian Peninsula by 2021. On the contrary, this population experienced an increase of 2.6%. Breeding pairs and years are presented in the axis.

The Egyptian vulture is a medium-sized, cliff-nesting, trans-Saharan migrant raptor that defends long-term established territories during the breeding season. Most territories hold a single nest (rarely 2–3 nests situated in the same or adjacent cliffs) that is occupied year after year over long periods of time. The long-term monitoring of marked birds shows that territories are reoccupied every year in early March by their previous owners or, when one dies, by a replacement bird [10]. Most territories hold a single nest (rarely 2–3 nests situated in the same or adjacent cliffs) that is occupied year after year over long periods of time. The dispersal of breeders is rare (only in 7.5% of 203 breeding attempts did one of the birds change territory). In the few areas where species numbers have recovered, most of the territories consist of recolonizations of vacant territories (the so-called extinct territories referred to throughout the text). Therefore, territorial extinctions occur not as a result of breeder dispersal, but because an external cause has affected the territory owners and/or the territorial 'attractiveness' for recruitment.

Despite being a territorial breeder, this species may forage and roost socially. This is especially true for non-breeding birds, which also migrate to and from Europe and gather in communal roosts during the breeding season. These roost sites are located near predictable food sources such as rubbish dumps or muladares (places traditionally used by farmers for dumping dead livestock).

In Andalusia, a total of 269 wind turbines from 20 wind farms were monitored from 2008 to 2022 to determine Egyptian vulture collisions. Eleven collided individuals of the Egyptian vulture were found during the non-migratory period, which we assume belonged to local population. These wind farms were constructed and began operation between 2006 and 2007, being the same wind farms studied for estimated Egyptian vulture mortality [10]. According to environmental impact (EID) regulations, facilities were required to develop surveillance programs [16,33]. The main goal of these programs is to document all the mortalities caused by the collisions of birds with turbine blades. These programs have been conducted annually every day of the year (365 days) from dawn to dusk (between 8 and 14 daylight hours in winter and summer, respectively) by 12–17 trained observers who are

interconnected by cellular telephones. Mortality searches are carried out at all turbines on a daily basis, with a fixed search effort to find dead birds or bats. The observers are evenly distributed throughout the area covered by the wind farms. With this high search effort, we do not need to apply corrections for search efficiency and scavenger removal, particularly in the case of a large bird like the Egyptian vultures. Anyway, our main goal was to detect relative trends in mortality. These data enable us to determine how mortalities were distributed among the different wind farms and their turbines.

After 2007, the selective stopping of turbines by observers when dangerous situations are detected became mandatory in Andalusia [18]. In these cases, the observers telephone the wind farm control office to switch off the turbines involved in the risk, stopping the turbine within a maximum of 3 min. All 20 wind farms studied have been required to carry out this stopping procedure since 2008 for all medium to large-sized birds that were observed in a risk situation. As a result, we were able to obtain data from the 269 wind turbines from 2008 to 2022 (14 years). Data were recorded according to the protocol designed by the Migres Foundation and collected by the Andalusia Environmental Ministry. We use these data as a proxy to detect a potential change (increase or decrease) in Egyptian vulture mortality rates.

2.1. Supplementary Feeding

Supplementary feeding is a common practice to increment the reproductive output in raptors and other bird species, either for experimental or for conservation purposes (e.g., California condor, sparrowhawk, various vulture species, common kestrel, and bearded vulture) [34–40]. Despite the widespread use of this technique over the past 50 years, particularly in endangered species, its application in conservation has only recently been critically discussed [41], revealing important differences in the evaluation of the technique. Some claim major beneficial effects at the population level, but others report little or no effect [39,42,43].

Significant positive effect of supplementary feeding in the case of the Egyptian vulture has been reported [40,44,45]. Typically, food is placed every day or two, close to the nests of selected pairs for part or all of the breeding cycle from before laying to independence of young, depending on the objective. The usual aims are to increase the size of the clutch or prevent nestling deaths by increasing the nutritional condition of adults.

2.2. Simulations

We conducted a simulation using Vortex 10, including the same simulation inputs as other authors [10], but substituting the values they used for both productivity and adult mortality recorded during the 2004–2022 period (Table 1). Following the criteria proposed by other authors [46], simulations were run during twice the life span of the species, 50 years in this case. We conducted simulations to analyze the evolution of predicted and observed population under different scenarios. We used the Vortex simulation software (Vortex, version 10.0.76). Vortex is an individual-based model for population viability analyses (PVAs). It models population dynamics as discrete, sequential events that occur according to probabilities defined by the user, and it can model constant or random variables that follow specified distributions. The events used for modeling describe the typical life cycle of sexually reproducing diploid organisms. This method is particularly appropriate for species showing low fecundity, long life span, small population size, estimable age-specific fecundity and survival rates, and monogamous breeding, as in the species and populations we modeled here. In fact, Vortex has already been used to analyze the viability

of populations of Egyptian vultures [10]. We conducted several simulations for different scenarios, performing 1000 replicates for each one.

Table 1. Differences in baseline conditions according to period used for estimates. For all the studied period (2004–2022), mean productivity was 0.864, which is 1.36 times greater than estimates used by [10]. Mortality from wind turbines had been 1.4 times lower than the one predicted by [10]. For all the study period (2004–2022), the mean mortality for poison was 0.002, which is 9.5 times lower than the one used in the simulation by [10]. Period 2004–2008 was the time period used to estimate demographic parameter by other authors [10].

Time Period	Fecundity (SD)	Mortality by Wind Turbines (SD)	Poison-Related Mortality (SD)
Period 2004–2008	0.633 (0.074)	0.80 (1.095)	0.019 (0.02)
Period 2009–2022	0.947 (0.460)	0.50 (0.650)	0.009 (0.09)
Total 2004–2022	0.864 (0.418)	0.57 (0.768)	0.002 (0.02)

3. Results

3.1. Predicted Versus Observed Population Trajectories of Egyptian Vultures Subsection

The Egyptian vulture in Spain, whose extinction in the Iberian Peninsula is due, among other causes, to mortality in wind farms, was predicted by 2020 (according to [10]); and yet, 14 years after this publication, its national (and European) population remains stable and even slightly increasing (+2.6%) [30]. According to [10], considering wind-farm-induced mortality in the species, all simulations performed showed highly negative stochastic growth rates (see Figure 3 [10]), with values lower than 0. In fact, their results predicted complete extinction of the Southern population by 2018 (Figure 1) and, some years later, by 2021, the complete extinction of the species from the Iberian Peninsula (Figure 2).

In the Egyptian vulture metapopulation, i.e., the Iberian population, and following the results of their simulations (see Figure 3 in [10]), the predicted negative slope was −0.8683, decreasing from 1529 breeding pairs in 2008 to 0 pairs in 2021. However, the real observed trajectory of the Iberian population has shown positive slope of +0.7860, increasing from 1529 breeding pairs in 2008 to 1612 pairs in 2021.

A similar pattern can be found in predicted versus observed trajectories for the Andalusian population, which they called the Southern population (see Figure 3 in [10]). The predicted slope was −0.9386, decreasing from 33 breeding pairs in 2008 to 0 pairs in 2021. However, the observed slope has been −0.6747, decreasing from 33 breeding pairs in 2008 to 28 pairs in 2021.

These differences between predicted and observed trajectories of populations offer a unique opportunity to review the initial variables included and the construction of the PVA to evaluate the underlying causes of those differences.

3.2. Change in Baseline Conditions

3.2.1. Mortality by Wind Farms

In [10], four years of data were used to estimate mortality rates due to wind farms (2004–2008) for the Andalusian population, and intervals of confidence were not considered for simulations. Within this period of data, the mean mortality of Egyptian vultures due to wind farms obtained was 0.8/year (Table 1). Considering the data now available, from 2009 to 2022, the real mean has been 0.50/year (Table 1). Consequently, mortality from wind turbines recorded during these years has been 1.6 times lower in the Southern population than the one predicted by [10]. Considering the full studied period, from

2004 to 2022, the mean has been 0.57/year (Table 1). Consequently, mortality from wind turbines recorded during these years has been 1.4 times lower in the Southern population than the one predicted by [10]. Those differences between estimated parameters, which seriously affect predictions, partially explain the differences found between predicted and observed trajectories.

3.2.2. Productivity

We have been providing supplementary feeding in Andalusia since 2017. Supplementary feeding is aimed at particular territorial pairs within the Southern population, and it is performed for only a limited period each year. The results showed an important variation in mean fertility over the years. Other authors [10] recorded a mean productivity of 0.633 during the period 2004–2008 (Table 1). Considering the data now available from 2009 to 2022, the mean has been 0.947 (Table 1), that is, 1.49 times greater than the one used by [10] in their predictions. For the full studied period (2004–2022), mean productivity was 0.864, which is 1.36 times greater.

3.2.3. Mortality from Poisons

Unfortunately, the use of poisoned baits is still a widespread practice in Spain and Europe to control natural predators of game and livestock species. This nonselective method poses a deadly threat to many threatened species in Andalusia such as the Spanish imperial eagle, the bearded vulture, or the Egyptian vulture. Despite the complexity of the actions required for its eradication, the measures implemented under the Andalusian Strategy against Poison in 2004 have led to a 50% reduction in the use of poisons in the region, from 205 cases detected in 2006 to 94 in 2012, and only 21 cases in 2019 (89.75% reduction). These measures placed Andalusia at the forefront of the fight against poison in Europe.

Adult poison mortality was 0.019 during the 2004–2008 period in Andalusia, decreasing to 0.009 during the period 2009–2022. For all the study period (2004–2022), the mean mortality for this cause was 0.002, which is 9.5 times lower than the one used in the simulation by [10].

3.3. Distribution of Simulated Demographic Stochasticity and Environmental Variability

Demographic stochasticity is the random fluctuation in the observed birth rate, death rate, and sex ratio of a population resulting from stochastic sampling processes. Environmental variability, on the other hand, is the annual fluctuation in probabilities of birth and death arising from random fluctuations in the environment. Demographic stochasticity is commonly modeled in VORTEX and other programs as binomial or, optionally, beta distributions, while environmental variation is modeled frequently as a normal distribution. Mortality rates, especially due to human infrastructures, tend to usually show non-normal distributions, following a strong Poisson distribution instead. Remarkably, the spatial pattern of mortality found in wind farms [16,18,21], resembling that caused by other anthropogenic infrastructures such as power lines [47] or roads [48], shows that few pylons, road sections, or wind turbines typically account for the majority of casualties. Therefore, the identification and subsequent application of mitigation measures in mortality hotspots would dramatically reduce overall mortality [47,49].

In fact, using homogeneity tests, the annual mortality of Egyptian vultures from the wind farm in the Andalusian population during 2004–2022 follows a Poisson distribution (lambda = 0.57, Kolmogorov–Smirnov d = 0.56; X2 = 0.535; $p = 0.464$) and not a normal one (Kolmogorov–Smirnov d = 0.35; X2 = 105.419; $p < 0.001$, see Figure 3). These particular distributions of mortality at man-made infrastructures tend to be spatially highly concentrated. An additional

factor affecting the predictions of [10] has been that data used showed a higher variance than expected in a normal distribution (mean = 0.8; variance 1.2), suffering over dispersion.

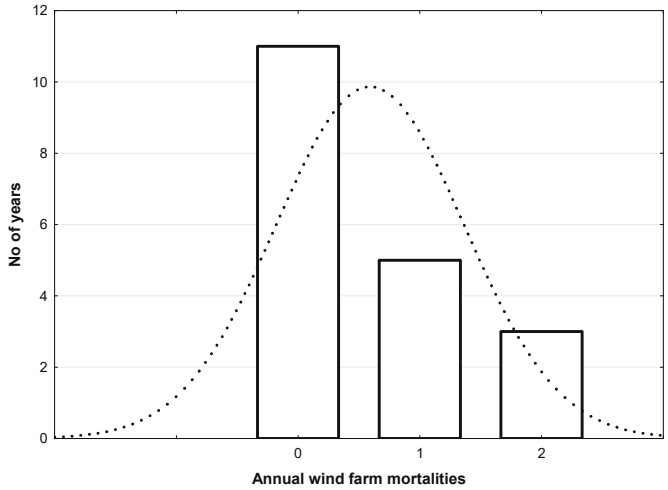

Figure 3. Wind farm annual mortality of Egyptian vultures in Andalusian population during 2004–2022. This mortality follows a Poisson distribution (lambda = 0.57, Kolmogorov–Smirnov d = 0.56; X^2 = 0.535; p = 0.464) and not a normal one (dotted line; Kolmogorov–Smirnov d = 0.35; X^2 = 105.419; $p < 0.001$).

3.4. Estimation of the Number of Pairs in Risk Areas

From 2004 to 2008, the authors in [10] found two territorial and three non-territorial (two young and one individually marked 6-years-old floater) birds dead at wind farms located in the Andalusian population. The closest distance between the breeding territories suffering from these casualties and the wind farms was 6.37 and 14.57 km, so they decided to use 15 km as a guide radius to obtain wind farm risk zones, assuming that all breeding pairs located within an area of 15 km radius around the wind farms, anywhere in Spain, must have the same risk of collisions. That included 389 pairs in Spain and 7 in the Andalusian population.

According to the paragraph above, these particular distributions of mortality at man-made infrastructures like windfarms tend to be spatially highly concentrated. It has been demonstrated that mortality does not depend on the wind farm but on the exact location of individual wind turbines [16,18,21,22]. According to [22], soaring birds do not move at random over the area, but follow some trajectories more than others. These preferred trajectories were determined by the wind speed, which was in turn related to the underlying topography of individual wind turbines. Consequently, certain locations of wind turbines could show a high collision risk even if there is a relatively low density of birds crossing the area, whereas other locations could be safer even with higher densities of birds in the wider area. This means that there is no clear relationship between predicted risk according to bird densities and actual recorded bird mortality at wind farms [16]. Risk assessment studies incorrectly assumed a linear relationship between the frequency of observed birds and fatalities. However, it is known that bird abundance and bird mortality through collision with wind turbines are not so closely related; this result challenges a frequent assumption, such as that formulated by [10], linking wind farm mortality risk to the distances of Egyptian vulture nests. In this case, predictions would be overestimating mortality rates for more than 400 pairs.

3.5. Simulation Results

The probability of extinction of the 100 simulated populations using the new baseline parameters was PE = 0.00. The simulated population showed a stable evolution and even a slightly increasing trend, with a positive stochastic population growth rate (rs = 0.023) and a positive deterministic population growth rate (rd = 0.024), with a $\lambda > 1$ (lambda = 1.024), again showing the trend towards a slight increase over years (Figure 4).

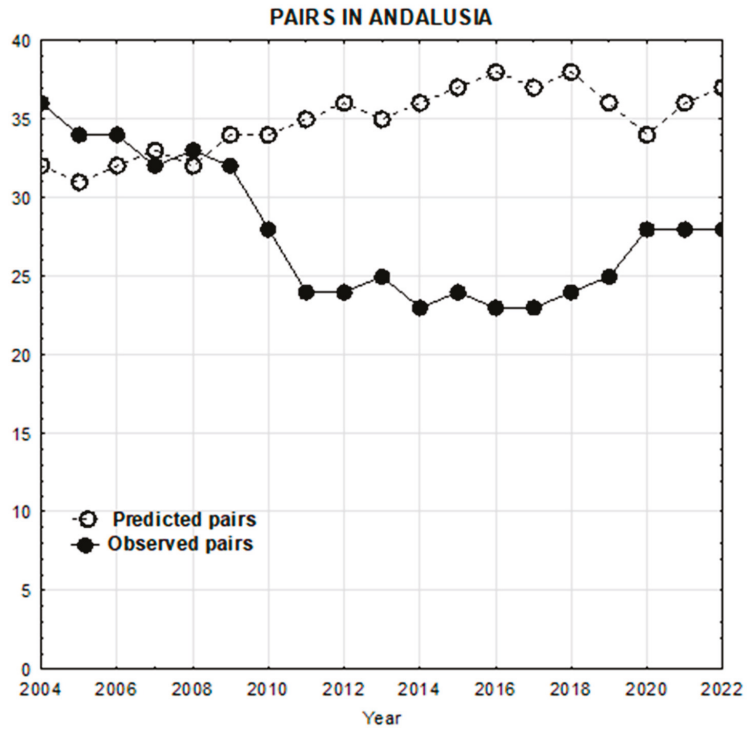

Figure 4. Predicted (according to new simulations) versus observed trajectories for Andalusian population of Egyptian vultures. As we can see, using the new values for productivity and mortality, both trajectories do not differ dramatically.

4. Discussion

Here, we reviewed previously published PVAs [10] warning of the imminent extinction of Egyptian vulture populations in Spain, and particularly in Andalusia. Real and additional data have shown that the PVA predictions had not come true, and, in fact, the conservation status of the species in question has improved.

According to our results, variation in baseline conditions was one of the reasons for these discrepancies between predicted and observed trajectories. Excellent results of the Andalusian Strategy against Poison, which achieved an 89.75% reduction in mean mortality for this cause, which is 9.5 times lower than the one used in the previous simulation [10], is one of the factors explaining differences between predicted and observed trajectories. Increase in fecundity, which was in fact 1.36 times greater than predicted, is in part due to supplementary feeding programs. Mortality from wind turbines recorded during the 2004–2022 period has been 1.4 times lower than the one predicted by [10] for the Southern population. Those differences between estimated parameters seriously affect predictions, partially explaining the differences found between predicted and observed trajectories.

In PVAs, the results depend sensitively on estimated parameters. Short time series or poor fits of the model to data lead to wide confidence intervals for the predicted trajectories of the simulated population. In many cases, the size of the confidence interval is so wide that estimates may become meaningless [9]. For the assessment of PVAs, it is essential to

account for imprecision in parameter estimates and its consequences for risk assessment. A variety of tools are available, such as confidence intervals on the risk of extinction within a given time horizon.

Other authors [10] alerted to the potential negative effects of wind farms on the conservation of many endangered species. They predicted future extinction scenarios that never happened. According to our current knowledge, the reasons beneath these differences between predicted observed population trajectories are (i) the amount of initial data, with only four years of data to simulate and generate 100-year predictions, (ii) changes in baseline conditions, with 1.4 times more productivity and lower mortality than estimated, (iii) not considering the actual distribution and its variance in mortality rates by collision in wind farms, and (iv) overestimation of the number of pairs in risk areas, assuming a clear relationship between predicted risk according distances and the actual recorded mortality at wind farms, when the abundance and mortality through collision with wind turbines are not linearly related. Abundance is a covariate, not an explanatory variable, in mortality at human-made infrastructures.

According to [10], current strategies implemented by power companies, based on vigilance of risky areas such that turbines are stopped when birds approach them, are completely inefficient in cases of solitary territorial birds that are rarely detected by observers [10]. However, this strategy, now called a turbine shutdown system, has achieved a 61.7% reduction in mortality of soaring birds and 92.8% specifically of collisions of griffon vultures, showing a net-beneficial application elsewhere [18]. In fact, it seems that this technique, perhaps in conjunction with supplementary feeding programs, was good enough to decrease the mortality of wind turbines by 1.4 times in the case of the Egyptian vulture.

No single wind turbine or wind farm has been removed in Spain to prevent Egyptian vulture collisions from 2008 to 2022, as far as we know, providing no support to an alternative explanation difference between predictions and observed trajectories—i.e., a decrease in the number of risk areas, which would in turn decrease mortality. In fact, in 2009, 19,148.8 MW were installed and operating in Spain, increasing to 29,813 MW by 2022, an increase of 10,664 MW [50]. What really changed during the years, after the predictions in [10], were productivity and adult mortality. The effort of previous authors [10] was important to send a warming message about the status of Egyptian vulture populations, encouraging mitigation actions including both poison and collision with wind turbines, but is also a good example of the limitations of the simulations as a conservation tool. With only four years of data, a long-term simulation is always dangerous due to small sample size to determine the means and variance of key demographic parameters. In this case, the imprecision in those demographic parameters was so high that the simulation results become meaningless.

It is clear that scientists' predictions must be rigorous and based on scientific evidence, but, even more, it is crucial to review scientific predictions after a reasonable time. However, the quality of published PVA predictions is rarely assessed. This is worrisome, because this is the only way to keep learning and improve our ability to make more accurate predictions.

5. Conclusions

This manuscript addresses, in our opinion, an important and often overlooked aspect of conservation science: the need to reassess past population viability predictions in light of subsequent reality. The Egyptian vulture in Spain could be a strong and timely case study. By confronting the forecasts made in 2009 [10] with actual monitoring data, we clearly show that the predicted extinction has not occurred and that, in some cases, population trends have been stable or even slightly positive. This is a valuable message, both for the

specific conservation context of the species and for the broader methodological debate on predictive models. The study's strengths lie in the long-term dataset used, the contrast between modeled and observed trajectories, and the identification of key differences in baseline conditions—such as reduced poisoning, lower wind farm mortality, and higher productivity—that help explain the discrepancy between the 2009 forecasts and reality.

This paper also addresses the question of scientific accountability. This case illustrates not only the limitations of PVA when based on short or incomplete datasets, but also the consequences of publishing forecasts that prove to be seriously inaccurate, especially when they influence conservation policy, funding, and public perception. We may wish to reflect explicitly on the responsibility of scientists to revisit their predictions, acknowledge when they are wrong, and ensure that conservation decisions are based on the best available evidence.

One clear conclusion that we can learn from this study is that PVA predictions must be confronted with real population trajectory when available. The final objective of any PVA is to determine if demography indicates a serious and imminent risk of extinction, but it is not very clear what criteria would be used to define what constitutes such a risk. A major problem with defining a risk of extinction is that, by any criteria, this definition is limited in time. Even taking this into account, we still need some objective criteria to decide when a population is at risk in order to obtain the public support for a long-term conservation action.

The main point for us is not to secure the population indefinitely, but to decide when the population is not at risk. Using simulations and objective criteria, like probability of extinction below 1% and positive trend over twice the lifespan of the species, we can make predictions about the length of the predictions. Predicted trajectories of the simulations can be used to check annually if the evolution of the new population is over, on, or under expectations. Adjusting the simulated period to twice the life span of the species allows us to compare species with different life histories in comparable units of time.

The original assessment [10] has limitations stemming from initial data scarcity, shifts in baseline conditions, failure to account for the actual distribution and variability of mortality risk from turbine collisions, and an overestimation of the number of breeding pairs in risk areas. Evaluating the predictive accuracy of PVAs by contrasting forecasts with observed trends is an essential exercise for model validation, adaptive management, early detection of unforeseen threats or positive actions, and the refinement of future PVAs. These differences between predicted and observed trajectories of populations show the limitations of simulations as a conservation tool and offer the opportunity to evaluate the used PVAs and the shortcomings that affected the assessment of the real trajectory of the species, such amount of initial data—with only four years of data to simulate and generate 100-year predictions—the distribution and variance of mortality rates by collision in wind farms, and the overestimation of the number of pairs in risk areas. Additionally, a clear relationship was assumed between predicted risk based on distances and the actual recorded mortality at wind farms, even though these factors are not closely related.

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Recommendations on How to Use Flight Initiation Distance Data in Birds

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Simple Summary: Data on flight initiation distance (FID) is an important tool in bird conservation to reduce the disturbance caused by human recreational activities. FID vary substantially between species and areas, and should be investigated in all areas before mitigation action or new constructions are decided. Instead of using mean or median FID values, I recommend the construction of a more accurate graphical relationship between the proportion of birds that flee at different distances from an approaching person. This will give detailed and necessary information to nature managers.

Abstract: Birds and other wildlife are negatively affected by many anthropogenic activities, including human recreational activities, which are often not considered in area planning. Here, I present factors affecting the flight initiation distance (FID)—the distance to an approaching human at which birds flee—for 1075 different flocks of waterbirds. The FID varied greatly between groups of birds and species. For some bird groups and species, the FID was longer in rural areas than in urban areas and increased with flock size and with disturbance from canoeing. In addition to the differences in FID between species and groups of species, there are two important conclusions from this study: (1) a graphical relationship between the proportion of birds that flee at different distances from an approaching person gives more information than mean or median FID values and should be used by nature managers, and (2) the FID should be investigated in each area before mitigating actions or new constructions are decided, considering all the factors affecting it. A global database with a mixture of FID values from a huge number of areas is valuable for some purposes but can be misleading for individuals in a specific area.

Keywords: alert distance; canoeing disturbance; dog disturbance; drone disturbance; FID; flock size; human disturbance; nature management; shorebirds; urban–rural; waterbirds

1. Introduction

Birds are important components of ecosystems, providing essential ecosystem services [1], and their diversity is positively related to human mental health [2]. In addition, birds are monitors of environmental change that can inform us about changes in the natural environment [3,4]. As they are widely distributed, relatively easy to survey, and responsive to environmental changes, we know more about their taxonomic, functional, and phylogenetic diversity; geographic distributions; ecology; conservation status than for any other comparable group of organisms [5], and we often know why some bird populations vary in abundance and distribution. In some parts of the world, bird populations are extensively monitored to follow their trends, especially in Europe [6] and the USA [7]. The monitoring results show that many bird populations are declining. For example, there

are 29% fewer breeding birds in the USA now compared to 1970 [8], and there has been about an 18% decline in the number of breeding birds in the European Union (EU) since 1980 [9]. Anthropogenic activity—either directly or indirectly—is the main explanation, and worldwide, one in eight bird species is threatened with extinction [10]. An important factor in conservation is the disturbance caused by human recreational activities, which is the focus of this publication.

Habitat destruction, fragmentation, and degradation have led to reduced bird populations through the loss of nesting sites, lower food availability, and increased predation risk [5,11–13]. Bird areas and their quality are reduced by, for example, urbanization and the expansion of roads, industrial areas, and buildings [14–17]; agricultural expansion and intensification [18–23]; drainage [24]; deforestation and forestry [12,25]. Other negative anthropogenic factors include pollution [12,26,27], climate change [28–33], wind farms and other kinds of energy production [34–42], collisions with buildings, powerlines, and cars [16,17,43–49], invasive alien species [11,12], and hunting and fishing [12,26]. The ultimate factors behind these and other destructive anthropogenic impacts on birds are the increases in human populations and the per capita rate of consumption [5].

The factors influencing bird populations mentioned above are not exhaustive. Another important factor is the disturbance caused by mere human presence in the environment. There is a substantial amount of disturbance from people in some important bird areas [50,51]. Birds should flee when approached by a possible predator; for example, a human that is viewed as a predator [52]. However, if a bird flees from an approaching animal that is not dangerous, this is a waste of time and energy. This might reduce the energy resources needed for other important activities and thus potentially reduce reproductive success and population size. Therefore, natural selection should result in an optimal flight initiation distance (FID)—the distance at which animals flee from an approaching danger—because of cost–benefit trade-offs. The FID will probably vary according to the kind of danger that is approaching and individuals' earlier experiences at different locations. This variation minimizes the costs of disturbance and maximizes the chances of survival. The FID is, therefore, naturally influenced by human presence and many other different factors [53–61]. For example, many waterbirds are negatively affected by human presence as they are scared away from otherwise important feeding, roosting, or breeding areas. In addition, human presence changes birds' activity patterns in a negative way [50,51,61–64]. In the last 50 years, shorebirds (44 species) in the USA have declined by 37.4% [8].

The FID is an important trait because escape behavior is vital for the survival of many species, and in the practical management of wild birds, knowledge about the vulnerability of different species to disturbances is vital. As birds' responses to human disturbance are highly variable and may depend on many factors, FID values in one region might not necessarily be transferable to other regions. As a novel application of the FID, I use the relationship between the proportion of fleeing birds and birds that stayed at different distances from pedestrians to improve the evaluation of the negative effects of human presence. In addition, I investigated different factors affecting the FID in waterbird groups (swans, geese and shelducks, dabbling ducks, diving ducks, waders, and gulls) and at the species level (Eurasian teals *Anas crecca*, mallard *Anas platyrhynchos*, common eiders *Somateria mollissima*, common goldeneye *Buchephala clangula*, and Eurasian oystercatcher *Haematopus ostralegus*) depending on data availability. Due to individual variations in the FID between species, determining the FID at the species level is important for conservationists.

2. Materials and Methods

2.1. Field Methods

The study area was in the middle part of Norway (Figure 1) and included 35 freshwater and saltwater areas of various sizes and distances from urban areas that were investigated between 2001 and 2019. All urban areas in this study were outside the centers of small cities and should be considered as suburban areas. Many areas were included to reduce the probability that the same birds could be studied several times. One or two researchers walked directly toward a focal bird flock (1–700 individuals) to avoid potential variations in the FID caused by a change in direction and because a tangential approach might reduce the FID compared to walking directly toward the birds [59,65,66]. I also started my approach far away from the birds in an open landscape when they were engaged in normal activities—most often searching for food—to avoid possible problems related to the positive relationship between the researcher's starting distance and FID [67,68]. This was performed in an open landscape to ensure that the birds detected us at a long distance and that no birds had a short FID because they were startled by us [69]. The sailboat, canoe, and drone were directed directly against the birds.

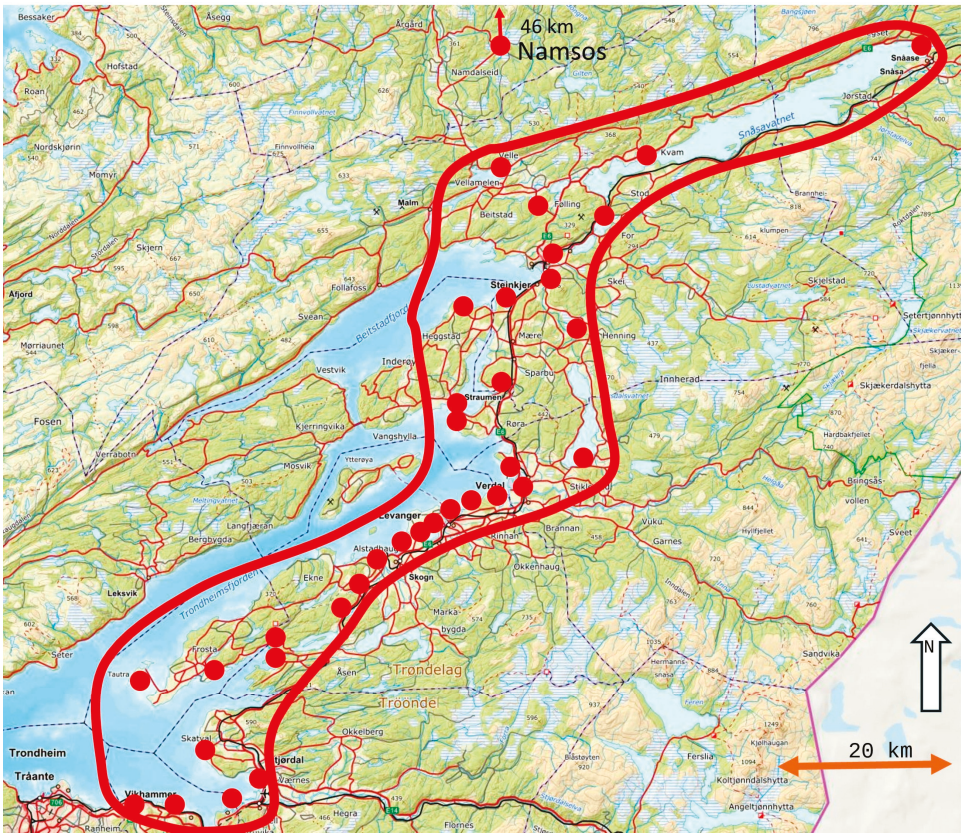

Figure 1. The study area in the middle part of Norway (north of Trondheim), showing each of the study sites, except one site situated further north, as illustrated by the arrow.

Altogether, the FID was measured for 1075 different flocks of waterbirds: swans and geese (n = 87), dabbling ducks (n = 323), diving ducks (n = 386), mergansers (n = 42), waders (n = 137), and gulls (n = 100). Included in the analyses are anthropogenic disturbances from humans without a dog (n = 767 bird flocks), humans with a leashed dog (n = 140), the use of a canoe and sailboat (n = 133), and the use of a drone (n = 35). A flock is defined as one or more birds of the same species separated by about 50 m or more from other birds

of the same species. The exact number of flocks in each size category is given in Table 1. Most birds were approached while they were searching for food because loafing birds may have different FIDs to birds searching for food both within and between species [70]. The FID was measured by the researchers (one or two) walking slowly (about 0.7 km/h) and directly against the flock of birds. This standardization is necessary as fast movements increase the probability that the birds will flee [51]. The researchers always observed the birds to follow their behavior; however, the FID might increase if we looked intently at the birds compared to looking in another direction [71].

Table 1. Variables included in the generalized linear mixed models (GLMM) analyses. N is the number of flocks with FID as the target variable.

Variable	Value	Comments	N
Target Variable			
FID	Continuous	Log_{10}FID	907
Fleeing method	1–2	1 = swimming or walking, 2 = flying	227, 353
Explanatory variable			
N of researchers	1–2	1 = one person, 2 = two people	720, 187
Open-narrow water	1–2	1 = open area, 2 = narrow (<50 m to the opposite shore)	664, 243
Flock size	1–7	1–4 = exact number of birds, 5 = 5–10 birds, 6 = 11–30, 7 = 31–700	327, 174, 78, 49, 126, 86, 57
Disturbance by dog	1–2	1 = with leashed dog, 2 = without dog,	140, 767
Rural–urban	1–2	1 = rural, 2 = urban	368, 539
Random effect			
Period	1–2	Winter and early spring, autumn	568, 507

The researchers stopped and measured the distance to the focal bird flock when a change in behavior was registered—for example, when the birds stopped feeding or became alert or moved away—to roughly measure the distance. If the flock consisted of more than one bird, special attention was paid to the closest bird, and distance measurements were taken with reference to this bird. This bird also generally seemed to change behavior first. The slow walking and stopping behavior of the researchers is similar to that of birdwatchers. Some birds (4 of 20 species) have been found to have a longer FID as a response to this behavior compared to their FID to people who are only walking without stopping and looking in binoculars [72]. Early in the investigation period, some alert distances were noted; that is, the distance from the researcher when the birds stopped their normal activity. When the birds fled, the researchers stopped walking and, for about half of the registrations, noted how far away the birds moved from their starting point. The researchers retreated, and, in a few cases (n = 90), they observed exactly when the fleeing birds moved away and when they came back to the place they started from. However, many flocks did not return. All distance measurements were made with laser instruments (Swarovski EL Range 8 × 42, Austria or Nikon Laser Rangefinder Prostaff 5, Tokyo, Japan).

The fieldwork was performed throughout the day in good light conditions in winter and early spring (January n = 36 flocks, February n = 114, March n = 87, April n = 195, May n = 73, and December n = 63), and autumn (August n = 78, September n = 200, October n = 118, and November n = 111). No strongly colored clothing was worn; it was normally green field clothing.

The drone (DJI Phantom 2 Vision +) was operated with a speed of 2 m/s and an approximately 10 m flight height above the birds situated on the ground or on the water,

away from the other experiments. FID measurements using a canoe and sailboat were only performed in one area, and these measurements were analyzed separately and not included in the generalized linear mixed model (GLMM) analyses. This area was open, quite rectangular, and about 1 × 2 km wide, meaning the birds should have been able to detect the boat long before they decided to flee. All measurements were performed in good weather conditions with little wind and no or very little precipitation. No breeding birds were included in the experiments.

The fleeing method was categorized as flying away or walking/swimming away. Walking and swimming were combined because both running and swimming birds with a similar mass use a comparable amount of energy at maximum speed, while the energy consumption during flight is approximately 2.5 times greater. This difference is caused by the higher mass of the flight muscles compared to the leg muscles, as well as their greater oxidative capacity [73].

2.2. Statistics

Due to non-normal data, the differences in FID between groups of birds or between species were tested using the non-parametric Mann–Whitney U-test (MW U-test). Likewise, the relationships between FID values were tested with non-parametric Spearman rank correlation tests (r_s). The variables included in the GLMM analyses are given in Table 1. The maximum correlation between the six explanatory variables (Table 1) was 0.343, far below the recommended upper limit of 0.7 [74].

Generalized linear mixed models (GLMMs) were used [75] because they remove the variability in responses that is associated with random factors other than the conditions of experimental interest, thus reducing the Type I error rate [76]. GLMMs may be the best tool for analyzing non-normal data that contain random effects [77]; however, the FID values were normalized using $\log_{10}$ in our GLMM analyses. From the global model with $\log_{10}$FID as a target variable [78], I selected various candidate models with the explanatory variables included without interactions by using backward stepwise removal of explanatory variables and Akaike's Information Criterion (AIC). The best models had a difference in the AIC values (△AIC) below the upper recommended value of 2, while models with △AICs > 2 compared to the optimal model were excluded [78,79]. In the GLMM analyses with $\log_{10}$FID as the target variable, I used a normal distribution and the identity link function. With the fleeing method as the target variable, I used a binary probability distribution with the logit link function and added $\log_{10}$FID as an explanatory variable. The period was included in the GLMM analyses as a random factor because all registrations were outside the breeding and main molting seasons, and potential differences in FID during autumn, winter, and early spring are of minor interest in this investigation. However, this potential variation should be compensated for. An attempt to include site as a random factor was not accepted by SPSS (ver. 30), probably because some sites had very few FID observations.

Due to the low number of measurements for each species, the effect of the drone on the FID was analyzed separately and not included in the GLMM analyses. The drone experiments were performed in a few places with a minimum of 5 km between each area, and these areas were not used in the FID experiments with pedestrians.

Using graphical tools [79], the histogram with Regression Standardized Residuals and Frequency showed a normal distribution [76] for the $\log_{10}$FID values. Data exploration [77–79] revealed no outliers. The effects of the use of a canoe and sailboat on FID were combined because there was no significant difference between them (MW U-test: $z = -0.738$; $n = 117$ and 16, respectively; $p = 0.460$), and they were analyzed separately from the GLMM because they were present in only 1 of the 35 areas. Likewise, the effects of an

approaching drone (n = 35) were analyzed separately due to the lack of data and the low number of tested areas.

Statistical tests were two-tailed with an α-level of 0.05 ($p < 0.05$). I am aware of the ongoing discussion about null hypothesis and statistical testing (NHST) versus IT-based inference [80], but I found the data to be suitable for NHST methods [78]. SPSS was used for all statistical calculations.

3. Results

3.1. FID Variation Between Different Waterbirds

The six different groups of birds exhibited clear differences in flight initiation distance (FID) when the researchers approached via walking on land without a leashed dog (Figure 2). Waders and gulls had similar FIDs, shorter than the other groups. The FID ($\log_{10}$FID) was significantly shorter for waders than for mergansers (MW U-test: Z = −3.221; n = 108 and 21, respectively; p = 0.001) and for gulls than for mergansers (Z = −3.300, n = 81 and 21, $p < 0.001$). Mergansers and diving ducks had similar FIDs (Z = −0.642, n = 21 and 260, p = 0.521). Dabbling ducks had a significantly longer FID than diving ducks (Z = −6.250, n = 236 and 260, $p < 0.001$). Swans/geese and dabbling ducks had similar FIDs (Z = −0.068, n = 61 and 236, p = 0.946).

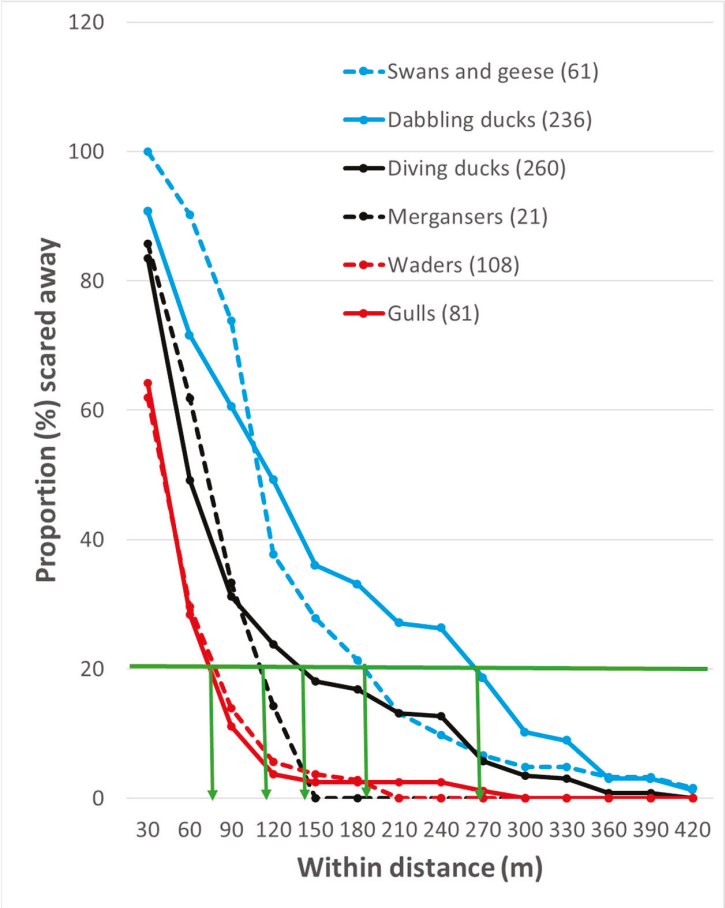

Figure 2. Proportion (%) of bird flocks belonging to different bird groups that fled when approached by pedestrians on land (without a leashed dog) at various distances. The number of flocks of each group is given in brackets. The green line indicates the point at which 20% of the birds fled, and the green arrows show the distance from the human at which this occurred for different bird groups.

Figure 2 shows the proportions of birds that were scared away when humans were at different distances from them. It is possible to use this figure to observe the effects of human presence in more detail than when using mean and median values only. As an example, a horizontal line is shown in the figure to indicate when 20% of the birds were scared away, with the arrows from the interception of this line and the lines for different groups of birds indicating at what distance this occurred. In the example, 20% of the waders and gulls were scared away when humans were present at around 75 m away; mergansers and diving ducks around 120–140 m, and swans and geese, and dabbling ducks around 190–270 m away. If the accepted proportion of birds scared away is lower than 20%, the distances to humans are greater.

The mean FIDs for different groups of birds and some of the species are given in Figure 3 when the disturbance was a pedestrian on land without a dog. The mean FID was about 150 m for swans, geese, and dabbling ducks, less than 100 m for diving ducks and mergansers, and just above 50 m for waders and gulls (Figure 3). At the species level, there were also quite large differences (Figure 3). Eurasian teals were shy and moved away when humans were about 225 m away, while mallards and common goldeneyes had a mean FID of about 130 m, and common eiders and Eurasian oystercatchers had a mean FID of about 50 m. If this mean value of 50 m is taken to create a buffer against human presence for waders, nearly 40% of them would be scared away from the area (Figure 2).

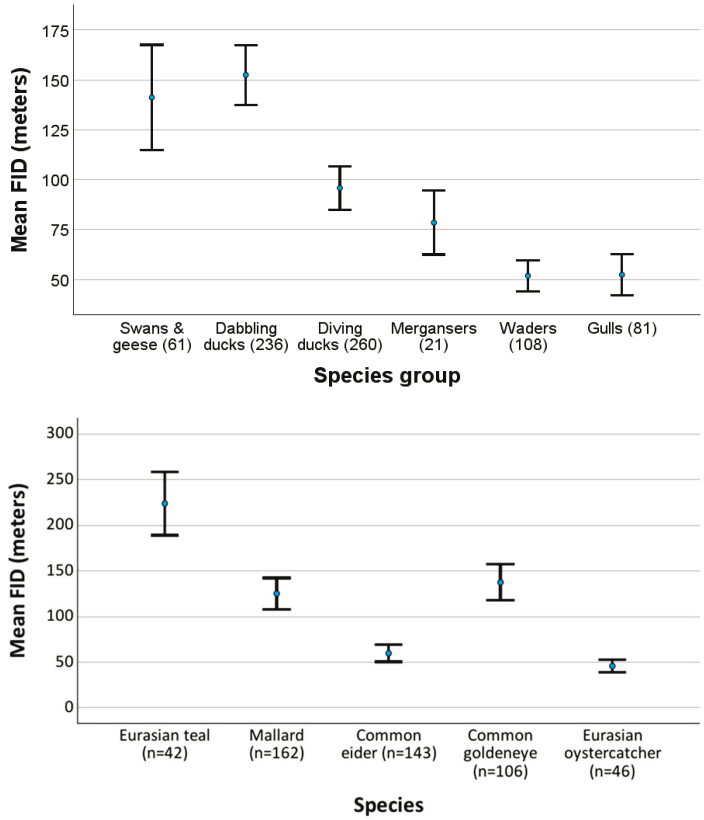

Figure 3. Flight initiation distance (FID) (±2 SE) for six different groups of waterbirds (**upper**) and five species (**lower**). The number of flocks is given in brackets. All birds were approached by a pedestrian on land, without a dog.

3.2. Factors Affecting the FID and Fleeing Method

The next best model in the GLMM analyses with $\log_{10}$FID as a target variable, with an △AIC = 1.0 compared to the optimal model, included all explanatory variables in Table 1, while the optimal model excluded the variable narrow or wide area. These two models

were the only relevant models. The model containing all explanatory variables was used. Tables 2 and 3 show the results of the GLMM analyses of the factors included in this model affecting the FID for both groups of birds and at the species level, respectively. Due to the low number of observations (n = 26), I did not run the GLMM analysis for mergansers. Two researchers approaching together (compared to only one researcher) led to a shorter FID for dabbling ducks when all other variables in the analysis were taken into consideration (Table 2). This effect was not significant for any of the other four groups. For species with at least 50 observations, two researchers approaching them resulted in a shorter FID for Eurasian teals and mallards but not for common eiders, common goldeneyes, or Eurasian oystercatchers (Table 3).

Table 2. p values in the GLMM analyses with explanatory factors possibly affecting $\log_{10}$FID in different groups of waterbirds. If significant, the direction of the change is given by + or −. The effects of the explanatory variables (value written in brackets) are compared with the lowest value (=1) (see Table 1). For flock size, six different categories were compared with one individual and the lowest p-value is given. Significant results for the explanatory variables are in bold and * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ for explanatory variables.

Variable	Swans and Geese	Dabbling Ducks	Diving Ducks	Waders	Gulls
Corrected model	0.898	**+<0.001**	**+<0.001**	0.587	0.144
Intercept	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
N of researchers (=2)	0.795	**−<0.001** ***	0.395	0.817	0.055
Open/narrow (=2)	0.257	**−0.014** *	**−<0.001** ***	0.510	**+0.014** *
Flock size (2–7)	Min0.693	Min0.068	Min**+0.002** (a)**	Min0.165	Min**+0.032** *
Disturbance (=2)	0.420	**+0.006** **	**+<0.001** ***	0.270	**−0.027** *
Rural–urban (=2)	0.607	0.449	**−0.003** **	0.927	0.566
Random effect	0.997	0.494	0.487	0.573	0.716
N=	67	277	306	127	93

(a) Flocks of diving ducks with three individuals had a significantly longer FID and those with two individuals had a near-significant longer FID than flocks of one individual (p = 0.082).

Table 3. p values in the GLMM analyses with explanatory factors possibly affecting $\log_{10}$FID in waterbird species with a minimum of 50 observations. If significant, the direction of the change is given by + or −. The effects of the explanatory variables are compared with the lowest value (=1) (see Table 1). For flock size, six different categories are compared with one individual and the lowest p-value is given. Significant results for the explanatory variables are in bold and * = $p < 0.05$, and *** = $p < 0.001$ for explanatory variables.

Variable	Eurasian Teal	Mallard	Common Eider	Common Goldeneye	Eurasian Oystercatcher
Corrected model	**+0.002**	**+<0.001**	**+<0.001**	**+<0.001**	**+0.004**
Intercept	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
N of researchers (>1)	**−<0.001** ***	**−<0.001** ***	0.951	0.121	0.708
Open-narrow (=2)	0.464	**−<0.001** ***	**−0.011** *	0.939	**−<0.001** ***
Flock size (2–7)	Min0.181	Min0.075	0.319	Min**+<0.001** (a)***	**Min −0.019** (b)*
Disturbance (=2)	0.441	0.169	0.236	**+<0.001** ***	0.068
Rural–urban (=2)	0.335	0.380	**−<0.001** ***	0.264	0.273
Random effect	0.911	0.597	0.604	0.512	0.710
N=	45	201	163	130	55

(a) Flocks of common goldeneyes with three individuals had a significantly longer FID and those with a flock size category 5 (5–10 individuals) tended to have a longer FID than single individuals (p = 0.055). (b) Eurasian oystercatcher flocks with two individuals had a shorter FID than one individual.

Narrow water areas led to a shorter FID compared with wide areas for both dabbling ducks (including mallards) and diving ducks (including common eider), but the opposite was true for Eurasian oystercatchers. For diving ducks, including the common goldeneye and gulls, for some flock sizes greater than one individual, there was a longer FID compared with one individual, while the FID was shorter for flocks of two individuals compared with one individual for Eurasian oystercatchers. When the researchers were not accompanied by a dog, both dabbling and diving ducks, including common goldeneyes, had a longer FID compared to when the researchers were accompanied by a leashed dog, and again, the opposite was true for Eurasian oystercatchers. In suburban areas, a shorter FID was found only for diving ducks, including the common eider, compared to rural areas.

The FID test with canoe and sailboat was only performed in one area, and the results are presented using diving ducks as an example in Figure 4. There was no significant difference in FID when the researcher was accompanied by a leashed dog compared with the researcher without a dog (MW U: z = −1.178, n = 23 and 118, p = 0.239) in this area. The use of a canoe and sailboat significantly increased the FID both for the researcher accompanied by a leashed dog (MW U: z = −6.560, n = 70 and 23, $p < 0.001$) and the researcher without a dog (MW U: z = −10.099, n = 70 and 118, $p < 0.001$). Canoeing and the use of a sailboat led to similar FIDs (see Statistics). The finding of a similar FID when the researcher was or was not accompanied by a leashed dog is opposite to those when I used the whole dataset (Table 3); however, in the GLMM analyses (Table 3), it was accounted for the other variables in the models.

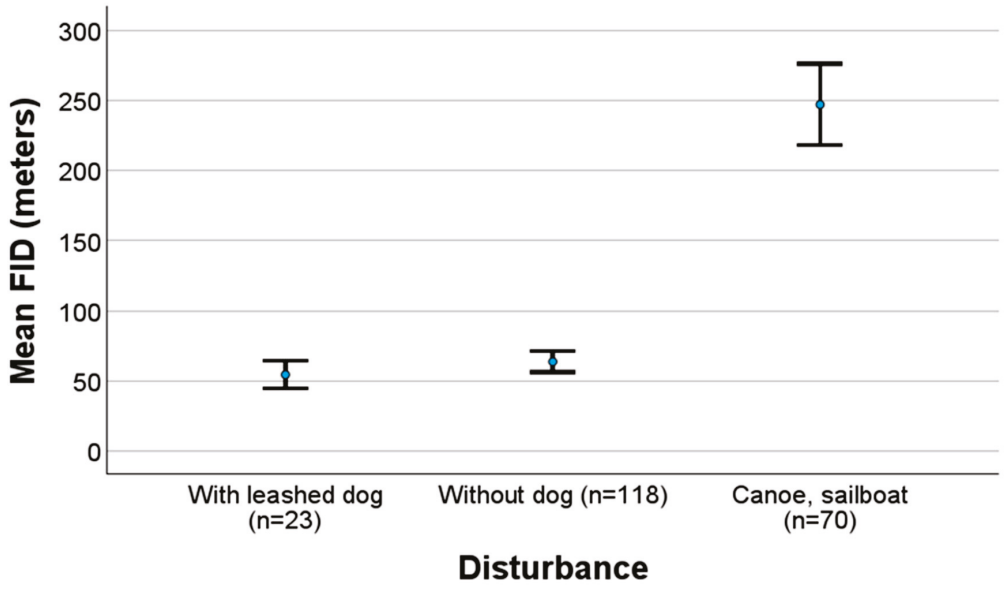

Figure 4. FID (±2 SE) for diving ducks with two kinds of disturbances from a pedestrian and from the use of a canoe/sailboat in the area where the effects of canoeing and the sailboat were investigated.

The FID was shorter when a drone approached whooper swans *Cygnus* cygnus, pink-footed geese *Anser brachyrhynchus*, graylag geese *Anser anser*, common shelducks *Tadorna tadorna*, mallards, tufted ducks *Aythya fuligula*, common eiders, common goldeneyes, and red-breasted mergansers *Mergus serrator* compared with the FID when one or more people approached the same species by walking towards them without a dog. It was not possible to compare the mean values of $\log_{10}$FID for all species due to the lack of data, but the difference was near-significant for pink-footed geese (p = 0.054) and significant for mallards (p = 0.038) and common goldeneyes (p = 0.021). Despite the lack of statistical tests, it is apparent that in general, swans, geese, and ducks are less afraid of an approaching drone

than an approaching pedestrian. Eurasian oystercatchers had a near-identical FID when the two disturbances were compared, and the common gull *Larus canus* tended ($p < 0.1$) to have a shorter FID when a drone was approaching compared to a pedestrian. In the tests, equal variances were not assumed.

In the GLMM analysis with the fleeing method as the target variable, the best models excluded the explanatory variables of open/narrow water (△AIC = 4.7 from the optimal model) and flock size (△AIC = 2.9). The next best model, with an △AIC = 1.5 from the optimal model, excluded rural/urban areas, while the optimal model excluded disturbance. Two relevant models had △AICs < 2 from the optimal model. I ran the analyses including all variables in the next best model (Table 1, only excluding open/narrow and flock size), and found that relatively more birds flew away compared to swimming or walking away in rural areas compared to urban areas ($p = 0.036$). All the other variables included in the final model, including $\log_{10}$FID ($p = 0.328$), had no significant effects on the fleeing method.

The fleeing method was analyzed for all birds belonging to the six bird groups; the disturbance was a researcher without a dog ($n = 500$). There were clear differences in the proportion of the individuals that flew away between the different groups of birds or species compared with the proportion that swam or walked away. In the smaller wader and gull species, 82% and 81%, respectively, flew away, while 67% of the dabbling ducks and only 26% of the diving ducks flew away. Mergansers exhibited similar behavior to dabbling ducks (73%, but there were fewer observations), and 46% of swans and geese flew away. The proportion of individual species that flew away was similar to that of the bird groups they belonged to (data not presented).

For swans/geese, dabbling ducks, and diving ducks, the FID was significantly longer in birds that flew away compared to birds that swam or walked away from the human disturbance (Table 4). In the other bird groups, there was no such tendency, but the number of measurements for mergansers was too low to draw a meaningful conclusion.

Table 4. Results of the MW-U test comparing the FID when birds of different groups flew or walked/swam when fleeing from an approaching human without a dog. + in front of the *p* value means flying birds had the longest FID. Significant results are in bold and * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

Bird Group	z	*n*	*p*
Swans and geese	−2.064	50	**+0.039** *
Dabbling ducks	−5.859	190	**+<0.001** ***
Diving ducks	−2.970	100	**+0.003** **
Mergansers	−0.215	12	0.830
Waders	−0.393	77	0.695
Gulls	−0.881	71	0.378

3.3. The Relationship Between FID and Other Behaviors

Before the birds started to move away from the researcher, they showed alert responses, which most often meant that they stopped feeding. There was a significant positive correlation between the alert distance from the researcher and the FID in all tests with sufficient data (Table 5). It is also interesting to note that dabbling ducks, diving ducks, mallards, and common eiders with a long FID moved significantly further away from their starting point than birds with a shorter FID (Table 5). That means that shy birds with long FIDs lose more feeding time and use more energy than more confident birds.

Table 5. Spearman correlations (R_s) between the FID and the distance at which the birds stopped normal activity, how far the birds moved from their starting point, and the length of time it took before the birds returned to the starting points when birds were approached by pedestrians on land with or without a leashed dog. No data means that $n < 10$, and * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. Non-significant is written as ns, and for near-significance, the p value is given. Significant results are written in bold.

	Stopped Normal Activity	Distance Moved	Time to Return
Swans and geese	**0.818, *n* = 17** ***	0.082, $n = 44$, ns	No data
Dabbling ducks	**0.974, *n* = 54** ***	**0.463, *n* = 117** ***	0.488, $n = 15$, $p = 0.065$
Diving ducks	**0.938, *n* = 94** ***	**0.269, *n* = 177** ***	−0.181, $n = 64$, ns
Mergansers	Lack of data	0.208, $n = 19$	No data
Waders	**0.916, *n* = 29** ***	0.159, $n = 64$, ns	No data
Gulls	No data	−0.084, $n = 54$, ns	No data
Eurasian teal	No data	0.394, $n = 10$, ns	No data
Mallard	**0.973, *n* = 52** ***	**0.479, *n* = 100** ***	0.488, $n = 15$, $p = 0.065$
Common eider	**0.967, *n* = 51** ***	**0.243, *n* = 94** *	−0.274, $n = 49$, $p = 0.056$
Common goldeneye	**0.898, *n* = 41** ***	195, $n = 72$, ns	−0.082, $n = 14$, ns
Eurasian oystercatcher	**0.797, *n* = 11** **	0.033, $n = 28$, ns	No data

There was no significant correlation between FID and how many minutes it took before the birds came back to where they fled from for all measurements combined ($n = 90$), but the number of data is low. Near-significant correlations ($p < 0.1$) were found for dabbling ducks, mallards, and common eiders, but the directions of the relationships were not consistent (Table 5). Birds that did not return during the registration period, either because they started to search for food in another area or because they went out of sight, were not included.

4. Discussion

4.1. FID Variation Between Different Waterbirds

I found the longest FID for swans/geese and dabbling ducks, while the FID was shorter for diving ducks and mergansers and the shortest for waders and gulls. This is in accordance with other findings that vegetarian birds that consume immobile food have a longer FID than birds that consume mobile food, which can be difficult to catch [70]. My findings are also mainly in accordance with earlier investigations demonstrating that larger birds have a longer FID than smaller birds [81–86]. However, within dabbling ducks and diving ducks, the smallest species had the longest FID, which is in accordance with one investigation that showed that large birds are more tolerant than small birds [87]. The authors suggested that the reason for their result is that larger bird species might be under intense pressure to be more tolerant of humans due to the high energetic costs of unnecessary escape. In addition, it is proposed that larger birds may be less likely to be killed by predators because of their larger body size and their relatively larger brains with potentially greater cognitive abilities that might be more able to assess risks [88]. However, these possible explanations should also be valid in most other areas. In the areas investigated in this study, there are many hunters that hunt geese [89] and ducks, and there is a dense population of white-tailed eagles *Haliaeetus albicilla* that also hunt the largest birds. The high energetic expenditure during flight for waders [90] might explain why the FID was shorter for this group of birds than most other groups (Figures 2 and 3).

I did not use any statistical tool, for example, Moran's I, to demonstrate possible spatial autocorrelation. There was a lot of variation in FID between areas, and the FID in one area did not seem to affect the FID in other areas. Even neighboring marine areas differ

substantially because of variations in salinity, exposure to wind and waves, and substrate, and therefore also species composition and FID values.

4.2. Factors Affecting the FID and Fleeing Method

For dabbling ducks (both Eurasian teals and mallards), when only one researcher approached, the FID was longer than for two researchers (Tables 2 and 3). This is opposite to what was expected. The reason for this might be because two or more people walking together is very common in the investigated areas, and one person alone is typical behavior of hunters of these species.

Shy birds have a long FID, and it is logical to expect that they will fly away from a human disturbance more often than walking or swimming away. For all birds combined, there was no such relationship, probably because the shyest bird groups consisted of heavy birds with high flying costs, and they could take advantage of their long FID by swimming or walking away to save energy. Most smaller birds, such as gulls and waders, saved energy by allowing an approaching human to come close, but they had to fly away when the threat felt too dangerous. Within each of the three most shy bird groups (Figure 2), where the birds are more similar in size, there is a significant positive relationship between the FID and the proportion of the birds that flew away (Table 4). To conclude, saving energy seems crucial when a bird decides to fly or swim/walk away from an approaching pedestrian. A long FID makes it possible to escape by less costly swimming or walking (swans/geese, dabbling and diving ducks); alternatively, a short FID may mean that the birds do not have to flee at all, but they must escape by costly flying if fleeing becomes necessary (gulls, waders). These two alternative strategies are probably affected by other factors, such as a combination of preferred food [70] and a relatively large body mass, making the proportion of diving ducks that flew away very low compared with other groups. Further research is recommended on this topic.

In narrow areas with <50 m to the opposite seashore, nearly all significant results showed that the FID was shorter here than in more open areas. This was true for dabbling ducks (including mallards), diving ducks (including common eiders), and Eurasian oystercatchers. This is probably because these narrow areas are not used by shy birds that normally would use a lot of energy by flying away more often relative to more confident birds. The FID of gulls was increased in narrow areas, probably because their short FID enables relatively shy individuals to stay in narrow areas. As expected, others have found that birds closer to the path were more likely to fly away as people approached than birds that were further away [91]. My findings of a short FID in narrow areas are opposite to those of an investigation in which the FID was investigated by use of a motorboat in lakes and rivers [68].

In the literature, the relationship between flock size and FID remains unclear [92]. Many studies have demonstrated that the FID increases with an increase in flock size [57,68,83,84,93,94]. This is not always the case, as flock size was found to have little effect on FID in a review paper featuring 99 bird species, but there was a positive relationship in waders [92]. However, other investigations have found that FID increases with flock size in only one of nine waders [95] and in two of five waders [96]. In the present publication, there were not many significant relationships between flock size and FID (Tables 2 and 3). Only diving ducks, including common goldeneyes, and gulls had longer FIDs for some flock sizes that were larger than one individual. Eurasian oystercatchers had a shorter FID when there were two individuals in the flock compared to one in this study and considering the contrasting results of other investigations, further research is recommended for waders. There may be several explanations for the lack of generality in the finding of the effect of flock size on FID. For example, larger flocks have more individuals that can detect a

predator, and therefore it is reasonable to expect that the FID will increase with flock size. There will be an opposite effect on the FID from dilution or safety in numbers, meaning that the probability that a certain bird is taken by a predator gradually reduces with flock size compared to a single bird, and thus, the FID might be reduced.

Dabbling and diving ducks, including common goldeneyes, had an increased FID when a pedestrian was not accompanied by a dog compared with when they were accompanied by a leashed dog (Tables 2 and 3). For some of the investigated waders, a dog on a leash increased the FID [95], as observed for Eurasian oystercatchers in this investigation (Table 3). However, free-ranging dogs are found to scare birds more than leashed dogs [97]. In one publication, free dogs scared birds in a similar manner to construction vehicles and military jets, and more than gunfire and passing cars [98]. The waders did not return to their feeding ground after five minutes when disturbed by humans, but gulls did, and waders responded especially strongly if disturbed by a dog [63]. Western snowy plovers *Anarhynchus nivosus*, on their wintering grounds, are more likely to fly away from dogs, horses, and crows than from humans and other shorebirds [99].

In the suburban areas included in this study, I found that the FID was shorter only for diving ducks, including common eiders. In addition, other investigations show that birds adapted to human presence in urban areas are more tolerant (shorter FID) than birds in rural areas [55,83,87,100–107]. Despite this habituation, birds tend to overestimate rather than underestimate the risk associated with human presence [61]. Except for city parks in which some people feed the birds, birds will also fear people close to them and eventually flee. Variation in the number of people using trails was not found to influence the number of waders [108]. This might be because the FID for waders is relatively short, and peoples' movement patterns when following tracks are predictable. However, the number of plovers was found to be lower near trailheads than elsewhere [99]. I expected to find a stronger urban/rural effect on FID in this investigation. This might be because the cities in this study are small and because the investigated urban areas are outside the densest part of the city in the suburban environment; and thus, the number of pedestrians here is not very high (but higher than in the investigated rural areas). Within cities, birds have a shorter FID in areas with a high pedestrian density [101], and urbanized birds have shorter FIDs than suburban birds [87].

Little is known about FID and recreational canoeing [109]. My investigation in an open area showed that the FID increased with the use of canoes and sailboats compared with pedestrians (Figure 4), in contrast to an investigation with paddling in a river [109]. When compared with pedestrians, a longer FID with the use of a boat was also found in pied avocet *Recurvirostra avosetta*, while a shorter FID was found in Eurasian oystercatchers [96]. More investigations are needed to compare the FID in the presence of canoes, paddling, and sailboats with that for pedestrians.

There are many investigations about the effect of an approaching drone on waterbirds [110]. These investigations have shown mostly shorter FIDs, similar to the results of this study, and drones are recommended for many waterbird censuses [111–113]. However, a considerable disturbance in waterfowl has also been observed, and it is recommended that drones should be forbidden in certain areas to minimize this potential disturbance [114].

4.3. Effects of Human Disturbance on Population Sizes

When birds are scared away from important feeding areas, the reduced food intake and extra energy expenditure can reduce their condition, survival, and population size. European birds with a long FID have more negative population trends than birds with a short FID [115]. When waders are forced to make 10 flights from their roosts per day,

their daily energy expenditure increases by 4.5% and 7.8% (knots *Calidris* spp. and sand plover *Charadrius* spp., respectively), which may reduce energy reserves to levels below the thresholds that can be replenished by normal intake rates and thus negatively affect survival [116]. Another investigation showed that the effects of disturbance are dependent on the number of pedestrians, and very few pedestrians per day do not seem to have any effect on waders' energy budget [82], with the authors calculating that the number of disturbances needs to be about 100–300 per day for different wader species before the feeding time is reduced by 10%, thus contrasting the investigation on knots and plovers referred to above.

Waders inhabiting tidal areas do not feed at full tide, and during the winter, waders the size of redshank *Tringa totanus* and smaller use 90–100% of daylight time, while larger waders use 50–80% of daylight to search for food when feeding grounds are exposed [117]. It is, therefore, reasonable to believe that disturbances that reduce feeding time and increase energy expenditure have a negative effect on birds. Piping Plovers *Charadrius melodus* on a beach with few people devote more time to foraging and less time to vigilance than birds at a beach with more people [62], which was also observed for waterbirds [91]. Sanderlings *Calidris alba*, feeding on the beach and running or flying away from people up to five times per minute, were searching for food during the evening and night when human disturbance was low [93]. Individually marked oystercatchers do not increase their feeding rate to compensate for lost feeding time; instead, they extend their feeding time by remaining longer on the mussel bed. These oystercatchers were able to compensate for short time losses of 30–60 min through disturbance [118].

To evaluate the negative effects of human disturbance, it is necessary to take into consideration the alert time, feeding time, and fleeing method, as well as the fact that the other area might have less food than the area they flee from. This investigation shows a positive correlation between FID and alert distance, fleeing distance, and a longer wait before they came back to their feeding grounds (Table 5). A positive relationship between FID and fleeing distance was also found by others [119]. This implies that shy birds with a long FID lose a lot of time and energy. However, extra loads in a period might be camouflaged by higher feeding rates or longer feeding periods, as referred to above, and therefore, individually marked birds must be investigated for a long time to unmask the negative effects of disturbance on population sizes. Experiments have shown that parent birds that were given extra loads managed to raise their nestlings to fledge but were less able to raise their fledglings to independence than control parents [120]. Such mechanisms make it challenging to quantify the impact of human disturbance in stopover and wintering sites on shorebird population sizes. So far, surprisingly little has been uncovered in this field [121].

4.4. Could a Worldwide FID Database Be Useful?

From the literature, it is possible to obtain FID information from a huge number of bird species around the world. In fact, a review paper published a database of 34,775 FIDs from 50 studies representing 650 non-nesting bird species from 2009 to 2015 [122]. The FID values in that publication were, in general, much lower than in this investigation, probably because investigations in all kinds of areas were included in the worldwide list, for example, parks in cities with many more people than in my investigated area, and thus it includes individuals habituated to human presence. However, large-scale FID databases can provide valuable general trends, such as the database containing 10,249 observations for 842 bird species inhabiting open tropical ecosystems in Africa, South America, and Australia [123] and that summarizing the analysis of latitudinal variation in FID using observations of 12,495 FIDs of 714 populations of 159 species [55].

The FID is influenced by a lot of different factors, as shown in this and other publications. In addition, the FID has been found to be positively correlated with the abundance of predators [55], the number of blood parasites [56], genetic variation [124], and relative eye size [125], for example. The great variability in FID, caused by different factors that might vary geographically [55], suggests that wildlife managers should be somewhat conservative in developing buffer zones [53]. In my opinion, it is not appropriate to use previously published FID data for a given species as guidelines for setting buffer zones in a certain area. Each area should be investigated to determine the FID for different species at that actual site and the proportion of each species that is scared away at different distances by disturbances and to decide what level of disturbance is acceptable (Figure 2). If the area is used by rare or red-listed species, the acceptable level of birds scared away should be zero or close to zero. FID investigations should be conducted even though shy bird species with a long FID are consistently shy also in other areas, while more tolerant species are consistently tolerant [53]. Not all species are investigated with regard to their FID, leading to a taxonomic bias in available FID values, and the available values are not often used by decision-makers [86]. This conclusion supports my recommendation that new site-specific investigations should be carried out in each actual area, with the relevant type of disturbance.

4.5. Conclusions

Graphs showing the relationship between different birds' probability to flee in relation to the distance from pedestrians (Figure 2) give more detailed information about bird behavior than median or mean FIDs. I, therefore, recommend the construction of similar graphs for each area to evaluate mitigation actions or whether a trail should be constructed or not. As far as I know, such graphs have not yet been constructed for this purpose; however, they have been constructed for a few bird species to evaluate the effects of pedestrians alone compared with pedestrians with dogs [97].

Nature managers and politicians should consider the negative effects of human presence on seashores and in and along lakes and rivers. On seashores, human recreational activities, such as fishing, walking, jogging, swimming, horseback riding, vehicle driving, dog walking (on and off leash), and kiting, occur mostly in the same zone of the beach used by waders, gulls, and terns [126]. In areas accessed by people, the closure of important wintering bird areas for humans has resulted in increased breeding populations [121,127], and appropriately constructed bird hides have reduced the disturbance for birds [128]. In areas where new activities are planned—for example, new trails along important areas for waterbirds—the species, their abundance, their FID, and the area's ecological functions for the different species should be investigated. I recommend that (1) in each actual area, the FID is investigated by following the methods described in this and other publications; (2) a diagram is constructed to show the relationship between the distance from the birds and the proportion of birds of each species that flee (Figures 2 and 3) a buffer zone is established based on the results during the time of the year when the birds are most vulnerable, as well as on their red list status. Thereafter, it should be determined whether the trail or other physical developments should be constructed or not.

In the literature, human disturbance is listed as an important conservation issue confronting shorebirds [121]. To reduce the conflict between human recreational activities and important bird feeding, roosting, and nesting areas, a map of all actual areas is necessary [129]. The species that are using the area and how the area is used (breeding, feeding, loafing, roosting) should be investigated. Wildlife managers should use the FID more than they do today to develop buffer zones to reduce human impacts on wildlife [130]. However, the FID varies between species and between sites [53], as found in this and

other investigations. The great variability in FID implies that wildlife managers should be somewhat conservative in developing buffer zones. They can use previously published FID data for a given species as guidelines for setting buffer zones [53,85], but only if new investigations are not possible. Despite the challenging task of demonstrating the negative effects of human recreational activities near wetlands on bird conditions, survival, and populations, researchers should prioritize obtaining such important knowledge. Perhaps, in the future, fewer tracks will be constructed near the shorelines, wetlands, and rivers, without any concerns about the bird's well-being.

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Feeding Habits of European Ground Squirrels in Anthropogenic Habitats in Central Macedonia, Greece

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Simple Summary: The endangered European ground squirrel in northern Greece is threatened by habitat loss and fragmentation. To support conservation efforts, this study investigated their diet and their seasonal change in a human-modified area in Central Macedonia. From 2022 to 2023, we recorded the plants that ground squirrels consumed and found that certain genera, like *Cynodon*, *Carex–Cyperus*, *Salvia*, *Solanum*, and *Plantago*, were most common. The squirrels mainly ate rhizomes and leaves, adjusting their diet based on what was available. During periods of food scarcity, they adjusted their diet to include less common or toxic plants. These findings show the species' ability to adapt its diet in changing environments, and offer useful information for conservation and habitat management.

Abstract: The European ground squirrel (*Spermophilus citellus*) is an endangered mammal that inhabits open habitats with low vegetation in central and southeastern Europe. Its southernmost range includes northern Greece, where populations are declining due to habitat degradation and fragmentation. Limited behavioural research on Greek populations underscores the need for studies to support conservation efforts. The present study examined the species' diet and seasonal changes in food consumption in an anthropogenic area of Central Macedonia, Greece. Between 2022 and 2023, feeding behaviour was documented recording plant parts, taxa, and feeding durations, while plant availability was assessed through surface sampling. The results revealed that *Cynodon*, *Carex–Cyperus*, *Salvia*, *Solanum*, and *Plantago* were the dominant plant genera in the species' diet, while rhizomes and leaves were the most frequently consumed plant parts. Seasonal dietary shifts were observed, with ground squirrels selecting specific plants and plant parts based on availability. Under resource-limited conditions, they adapted by consuming nutritious rhizomes or the less-common, but toxic, *S. elaeagnifolium*. These findings highlight the species' dietary adaptability to Mediterranean and human-modified environments, providing valuable ecological insights and informing habitat conservation and improvement strategies.

Keywords: *Cynodon dactylon*; dietary flexibility; foraging preferences; ground squirrel conservation; human-modified environment; Mediterranean; *Solanum elaeagnifolium*; *Spermophilus citellus*

1. Introduction

As anthropogenic habitats rapidly expand globally [1], wildlife in human-modified environments faces numerous challenges, including habitat fragmentation, increased disturbance, and landscape and vegetation structure alterations [2]. These changes significantly affect food availability, which is vital for maintaining physical condition, reproduction, and overall fitness [3–5]. In response, wildlife often adapts by incorporating food sources that may not be preferred in natural settings. Foraging decisions in such environments involve trade-offs in timing, location, and diet as animals balance predation risk, energy consumption, and opportunity costs [6–8]. This is particularly relevant for hibernators, who must accumulate sufficient energy during a limited active period to survive hibernation, often prioritizing food acquisition over other activities [9–11].

The European ground squirrel (*Spermophilus citellus*) is a semi-fossorial and obligate hibernator that has successfully adapted to human-modified habitats [12,13]. The species is endemic to central and southeastern Europe, with its southern range limit extending into northern Greece. There, the species has adapted its activity patterns, foraging, and vigilance behaviours to the arid climatic conditions of the Mediterranean climate [14,15]. In this region, the species faces population decline and habitat loss, resulting in smaller and more-fragmented populations, and is classified as endangered (EN) on the IUCN Red List [16–18].

Understanding the dietary habits of small mammals in human-modified environments is crucial for their conservation. Diet influences survival, reproduction, and adaptability, determining how well a species can persist in changing landscapes. In degraded and fragmented habitats, resource availability fluctuates, making dietary flexibility essential for coping with seasonal and environmental challenges. However, no thorough studies on the feeding ecology of European ground squirrels in Greece have been conducted thus far to address these challenges. Most previous studies have been conducted in the natural habitats of northern-ranging populations of the species (in Hungary, Romania, and Serbia), reporting a diverse plant-based diet, consisting primarily of green plant parts, flowers, and seeds [19–22]. The most representative species are *Achillea* sp., *Festuca* sp., and legumes, with *Achillea millefolium*, various clover species (*Trifolium arvense*, *T. campestre*, *T. medium*, *T. repens*), and dicotyledons, such as *Plantago lanceolata* and *Pimpinella saxifraga*, being particularly important [20,21]. The main plant parts consumed are grass leaves and seeds (Poaceae), dandelion radix (*Taraxacum* sp.), and *A. millefolium* seeds [19,22]. Moreover, insects constitute a significant part of the diet, especially for pregnant females, and small mammals, birds, and reptiles are occasionally consumed [22–24]. The species is also known to forage on cultivated crops, cereals, and fallen fruits, further demonstrating its dietary flexibility in human-modified environments [25,26].

As similar data are lacking for the southernmost populations, this study aims to explore the dietary habits of *Spermophilus citellus* at the southernmost edge of its distribution in Central Macedonia, Greece, where human-modified environments under a Mediterranean climate represent the primary habitat of this species [16]. The study seeks to assess selection and seasonal shifts in plant parts and taxa, and to provide insights into the species' adaptations to human-modified environments in Mediterranean ecosystems. This information will help to elucidate the ecological ways in which the species has adapted to anthropogenic pressures, guiding conservation strategies to mitigate further population declines. Moreover, identifying key food sources may help to inform habitat restoration efforts, ensuring the preservation of critical plant species and improving ecosystem management.

2. Materials and Methods

2.1. Study Area

Fieldwork was conducted at a European ground squirrel colony present at the School of Agriculture of Aristotle University of Thessaloniki (N 40° 32′, E 22° 59′), located in Thessaloniki, Greece (Figure 1). All observations were carried out within an approximately 0.62 ha fenced area, characterized by low vegetation that is regularly mowed. The site is located near a residential zone with significant anthropogenic influence. Previous studies have reported a relatively high population of European ground squirrels in this colony, with an average abundance of 6.9 ± 6.3 individuals (mean ± SD, late spring census) and an average density of 43.3 ind/ha (late spring census) [15].



Figure 1. The location of the study area (Thessaloniki, Greece) within the species' distribution area [18].

2.2. Assessment of Plant-Based Food Availability

Vegetation composition and cover were assessed using the quadrat method, with monthly samples in five randomly placed 1 × 1 m (1 m^2) quadrats. Plant taxa were identified, and cover density was calculated as the mean percentage cover across all quadrats. Identification was performed primarily at the genus level, with species-level identification for distinct taxa, like *Cynodon dactylon* and *Solanum elaeagnifolium*. Differentiating between *Carex* and *Cyperus* was challenging in mowed areas, so both were grouped as *Carex–Cyperus*. To explore seasonal diet variation, data were grouped into four periods based on the species' life cycle: (i) period A (March–April): emergence and reproduction; (ii) period B (May–June): gestation and lactation; (iii) period C (July–August): independence of young; (iv) period D (September–October): preparation for hibernation. For each period, the vegetation composition was calculated as the mean over two months.

2.3. Behavioural Data Collection

Our study adhered to the guidelines of the IUCN Position Statement on Research Involving Species at Risk of Extinction [27]. Behavioural data collection covered the entire active period of the species, from March (emergence from hibernation) to October (entry into torpor) [14], but was split between two years: July to October 2022 and March to June 2023. Observations were conducted using binoculars from fixed locations selected to offer maximum visibility across the colony. Only adult individuals, identified by their size

and activity, were sampled. As European ground squirrels are diurnal, all observations occurred between 07:30 and 19:30, with slight monthly time adjustments for seasonal daylight variations. Within each month, we collected data during three daily 12 h sessions.

Foraging behaviour was observed using the ad libitum focal sampling method [28]. An arbitrarily selected individual was continuously monitored for 10 min per session, while foraging, with observations terminated either when the 10 min session ended or when the individual was lost from sight (e.g., behind vegetation or inside a burrow). We then shifted to the next available adult individual in sight, paying particular attention to equally sampling all available individuals. During data collection sessions, the food type and the feeding bout duration (in seconds) were recorded. A bout began when food from a specific plant category was acquired, handled, and ingested, and ended when the individual switched to another behaviour (e.g., movement or vigilance) lasting more than 3 s, or changed food type. Based on studies of intermittent locomotion and vigilance in rodents [29], we used the 3 s threshold to define pauses. Data independence was ensured by limiting observations to 10 min per individual, applying the 3 s threshold, and sampling individuals from different areas in the colony [28,29].

During foraging observations, we also recorded data on different food types (plant or animal matter), and the different parts and genera (or species) of the plants consumed (see Table 1).

Table 1. Variables and definitions of food types and plant parts (based on Crang et al. [30]).

Food Type	Food Part	Definition
Plant matter	Leaves	Lamina, petiole, and sheath
	Stem	Main vegetative axis and lateral branches from which leaves and reproductive organs develop
	Seeds	Includes solitary seeds and those formed on seedheads
	Flower	Includes solitary flowers and inflorescences
	Root	Underground plant organ that functions in anchorage and absorption
	Rhizome	Modified underground stems that grow horizontally at shallow depths beneath the soil surface
	Leaves + Stem	Undistinguishable combination of leaves and stem
	Flower + Seeds + Leaves	Undistinguishable combination of flowers, seeds, and leaves
Animal matter	Animal	Invertebrate or vertebrate body part(s) that could be taxonomically identified by their remains

2.4. Statistical Analysis

As food parts differ in the time needed for their handling and consumption, we calibrated for this discrepancy by computing the weighted consumption of each food type and genus. This entailed multiplying the mean consumption duration of each food item by the total number of feeding bouts, and dividing by the total consumption duration across all food types. Non-parametric tests were applied to assess seasonal variation in food consumption and taxonomic composition; the Shapiro–Wilk test indicated deviation from normal distribution [31]. The G test of independence was used to compare food consumption across different food types and plant genera between seasons, followed by a post hoc pairwise G test for seasonal differences [31]. Both analyses were conducted using the RVAideMemoire package v. 0.9-83-7 [32] in R v4.3.2 [33]. Statistical significance was

set at $p < 0.05$. Ivlev's Index was used to evaluate the preference or avoidance of the five most-consumed plant genera, calculated as follows:

$$E = (r - p)/(r + p)$$

where r represents the proportion of food consumed, and p represents the available proportion of food [34]. The index ranges from -1 to +1, with values near +1 indicating strong preference and values near -1 indicating avoidance [34].

3. Results

3.1. Vegetation Dynamics and Structure

Monthly vegetation surveys identified a total of 28 plant genera in the study area (Figure 2). Overall, *Cynodon* (9.91%) and *Carex–Cyperus* (9.12%) were the most abundant monocotyledons, and *Solanum* (6.66%) was the most common dicotyledon. Despite high taxonomic diversity, most genera showed low coverage, reflecting the area's aridity. Seasonal changes revealed that period A had the highest diversity (23 taxa), with relatively low diversity in the periods that followed (period B = 14 taxa, period C = 11 taxa, period D = 14 taxa). Plant taxa coverage changed significantly during the different periods (Figure 2; G = 68.5, $p < 0.001$). Although *Veronica* was the dominant plant in period A (22.35% coverage), it significantly declined during the second period (0.75%) and disappeared altogether as summer progressed. *Plantago* showed considerable coverage in periods A and B (10.45% and 22.50%, respectively), but significantly decreased as summer progressed. *Cynodon* dominated in period B (26.32% coverage), but significantly decreased to 10.73% in period D. *Carex–Cyperus* showed variable coverage, with the highest values in periods C and A (21.00% and 11.35%, respectively). *Solanum* was generally underrepresented during most periods, but significantly increased to 19.43% coverage in period C.

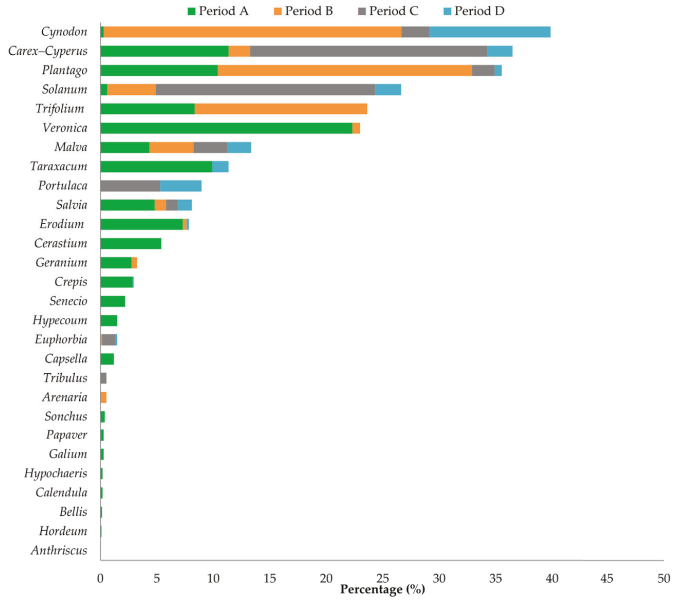

Figure 2. The percentage cover of plant genera recorded in the study area for the different study periods (A: March–April, B: May–June, C: July–August, D: September–October).

3.2. General Feeding Profile

In terms of food type, the overall food consumption patterns of *S. citellus* revealed the highest intake of rhizomes (38.90%), followed by leaves (28.80%) (Table 2). The latter were

consumed twice as often as rhizomes, albeit in threefold shorter feeding bout durations. Stems (12.20%) and seeds (11.10%) followed, while other food types (leaves + stems, inflorescences, and inflorescences + seed + leaves) accounted for less than 5%. Animal matter intake was negligible, being observed only once. Differences in food type consumption were significant ($\chi^2 = 111.7$, df = 7, $p < 0.001$).

Table 2. Overall mean duration of consumption (sec), total events (N), and weighted percentage of consumption (%) of each food type.

Food Type	Mean Duration (s)	Total Events (N)	Weighted Percentage (%)
Rhizome	73.5	668	38.90
Leaves	23.0	1583	28.80
Stem	29.7	518	12.20
Seeds	35.7	391	11.10
Leaves + Stem	43.7	109	3.77
Flower	17.9	262	3.72
Flower + Seeds + Leaves	21.4	85	1.44
Animal	60.0	1	0.05

Regarding taxonomic representation, the diet of the species was dominated by a few plant genera, with the weighted consumption percentages and average consumption times detailed in Table 3. *Cynodon* was the most consumed plant (49.40%), consumed more than twice as often as *Carex–Cyperus* (21.20%). *Salvia* also represented a considerable percentage of the diet (7.13%), with all other taxa having percentages below 5%. *Cynodon* required the longest bouts to be consumed (mean = 52.5 s), except for animal protein (snails, n = 1–60 s). Differences among the plant taxa consumed were significant ($\chi^2 = 650.3$, df = 24, $p < 0.001$).

Table 3. Mean consumption duration (sec), total number of events (N), and weighted consumption (%) per plant genus consumed.

Genus	Mean Duration (s)	Total Events (N)	Weighted Percentage (%)
Cynodon	52.5	1154	49.40
Carex–Cyperus	30.0	867	21.20
Salvia	40.1	218	7.13
Solanum	20.2	270	4.45
Plantago	18.4	285	4.26
Erodium	27.3	99	2.20
Morus	30.8	86	2.16
Malva	27.8	78	1.77
Taraxacum	18.2	118	1.75
Veronica	19.9	83	1.35
Euphorbia	45.7	36	1.34
Portulaca	33.2	28	0.75
Hordeum	48.7	18	0.71
Trifolium	14.5	28	0.33
Crepis	17.4	23	0.33
Geranium	15.1	20	0.25
Arenaria	19.5	14	0.22
Cerastium	18.4	10	0.15
Senecio	13.7	6	0.06
Gastropoda	60.0	1	0.04
Tribulus	40.0	1	0.03
Capsella	19.5	2	0.03
Muscari	30.0	1	0.02
Bellis	9.0	1	<0.01
Hypecoum	9.0	1	<0.01

3.3. Seasonal Variation in Dietary Patterns

Spermophilus citellus exhibited significant seasonal variation in plant food type consumption ($G = 328.8$, $p < 0.001$; Figure 3). In period A, seeds (38.16%) and stems (28.64%) were mostly consumed. This period was further characterized by minimal rhizome intake (0.48%), which increased significantly during periods B, C, and D (54.66%, 46.55% and 50,20%, respectively) (A vs. B: $G = 210.2$, $p < 0.001$; A vs. C: $G = 160.8$, $p < 0.001$; A vs. D: $G = 143.1$, $p < 0.001$). Leaf consumption was moderate during periods A (18.9%) and B (17.14%), but increased significantly as summer progressed (period C = 44.48%, period D = 36.95%) (A vs. B: $G = 114.4$, $p < 0.001$; A vs. C: $G = 153.3$, $p < 0.001$; A vs. D: $G = 81.2$, $p < 0.001$; B vs. C: $G = 180$, $p < 0.001$; B vs. D: $G = 101.2$, $p < 0.001$). After period A, seed consumption progressively declined to 6.86% in period B, and to almost no intake in the subsequent periods (A vs. B: $G = 327.8$, $p < 0.001$; A vs. C: $G = 652.2$, $p < 0.001$; A vs. D: $G = 326.4$, $p < 0.001$). A similar pattern was also observed for stem consumption, as intake decreased to 17.34% in period B, and to almost nothing in periods C and D (A vs. B: $G = 380.1$, $p < 0.001$; A vs. C: $G = 436.8$, $p < 0.001$; A vs. D: $G = 211.4$, $p < 0.001$).

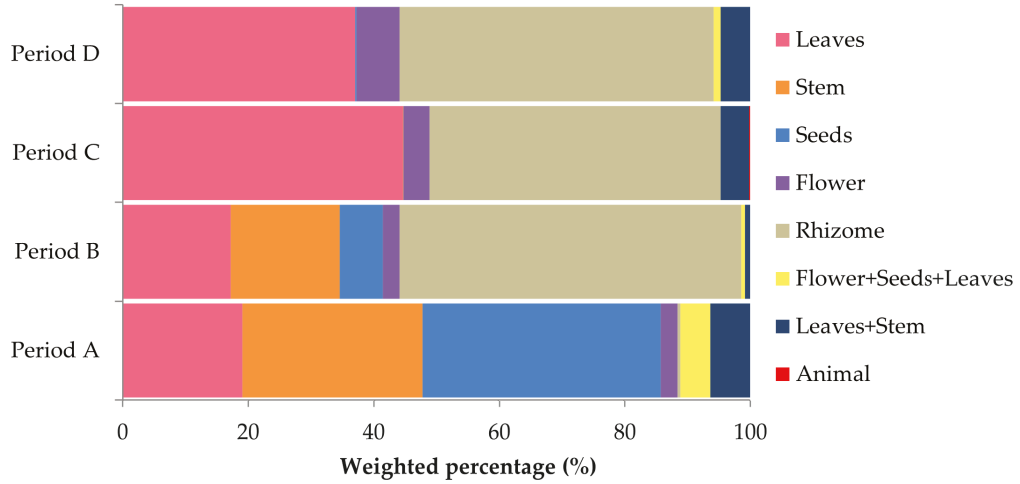

Figure 3. Variation in the consumption of plant food types across periods (A: March–April, B: May–June, C: July–August, D: September–October) by *S. citellus*.

Spermophilus citellus exhibited significant seasonal variation in plant taxa consump-tion ($G = 348.4$, $p < 0.001$; Figure 4). Although *Cynodon* was not consumed just after emergence, it dominated in periods B (73.44%), C (49.85%), and D (74.17%) (A vs. B: $G = 131.2$, $p < 0.001$; A vs. C: $G = 71.4$, $p < 0.001$; A vs. D: $G = 133.5$, $p < 0.001$). Intake of *Carex–Cyperus* prevailed in periods A (34.18%) and C (33.14%), but significantly decreased in periods B (6.71%) and D (6.87%) (A vs. B: $G = 249.3$, $p < 0.001$; A vs. D: $G = 245.3$, $p < 0.001$; B vs. C: $G = 134.6$, $p < 0.001$; C vs. D: $G = 230.5$, $p < 0.001$). *Salvia* represented one of the main food sources of *S. citellus* in period A (29.85%), but its intake significantly decreased in period B (0.26%), and ceased thereafter (A vs. B: $G = 439.3$, $p < 0.001$). Finally, consumption of *Solanum* was minimal in periods A and B, but moderately increased in periods C (9.72%) and D (9.94%) (A vs. C: $G = 128.3$, $p < 0.001$; A vs. D: $G = 131.5$, $p < 0.001$; B vs. C: $G = 117.8$, $p < 0.001$; B vs. D: $G = 120.9$, $p < 0.001$).

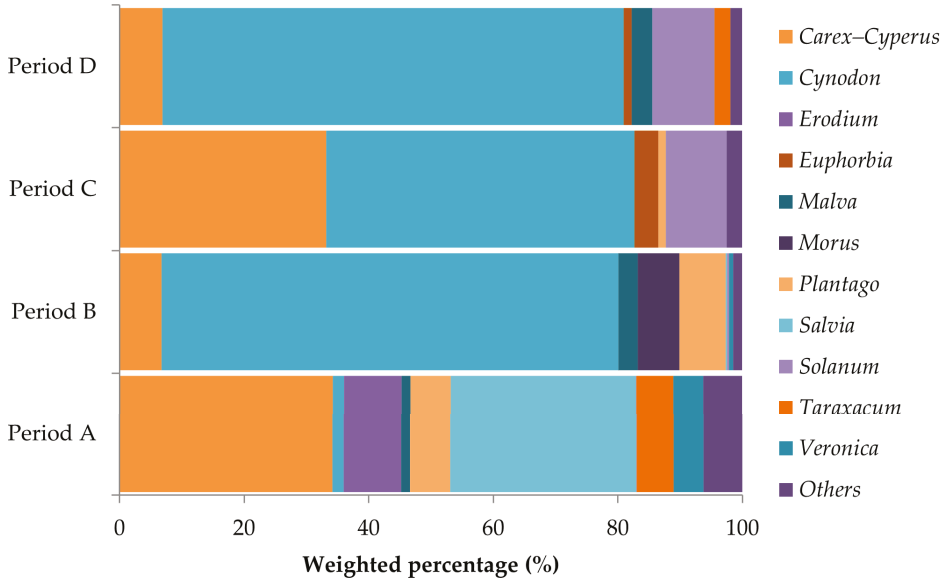

Figure 4. Variation in the consumption of plant genera across periods (A: March–April, B: May–June, C: July–August, D: September–October) by *S. citellus*.

3.4. Preference or Avoidance of Plant Taxa

Ivlev's Index for the five most-consumed genera by *S. citellus* revealed preferences and avoidances across periods (Figure 5). European ground squirrels showed a strong preference for *Cynodon*, particularly in period C (0.9), with an overall selection index of 0.66. *Carex–Cyperus* followed a similar trend, exceeding 0.5 in most periods, but remaining neutral in period C (Figure 5). *Salvia* was well preferred in period A (0.72) but was strongly avoided in periods C and D (−1). Nevertheless, the overall index of 0.55 suggested an overall preference. *Solanum* was avoided in periods A and B, but was preferred in period D, with an overall low avoidance (−0.20). In all periods, *Plantago* seemed to be variably avoided, with a negative overall index (−0.35).

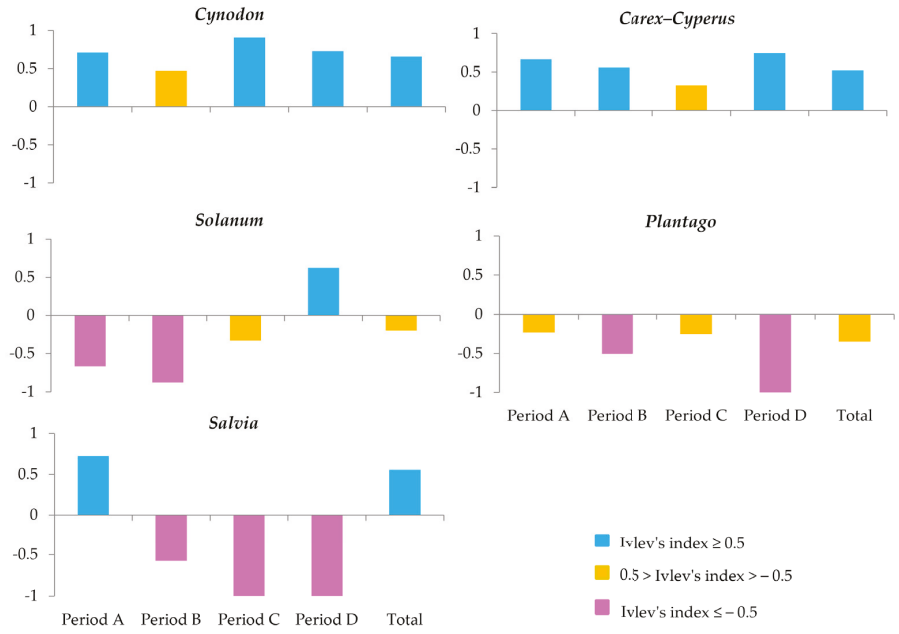

Figure 5. Ivlev's index for the five most-dominant plant genera in the diet of *S. citellus*, calculated per period and overall. Colour-coding indicates preference (blue), avoidance (purple), or neutrality (yellow).

4. Discussion

Dietary choices, including food processing and consumption, shape the nutritional specialization, physiology, anatomy, and foraging and vigilance behaviours of animals [2]. Diet analysis also informs key ecological processes, such as habitat selection and species competition. This study examined the dietary habits of the European ground squirrel *Spermophilus citellus* in a human-modified environment in a suburban area of Central Macedonia in Greece, through direct field observations.

In the study area, the vegetation survey recorded 28 plant genera, with taxa related to disturbed, human-modified habitats, such as *Cynodon dactylon*, *Plantago lanceolata*, *Solanum elaeagnifolium*, and *Carex–Cyperus*, being the most dominant [35–37]. However, as rainfall decreased and temperatures increased during the active period of *Spermophilus citellus*, this diversity significantly diminished, and the relative coverage of some key species, such as *C. dactylon*, decreased [35]. These changes were evident in the transition from *Veronica*, *Carex–Cyperus*, *Plantago*, and *Taraxacum* in early spring to *Cynodon* in summer and *Carex–Cyperus* and *Solanum* before hibernation in late autumn. *Cynodon* remained dominant due to strong interspecific competition [38], while *Solanum* thrived in dry months, due to its deep-rooted drought resilience [39]. These seasonal shifts indicate a highly disturbed ecosystem influenced by strong climatic shifts, human activities, and the presence of invasive plant species.

Overall, the diet of *Spermophilus citellus* mainly consisted of selected plant parts, with minimal animal intake. Rhizomes, rich in amylum and proteins, represented the primary food source, followed by leaves, stems, and plant reproductive structures. Rhizomes offer essential nutrients and are a drought-resilient food source, especially during periods when surface vegetation dries out [30] or is removed, as in the case of mowing in human-modified habitats, like the one in this study. Although leaves were more frequently consumed, rhizome foraging took nearly three times longer due to tougher tissues and excavation effort, especially in *Cynodon dactylon*, where rhizomes cluster underground. Young leaves, with higher levels of protein and lower levels of secondary metabolites [40], were preferred. Stems provide hydration and nutrients [41] and appear to be essential when energetic demands are high, such as during emergence, reproduction, and pregnancy. Despite its name (*Spermophilus* = seed lover), seeds were not predominant in the diet of the species, except for the period after emergence from hibernation. This intake perhaps underlines the importance of seeds as a source of protein and lipids, although the fleshy parts are usually lower in protein and lipids, but rich in sugars and water [42].

Dietary composition varied seasonally, with the highest plant diversity in the period of early activity and reproduction (period A), due to abundant vegetation. In the period of gestation and lactation (period B), rhizome intake increased as seed availability declined, and higher temperatures led to plant desiccation. During the periods of progressive independence of young individuals (period C) and preparation for hibernation (period D), leaves and rhizomes dominated, as summer drought and continuous anthropogenic impact (mowing) limited plant growth. Comparative studies show that ground squirrels generally prefer leaves, followed by flowers and seeds [43,44], although some species focus more on seeds [45]. *Spermophilus citellus* prefers leaves over seeds and flowers [20], and selectively forages on rhizomes from species of *Poa*, *Cynodon*, and *Plantago*, while previous studies have shown more moderate intake of rhizomes [19]. This study emphasizes the importance of rhizomes in the diet of the species in its southernmost range, indicating an adaptation to the Mediterranean climate and summer drought, as well as to a highly disturbed habitat, where rhizomes are the sole resource during periods of extreme aridity and frequent mowing. *Cynodon dactylon* is highly adapted to frequent trampling and

mowing, and these disturbances facilitate the species becoming dominant in semi-natural and human-made environments.

Animal consumption was minimal, with only one recorded instance in period C (July–August), when an individual consumed a snail (Gastropoda). Studies on other ground squirrel species, such as *Xerospermophilus tereticaudus* and *Ictidomys tridecemlineatus*, report higher intake of insects and animal matter in general [43,46]. In Serbia, fecal analysis of *S. citellus* showed that insect and gastropod consumption reached nearly 25% in August–September, though this may be an overestimate, due to the difficulty of digesting chitin [20]. The minimal invertebrate consumption observed in our study site may reflect the rarity of this food type in the diet of this colony, or potential underestimation due to observation limitations. It is always easier to observe the consumption of plant matter rather than the consumption of animal matter, as the latter usually requires different approaches for seizing and handling the prey.

The diet of *Spermophilus citellus* was dominated by grasses and sedges, with *Cynodon* and *Carex–Cyperus* exceeding 20% of plant consumption, reflecting their local abundance. Other frequently consumed taxa, such as *Salvia*, *Solanum*, and *Plantago*, also mirrored their availability within the study site, while minimally represented taxa contributed negligibly to the overall diet of the species. This profile suggests opportunistic foraging according to availability to minimize energy expenditure and predation risk. A similar dietary profile, where consumption of the most prevalent plants, including shrubs and invasive annuals, dominates, also characterizes other ground squirrel species, such as *Xerospermophilus mohavensis* and *Urocitellus mollis* in North America [43]. In contrast, the diet of *S. citellus* in northern Greece appears to differ from that recorded in previous studies [19–21]. For instance, in northern Serbia, Arok et al. [20] reported lower grass consumption, despite its abundance. These differences may stem from differential regional plant communities, as previous research focused on sites in eastern Europe, whereas the distinct Mediterranean climate and human-modified habitats of suburban areas in northern Greece appear to strongly shape dietary preferences.

Cynodon dactylon was the primary plant food for *Spermophilus citellus*, dominating its diet in three of four activity periods (B, C, D). Rhizomes were presumably preferred over leaves and stems due to their high carbohydrate content, supporting energy-demanding activities like burrowing [47]. Despite the high plant diversity in period A (emergence and reproduction), *S. citellus* still sought *C. dactylon*, possibly for its additional nutritional or medicinal properties [48]. During the late summer drought (period D), *C. dactylon* rhizomes remained a vital food source when most vegetation had dried (see also above). While earlier studies noted frequent consumption, they reported a preference for stem buds [19] and little rhizome intake. In some regions, *S. citellus* avoided *C. dactylon*, favouring *Festuca* spp., likely due to grazing competition [21].

Carex–Cyperus was the second most important food source for *S. citellus*, with peak consumption during periods of high vegetation cover (Periods A and C). Leaves were preferred, likely due to their softness and frequent mowing limiting plant growth. Despite being considered nutritionally inferior to grasses, Cyperaceae provide comparable nutrients and bioactive compounds with antimicrobial and antioxidant properties [49–51]. Unlike previous studies reporting rare consumption [19,20], *S. citellus* actively sought these taxa, even at low densities.

Salvia was an occasional but important food source for *Spermophilus citellus* during period A (March–April), with the species showing a preference for its energy-rich seeds. The species favoured its seeds, which are rich in phenolic compounds and polyunsaturated fatty acids, providing high energy, especially during post-hibernation and reproduction [52].

Despite being less abundant, this plant species was selectively foraged (Ivlev's Index > 0.5) post-hibernation and during reproduction [53]. However, it was avoided in later periods (Ivlev's Index = −1), possibly due to its strong odour from essential oils.

Solanum elaeagnifolium is an alien and invasive species in highly disturbed environments, and is toxic to many animals. It represented a moderate proportion of the diet of *Spermophilus citellus*, with increased consumption during the hotter and more arid periods (C, D). In period C, the density of this invasive plant peaked, and in period D, all other plant resources were scarce. *Spermophilus citellus* preferred *S. elaeagnifolium* in pre-hibernation (period D) (Ivlev's Index > 0.5), likely due to high energy and nutrient demands. During periods A and B, the plant was avoided (Ivlev's Index < −0.5). The leaves were mostly consumed, with occasional feeding on flowers. Leaves were preferred, presumably due to their lower toxin concentrations, particularly younger leaves, which also possess fewer spines [54]. The increased consumption of flowers may be attributed to their softer tissue, while toxin levels tend to rise in more mature plant structures. Frequent mowing most likely prevented seed production, excluding them from the diet, though *Spermophilus citellus* were observed to consume *Solanum* seeds in nearby areas. Previous studies did not detect consumption of *S. elaeagnifolium*, likely due to the minimal disturbance in the regions studied. The reliance of *S. citellus* on these food sources in human-modified habitats may reflect the adaptability of the species in similar habitats. However, further investigation is required to assess the potential adverse physiological effects associated with toxin ingestion [55].

Some plant genera, like *Plantago*, were also consumed, despite being either avoided or exploited according to availability. This could be related to overall low plant diversity, limiting food choices. *Plantago* leaves are rich in fibre but low in lipids and carbohydrates, making them less significant energy sources [56]. *Spermophilus citellus* primarily consumed *Plantago* during periods A and B, when its density was high, avoiding the need for more energy-demanding foods. Consumption of *Plantago* varied in other areas, depending on environmental conditions [19–21].

5. Conclusions

In human-modified environments, the survival of *Spermophilus citellus* largely depends on the plant community's composition and food availability. The dominance of *Cynodon dactylon* and *Carex–Cyperus* in the diet of the species is related to their abundance and nutritional content in grassland ecosystems. Ground squirrels in semi-urban environments, like that of the present study, rely heavily on human activities influencing plant communities. The invasive species *Solanum elaeagnifolium*, which competes with other plants, becomes a significant food source during droughts, although the effects of the toxins on the squirrels remain unknown. To support native species and provide more nutritious food, mechanical removal of *S. elaeagnifolium* is recommended. Additionally, reducing mowing during dry months would allow resilient plants to grow, helping to retain morning moisture, and planting more seed-bearing trees could increase food availability. These measures aim to improve the habitat by prioritizing and enhancing key food sources, aligning more closely with the ground squirrel's natural environment [57]. Ecosystem management that involves maintaining plant diversity and limiting invasive species is essential for the survival and conservation of *S. citellus* in both natural and human-modified ecosystems.

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Sexual Body Size Dimorphism in Small Mammals: A Case Study from Lithuania

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Simple Summary: We studied the differences in body size between males and females of 14 small mammal species in Lithuania. By measuring standard physical traits in voles, mice, and shrews from a large collection of long-term surveys, we updated information published 35 years ago and compared our results with data from other countries. Our results showed that males were larger than females in the yellow-necked mouse, root vole, and three other meadow voles, especially among adults. This pattern is consistent with Rensch's rule, which refers to how size differences between the sexes change with total body size. In contrast, females were larger than males in bank voles and four mouse species, although this was less consistent across age groups. Shrews and the smallest mouse species, the herb field mouse, showed no significant size differences between sexes. In some species, these size differences changed as the animals grew. We are adding data on less common species such as the sibling vole and northern birch mouse, which have not been studied extensively. Our research provides new baseline data for small mammals in the boreal mid-latitudes and serves as a foundation for future studies of how animals adapt to changing ecological conditions or climate change.

Abstract: We assessed the sexual size dimorphism (SSD), analyzing standard morphometric traits in juveniles, subadults, and adults, of 14 species of voles, mice, and shrews in Lithuania on the basis of long-term surveys, updating information published 35 years ago and in the context of data from other countries. ANOVA, *t*-tests, and a 5% threshold were used in the analyses. Male-biased SSD was observed in *Apodemus flavicollis* and *Alexandromys oeconomus*, which was subject to Rensch's rule, and in three other meadow vole species, with the strongest expression in adult individuals. Female-biased SSD was best expressed in *Clethrionomys glareolus* and was also identified in four mouse species, although patterns were less consistent across age groups. Shrews and the smallest mouse, *Apodemus uralensis*, exhibited monomorphic traits. The transition of dimorphism during postnatal development was observed in some species. We present an assessment of SSD in *Microtus rossiaemeridionalis* and *Sicista betulina*, the less common small mammals not analyzed in publications by other authors, and provide new baseline data for boreal mid-latitude small mammal species as a foundation for future studies on SSD and its adaptive significance under shifting ecological pressures or climate change.

Keywords: voles; mice; shrews; age groups; morphometric differences; long-term research

1. Introduction

Sexual dimorphism is generally defined as differences between males and females of the same species [1]. These sex-related differences are not limited to sexual organs but can also affect growth rates, maturation, life expectancy, behavior [2], and certainly external traits. The term "sexual size dimorphism" (hereafter SSD) is used to analyze differences in body size with respect to animal sex [1].

Previous studies considered mammals to be potentially sexually dimorphic, with males being larger [3], and the driving force for this phenomenon was considered to be male–male competition [4]. However, there are a number of mammalian species with female-biased SSD, defined as 38 out of 186 species in [5]. This has been explained by

higher energetic demands during pregnancy [6] and lactation [7], and by the fact that more body space is required to maintain eggs and embryos than to maintain sperm [4]. However, our knowledge of the physiological costs of reproduction in small mammals has been described as "rudimentary" [8].

If the size difference between males and females varies with total body size, this is an evolutionary regularity described as Rensch's rule [9]. This rule suggests that, specifically in mammals, male body size tends to increase more rapidly than female body size in larger species. Therefore, in smaller species, males and females tend to be more similar in size, or females may even be slightly larger. In general, insectivores and rodents have been characterized as following Rensch's rule, with males being larger [Lindenfors 2007], but other authors point out that not all small mammals follow Rensch's rule [10].

In some mammalian taxa, such as mustelids, smaller species are more dimorphic [11], although in general larger species of the same genus are more dimorphic. In small mammals, this regularity is not clear. While some authors point to better expression in larger species [12], dimorphism is usually not expressed [13]. While in rodents, males are usually the larger sex [10], there are exceptions in microtines [12,14].

In mammals, data availability has limited the ability to estimate SSD [15]. In the absence of statistics in the literature, researchers have used arbitrary values of differences between means expressed as percentages [16] or simply relied on the male:female ratio being greater or less than 1 [17]. Therefore, SSD has been better studied in ungulates and pinnipeds [3,18,19], with a limited number of dedicated studies in rodents and other small mammals [14,20]. It is possible that relatively small sex differences in rodents have not attracted attention [12].

Data on dimorphism can be found in scientific papers not devoted to SSD. Data on the body weight of 10 small mammal species from Central Europe show that five species of mice, voles, and shrews are female-biased, three species are similar in weight, and two species are male-biased [21]. The results of a comprehensive study of SSD across a wide taxonomic range of rodent species are presented in [12], which reports monomorphism and SSD for 172 species based on structural characteristics—body length or body weight. However, this publication does not take into account possible geographic variation and limits the data to single populations. The most variable species in terms of SSD are the voles of the genera *Clethrionomys* and *Microtus*. In these genera, female-biased and male-biased SSDs are found even within the same species [12,20].

Recently, the availability of more comprehensive data has led to the refutation of earlier findings regarding male-biased dimorphism [15]. The dataset includes 429 mammalian species and presents the distribution of male-biased, female-biased, and monomorphic species across 17 mammalian orders. According to this review, the number of species categorized as female-biased, monomorphic, and male-biased were as follows: 4, 32, and 18 in the family Cricetidae; 4, 15, and 20 in the family Muridae; and 0, 7, and 6 in the family Soricidae. The family Sminthidae (birch mice) was not analyzed [15].

Long-term data are essential in the study of SSD in small mammals, as there are cases where the established pattern of SSD is reversed in the same species. In the yellow-necked mouse (*Apodemus flavicollis*), male-biased SSD has been reported in different regions of Europe [12,21–23]. In Lithuania, this species was reported as monomorphic [24]. However, in Slovakia, a case was recorded where the SSD changed from male-biased to female-biased in one of the six survey years, as the population responded to an exceptionally high population density. Therefore, the body size of the species was considered to be plastic in terms of adapting to changes in the short term [25]. This was explained by (i) a faster growth rate of females compared to males of similar size, and (ii) inter-annual variation in growth rates indicating a dependence on population density [26].

In Lithuania, previous research on male and female size differences in small mammals was summarized in the book Fauna of Lithuania: Mammals [24]. Although no statistics of the comparisons were presented, at least it is possible to recalculate if the SSD meets the 5% criterion formulated in [27], see Discussion. Furthermore, male and female body mass and body length were presented for vole, mouse, and shrew species in publications devoted to the analysis of differences in tooth row length between males and females and in different age groups. The main drawback is that the data for males and females were pooled, so the papers do not give a true picture of SSD [28–30]. Finally, for root vole (*Alexandromys oeconomus*), it was shown that SSD is best expressed in adult age [31], although the sample size was quite limited.

We aimed to test Lithuanian small mammals on the issue of SSD, using standard morphometric body measurements (body mass, body length, tail length, hind foot length, and ear length), and the consistency of these differences in age groups (juveniles, subadults, and adults). This is the first study of its kind based on long-term data and covering all species of voles, mice, and shrews in the same country, and, therefore, can claim to be a reference for boreal mid-latitude small mammals.

We believe that dividing the sample into six age-sex groups gives a better understanding of SSD in small mammals and shows how SSD is developed in postnatal development. This could explain the presence of two different patterns of body condition index in small mammals (either increasing or decreasing from juvenile to adult), as this index is calculated from body weight and length [32]. From a practical point of view, data on SSD should be a basis for pooling data on both sexes together or analyzing them separately. Our conclusions are based on the long-term experience of small mammal trapping in Lithuania, which has provided large samples of most species in the country.

2. Materials and Methods

2.1. Study Site and Small Mammal Trapping

The study site is Lithuania, a country located in northern Europe along the Baltic Sea, whose fauna and flora share similarities with other countries at similar latitudes, especially in northern and eastern Europe. It covers an area of 65,286 square kilometers and has mostly flat terrain with the highest elevation being about 294 m. The country's climate is transitional between maritime and continental, with average temperatures ranging from −4.9 °C in January to 17.2 °C in July, and annual precipitation ranging from 570 to 902 mm, depending on location. Its vegetation includes mixed forests, coniferous and deciduous woodlands, meadows, pastures, and wetlands. Land use is divided into 57% agricultural land, 30% forests and shrubs, 4% water bodies, 3% swamps, and 6% other areas, supporting a population density of approximately 45.3 inhabitants per square kilometer.

For the analysis, we used data on small mammals caught in snap traps in the period 1975–2024 all over Lithuania (Figure 1). The main method used was trap lines with 25 traps, 5 m apart, set for three days and checked once or twice a day, i.e., in the morning or the morning and evening.

During this period, the trapping effort of 516,714 trap days resulted in the capture of 57,753 individuals of 20 small mammal species. Trapping occurred in 9 different habitats, including grassland, forest, wetland, shrub, commensal agricultural, disturbed, and riparian habitat, and if trapping occurred in several different habitats, then the habitat was designated as "mixed". As already mentioned, trapping effort was not equal in all habitats [32].

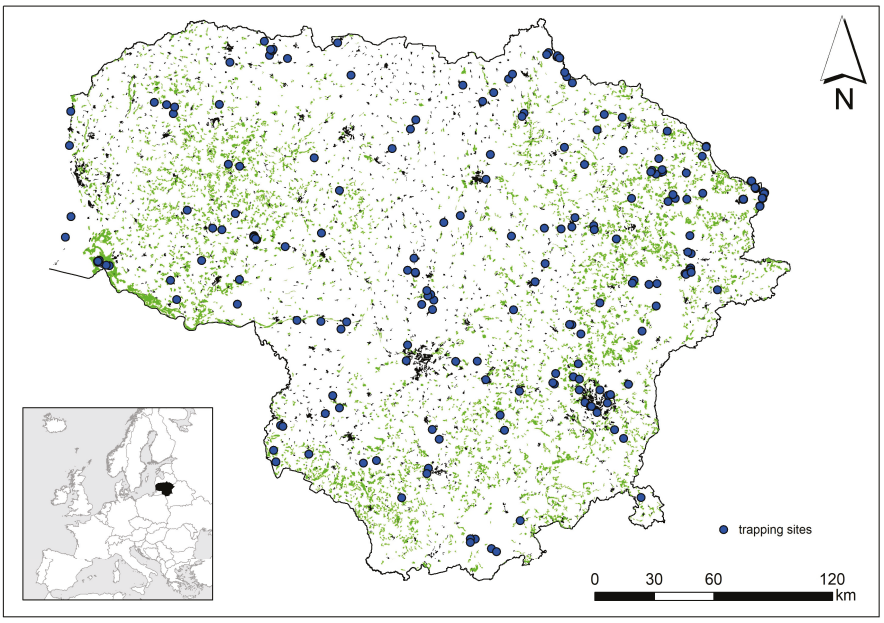

Figure 1. Trapping areas of small mammals in Lithuania, 1975–2024. Green color shows grasslands, black color shows urbanized areas. Lithuania's position in Europe is shown in the inset map.

2.2. Small Mammal Processing: Identification, Measurements, and Sample Size

No retrospective morphometric data are available for small mammal autopsies prior to the 1980s. In the 1980s–1990s, captured small mammals were processed in the field, while after the 2000s, captured individuals were kept frozen and transported to the laboratory for processing. Species were identified by external features, while *Microtus* voles were identified by differences in their teeth [24]. Genetic methods were used to identify sibling voles (*Microtus rossiaemeridionalis*) in the 1990s and 2023–2024. However, in most of the traps, we used *Microtus arvalis* sensu lato, as *M. rossiaemeridionalis* was not specifically identified. In addition, not all trapped individuals were measured for various reasons (body scraped, missing body parts, decomposition for autopsies, individuals not preserved for necropsy for a variety of reasons depending on the objectives of the study, etc.). Norway rat (*Rattus norvegicus*) and black rat (*R. rattus*) were not analyzed along with the other small mammals due to the difference in capture methods, the holding of individuals prior to necropsy, and the short collection period.

Three age groups (juveniles, subadults, and adults) were identified in necropsy in voles, mice, and shrews based on the development of their sexual organs [33,34], the degree of thymus atrophy [35], and only in a few cases on body mass. It has been shown that body mass varies greatly among age groups of small mammals and is, therefore, not the best indicator of age [36].

All non-breeding individuals with a well-developed thymus, filiform uterus in females, or testes in the abdominal cavity in males were defined as juveniles [37]. All non-breeding individuals with partially involuted thymus glands and inactive genitalia were defined as subadults [34]. All individuals with an involuted/atrophied thymus gland indicating reproductive activity (females: perforated or obstructed vagina, lactating, pregnant, corpora lutea in the ovaries [38] and/or placental scars in the uterus [39,40]; males: enlarged, scrotal testes, developed epididymis and seminal vesicles) or being post-reproductive were considered adults. Additional information on the identification of age groups is presented in [32].

Of the 29,203 individuals processed, 14,915 were males, 12,931 were females, and in four cases, both male and female sex organs were present in the same individual, so they were identified as hermaphrodites. The sex of 1353 individuals could not be determined. The sample composition of the dissected small mammals by species is shown in Table 1.

Table 1. Sample composition of dissected small mammals, 1980–2024, by sex and age.

Species	Adults		Subadults		Juveniles		Total
	Males	Females	Males	Females	Males	Females	
Common shrew (*Sorex araneus*) *	370	253	532	313	103	121	2549
Pygmy shrew (*Sorex minutus*) *	92	61	50	38	16	14	811
Water shrew (*Neomys fodiens*) *	21	18	13	13	5	0	101
Mediterranean water shrew (*Neomys milleri*) *	1	0	0	0	0	1	3
Hazel dormouse (*Muscardinus avellanarius*)	0	0	0	0	0	1	1
Northern birch mouse (*Sicista betulina*)	8	3	3	0	1	3	18
House mouse (*Mus musculus*)	110	74	97	24	70	65	440
Striped field mouse (*Apodemus agrarius*) *	456	333	755	331	966	931	3823
Yellow-necked mouse (*Apodemus flavicollis*) *	1585	1039	798	855	590	713	5666
Wood mouse (*Apodemus sylvaticus*)	2	0	0	1	2	0	5
Pygmy field mouse (*Apodemus uralensis*) *	4	16	9	14	14	16	74
Harvest mouse (*Micromys minutus*) *	18	40	60	34	108	78	350
Bank vole (*Clethrionomys glareolus*) *	1724	1796	2187	1329	1511	1759	10,398
Water vole (*Arvicola amphibius*)	0	1	2	2	5	0	10
Root vole (*Alexandromys oeconomus*) *	246	445	160	82	224	189	1356
Common vole (*Microtus arvalis*) *+	279	449	272	155	705	631	2510
Short-tailed vole (*Microtus agrestis*) *	117	210	135	58	120	77	719
Sibling vole (*Microtus rossiaemeridionalis*)	20	34	4	3	3	2	66

*—The total number of individuals captured (N) and the sum of identified age and gender numbers differ; +—sensu lato. In most studies, *M. rossiaemeridionalis* was not specifically identified.

In four listed species, *N. milleri*, *M. avellanarius*, *A. sylvaticus*, and *A. amphibius*, the sample size was minimal, 10 individuals or less. For *N. fodiens*, *S. betulina*, *A. uralensis*, and *M. rossiaemeridionalis*, sample size was about one hundred individuals or less, so some age/sex groups are under-represented.

Animals were weighed and measured prior to necropsy. Body mass (Q) was measured to the nearest 0.1 g using spring, weighing, or electronic scales. Body length (L), tail length (C), hind foot length (P), and ear length (A) were measured to the nearest 0.1 mm using mechanical or electronic calipers in a standard manner [41]. Over 80% of the measurements were taken by the same person throughout the study period, minimizing potential bias according to [42]. However, not all of the above measurements were taken on all individuals captured, depending on the species and the purpose of the capture. For example, in small mammal monitoring, most trapped individuals were at least weighed and measured for body length, whereas in biodiversity research, only species, sex, and age group were recorded for the most abundant species. This resulted in different sample sizes for the measured parameters, with some species being underrepresented (Table 2).

Table 2. Sample composition of weighed and measured small mammals by species. Abbreviations: Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length.

Species	Number of Individuals						
	Age	Gender	Q	L	C	P	A
Sorex araneus	2256	1871	2416	2321	1572	1565	1021
S. minutus	326	468	765	732	420	433	253
Neomy fodiens	93	78	100	100	64	92	72
N. milleri	2	3	3	3	1	2	2
Muscardinus avellanarius	1	1	1	1	1	1	1
Sicista betulina	18	18	17	17	17	17	16
Mus musculus	440	440	438	432	224	231	224
Apodemus agrarius	3785	3778	3578	3531	2012	2104	2053
A. flavicollis	5585	5576	5551	5508	3403	3522	3463
A. sylvaticus	5	5	5	4	4	5	4
A. uralensis	74	73	69	83	72	74	72

Table 2. *Cont.*

Species	Number of Individuals						
	Age	**Gender**	**Q**	**L**	**C**	**P**	**A**
Micromys minutus	346	338	343	341	265	275	271
Clethrionomys glareolus	10,341	10,317	10,158	10,015	6701	6602	6089
Arvicola amphibius	10	10	10	10	10	10	10
Alexandromys oeconomus	1350	1349	1340	1308	974	984	943
Microtus arvalis *	2491	2487	2460	2432	983	987	948
M. agrestis	718	718	714	698	459	459	439
M. rossiaemeridionalis	66	66	66	66	29	30	30

*—sensu lato. In most studies, *M. rossiaemeridionalis* was not specifically identified.

2.3. Data Analysis

The normality of the distribution of Q, L, C, P, and A measurements was tested for each sex/age group with a sample size of at least 15 individuals using an online calculator with almost unlimited sample size [43]. We used the Shapiro–Wilk test, which is particularly suitable for small samples. Based on the *p*-value, we identified three groups of samples: those with normal distribution ($p < 0.05$), those with near-normal distribution ($0.10 > p > 0.05$), and non-normal samples ($p > 0.10$).

Fulfilling the condition that our data are continuous and the samples are randomly drawn from the species populations, we used ANOVA and a series of *t*-tests on all five traits (Q, L, C, P, and A) to find out if there is an SSD in the age groups of the analyzed small mammal species. According to [44], the *t*-test can be used with approximately normal distributions when the sample size is greater than 30 per group. The *t*-test is quite robust to mild deviations from normality, especially if the data do not have extreme outliers or highly skewed distributions, both of which are true for our data. For small samples, we used the Bayes factor (bootstrap with 9999 replications).

To test whether the reversal of dimorphism during postnatal development is significant, we used a linear mixed effects model with body mass (Q) as the dependent variable, sex and age groups, and their interaction, as fixed factors, capture site as a random factor, and month of capture as a covariate. If there was a significant interaction between sex and age group in determining body mass, reversal dimorphism was considered significant.

We also checked whether the difference between males and females reaches the 5% threshold by calculating it as $(F - M)/F \times 100$, where F and M denote trait values in females and males, respectively. Negative values indicate larger trait sizes in males, positive values indicate larger trait sizes in females. In line with R.J. Smith's criticism [45], we do not use the M/F ratio for our data or for comparisons with other authors' samples.

Calculations were performed in Statistica for Windows, version 6.0 (StatSoft, Inc., Tulsa, OK, USA) [46] and in PAST version 4.13 (Museum of Paleontology, Oslo College, Oslo, Norway) [47]. The minimum confidence level was set as $p < 0.05$.

3. Results

3.1. Sexual Size Dimorphism in Lithuanian Small Mammal Species

Of the 18 small mammal species analyzed, the sample size was large enough for statistical analysis of male–female differences in 12 species, while in *S. betulina* and *M. rossiaemeridionalis*, the results may be dependent on the small sample size (see Table 1). Based on the significance of differences in five morphometric parameters, species were characterized as monomorphic or dimorphic, and in the latter case, with male-biased dimorphism or female-biased dimorphism (Table 3). The body mass variation in all the small mammal species studied is summarized in Appendix A (Table A1).

In addition, it was found that the statistical significance of the differences did not fully meet the 5% threshold—in several species where male–female differences were not significant, the 5% threshold of differences was met. These cases are discussed in the respective chapters.

Table 3. Age-related sexual size dimorphism in small mammal species from Lithuania based on five main morphological parameters. Significant differences indicated in bold type. Abbreviations: M—males, F—females, Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length.

Species	Age Group	Q	L	C	P	A
Sorex araneus	Adults	F > M	M = F	M = F	**M > F**	**F > M**
	Subadults	M = F	M = F	M = F	M = F	M = F
	Juveniles	M = F	F > M	M = F	**M > F**	M > F
S. minutus	Adults	M = F	M = F	M = F	M = F	M = F
	Subadults	M = F	F > M	M = F	M = F	**F > M**
	Juveniles	M = F	M = F	M = F	M = F	M = F
Neomys fodiens	Adults	M = F	M = F	**M > F**	M = F	M = F
	Subadults	M = F	M = F	M = F	M = F	M = F
	Juveniles			Males only		
Sicista betulina	Adults	M = F	F > M	M = F	M = F	M = F
	Subadults			Males only		
	Juveniles	M = F	M = F	M = F	M = F	M = F
Mus musculus	Adults	**F > M**	**F > M**	M = F	M = F	M = F
	Subadults	M = F	M = F	M = F	M = F	M = F
	Juveniles	M = F	M = F	M = F	M = F	M = F
Apodemus agrarius	Adults	**F > M**	**F > M**	**F > M**	**M > F**	M = F
	Subadults	M = F	**M > F**	M = F	**M > F**	**M > F**
	Juveniles	**F > M**	**F > M**	M = F	**F > M**	**F > M**
A. flavicollis	Adults	**M > F**	**M > F**	**M > F**	**M > F**	**M > F**
	Subadults	**M > F**	**M > F**	**M > F**	**M > F**	M = F
	Juveniles	F > M	**F > M**	**F > M**	**M > F**	M = F
A. uralensis	Adults	M = F	M = F	M = F	**M > F**	M = F
	Subadults	M = F	M = F	M = F	M = F	M = F
	Juveniles	M = F	M = F	M = F	M = F	M = F
Micromys minutus	Adults	**F > M**	M = F	M = F	M = F	F > M
	Subadults	M = F	F > M	M = F	M = F	**F > M**
	Juveniles	M = F	M = F	M > F	M = F	M = F
Clethrionomys glareolus	Adults	**F > M**	**F > M**	**F > M**	**M > F**	**F > M**
	Subadults	M = F	M = F	M = F	**M > F**	M = F
	Juveniles	M = F	M = F	M = F	M > F	**M > F**
Alexandromys oeconomus	Adults	**M > F**	**M > F**	**M > F**	**M > F**	**M > F**
	Subadults	**M > F**	**M > F**	**M > F**	**M > F**	M = F
	Juveniles	**M > F**	M > F	**M > F**	**M > F**	M = F
Microtus agrestis	Adults	M = F	**M > F**	M > F	**M > F**	M = F
	Subadults	M = F	M = F	M = F	M > F	M = F
	Juveniles	M = F	M = F	M = F	M = F	M = F
M. arvalis	Adults	**M > F**	**M > F**	**M > F**	**M > F**	**M > F**
	Subadults	F > M	**F > M**	M = F	**M > F**	**F > M**
	Juveniles	**M > F**	**M > F**	**M > F**	**M > F**	**M > F**
M. rossiaemeridionalis	Adults	M > F	M = F	M = F	M = F	M = F
	Subadults	M > F	M = F	M = F	M = F	M = F
	Juveniles	M = F	M = F	M = F	M = F	M = F

M = F—monomorphic species, $p > 0.10$ (shaded green); M > F—male-biased traits, $p < 0.10$ (no shading); F > M—female-biased traits, $p < 0.10$ (no shading); **M > F**—male-biased traits, $p < 0.05$ (light blue shading); **F > M**—female-biased traits, $p < 0.05$ (light orange shading); **M > F**—male-biased traits, $p < 0.01$ (medium blue shading); **F > M**—female-biased traits, $p < 0.01$ (medium orange shading); **M > F**—male-biased traits, $p < 0.001$ (dark blue shading); **F > M**—female-biased traits, $p < 0.001$ (dark orange shading).

3.2. Species with Male-Biased Sexual Size Dimorphism

Male-biased SSD was found in one mouse (*A. flavicollis*) and four vole species (*A. oeconomus*, *M. arvalis*, *M. agrestis*, and *M. rossiaemeridionalis*). Strong (greater than 5%) and significant male-biased SDD was characteristic of *A. oeconomus* (Figure 2) in all age groups, though not in all traits (Table 4). Significant, but of variable strength, male-biased SDD was characteristic of adult *A. flavicollis*. Ear length did not differ in other age groups of this species, and differences were less pronounced in juveniles (Table 4). Significant but of limited strength, not always reaching the 5% threshold, male-biased SSD was found in *M. arvalis*. Strong, exceeding the 5% threshold, but not significant SSD due to a smaller sample size was characteristic of *M. rossiaemeridionalis*, and male-biased SSD of limited strength and limited significance was found in *M. agrestis*. In the last three species, some parameters were female-biased, but neither strong nor significant, and only observed in immature individuals (Table 4).

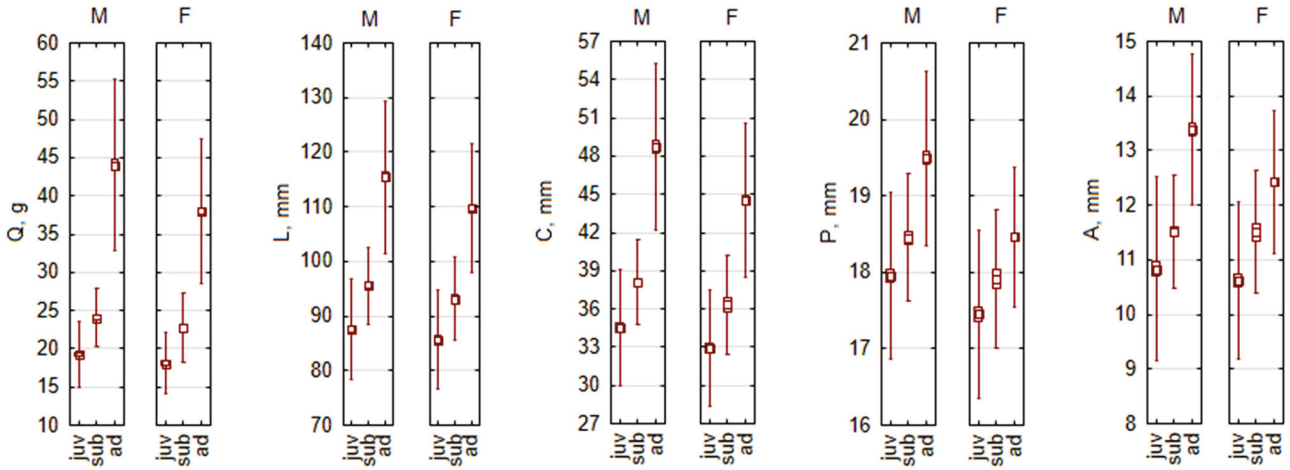

Figure 2. Male-based body size dimorphism by age groups in *Alexandromys oeconomus*, as best example of species with larger males. Abbreviations: M—males, F—females, juv—juveniles, sub—subadults, ad—adults, Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length.

Table 4. Percentage expression of sexual size dimorphism in male-biased small mammal species. Negative values indicate larger size in males, positive values indicate larger size in females. Abbreviations: Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length. Bold indicates reliable differences with $p < 0.05$.

Age Group	Parameter	*A. flavicollis*	*A. oeconomus*	*M. arvalis*	*M. rossiaemeridionalis*	*M. agrestis*
Adults	Q	**−11.2**	**−15.8**	**−5.0**	−12.2	−3.8
	L	**−3.8**	**−5.2**	**−2.6**	−3.6	−2.9
	C	**−2.2**	**−9.4**	−3.0	−3.4	−3.0
	P	**−3.4**	**−5.6**	**−3.4**	−2.1	**−2.7**
	A	**−2.9**	**−7.8**	**−2.8**	−2.1	−1.2
Subadults	Q	**−8.9**	**−5.9**	3.0	−15.5	0.1
	L	**−2.4**	**−2.6**	**1.7**	−3.6	−0.7
	C	**−1.4**	**−4.7**	1.2	5.5	1.8
	P	**−1.8**	**−3.0**	**−1.8**	−4.9	−2.3
	A	0.7	0.0	**6.3**	−7.4	−1.7
Juveniles	Q	2.8	**−6.3**	**−6.1**	−40.3	−2.9
	L	**1.5**	−2.1	**−1.2**	−21.1	0.4
	C	**1.9**	**−5.1**	**−5.1**	6.2	−3.0
	P	**−0.8**	**−2.9**	**−3.0**	−0.2	−0.2
	A	0.7	−2.0	**−3.6**	−20.5	0.8

In summary, consistent male-biased SSD in the adult age group was characteristic of all listed species and most characters. In subadults, some characters indicated female-biased SSD (Q and A in *M. arvalis*), while *A. flavicollis* and *M. rossiaemeridionalis* continued to show male-biased SSD. Juveniles of these species were the most variable in SSD. For example, *A. flavicollis* has more balanced dimorphism in juveniles, with some female-biased traits not strong, below the 5% threshold (Table 4). In Q, reversed dimorphism during postnatal development of this species was significant ($F_{2,6} = 45.9$, $p < 0.0005$).

3.3. Species with Female-Biased Sexual Size Dimorphism

Female-biased SSD was found in one vole and four mouse species, but not all of them were consistent in SSD expression across age groups and characters (Table 5). Adult individuals of the mentioned species show female-biased SSD in Q and L in all five species, in the consistently positive values. In two species, *M. minutus* and *S. betulina*, the differences in L were not significant. The highest female-biased SSD was a characteristic of adult individuals of *S. betulina*.

Table 5. Percentage expression of sexual size dimorphism in female-biased small mammal species. Positive values indicate larger size in females, negative values indicate larger size in males. Abbreviations: Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length. Bold indicates reliable differences with $p < 0.05$.

Age Group	Parameter	*A. agrarius*	*C. glareolus*	*M. minutus*	*M. musculus*	*S. betulina*
Adults	Q	**10.1**	**8.1**	**14.2**	**13.2**	17.9
	L	**1.8**	**2.2**	1.5	**4.7**	13.3
	C	**3.0**	**5.4**	1.9	1.9	−3.8
	P	**−0.9**	**−0.4**	−1.3	−1.0	4.3
	A	0.7	**1.8**	5.4	2.2	8.8
Subadults	Q	−0.5	0.1	5.4	0.1	
	L	**−1.8**	0.0	2.8	−2.3	
	C	0.0	−0.5	2.6	−1.5	
	P	**−2.0**	**−0.6**	0.4	−1.3	
	A	**−1.9**	−0.3	**10.6**	−4.1	
Juveniles	Q	**−1.7**	0.2	−2.7	2.2	27.6
	L	**−1.6**	−0.1	−1.2	2.8	3.2
	C	−0.5	−0.2	−2.5	3.2	5.5
	P	**−1.4**	−0.4	−0.1	−0.3	−4.8
	A	**−1.3**	**−1.2**	−2.9	1.9	−9.1

In non-adult animals of these species, SSD was inconsistent and less pronounced compared to adults, suggesting transitional growth patterns that vary among species. The five percent threshold was exceeded only for Q and A in *M. minutus*, and only the latter difference was significant.

The most variable SSD pattern was recorded in juveniles, with females of *S. betulina* and *M. musculus* being larger in Q, L, and C. In contrast, *A. agrarius*, *C. glareolus*, and *M. minutus* had slightly negative or neutral SSD in many parameters, suggesting that males and females are more similar in size at this stage. Thus, the juvenile age group showed the least consistent SSD among the species. In Q, reversed dimorphism during postnatal development of *A. agrarius* was significant ($F_{2,7} = 15.7$, $p < 0.005$). Considering Q in *M. minutus*, reverse dimorphism was strong but did not reach significance ($F_{2,2} = 1.8$, $p = 0.11$).

As an example of female-biased SSD, we present the distribution of morphological parameters in *A. agrarius* (Figure 3), which is present only in adult animals. A similar pattern is characteristic for adult *C. glareolus*—in both species, P was larger in males, by 0.8% and 0.4%, respectively, i.e., a small but significant difference (Table 5).

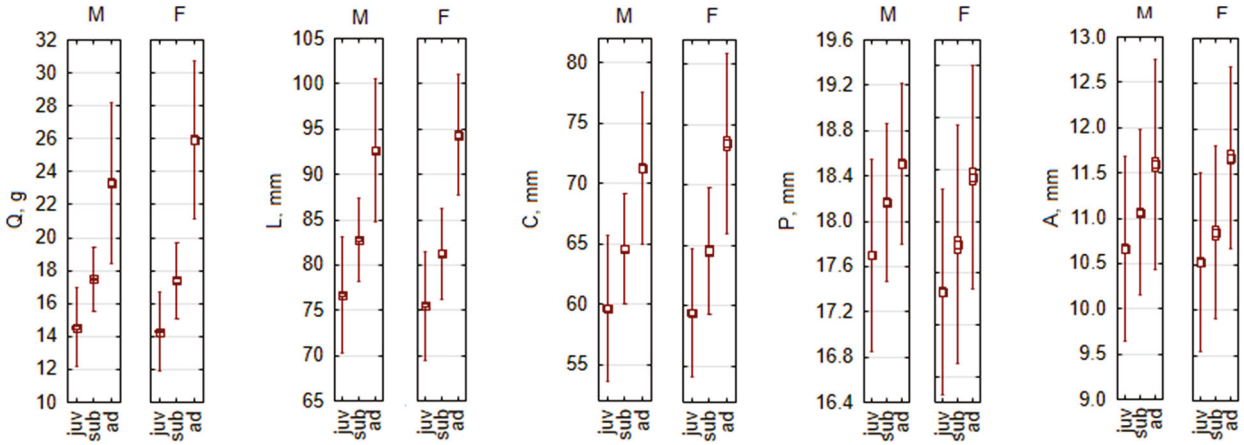

Figure 3. Female-based body size dimorphism by age groups in *Apodemus agrarius*, as an example of species with larger females. Abbreviations: M—males, F—females, juv—juveniles, sub—subadults, ad—adults, Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length.

3.4. Monomorphic Small Mammal Species

The four small mammal species, three shrews and one mouse, could be classified as monomorphic with minimal SSD for most parameters and age groups (Table 6). Some parameters, such as ear length (A) in *S. minutus*, show higher female-biased SSD values in adults and subadults, suggesting unique developmental or ecological factors at play for this species. Juvenile stages show more variability, likely reflecting growth differences that stabilize by adulthood.

Table 6. Percentage expression of sexual size dimorphism in monomorphic small mammal species. Positive values indicate larger size in females, negative values indicate larger size in males. Abbreviations: Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length. Bold indicates reliable differences with $p < 0.05$.

Age Group	Parameter	*S. araneus*	*S. minutus*	*N. fodiens*	*A. uralensis*
Adults	Q	3.1	0.9	−1.9	4.5
	L	0.3	1.3	0.0	1.4
	C	−0.5	0.9	**−6.4**	−1.3
	P	**−1.3**	0.2	−1.7	**−7.8**
	A	**13.8**	31.9	−4.9	−1.3
Subadults	Q	−0.9	3.5	0.8	−4.6
	L	0.6	2.9	1.0	−0.8
	C	0.4	−1.6	−0.6	0.8
	P	−0.3	0.4	2.3	−2.9
	A	3.2	**58.1**	−3.0	−1.7
Juveniles	Q	0.1	−10.6		−3.9
	L	1.7	0.9		−0.6
	C	−1.2	−2.0		3.3
	P	**−1.9**	−2.8		−0.4
	A	−26.5	5.7		1.1

Adult individuals generally had a low SSD, with most values close to zero. The high female-biased SSD in the ear length (A) of *S. minutus* and *S. araneus* is atypical.

Subadults and juveniles of these species showed mixed results, with generally low SSD values, consistent with their monomorphic classification. The most variable in SSD was the juvenile age group, which showed a male-biased SSD, suggesting that male juveniles in this species grow faster and therefore reach certain sizes earlier than females.

As an example of a monomorphic species with inconsistent distribution of morphometric parameters in age groups, we present *S. araneus*, with some significant differences and also some exceeding the 5% threshold (Figure 4).

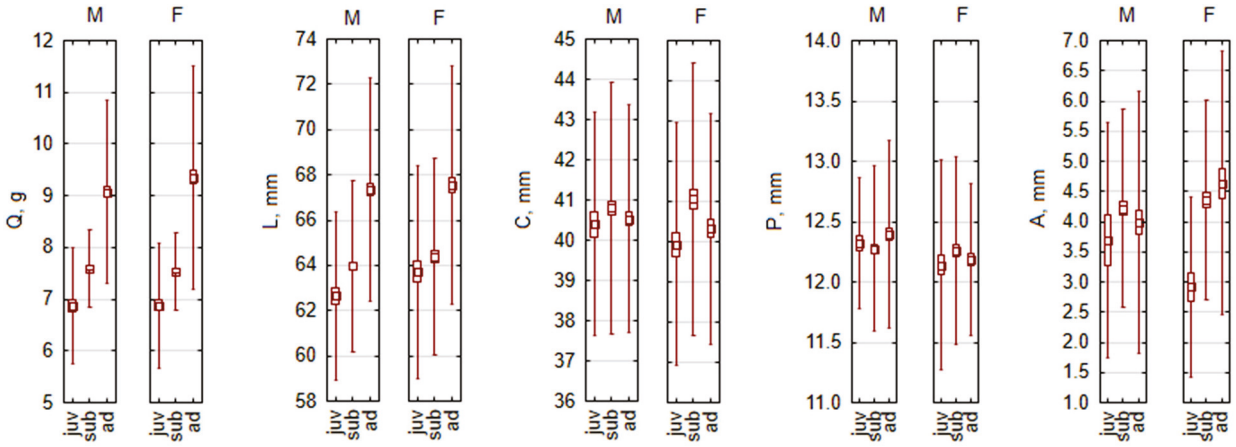

Figure 4. Sex-based body size dimorphism by age groups in *Sorex araneus*, as an example of monomorphic species. Abbreviations: M—males, F—females, juv—juveniles, sub—subadults, ad—adults, Q—body mass, L—body length, C—tail length, P—hind foot length, A—ear length.

4. Discussion

Studies of the differences in physical characteristics between males and females of the same species, referred to as SSD, are important for several reasons [48]. The results help to understand species evolution and shaping factors [49]. Sexual dimorphism is related to the mating system of species, so understanding SSD provides insights into reproductive success, mate choice, and parental investment [50]. In small mammals, particularly microtines, the importance of SSD in two morphological traits, body mass and body length, in relation to the species mating system has been discussed, with mixed results [51,52]. In North American voles, species of *Microtus* have been shown to have male-biased SSD, while species of *Clethrionomys* have largely female-biased SSD [27]. Our results confirm this (see Tables 4 and 5).

As an adaptation to different pressures, SSD may vary in different environments [53], with changes in male and female morphology along a north–south latitudinal gradient as shown for *A. uralensis* [54]. The relationship between SSD and trophic niche partitioning is well established in many different animal groups [49], with females selecting habitats and diets that differ significantly from those of males [1]. Competition for resources could lead to very rapid changes in SSD [55], although evidence for mammals is lacking. In extreme environments, such as Great Cormorant (*Phalacrocorax carbo*) breeding colony areas, the impact of avian activities affected the morphological parameters of *A. flavicollis* in less than a decade, confirming the possibility of rapid change under environmental pressure [56].

Three main factors in the evolution of SSD were identified: (i) correlation with body size, referred to as Rensch's rule, (ii) differences in dietary niche, and (iii) mating system and social organization of the species [57]. However, the scientific debate on these factors is still open. For example, small mammals, insectivores, and rodents mostly have male-biased SSDs [4], although there are examples of subterranean rodents [10], bats [58], canids [59], and other animal groups [9] that do not follow Rensch's rule.

There is a link between reproduction and SSD in small mammals, influenced by mating systems, ecological roles, reproductive investments, and environmental stressors. In flying squirrels (*Glaucomys volans*), larger females produce larger litters and have larger home ranges, allowing them to access more food resources [60]. A positive association between female body size and litter size has been found in many New and Old World voles [61], although such an association probably does not operate in shrews [62]. In Lithuania, we found positive correlations between female body mass and litter size in *A. flavicollis*, *A.*

agrarius, *M. arvalis*, *M. agrestis*, *A. oeconomus*, and *C. glareolus* [63]. However, the correlation could be obscured by the fact that most small mammals continue to grow throughout their lives [64,65].

Therefore, as noted by [5], inconsistent statements about SSD in many species are at odds with the best available raw data. Our results, while consistent with the general conclusion that SSD is rarely observed in shrews [57], show male-biased SSD in one mouse and four vole species, and female-biased SSD in one vole and four mouse species. Therefore, we made comparisons with small mammal body size data from publications to see if our results (see Tables 4–6) were compatible with other countries at similar latitudes (Table 7).

Table 7. Variation in percentage expression of sexual size dimorphism (SSD) in European small mammals. Positive values indicate larger character size in females, while negative values indicate larger character size in males. Our data show values of adult individuals. Abbreviations: Q—body mass, L—body length.

Species	Trait	SSD Expression (%) [Source]	Our Data
Sorex araneus	Q	−3.0 [15], 0.0 [24], 4.6 [15], 4.7 [21]	3.1
	L	0.0 [24]	0.3
S. minutus	Q	−15.6 [21], 0.0 [24], 0.3 [15], 10.8 [15]	0.9
	L	0.0 [24]	1.3
Neomys fodiens	Q	0.0 [24], 0.1 [15], 2.1 [15], 3.4 [21]	−0.9
	L	0.0 [24]	0.0
Sicista betulina	Q	1.2 [15]	17.9
	L		13.3
Mus musculus	Q	−19.6 [66], −18.7 [67], −11.8 [65], −9.8 [65], −9.4 [65], 4.5 [68], 7.1 [68], 24.2 [24]	13.2
	L	−3.2 [15], −1.8 [12], 3.1 [13], 5.7 [24]	4.7
Apodemus agrarius	Q	−21.0 [69], −6.7 [23], −2.4 [23], 1.5 [21], 1.8 [15], 7.2 [24]	10.1
	L	−7.4 [69], −4.0 [23], −2.4 [23], −0.8 [13], 1.2 [24]	1.8
A. flavicollis	Q	−17.7 [70], −16.6 [71], −8.6 [21], −7.0 [23], −6.6 [23], −6.2 [15], −6.2 [22], −1.5 [22], 0.0 [24]	−11.2
	L	−4.4 [71], −3.3 [12], −2.0 [23], −1.8 [23], 0.0 [24]	−3.8
A. uralensis	Q	−5.0 [23], −1.1 [54], 3.4 [21], 6.9 [15], 7.2 [21]	4.5
	L	−1.7 [54]. −1.6 [23], 1.0 [23]	1.4
Micromys minutus	Q	1.6 [15], 15.7 [13], 31.0 [24]	14.2
	L	0.2 [12], 7.8 [24]	1.5
Clethrionomys glareolus	Q	−22.7 [20], −19.5 [20], −18.3 [20], −15.0 [20], −11.7 [20], −9.6 [20], −8.6 [20], −5.4 [20], −4.8 [20], −3.7 [20], −3.1 [20], −2.7 [20], −0.9 [20], −0.7 [20], 0.0 [20], 0.8 [20], 1.6 [20], 2.9 [15], 4.4 [21], 5.5 [20], 5.8 [61], 6.9 [20], 8.3 [20], 9.1 [15], 10.0 [12], 10.0 [15], 10.0 [20], 11.2 [24], 15.6 [15], 15.7 [15]	8.1
	L	3.4 [24],	2.2
Alexandromys oeconomus	Q	−28.6 [15], −25.0 [61], −24.0 [15], −14.9 [72], −8.5 [73], 3.5 [73]	−15.8
	L	−3.1 [73], −3.5 [73], 2.3 [13]	−5.2
Microtus agrestis	Q	−29.0 [15], −22.2 [15], −16.2 [61], −15.1 [15], −15.1 [72], 0.0 [24]	−3.8
	L	0.0 [24]	−2.9
M. arvalis	Q	−17.4 [72], −2.7 [24], −0.8 [21], −0.2 [61]	−5.0
	L	−0.8 [24]	−2.6
M rossiaemeridionalis	Q		−12.2
	L		−3.6

References and comments: [13]—small sample size; [15]—different populations; [23]—adults and subadults presented separately; [24]—when stating that males and females do not differ in size and providing pooled data, we arbitrarily set the percentage to zero; [54]—pooled data from most of the range; [61]—alpine populations, three sites sampled for several years in spring and autumn, recalculated; [65]—30–35 days old individuals, geographically distant populations; [71]—male bias also for C, P and A; [73]—adults and subadults presented separately, male-biased also for C, P, and A.

Obviously, the classification in [12], where *M. agrestis* and *A. flavicollis* species were considered as male-biased SSD, *C. glareolus* as female-biased SSD, and *M. musculus* and *M. minutus* as monomorphic species, is not completely correct. As shown in Table 7, *M. minutus* had female-biased SSD and *A. flavicollis*, *M. agrestis*, and *M. arvalis* were male-biased. The remaining species (*S. araneus*, *S. minutus*, *N. fodiens*, *A. agrarius*, *A. uralensis*, *A. oeconomus*, and especially *C. glareolus*) show a wide variation from female-biased to male-biased SSD. Our results in Lithuania (see Tables 4–6) are in agreement with the data, discussion, and exclusions of other authors presented below.

In *Sorex* shrews, body mass is highly variable, while body length is poorly represented. In *S. araneus*, body mass varies from male-biased to female-biased values, with our data aligned at the higher end, but all values do not reach the 5% threshold. Body length is monomorphic in this species. Conversely, in *S. minutus*, the variation in body mass is substantial, from a male-biased to a female-biased SSD, exceeding the 10% threshold at both ends (Table 7). Our data show that this species in Lithuania was historically and currently monomorphic in body mass and length. Differences in other traits (Table 6) do not fit this picture and require additional temporal or spatial analysis. The third species, *N. fodiens*, should be considered monomorphic. Postnatal SSD changes in this shrew species are variable and require additional attention, as in our samples the proportion of individuals with unknown age or sex was high (see Tables 1 and 2). The other peculiarity of this group is the reduction in body size in winter, a phenomenon known as the Dehnel effect, which helps to balance their high metabolic demands with environmental constraints [74]. This seasonal change involves a decrease in body mass, skull size, and some internal organs, with body size typically increasing again in the spring. The other problem is that shrews do not fit Bergmann's rule, which states that larger body sizes are found in colder climates to conserve heat [75].

For *S. betulina*, our results show a strong female-biased SSD in both body mass and body length, in contrast to the monomorphic SSD suggested by [15]. The paucity of data in published sources and the comparatively small sample size in our analyses (see Table 2) should be noted. The species is not numerous in Lithuania and Poland [76], with even more fragmentation of the range in Western Europe [77]. Our data therefore contribute to the knowledge of species morphometry in general, not only for SSD.

As shown in [78,79], there is considerable variation in body size in *M. musculus*, and there does not appear to be a geographic cline in this species [24,65–68]. Sexual size dimorphism in *M. musculus* shows considerable variability across studies, ranging from a strong male bias to the strongest female bias reported in the older study from Lithuania [24]. Our data on body mass show a strong female-biased SSD, and in body length, fall within the range of previously reported values. In juveniles and subadults of this species, SSD was not expressed.

Body mass variation in *A. agrarius* shows a wide range across populations, from strongly male-biased SSD [69] to female-biased SSD, exceeding a 5% [24] or even 10% threshold (our data). Regarding body length, our results show that females are larger, in contrast to other authors [13,23,69]. Juveniles of *A. agrarius* had a male-biased SSD in body mass (−1.7%) and other measurements (see Table 5), suggesting a significant reversal of dimorphism during postnatal development.

Previous studies suggest a predominantly male-biased SSD in *A. flavicollis*, ranging from very strong [70,71] to neutral (Table 7). Our data are consistent with this male-biased trend and exceed the 10% threshold. Body length also shows a male-biased SSD, but the 5% threshold is not reached in any study. In subadult *A. flavicollis*, the male bias is less pronounced, but in juveniles of this species, body mass shows a female-biased SSD, indicating a statistically significant reversal of dimorphism at this stage. A case of reversion to female-biased SSD under exceptionally high population density was recorded in the West Tatra Mountains in Bulgaria [25].

Our data on *A. uralensis* indicate a moderate female-biased SSD, consistent with the upper range of previous findings, which ranged from −5.0% to 7.2% for body mass. The

variation in body length SSD is much smaller. During postnatal development, both Q and L change from male-biased in juveniles and subadults to female-biased in adults. Variation in SSD was also found in previous studies, with no clear conclusion as to whether males or females were larger [23,80–82]. Male-biased SSD was characteristic of higher elevation localities [54].

Compared to the extreme value published previously [24], our finding of a strong and significant female-biased SSD in body mass of *M. minutus* and less than 5% SSD in body length is consistent with the other observations (Table 7), but not with the classification of the species as monomorphic by [12]. Juveniles of *M. minutus* in Lithuania were male-biased.

The widest range of SSD is found in *C. glareolus*, from strong male bias (−22.7%) to significant female bias (15.7%). Our data (8.1%) reflect a female-biased SSD within this spectrum for body mass and an insignificant female-biased SSD for body length. In postnatal development, changes in SSD occur with maturation, as juveniles and subadults were characterized by neutral SSD values (see Table 5).

The four meadow vole species (*A. oeconomus*, *M. agrestis*, *M. arvalis*, and *M. rossiaemeridionalis*) share the following characteristics in SSD: (i) all species exhibit male-biased SSD in adults, indicating that males tend to be larger than females for all traits examined, and (ii) in all these species, SSD changes during postnatal development, with juveniles and subadults being less male-biased.

The strongest male-biased SSD is characteristic of *A. oeconomus*, especially for body mass, which exceeds 15% in adults and 5% in other age classes. For body length, our data and those of [23] contrast with the report of female-biased SSD in [13]. In *M. agrestis*, male bias in body mass and body length, which does not reach the 5% threshold, contrasts with much stronger male bias reported by other authors (see Table 7), but is more consistent with previously reported monomorphism of this species in Lithuania [24]. In *M. arvalis*, where male-biased SSDs of varying strength (from −17.4% to −0.2%) have been reported, our results are in the range of the others. Finally, there are no data to compare strong male-biased SSD in *M. rossiaemeridionalis*, which we report. Males of this species were heavier than females in all age groups, especially in juveniles (see Table 4).

There are three methodological problems in studies of SSD in small mammals related to (i) age classification, (ii) the method used, and (iii) sample size.

Classification of age categories according to body mass, body length, fur condition, reproductive condition, and capture history in *C. glareolus* and *A. flavicollis* [36] defined *C. glareolus* less than 15 g and 85 mm and *A. flavicollis* less than 100 mm as juveniles. Based on reproductive parameters and thymus involution, we found 13 adults in *C. glareolus* with body mass less than 15 g and 69 adults with body length less than 80 mm. The same is true for *A. flavicollis*—the body mass of adults in our sample started from 15 g and the body length from 71 mm. The hibernation of small mammals observed in Lithuania [83] questions whether all hibernated individuals are adults [36,84], therefore, we assumed that reproductive parameters and thymus involution are the best parameters for age determination in small mammals.

Various methods were used to quantify SSD. This can be the slope of the reduced major axis of a regression of male size on female size, as recommended in [85], or a regression and residuals [45]. However, these methods require raw data, so regressions are rarely available for retrospective samples. Using a rate such as F/M on a linear scale eliminates the problem of spurious correlation or non-normality [45], leaving only the question of threshold. The 5% threshold [27] is a practical standard that helps researchers compare SSD across studies and assess its evolutionary and ecological implications. There are publications using lower thresholds for inferring differences, starting at 1% [86] and going as high as 10% [87]. Statistical significance in trait values between males and females based on *t*-tests, ANOVA, and post hoc has also been used, e.g., in [20,22,23]. In our study, we used a combination of ANOVA, *t*-tests, and F/M ratio with a 5% threshold for differences.

The sample sizes, which in our sample were larger than those cited, should be noted: *S. betulina* 11, *N. fodiens* 39, *A. sylvaticus* 20, and *A. uralensis* 33 individuals in [15], *A. oeconomus*

8, *A. agrarius* 18, and *M. minutus* 9 individuals in [13]. Therefore, our conclusions are better based on the actual data.

We illustrate the complexity of the SSD problem by considering a group of small mammals, mice of the genus *Apodemus*. In the broader ecological context, the comparison of *Peromyscus* and *Apodemus* as ecological equivalents is hampered by a lack of information, environmental heterogeneity, and methodological inconsistencies [88]. Nevertheless, many *Apodemus* species are considered to be dimorphic with larger males. Surprisingly, this is not always the case for the largest species, the Japanese field mouse (*Apodemus speciosus*), especially in isolated island populations [89–91]. Craniometric traits in *A. agrarius*, *A. sylvaticus*, and *A. flavicollis* from Bulgaria [92,93] not only confirmed the presence of SSD in these species but also emphasized the relevance of SSD in ecological and evolutionary contexts. Sex and body mass differences have also been linked to sexually dimorphic parasitism in *A. sylvaticus*, with potential implications for health and reproductive success [94].

Therefore, data on SSD in various small mammal species are important for advancing our understanding of rodent morphology, ecology, and evolutionary processes.

5. Conclusions

Our main results are the identification and numerical evaluation of male-biased SSD in *Apodemus flavicollis* and four vole species and female-biased SSD in *Clethrionomys glareolus* and four mouse species, and the characterization of adults as the age group with the most pronounced SSD. Several species showed transitions in dimorphism patterns during postnatal development. The strongest male-biased SSD was found in *Alexandromys oeconomus* and *Apodemus flavicollis*, the largest meadow vole and mouse species in the country, respectively, suggesting that these groups are subject to Rensch's rule.

We present the evaluation of SSD in *Microtus rossiaemeridionalis* and *Sicista betulina*, the less common small mammals not analyzed by other authors. Furthermore, this study is a call for new analysis to determine whether there have been temporal changes in body size and SSD in Lithuanian small mammals during the last five decades that can be related to climate change.

Our analysis of SSDs for 14 species of small mammals, based on a large sample of long-term surveys, updated information published 35 years ago and showed that it is not advisable to rely on (especially older) SSDs from neighboring countries because of geographical or methodological differences.

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Institutional Review Board Statement: The study uses historical material on small mammal trapping and material collected for other projects. It was conducted in accordance with Lithuanian legislation (the Republic of Lithuania Law on the Welfare and Protection of Animals No. XI-2271, "Requirements for the Housing, Care and Use of Animals for Scientific and Educational Purposes", approved by Order No B1-866, 31 October 2012 of the Director of the State Food and Veterinary Service (Paragraph 4 of Article 16) and European legislation (Directive 2010/63/EU) on the protection of animals, and was approved by the Animal Welfare Committee of the Nature Research Centre, protocols No GGT-7 and GGT-8).

Informed Consent Statement: Not applicable.

Data Availability Statement: This is an ongoing research, therefore, data are available from the corresponding author upon request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Body mass variation (mean ± SD, g) by age and sex in small mammals of Lithuania.

Species	Juveniles		Subadults		Adults	
	Male	Female	Male	Female	Male	Female
Sorex araneus	6.9 ± 1.1	6.9 ± 1.2	7.6 ± 0.7	7.5 ± 0.8	9.1 ± 1.8	9.4 ± 2.2
S. minutus	2.6 ± 0.4	2.4 ± 0.5	2.9 ± 0.5	3.0 ± 0.4	3.4 ± 0.7	3.4 ± 0.9
Neomy fodiens	12.6 ± 3.7	–	14.1 ± 0.8	14.2 ± 1.5	15.8 ± 1.4	15.5 ± 1.4
Sicista betulina	5.5	7.6 ± 2.1	8.7 ± 2.4	–	8.2 ± 0.8	10.0 ± 4.9
Mus musculus	10.2 ± 2.1	10.4 ± 2.3	14.2 ± 2.5	14.2 ± 2.1	16.7 ± 3.1	19.3 ± 4.0
Apodemus agrarius	14.5 ± 2.4	14.3 ± 2.4	17.5 ± 1.9	17.4 ± 2.3	23.3 ± 4.9	26.0 ± 4.8
A. flavicollis	21.7 ± 5.9	22.3 ± 5.4	31.2 ± 5.6	28.7 ± 4.6	40.7 ± 7.5	36.6 ± 6.5
A. uralensis	13.0 ± 1.8	12.5 ± 1.6	13.6 ± 1.4	13.0 ± 2.4	17.4 ± 2.9	18.2 ± 4.7
Micromys minutus	5.8 ± 0.9	5.7 ± 0.9	6.3 ± 1.1	6.7 ± 1.2	8.9 ± 1.4	10.3 ± 1.7
Clethrionomys glareolus	15.3 ± 2.4	15.4 ± 2.1	17.7 ± 1.7	17.7 ± 1.7	21.6 ± 2.9	23.5 ± 3.7
Alexandromys oeconomus	19.3 ± 4.4	18.1 ± 4.1	24.1 ± 3.8	22.7 ± 4.5	44.0 ± 11.2	38.0 ± 9.5
Microtus arvalis	15.0 ± 2.4	14.1 ± 2.2	18.4 ± 2.2	18.9 ± 4.2	28.0 ± 6.9	26.6 ± 6.1
M. agrestis	18.8 ± 4.0	18.3 ± 3.9	22.0 ± 2.6	22.0 ± 2.8	32.3 ± 7.2	31.1 ± 6.0
M. rossiaemeridionalis	10.0 ± 5.9	7.2 ± 0.9	20.7 ± 1.6	18.3 ± 1.6	32.5 ± 8.6	27.8 ± 6.9

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Article

Applying a Method to Estimate the Breeding and Non-Breeding Population Fractions of the Globally Threatened Red-Spectacled Amazon

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Simple Summary

Information on individuals from all stages of life is crucial for advancing knowledge of the ecology, evolution, and conservation of species. However, the life cycles of many species include cryptic stages that are often overlooked in population studies, such as the non-breeding fractions in bird populations. Here, we present a simple method applicable to species that show phenotypic differences between adults and juveniles. By quantifying age proportions, total population size, and productivity, we can infer the sizes of the non-breeding and breeding fractions. We applied this approach to a threatened parrot species whose global population concentrates in a small region of Southern Brazil after breeding. Our results indicate a low proportion of juveniles (14%) and a high proportion of non-breeders (80%) within the adult population, from a global population ranging between 16,000 and 20,000 individuals. This method can be applied to long-term monitoring of this and many other species, improving our understanding of their conservation challenges and informing effective management strategies.

Abstract

Population size estimates are essential for investigating numerous aspects of the ecology, evolution, and conservation of wildlife. However, the life cycles of many species include cryptic life stages that are difficult to detect or sample, such as the non-breeding fractions typical of many bird populations. Quantifying the proportions of breeders and non-breeders is crucial to better assess their conservation status and population trends. We propose a simple method applicable to species that show phenotypic differences between adults and juveniles. By quantifying the proportion of ages, the size of the whole population, and its productivity, the size of the non-breeding and breeding fractions can be inferred. We applied this approach to the red-spectacled amazon, *Amazona pretrei*, a threatened parrot endemic to the Brazilian Atlantic Forest. The entire global population aggregates in winter in a few localities to feed on the seeds of the Parana pine *Araucaria angustifolia*, a critically endangered tree whose range has declined >97% due to massive exploitation. From a global population of ~16,000 individuals in 2015 and ~20,000 individuals in 2017, our methodology allowed us to estimate a low proportion of juveniles (14%) and a large proportion of non-breeders (80%) within the adult population, with narrow confidence intervals. These

proportions did not change between years, but the estimated number of breeding pairs increased, from ca. 1300 to 1700 pairs, in parallel with the global population increase. Our methodology, with its possible improvements to reduce uncertainty in estimates, can be applied to the long-term monitoring of this and many other species, to better understand their conservation challenges and inform effective management strategies.

Keywords: *Amazona pretrei*; *Araucaria angustifolia*; cryptic populations; extinction risk; non-breeding populations; population size; population monitoring; Psittaciformes; red-spectacled amazon

1. Introduction

Determining the size of animal populations is crucial for numerous studies on their ecology, evolution, or conservation. In fact, estimates of population sizes inform a range of decision-making processes such as the IUCN Red Listing, which in turn underpins many conservation prioritization processes [1]. Estimating population sizes is a challenge, and in the case of birds, most studies focus on breeding populations or whole population sizes. However, little attention has been given to the non-breeding fractions of wild populations, despite their increasingly recognized importance in population size and in improving our understanding of population dynamics and conservation challenges [2]. For example, breeding and non-breeding fractions of a single population may differ in the threats they face [3,4], which may be reflected in different population dynamics [5,6]. On the other hand, IUCN uses, among other criteria, estimates of the number of mature individuals as a proxy of effective population sizes (N_e) for identifying globally threatened species to be included in the Red List [7,8]. This information most often relies on very crude estimates and inter-species extrapolations, given the difficulties for obtaining the size of non-breeding populations, to the extent that these fractions have been termed "cryptic" or "ghost" populations [9,10]. Inaccuracies in estimating the breeding to non-breeding population rates [9] may lead to misleading conservation status and actions [4]. Therefore, a great effort is needed to advance our knowledge of population structures, not only through increasing monitoring efforts, but also by relying on newly proposed tools, such as population modeling [11,12] and non-invasive genetic tagging [13].

In the case of species with phenotypic differences between juveniles and adults, simple population surveys conducted after the breeding season, combined with information on population size and breeding success, may offer reliable information on the proportions of adults/juveniles and breeding/non-breeding adults, as exemplified for a restricted-ranged macaw species [4]. Populations of parrots (order Psittaciformes) should be expected to include a large fraction of non-breeders, since they usually are long-lived species with deferred sexual maturity [14]. In fact, in three parrot species studied, it was found that the fraction of the non-breeding population was greater than the breeding fraction [4,15,16]. However, much more information is needed before patterns on breeding to non-breeding population ratios can be generalized. This is especially necessary in the case of parrots, one of the most diverse groups of birds (with around 400 species), yet also among the most threatened with extinction [17,18], and for which information on their abundance and population sizes remains scarce [19].

Here, we essayed a simple method applicable to species that show phenotypic differences between adults and juveniles, to estimate age proportions and breeding to non-breeding population fractions in the red-spectacled amazon (*Amazona pretrei*). This is a globally threatened parrot, endemic to the Atlantic Forest of southern Brazil. The species is

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listed by IUCN as Vulnerable worldwide [20] and in Brazil [21], due to a large population decline and range contraction. The red-spectacled amazon is distributed over only about 24,600 km^2, and its distribution contracts even further in winter [20,22], when the species aggregates in a few localities to feed almost exclusively on the seeds of the Parana pine *Araucaria angustifolia* [23,24]. The Parana pine is listed by IUCN as critically endangered, given that its range declined by more than 97% in the last century, due to massive exploitation for timber, generating a mosaic of forest patches [25]. These parrots are the main legitimate dispersers of the seeds of the Parana pine, so the conservation of the two species is closely intertwined [26]. However, seed predation by non-native mammals reduces the potential regeneration of Parana pine forests as well as food supply for their native consumers [27]. Although the population of red-spectacled amazon has remained relatively stable in recent years [20,22], less than 1% of its year-round distribution is covered by protected areas [28]. Moreover, niche modeling predicts drastic decreases for breeding (63%) and wintering (91%) ranges by 2060 [28]. Given the species' precarious conservation status, it is important not only to estimate total population size but also to quantify its breeding and non-breeding fractions in order to better understand threats and identify appropriate management actions [4]. We demonstrate how these demographic components can be derived for the global population of the species by leveraging plumage-based age distinctions between adults and juveniles (Figure 1) in combination with long-term monitoring of population size and breeding parameters.



Figure 1. Juvenile red-spectacled amazons (indicated with red arrows) show much smaller plumage red patches in the head and wings than adults, both in flight and when they are perched. Photographs: Archive Projeto Charão.

2. Materials and Methods

2.1. Study Species and Study Area

The red-spectacled amazon is a medium-sized parrot, with adults showing large red patches in the forecrown to lores and around the eyes, as well as on the bend of the wing to carpal edge. Juveniles have a distinctive plumage, with much smaller red patches restricted

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to the forehead and the bend of the wing [29]. These age-related plumage differences are readily discerned in the field, whether birds are perched or in flight (Figure 1).

Pairs are monogamous and breed dispersedly in cavities of a variety of tree species [30]. A few months after chicks fledge, all individuals concentrate in forests of Parana pine, where they spend the winter in large flocks, feeding mostly on the large seeds of this critically endangered tree species [24]. The winter distribution of red-spectacled amazon has contracted recently [28], and for the past two decades, nearly the entire population concentrates during May-June in the small municipalities of Painel, Bocaina do Sul, and Urupema, in the Serra Catarinense region of Santa Catarina state, Brazil ([23,31], N.P. Prestes and J. Martinez unpubl. data) (black square in Figure 2). During this period, the parrots gather in a large communal roost, facilitating census and allowing annual assessments of the global population size since 1995 ([22,23,31], J. Martinez and N.P. Prestes, unpubl. data).

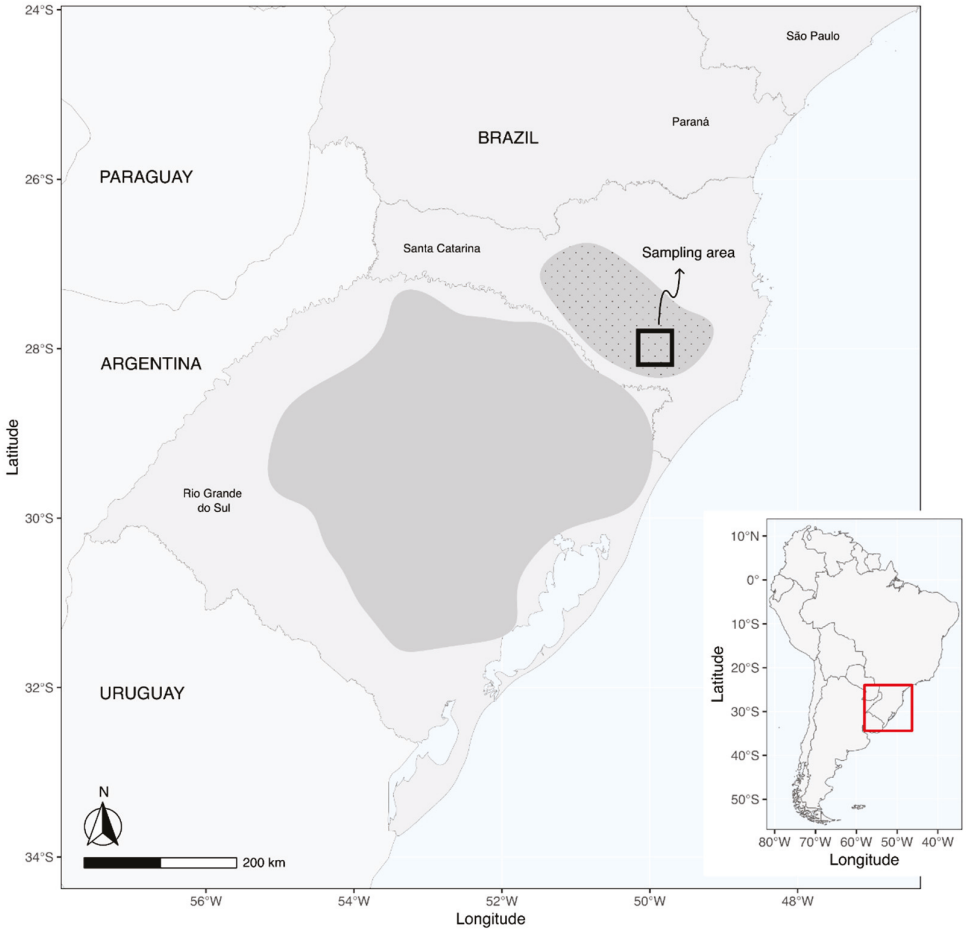

Figure 2. Global breeding (solid gray polygon) and non-breeding (wintering) range (dotted gray polygon) of the red-spectacled amazon (*Amazona pretrei*), according to the IUCN Red List [20]. The black square shows the study area, mostly covering the municipalities of Painel, Bocaina do Sul, and Urupema, where the world population concentrates during May and June in recent decades.

2.2. Field Surveys

Field work specific to this study was conducted in May 2015 and 2017 in the Serra Catarinense (Figure 2). Censuses of amazons gathering at the large communal roost were systematically carried out every year since 1995 by NPP and JM, with the help of experienced volunteers, and results were published elsewhere [22,23,31]. This is a clear example where entire parrot populations can be counted when they congregate in a few communal roosts that are reused year after year [32]. Briefly, the observers positioned

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at four fixed points, sited at North, South, East, and West advantage points surrounding the roosting site, and counted all parrots arriving at the roost with the help of binoculars. Observers recorded the arrival time, flock size, incoming flight direction (N, S, W, E), and their destination within the roosting site. Roost surveys began one hour before sunset and extended for thirty minutes afterward. At the end, the observers compared the information regarding the arrival times and flight directions of the flocks to avoid double-counting instances. These censuses were repeated for five consecutive days (between 1 and 5 May), and the maximum value obtained was taken as the global population size.

Poor light conditions (i.e., twilight) during roost censuses hindered differentiation of age-related plumage patterns. We thus relied on roadside car surveys, which maximize the probability of encountering flocks of parrots that are scarce and scattered in the landscape [33]. JM first designed, and traveled with the rest of the authors on 24 May, an itinerary of 105 km of unpaved roads that covered well the main foraging areas of the species in the Serra Catarinense. Thereafter, this itinerary was surveyed by a team (FD, VZ, FH, and JLT) by driving a car at low speed (20–40 km/h) between 25 and 28 May. These roadside surveys were conducted within a radius of approximately 50 km around the communal roost. The location of the communal roost and of these surveys covering the foraging areas is not detailed in Figure 2, as this is sensitive information whose disclosure could negatively affect the conservation of the species. Roadside parrot surveys allow for sampling large areas during a short period of time, thus reducing the probability of observing the same individuals twice [33]. The probability of resampling the same individuals also seems very low, considering that we sampled only 4–7% of the global population to obtain the age proportion (see Section 3).

We stopped the car each time we saw or heard amazons to locate them and identify the age of individuals observed. In these cases, one or two observers spotted perching birds with binoculars and a 20–50× telescope, while another observer attempted to take photographs of flying flocks with a 300–400 mm camera lens, and another recorded the data. We recorded the age of observed birds in situ, while we further identified the ages in photographs on computer screens. Following this successful experience, in 2017, we only took photos of different parrot flocks to estimate the age ratio on the same dates.

2.3. Data Analysis

We estimated productivity—defined as the average number of fledglings per breeding attempt, including failures—by monitoring 49 breeding events over 24 years (period 1994–2017) ([30,34], N.P. Prestes and J. Martínez, unpubh. Data), yielding an average of 1.63 (SD = 1.42, range = 0–4, 95% CI: 1.23–2.02) fledglings per nest. Attending to this value, we tested the statistical significance of the proportion of adults observed during the field surveys against the expected value of 1.23 adults per juvenile bird (i.e., 2 adult parents per 1.63 fledglings). We extrapolated the obtained age proportions to the whole population count to estimate the total number of juveniles and adults. We estimated the number (and proportion) of adult breeders as 2 parents × (number of juveniles/average productivity), and the number (and proportion) of breeding pairs as the number of estimated breeding adults divided by 2. We calculated the number (and proportion) of non-breeding adults as the total number of adults − number of breeding adults. We assessed the uncertainty of estimates obtained for age proportions, breeding and non-breeding fractions, and number of breeding pairs as their 95% confidence intervals (95% CI). We performed separate calculations for 2015 and 2017. We used Yates' corrected Chi-square tests for testing differences in proportions between observed and expected ages and between years.

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3. Results

In 2015, we estimated the global population of red-spectacled amazons at 15,685 individuals, of which we were able to identify the age of 1097 individuals (7% of the total) belonging to 60 different flocks, and composed mostly by adults (N = 948). The obtained proportion of adults in the population (86.42%) showed a low margin of uncertainty (95%CI: 84.39–88.44%) and differed significantly from the expected proportion ($\chi^2 = 149.36$, df = 1, $p < 0.0001$), considering the expected value of 1.23 adults per juvenile bird (i.e., 2 adult parents per 1.63 fledglings). The proportion of juveniles was 13.58%, also showing a low margin of uncertainty (95%CI = 11.56–15.61%).

Attending to proportions obtained above, 2130 of 15,685 individuals would be juveniles (95%CI: 1813–2448 individuals), and 13,555 of them would be adults (95%CI: 13,460–13,872 individuals). Considering the average productivity of 1.63 juveniles per breeding pair, and the estimated number of 2130 juveniles, there would be 1307 breeding pairs (95% CI: 1112–1502 breeding pairs), equaling to 2614 breeding adults (95%CI: 2224–3004 breeding adults) in the whole population. Therefore, the breeding fraction of the adult population would be 19.28% (95%CI: 16.03–22.69%), and the non-breeding fraction of adults would reach 80.71% (95%CI: 77.30–83.97%). These proportions remained similar when obtained by applying the extreme values of the 95%CI of the productivity recorded for the species (Table 1).

Table 1. Estimated global population size, number of individuals sampled for age determination, percentage of juveniles and adults recorded among them, and the proportion of non-breeding and breeding adults, and the number of breeding pairs estimated in 2015 and 2017. Numbers in parentheses represent the 95% confidence intervals (CI). We obtained the estimates of non-breeding and breeding fractions by applying the average, lower, and upper 95%CI values of productivity (number of fledglings/nest) recorded for the species.

Productivity	**Average = 1.63**	**Lower 95% = 1.23**	**Upper 95% = 2.02**
2015			
Global population	15,685		
Population sampled	1097		
% Juveniles	13.58 (11.56–15.61)		
% Adults	86.42 (84.39–88.44)		
% Non-breeding adults	80.71 (77.30–83.97)	74.41 (69.93–78.75)	84.45 (80.19–87.05)
% Breeding adults	19.28 (16.03–22.69)	25.58 (21.25–30.07)	15.55 (12.95–19.81)
Number of breeding pairs	1307 (1112–1502)	1732 (1474–1990)	1054 (898–1212)
2017			
Global population	20,128		
Population sampled	918		
% Juveniles	14.49 (12.21–16.76)		
% Adults	85.51 (83.24–87.79)		
% Non-breeding adults	79.21 (74.11–82.93)	72.45 (65.70–77.40)	82.23 (79.11–86.24
% Breeding adults	20.79 (17.07–25.89)	27.55 (22.60–34.30)	16.77 (13.76–22.89)
Number of breeding pairs	1789 (1508–2070)	2371 (1997–2742)	1443 (1216–1670)

In 2017, the estimated global population reached 20,128 individuals. We were able to determine the age of 918 individuals (4.56% of the total), again finding a proportion of adults (85.51%, 95%CI: 83.24–87.79%) and juveniles (14.49%, 95%CI = 12.21–16.76%) that

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differed significantly from those expected given the average productivity of the species ($\chi^2 = 119.47$, df = 1, $p < 0.0001$). These age proportions did not differ from those recorded in 2015 ($\chi^2 = 0.269$, df = 1, $p = 0.604$). Consequently, the non-breeding and breeding adult population fractions remained almost identical (Table 1), with the number of breeding pairs (1789, 95%CI: 1508–2070) increasing in parallel with the overall population size. As in 2015, the proportions of breeding and non-breeding adults remained similar when obtained by applying the extreme values of the 95%CI of the productivity recorded for the species (Table 1).

4. Discussion

Few attempts have been made to estimate the non-breeding fraction of bird populations, despite its key importance in ecology and conservation [2,12]. To our knowledge, breeding to non-breeding ratios were previously obtained for only three species of Psittaciformes. For those species, it was possible to quantify both the number of breeding pairs and the total population [4,15,16], as they nest colonially on cliffs and aggregate at predictable communal roosts, which facilitates population censuses [32]. Here, we present a simple method, applicable to species with phenotypic differences between adults and juveniles, which allowed us to infer the size of the non-breeding fraction by quantifying the proportion of ages, the size of the whole population, and its productivity. This method is especially valuable for species for which censusing breeding populations is difficult or logistically unfeasible, requiring the use of indirect methods [32,35], such as the red-spectacled amazon, whose pairs nest in a scattered manner and at low densities across large forest areas [23,30,34].

Our results show a low percentage of juveniles in the population and a large proportion of non-breeding adults. These findings are consistent with the life-history theory, since most parrots are long-lived species, with a slow breeding strategy, deferred sexual maturity, long life spans, and a relatively long period of senescence [14]. Therefore, an important fraction of the adult population is expected to be composed of non-breeding individuals, which may include immature birds with adult plumage, unpaired mature birds, and even senescent ones (i.e., infertile old individuals). The non-breeding fraction of adults obtained (ca. 80%) is similar to that obtained for the red-fronted macaw *Ara rubrogenys* [4] and Lear's macaw *Anodorhynchus leari* [15], but much higher than that obtained for the burrowing parrot *Cyanoliseus patagonus* (ca. 40–50%) [16]. Although information from wild populations is lacking, these results agree with data from captive birds, which indicate a higher age of first reproduction and longer senescence periods in macaws and amazons than in other smaller-sized, shorter-lived parrot species [14]. Future studies focused on several species covering a greater variability of life histories [14] are needed to test the hypothesis that species with longer life expectancy exhibit a higher proportion of non-breeding adults.

Besides the life history traits of each species, ecological conditions may also increase the non-breeding fraction of populations. Many parrot species are obligate secondary cavity nesters, and habitat loss may reduce breeding densities through a reduction in tree cavities and an increase in interspecific competition for nest sites [36]. The drastic reduction in forests inhabited by red-spectacled amazons in recent decades [20,25,28] may have limited the availability of nest cavities in the remaining old-growth forests and thus increased the fraction of non-breeding adults. However, this fraction could have been unchanged given that the global population of the species also apparently declined in parallel to habitat loss [20]. The addition of artificial nest boxes may serve as an experiment for testing whether nest sites constitute a limiting resource for breeding populations [36,37]. The installation of nest-boxes in six regions within the current breeding distribution of the red-spectacled amazon has shown a low percentage of occupation by this species (3.6%) [38], suggesting

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that breeding numbers are not strongly limited by nest-site availability. Similarly, the apparent increase in the number of breeding pairs between 2015 and 2017, in parallel with a global population increase that has continued in subsequent years ([22], N.P. Prestes and J. Martínez, unpubh. Data), does not suggest a strong limitation of nesting sites at present.

The reliability of population structure estimates may be strongly influenced by the strength of surveys related to the overall population size. In our case, we were able to age approximately 1000 individuals each year (i.e., 4–7% of the global population), with a low probability of pseudoreplication given our sampling design, resulting in reliable estimates of the juvenile-to-adult ratio with narrow confidence intervals. The high consistency of the age proportions obtained over two years with different population sizes generates additional confidence in the methodology used. On the other hand, the proportion of breeding versus non-breeding adults is markedly influenced by estimates of productivity (number of fledglings per breeding attempt). The relatively low number of breeding attempts we monitored may increase the uncertainty in our estimate. Ideally, robust information on breeding parameters should be obtained from the breeding season preceding the population survey; however, achieving large sample sizes within a single year is challenging for threatened species such as our study model. Nonetheless, our productivity estimates were derived from 24 years of breeding monitoring, likely capturing interannual variations, and thus may approximate the true parameter value reasonably well. However, our methodology does not account for juvenile mortality between fledgling and survey time, which may be higher than adult mortality during the same period [6]. By underestimating the proportion of juveniles, this limitation could lead to negative bias in the estimates of breeding adult fractions. Finally, roost monitoring programs can also be refined to account for the uncertainty in counts and population size estimates [39]. Improving the quality of reproductive and population parameters would further increase the robustness of age structure and breeding-to-non-breeding adult fraction estimates. Even recognizing the methodological limitations inherent in each population studied, the systematic replication of surveys over multiple years can provide reliable insights into long-term changes in the population structure of species.

5. Conclusions

Our proposed methodology can be applied to any species that exhibits age-related plumage dimorphism and for which estimates of the entire population size and breeding success are available. In this study, this method allowed us to obtain reliable estimates for the global population of a parrot species, facilitated by its restricted geographic range and the concentration of individuals in a few well-defined communal roosts. However, this approach can also be applied to local populations of other species, provided that they function as closed populations and that age classes are not spatially structured (e.g., juveniles and/or non-breeders concentrate out of the surveyed breeding population).

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Estimating an Elephant Population Size Through Local Ecological Knowledge

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Simple Summary: In a mountainous area of northwest Namibia, known as the Northern Highlands, community game guards have many years of experience in monitoring local wildlife. But until our study there had been no published research specifically on the elephants in the Northern Highlands. The area is highly remote, and the steep, rocky landscape makes traditional monitoring methods difficult for this small population of elephants, both in terms of on-the-ground tracking and aerial surveys. We identified that an alternative approach of estimating the size of the elephant population could be to gather local ecological knowledge of game guards. From this method, we estimated that there are between 78 and 212 elephants in the Northern Highlands, with a best estimate of 128. We identified how the method could be improved and conclude that it would be applicable for longer-term ecological monitoring of trends in population. This cost-effective approach would supplement other approaches such as aerial surveys that can only provide a snapshot of the population estimate once every five years, because of the high costs. The long-term monitoring would be important to inform the planning of adaptive strategies for the protection of elephants, an endangered species.

Abstract: In planning and monitoring measures to protect wildlife in an area, it is important to have a reliable baseline estimate of population size and trends. There has been minimal published information on a small population of elephants, a keystone and endangered species, in a large area west of Etosha National Park in Namibia, known locally as the Northern Highlands. It is a highly remote, mountainous area in which it is difficult to count elephants. It is semi-desert, where the protection of wildlife is at increasing risk from climate change events, and research on the elephant population is a priority. We interviewed 34 community game guards in the Northern Highlands, focusing on the number of elephants and distinguishing features in known groups. Based on the collated knowledge, and analysis to reduce double counting of known groups, we estimate that there are between 78 and 212 elephants in the Northern Highlands, with a best estimate of 128. The wide range is an indication of the current uncertainties in the method. However, we conclude that this low-cost method, if adapted based on lessons from this pilot study, would be applicable for longer-term ecological monitoring in areas that have a low population density.

Keywords: community game guards; elephants; Kunene; local ecological knowledge; Namibia; population estimates

1. Introduction

According to the IUCN African Elephant Database and the most recent IUCN African Elephant Status Report, the African savannah elephants (*Loxodonta africana*) in the Kunene Region, outside of Etosha National Park, are estimated to be about 1.5% of the Namibian elephant population, spread over a very large area of at least 41,000 km^2 [1,2]. This range includes an area sometimes referred to locally as the Northern Highlands, which is about 12,000 km^2 (Figure 1). It is a remote, mountainous terrain with steep, rocky slopes and

altitudes up to about 1800 m above sea level. Further to the west of the Northern Highlands, the landscape drops, with an escarpment towards the Skeleton Coast.

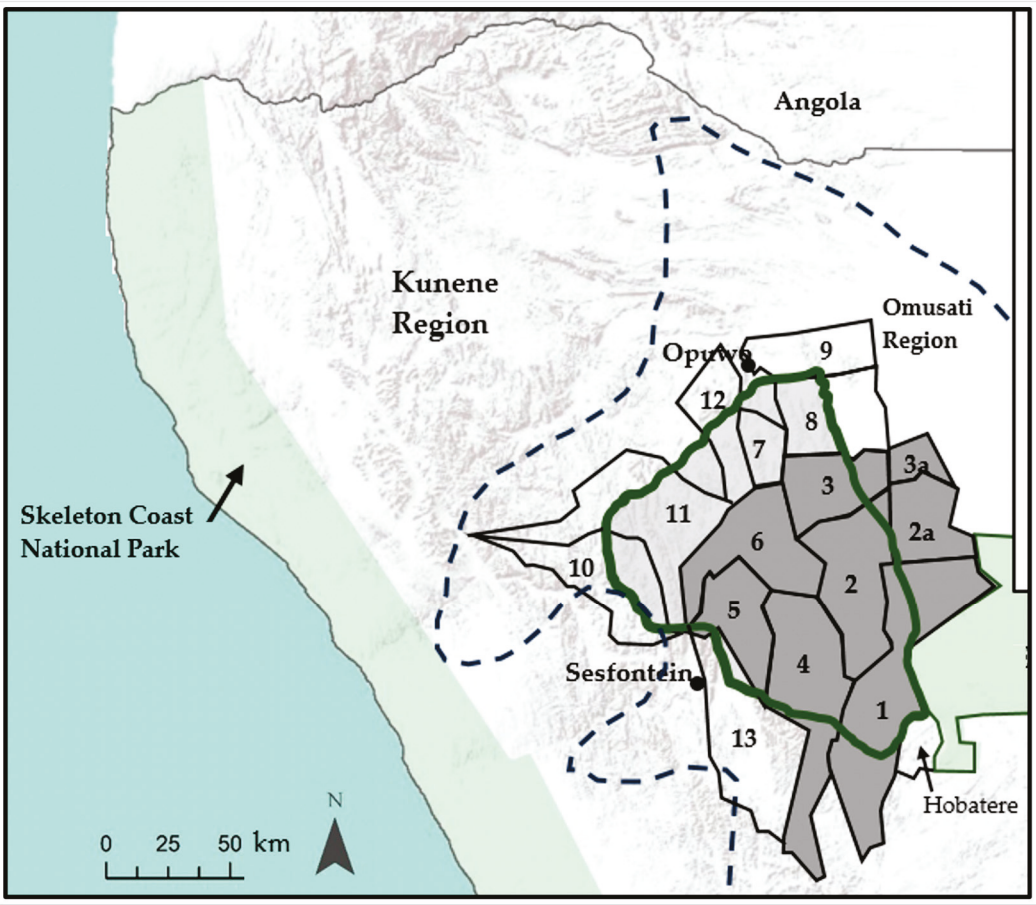

Figure 1. Conservancies in the Northern Highlands of Namibia, highlighting the main six conservancies covered in this study, as well as the elephant range. Key 1: Ehi-Rovipuka; 2: Orupupa; 2a: Additional area monitored by Orupupa; 3: Otuzemba; 3a: Additional area monitored by Otuzemba; 4: Omatendeka; 5: Ozondundu; 6: Okangundumba; 7: Otjombande; 8: Okongoro; 9: Otjindjerese; 10: Otjikondovirongo; 11: Ombujokanguindi; 12: Okatjandja Kozomenje; 13. Anabeb. The thick green line encompasses the Northern Highlands, whereas the conservancies covered in this study are coloured dark grey. According to [1] the range of elephants is within the dashed line.

The Northern Highlands is on the edge of the Namib Desert, which runs along the length of the Namibian coast. The Highlands has low rainfall (typically 50 to 200 mm per year) [3,4]. The rainy season tends to be from January to April. However, some areas receive no rainfall for several years at a time. When it occurs, rainfall tends to be highly localised and intense, in places mobilising large quantities of sediment leading to soil erosion. This process has been exacerbated by anthropogenic activities in terms of overgrazing of livestock in the area [5–7], highlighting the dynamic and complex relationship between abiotic and anthropogenic stressors (climate and human activity). Vegetation is sparser just to the west of the Highlands, and game species tend to migrate with the rain to search for fresh grasslands. While much of northwest Namibia is not fit for extensive agricultural development [7,8], livestock farming continues to be the main focus of the community for their livelihoods. Access to grazing and water resources are major priorities in terms of local livelihoods and culture. The potential risk of climate change events is likely to increase the competition between humans (and their livestock) and elephants for natural resources. It is important to have a reliable estimate of the population of elephants

as a baseline for monitoring the performance of conservation measures, and to have a cost-effective method to monitor trends in population.

Reliable estimates of elephant numbers in northwest Namibia are difficult to obtain because of the very low density of elephants and the large range covered by the species. In the Northern Highlands, the mountainous terrain and difficult access constrain monitoring activities such as foot patrols and aerial surveys [9–11]. The desert-adapted elephants to the west of the Northern Highlands, nearer the Skeleton Coast, have been extensively studied in the lower Hoanib and lower Hoarusib Rivers [12–18]. These small populations are currently well-known in terms of identification and knowledge of individual groups and some individual elephants, with researchers carrying out almost annual monitoring visits since 2006 and having the benefit of vehicles and equipment (e.g., binoculars, cameras) [17,18]. Until recently, there have been minimal research and publications on the elephants in the Northern Highlands.

Elephants in northwest Namibia often move into the villages to drink at the community water points, and to eat at community vegetable gardens, and there are challenges with human–elephant conflict in northwest Namibia [10,19–21], including in the Northern Highlands [22]. The African savannah elephant, a keystone species, is now classified as Endangered in the IUCN Red List of Threatened Species [23]. Namibia's National Elephant Conservation and Management Plan includes the strategic objectives to plan local interventions to protect the elephants and their habitats in northwest Namibia, including to reduce the incidents of human–elephant conflict [10]. The plan identifies that research is needed to obtain more reliable estimates of the number of elephants in northwest Namibia.

The estimate of the elephant population in northwest Namibia in the most recent IUCN African Elephant Status Report was 314 elephants [1]. This was based on the results of an aerial survey in 2011 of part of the northwest [24], which concluded with a range of elephants with a 95% confidence limit of 221 to 529. An aerial survey in northwest Namibia in 2016 estimated a wide range in the potential elephant population and recommended that, when planning elephant management, a precautionary approach would assume an estimate of 416 elephants in the northwest [25]. These estimates for northwest Namibia compare to the estimated population in Etosha National Park of 2911 elephants [1].

Aerial surveys have indicated that the population of elephants is likely to have been growing in northwest Namibia since the late 1990s [11,24,26]. However, the high costs of the aerial surveys mean that they have not been carried out frequently, and there were only three aerial surveys between 2003 and 2023. Also, there are high uncertainties in the estimated populations from the survey observations [10]. This is mainly because of the very low density of elephants over such a large range area, meaning that it is difficult to scale up sample counts with confidence.

The Northern Highlands makes up part of the elephant range in northwest Namibia and most of the area of the Northern Highlands is covered by community conservancies (Figure 1). The conservancy programme has been implemented by the Government of Namibia since the 1990s, then an innovative approach to adapting conservation strategies to the local situation in remote communal areas. One of the benefits of the conservancy programme is the employment of community game guards. Each conservancy tends to employ between five and ten game guards, and some of the game guards have had over 20 years of experience in their roles. They are responsible for recording wildlife sightings and incidents of human–wildlife conflict in Event Books (Figure 2a). Their role also includes raising awareness in communities on the benefits of conservation of habitats and wildlife, in line with the core objectives of the conservancy programme. The game guards have received training from the Ministry of Environment, Forestry and Tourism (MEFT) and the main NGOs involved in community-based wildlife conservation in Namibia (Namibian Association of CBNRM Support Organisations (NACSO), IRDNC Integrated Rural Development and Nature Conservation (IRDNC) and WWF Namibia). They carry out several foot patrols each week, during which they usually do not see elephants, but they will often observe evidence of elephant movements and numbers, such as through

footprints, dung and tree species that have been browsed by elephants (Figure 2b,c). They do sometimes observe the elephants when they visit community water points and vegetable gardens, although often that happens at night. The game guards therefore have much useful local ecological knowledge on the elephants in the Northern Highlands, which could inform planning of conservation measures.

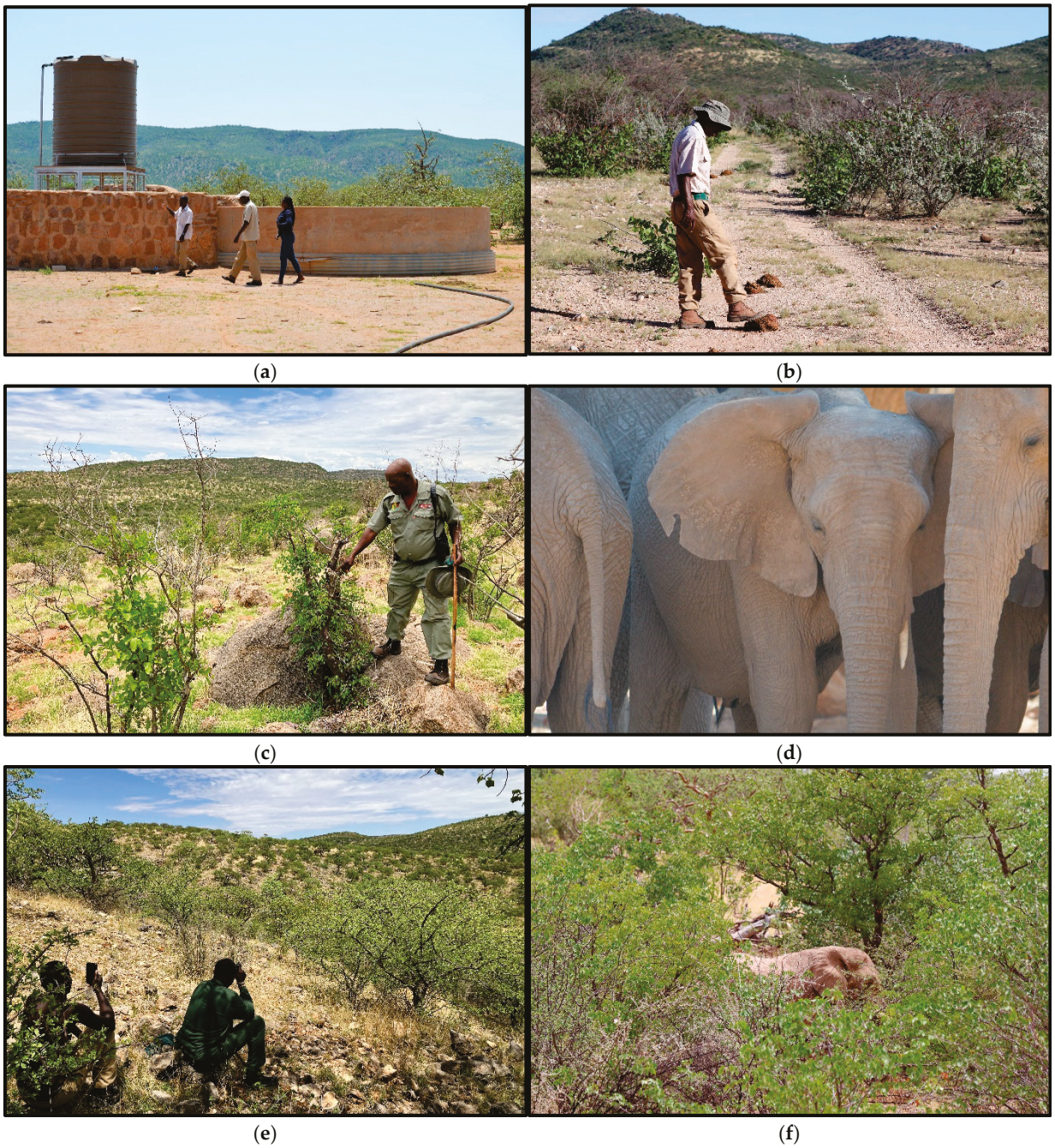

Figure 2. (**a**) Game guard meeting community members to investigate damage by African savannah elephants at a water point in Ekoto, Orupupa Conservancy; (**b**) game guard observing evidence of elephant movements on patrol; (**c**) game guard on patrol identifying a tree partly eaten by elephants as evidence of elephant movements; (**d**) an elephant in the lower Hoanib with a missing tusk and torn ear, as an example of distinguishing features that can help identify individuals or groups of elephants; (**e**) game guards observing an elephant in Ozondundu Conservancy; (**f**) elephant in Omatendeka Conservancy in the shade of trees near the main river bed, demonstrates that it is difficult to observe and count elephants.

The objective of this study was to test an alternative approach to ecological monitoring through estimating the elephant population in the Northern Highlands based on local ecological knowledge, and to assess whether such a method might be applicable in the future for monitoring population trends over the longer term. There have been some positive conclusions on the usefulness of local ecological knowledge from other studies on elephants [27,28].

2. Method

This study was carried out by a mix of researchers and practitioners. It involved a total of six months in the Northern Highlands study area, in 2021, 2022 and early 2023. The earlier scoping activities in the study area in 2021 and early 2022 were important to get to know the local situation and plan the method. Scoping activities included consultations with conservancy chairpersons and game guards for ten conservancies. Consultation was also carried out with the Ministry of Environment, Forestry and Tourism to align the research with the objectives and current priorities in Namibia's National Elephant Conservation and Management Plan [10].

The core research work involved semi-structured interviews. These were carried out over three months (December 2022 to February 2023) with 34 individual game guards in eight conservancies in the Northern Highlands: Ehi-Rovipuka (2 game guards), Orupupa (6), Otuzemba (6), Omatendeka (8), Ozondundu (6), Okangundumba (4), Otjombande (1) and Okongoro (1) (Figure 1). These conservancies were selected because our findings from the scoping activities were that the main conservancies with elephants are in the southern, central and eastern conservancies in the Northern Highlands. The game guards interviewed had between 1 and 30 years of experience, with an average of 10.3 years in their role. The game guards were selected with the agreement from conservancy chairpersons. The game guards interviewed were mainly from the highland areas in the six conservancies. The interviews included all game guards in Orupupa and Ozondundu, all in the highland area of northern Omatendeka, all game guards in Okangundumba except one game guard in the west of the conservancy where elephants are not commonly observed, the core team of game guards in Otuzemba (excluding trainee game guards that had recently been recruited) and two game guards from the more mountainous part of northern Ehi-Rovipuka.

Topics discussed during the semi-structured interviews included the elephant population, elephant movements and potential reasons for their movements, preferred browsing vegetation of elephants and other aspects of elephant behaviour. The most detailed component of the interviews with the game guards involved semi-structured discussion on known groups of elephants (game guards tend to refer to herds of elephants as "groups"). Game guards were asked about their perception of the number of elephants in a known group and any distinguishing features in the group (for example broken tusks, torn ears, etc., of the matriarch or another specific elephant in the group: Figure 2d). Other questions on each group included the main places that they have observed the group; the estimated time spent by the group in the conservancy per year; the number of years that the game guard had been aware of the specific group; and their perception of the typical movements of the group.

The aim was to develop an estimate of the elephant population in the Northern Highlands based on the knowledge of the game guards. For this exercise, we defined a "known group" as a group of elephants that is known by one individual game guard. This means that there would likely be much double counting where many of the 34 game guards interviewed would each know the same group. The questions on the size of group and distinguishing features would help to eliminate double counting by identifying when game guards know the same group. Box 1 provides the part of the semi-structured interview specifically on each known group of elephants.

Box 1. Questions on each group of elephants known by each game guard

1. Group reference number: (include name if already named by community)
2. Type of group
   - Female-led group (herd)
   - Informal group of males
   - Individual male
3. Main areas where the group/individual spends time (note if the group is mainly in another conservancy, and note which conservancy)
4. Estimated number of elephants in group (estimate by the game guard)
5. Basis for the estimate (e.g., sightings by game guard, reports from villagers, etc)
6. Distinguishing features of the group that allows it to be identified (e.g., features on matriarch or larger elephants, etc.)
7. How long have you been seeing (and able to identify) the group?
   - Less than 2 years
   - 2 to 5 years
   - More than 5 years
   - Don't know
8. Overview of movements of elephant group (distance, direction, time of year)
9. Direction of movements in/out of conservancy (if applicable).
10. Amount of time spent outside conservancy per year
11. Perception on reasons for movements of group
12. Problems with human-elephant conflict with this group / individual (High/Medium/Low)
13. Examples of damage caused by herd
14. What is your judgement of the overall uncertainty of the estimated number of elephants in this group? (A, B, C, D, E)
15. Interviewer's judgement of uncertainty. (A, B, C, D, E)

Many of the times that game guards are close to elephants are at night at village water points, and it is difficult to count the elephants (game guards do not have spotlights, and need to keep a distance for safety reasons: Figure 2e). In the daytime, sometimes elephants are difficult to count when they are spending time in the shade of the taller trees (Figure 2f). In addition, the number of elephants in a group varies, as adult male elephants tend to move in and out of the groups. Game guards know more about some groups than others, some through their own observations and some from feedback about the elephants from community members. Therefore, we developed a simple uncertainty rating and asked game guards to assign a rating to each estimate of number of elephants in each group. The uncertainty ratings for the estimates by game guards of the number of elephants in a group were defined as follows:

A. Estimate based on many sightings and observations by the game guard interviewed, verified with much feedback from other game guards and local people, with high confidence.
B. Estimate based on some sightings and observations by the game guard interviewed, verified with much feedback from other game guards, and with some confirmation by local people, with good confidence.
C. Estimate based on some sightings and observations by the game guard interviewed, some feedback from game guards and local people, with medium confidence.
D. Estimate based on a few sightings and observations by the game guard interviewed, some feedback from local people, with low confidence.
E. Estimate based on only feedback from local people, no sightings and observations by the game guard interviewed or other game guards, minimal confidence.

3. Results

Our approach for the analysis of known groups was to develop an estimate of the elephant population by identifying the likely number of elephant groups that are more permanently in the Northern Highlands and the likely size of each group.

The 34 game guards identified a total of 113 known female-led groups, plus 45 individual males or informal groups of males. Out of the 34 game guards, 16 said that they know four or more female-led groups (Figure 3), but no game guard has knowledge of more than six female-led groups. Individual game guards know 3.3 female-led groups on average.

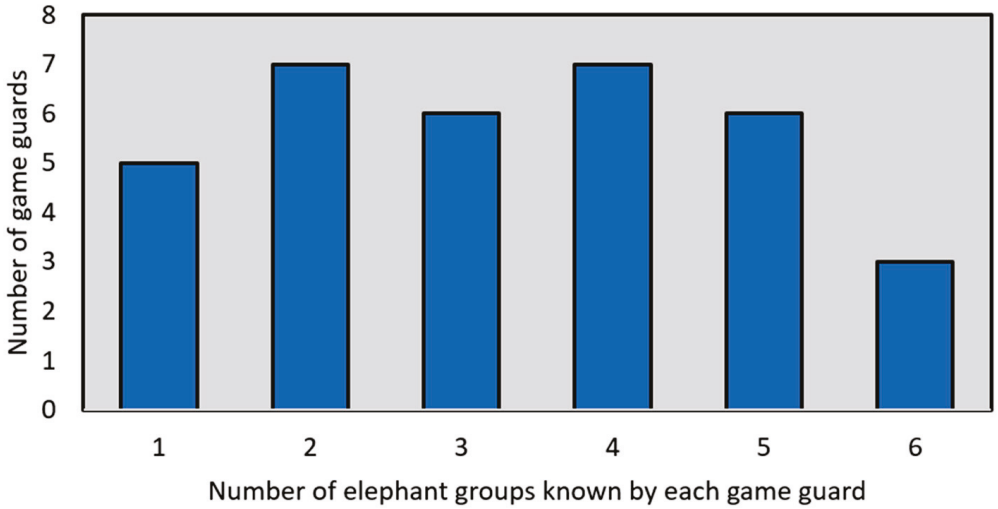

Figure 3. Number of African savannah elephants (*Loxodonta africana*) groups known by game guards in the Northern Highlands of Namibia.

Our judgement, based on interviews with the game guards and on much time spent in the study area, is that the number of large groups (e.g., of ten or more elephants) is likely to be between three and ten. The 34 game guards in total identified 99 known groups of ten or more elephants, which is an indication of the amount of double counting. Our assumption that there are three to ten larger groups is supported by the results of the aerial survey in 2016, during which four female-led herds of elephants were observed in the Northern Highlands study area [25].

For our analysis we focused on the groups that were best known by game guards. Sixty-one known groups were given a Category A rating of uncertainty by game guards (Box 1). Using the plot of these known groups in Figure 4, we divided the set of known groups of Category A into subsets of similar group size. We analysed each subset through visual inspection of the tables of data. For example, we started with a subset of known groups perceived to be containing 16 to 22 elephants. This was because three experienced game guards from Otuzemba Conservancy named a group of 19 elephants that they know (the "Topoti" group). This was the only female-led group to have been given a name, and the game guards have common knowledge of distinguishing features such as a large female elephant in this group with unusually long tusks, one elephant with a torn ear, and one with a large hole in its foot showing in footprints. Splitting the data set of known groups into subsets was useful to eliminate double counting. For example, most of the game guards that know a group of between 16 and 22 elephants only know one group around that size, and we conclude that there is likely to be just one group of about 20 elephants in the Northern Highlands.

Several game guards each know two groups of about 30 elephants (one group is likely to have a little less than 30 elephants and one group a little more (Figure 4)). The different distinguishing features noted by game guards gave some confidence that there are two groups around that size. There had also been reports from game guards on at least two occasions when about 60 elephants have been observed at a water point (e.g., Omunuandjai

in Okangundumba Conservancy and Otjitoko in Otuzemba Conservancy), implying that there are two groups of about 30 elephants, which might sometimes visit water points at the same time.

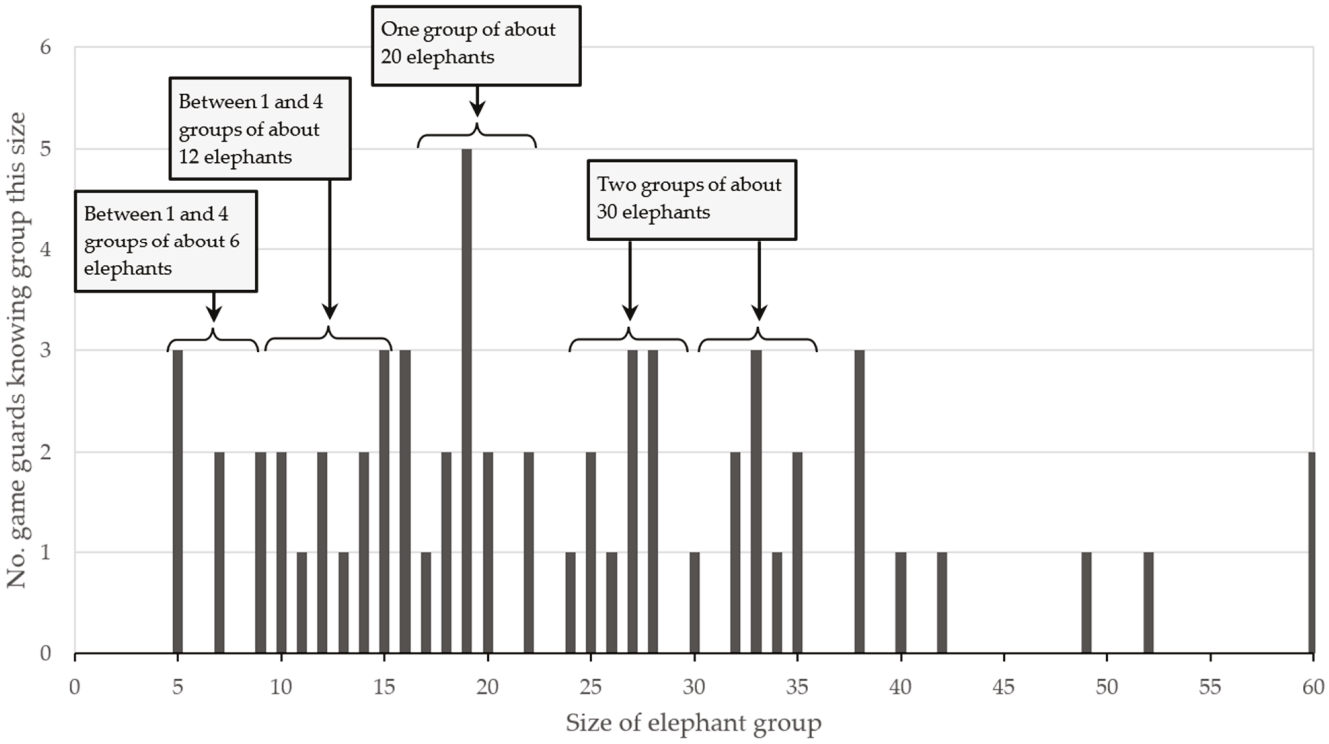

Figure 4. Number of game guards knowing groups of a specific size for the 61 known African savannah elephants (*Loxodonta africana*) groups in Namibia's Northern Highlands with Category A level of certainty.

For the elephant herds with lower group sizes, the distinguishing features noted by game guards were not sufficient to make an informed conclusion about the number of groups in the Northern Highlands. Based on the discussions with game guards over the time period in the field, we assumed that there are between one and four groups of around twelve elephants (in a range of nine to fifteen elephants), and between one and four groups of around six elephants (in a range of five to eight elephants).

Adult male elephants often associate in groups. The male elephants tend to keep more distance between them than female-led groups [29,30], and male groups are sometimes observed by game guards when they come together at water points. During the interviews, game guards described several individual male elephants and informal known groups of up to four male elephants. Based on an assessment of the information from game guards on known male elephants during the interviews, we estimate that there are between 10 and 20 adult male elephants moving as individuals or groups in the Northern Highlands. There are uncertainties in this assumption, partly because adult males join female-led groups at times.

From our analysis of the likely number of elephant groups in the Northern Highlands and the likely size of each group, we estimate is that there are between 78 and 212 elephants in the Northern Highlands, with a best estimate of 128 elephants (Table 1). In addition, there are some groups and individuals leaving Etosha National Park for short time periods but spending most of their time in the park, as described by game guards from Ehi-Rovipuka and Orupupa, the conservancies nearest to the park (Figure 1).

Table 1. Summary of analysis for estimated number of African savannah elephants (*Loxodonta africana*) in the Northern Highlands study area, based on known groups identified by game guards.

Group Type	Group Size	Range in Number of Groups of This Size	Range in Total Number of Elephants	Best Estimate of Number of Groups	Best Estimate of Number of Elephants
Female-led	30	1–2	30–60	2	60
Female-led	20	1–3	20–60	1	20
Female-led	12	1–4	12–48	2	24
Female-led	6	1–4	6–24	2	12
Individual or groups of males			10–20		12
Estimated total no. of elephants			78–212		128

4. Discussion

4.1. Importance of Knowing the Elephant Population

There are increasing stresses to the Northern Highlands habitats and landscape, with the contribution of over-grazing to erosion from high rainfall events, and longer-term droughts also from climate change, both leading to increased conflicts between humans and wildlife for local resources. A reliable estimate of the current number of elephants in the Northern Highlands, and knowledge of the trends in the population, is important as the baseline for adapting conservation strategies and planning measures to protect the elephants, reduce human–elephant conflict and improve safety for the local people. Knowledge to be able to identify individual herds (groups) of elephants, and individual males, would also be useful because common feedback from game guards is that only a proportion of groups and individual males cause incidents with human–elephant conflict. With the coverage of the mobile phone network improving in the area, early warning communication systems would be useful to inform local villages of certain elephants being in the area. Information on specific herds and individual males would also be useful to help plan GPS collaring programmes, which is part of the action plan for the northwest in the Government of Namibia's National Elephant Conservation and Management Plan.

4.2. Contributing to Knowledge on Elephant Movements

One of the interesting points identified from our analysis confirms that known groups are moving long distances all around the Northern Highlands, as concluded in earlier studies using GPS collars in northwest Namibia [31]. This is because the same known groups of about 20 and 30 elephants have been identified by game guards in different locations across several conservancies. For example, the same group of about 19 elephants has been observed in Otjitoko in Otuzemba Conservancy and Okakuyu in Ozondundu Conservancy, which are over 80 km apart on a direct route.

4.3. Estimated Population of Elephants

The interviews were carried out in late 2022 and early 2023. The estimated population of 128 elephants (range 78 to 212) can be considered to represent the situation in 2022. Although the known groups identified, on which this estimate is based, were mainly from game guards in six conservancies (highlighted in Figure 1), we consider that this estimate represents the population in the Northern Highlands area in Figure 1. For example, the game guards in Otjombande and Okongoro Conservancies indicated that they observe fewer elephants than those in Otuzemba and Orupupa, and that elephants in those conservancies visit from Otuzemba (and sometimes from Omusati Region in the east) for shorter time periods relative to the groups in the main six conservancies in the study. The indications from wider consultation during our field work were also that elephants in Otjikondovirongo and Ombujokanguindi Conservancies only visit from the southwest for short time periods. Therefore, we consider that including elephants known in those other conservancies would likely be double counting and these elephants are already covered in the above estimate.

4.4. Uncertainties in the Estimates of Elephant Populations

There are several uncertainties with the method used to estimate the population from local ecological knowledge on known groups. Based on the interviews and the time spent in the study area, we conclude that there are between three and ten large groups of elephants in the Northern Highlands (e.g., of ten or more elephants in a group). However, the uncertainties relate more to the number of elephants in each group. The game guards tend mostly to see elephants at night, when they are difficult to count. Even in the day, counting of larger groups is difficult because of the vegetation coverage and the fact that game guards need to keep a distance for safety reasons. The number of elephants in a group also often changes, with adult male elephants moving in and out of groups at different times. In particular, the game guards do not have binoculars and spotlights.

The wide range in our estimate from known groups, of 78 to 212 elephants (best estimate 128 elephants), demonstrates the current uncertainties in the method. However, this compares to an estimate of 84 to 325 elephants (best estimate 115 elephants) in the Northern Highlands based on the average elephant density from the reports of two aerial surveys of northwest Namibia [25,26]. It is understood that an aerial survey has been carried out in early 2024. When the results of that survey are available, it would be interesting to compare the estimated elephant population with the findings from this study.

4.5. Improving the Method

As would be expected when testing a method, our experience during this study is that the interview questions on groups could be improved, with more precise and detailed questions on distinguishing features. Despite the uncertainties, the method provides low-cost estimates that are based on many years of experience of game guards, compared to high-cost aerial surveys that provide a snapshot estimate once every few years (and there is uncertainty in the estimates from aerial surveys because the elephant density is low). Overall, we conclude that the method could be applied with confidence for long-term ecological monitoring if the game guards were implementing a structured approach over time, using binoculars, spotlights and cameras (with zoom lenses) so that they could more easily identify distinguishing features. Game guards would need to be provided with training. An established NGO, Elephant-Human Relations Aid (EHRA), carries out elephant tracking for the small population of desert-adapted elephants in the Ugab River catchment, over 200 km to the south of the highlands. EHRA has developed an elephant identification protocol [32], which includes a structured approach to identify groups and individual elephants. EHRA trains community game guards in the Ugab area, including in elephant identification methods [33]. Although there are more elephants and more game guards over a much larger area in the Northern Highlands than the Ugab catchment, there is an opportunity for the EHRA training programme to be rolled out in the Northern Highlands, subject to funding, to set up a more structured approach over time, implemented by the community game guards using the EHRA protocol. Improved estimates of the elephant population in the Northern Highlands, from a revised and more structured method that takes into account the lessons from this study and includes statistical analysis, would contribute to planning adaptive measures to protect the elephants, including to reduce human–elephant conflict, at this time when climate change is putting increasing stresses on natural resources in the semi-desert Northern Highlands.

4.6. Combining Local Ecological Knowledge with Developing Monitoring Technology

The collation of local ecological knowledge is a growing trend in monitoring species, from specific studies, for example, in Cameroon and Botswana that included local knowledge on elephants [27,28], to wider programmes of citizen science, many of which take advantage of web-based technology on smart phones. The future implementation of approaches discussed above for the game guards in Namibia should keep track of the potential to combine with more modern monitoring techniques that are being researched and developed using technology. Camera traps would also be useful for game guards, but a

limitation is the low number of elephants over a large area in the Northern Highlands. The elephants move around to different water points, and their visits to water points that would have camera traps would not be frequent, making identification from the photographs difficult. Monitoring technology using AI has the potential to improve the accuracy and efficiency of studies on elephant populations [34]. AI recognition software attached to camera traps has been tested for elephant monitoring [35,36], but in areas with low elephant population density, such as the Northern Highlands, there would unlikely be a sufficient number of images taken for the technology to identify elephants. Elephants also tend to break camera traps. Satellite technology for counting elephants also has applicability [37], but the low density of elephants in the Northern Highlands, as well as the tree cover along the riverbeds, provide constraints to this approach.

5. Conclusions

In planning and monitoring measures to protect wildlife in an area, especially those areas that are remote and least accessible, it is important to have a reliable baseline estimate of population size and trends. Until we initiated our study, there had been minimal published information on the population size, let alone population size changes, of the African savannah elephants in Namibia's Northern Highlands. The elephants here are a keystone species and form a distinct population of this globally threatened species. Given the aridity of the area, future climate change may disproportionately affect this population, and reliable estimates of current and future population sizes are essential for effective management. The involvement of personnel engaged in conservation is important in testing new methods for monitoring species. We identified that using local ecological knowledge from game guards is an effective method initially to obtain a first estimate of population sizes of these elephants, and secondly that the method could be improved and adapted so that it would be applicable for longer-term ecological monitoring of trends in the population. This cost-effective approach would supplement other approaches such as aerial surveys that cannot be frequently repeated, and can only provide a snapshot of the population, because of the high costs. The long-term monitoring would be important to inform the planning of adaptive strategies for protection of the elephants, as well as other species living at low population densities over vast areas.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data table of results can be obtained by emailing the corresponding author. Due to sensitivity of the data, the exact locations of the elephants will not be revealed.

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Spatial Distribution Patterns, Environmental Drivers, and Hotspot Dynamics of the European Rabbit on a Mediterranean Island: Implications for Conservation and Management

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Simple Summary: The European rabbit is regarded as an agricultural pest on the island of Lemnos, Greece. Over the past three decades, its population on the island has rapidly expanded, resulting in significant ecological disruptions and economic losses. To gain a clearer understanding of its distribution and habitat preferences, we conducted field surveys across the island and utilized spatial analysis techniques to identify high-density areas (hotspots) and the environmental factors driving its presence. Our findings revealed that rabbits are highly concentrated in fertile lowland plains, particularly in areas with cultivated and abandoned fields. Notably, more than half of the high-density areas overlapped with conservation zones, and habitat preferences were strongly influenced by factors that support burrow construction, provide shelter, and ensure abundant food resources. Spatial modeling further revealed that habitat conditions in one area significantly affect rabbit densities in neighboring regions, emphasizing the interconnected nature of their populations. Our findings provide essential information to guide targeted management strategies that may balance agricultural needs with conservation priorities in insular environments.

Abstract: The European rabbit (*Oryctolagus cuniculus*) presents a significant conservation and management challenge in Greece. While it has been listed in national biodiversity assessments, its population dynamics on the island of Lemnos demonstrate the characteristics of a highly adaptable and rapidly expanding species, exerting substantial ecological and economic impacts. Addressing this issue requires a spatially explicit understanding of its distribution patterns and habitat preferences, particularly given its extensive population growth over the past three decades. To this end, we conducted 40 field surveys across the island, documenting 1534 presence records of the species. We applied Kernel Density Estimation, Getis-Ord Gi *, and Anselin Local Moran's I to identify the spatial distribution patterns and significant hotspots. A spatial lag model was used to quantify hotspot intensity and clustering dynamics, while abiotic, biotic, and anthropogenic factors were analyzed to assess habitat associations. Our results revealed that rabbit hotspots are predominantly

concentrated in fertile lowland agroecosystems, with nearly 60% of high-density areas overlapping conservation zones. Soil and field conditions, grazing-supporting landscapes, and arable and subsidized agricultural areas emerged as significant predictors of *O. cuniculus* presence. The observed spatial dependencies indicated that while hotspot intensities and clustering dynamics are influenced by the conditions in neighboring areas, habitat characteristics remain fundamental in shaping their distribution, highlighting the broader landscape-scale spatial patterns affecting rabbit populations. These findings underscore the necessity of adopting spatially informed management strategies that mitigate agricultural impacts while accounting for interconnected spatial dynamics, providing a foundation for informed decision-making to manage rabbit populations while balancing conservation and agricultural priorities.

Keywords: *Oryctolagus cuniculus*; spatial distribution; spatial clustering; hotspots; spatial lag models; Lemnos Island; Greece

1. Introduction

Species introductions beyond their native ranges, either through intentional or accidental means, have emerged as a significant catalyst for global biodiversity loss and ecosystem transformation in recent years [1,2], with particularly pronounced impacts observed in fragile ecosystems [3,4]. Among these, islands stand out as being exceptionally vulnerable to the effects of introduced species [5–9] due to their geographic isolation, evolutionary uniqueness, and ecological fragility [10,11]. This inherent vulnerability is further magnified by several interrelated ecological factors: high levels of species endemism [12], low species richness [13], reduced complexity in trophic relationships [14], scarcity or absence of predators [15], and the prevalence of native species [16,17], all of which collectively heighten the susceptibility of island ecosystems to severe disruptions, such as predation, competition, and herbivory, by introduced species [18–21].

On islands, the introduction of mammalian terrestrial predators such as red foxes (*Vulpes vulpes*) [22], black rats (*Rattus rattus*) [23], brown rats (*Rattus norvegicus*) [23], and domestic cats (*Felis catus*) [24,25] has been particularly detrimental, leading to ecosystem disturbances, significant declines in native species populations [26], and, in some cases, localized extinctions [27]. Compounding these predatory pressures, introduced herbivores such as feral goats (*Capra hircus*) [28], cattle (*Bos taurus*) [29], deer (Cervidae) [30], and European rabbits (*Oryctolagus cuniculus*) are recognized as some of the most ecologically disruptive taxa [31] due to their generalist feeding habits and the ability to thrive in diverse environmental conditions, which allow them to rapidly colonize and dominate island ecosystems [32]. As a result, many islands have been pushed beyond ecological tipping points, where natural recovery becomes exceedingly difficult, if not impossible [19].

Among the human-mediated introduced species, the European rabbit (*O. cuniculus*) stands out as one of the most widespread and ecologically disruptive herbivores in island ecosystems [31]. Native to the Iberian Peninsula, *O. cuniculus* has been deliberately introduced to over 800 islands worldwide [33], primarily for hunting, meat production, and the fur trade [34,35]. Its ability to establish populations in non-native environments [34] is largely attributed to a suite of biological traits, including a generalist diet, high grazing efficiency, reproductive potential, and the ability to construct burrows, which provide shelter and create microhabitats that facilitate survival in challenging conditions [36,37].

As selective herbivores, rabbits exert significant pressure on island ecosystems, primarily through the overgrazing and selective browsing of plant species [21,38–40] suppressing native vegetation regeneration and altering plant community dynamics [31,41]. This selective feeding behavior frequently promotes the dominance of less palatable or invasive plant species, further destabilizing native plant assemblages and disrupting ecological balance on island ecosystems [21,42]. Beyond such direct impacts on vegetation, rabbits also serve as ecosystem engineers, reshaping soil composition, nutrient cycling, and hydrological regimes [43,44]. Such alterations often trigger cascading ecological effects, disrupting habitat structures and resource availability for species dependent on intact vegetation for food, shelter, or reproduction [41]. Additionally, rabbits also impose significant economic burdens on agriculture, causing extensive crop damage and necessitating costly pest control measures [45–48].

This combination of biological adaptability and ecological impact has positioned the European rabbit as one of the most paradoxical species in conservation biology [34]. While globally recognized as Endangered on the IUCN Red List due to severe population declines in its native range [49], the species simultaneously ranks among the top 100 worst invasive species worldwide [50]. This dual status reflects its contrasting ecological roles; a keystone species in its native range [51,52] and an invasive herbivore causing substantial ecological and economic damage to the island ecosystems it invades.

In Greece, *O. cuniculus* presents a compelling example of an introduced species that has become ecologically established, particularly on the Aegean and Ionian islands where it was likely introduced centuries ago by Phoenician traders [33]. The species has now been included in national biodiversity assessments [53], yet its population dynamics on Lemnos Island have followed a different trajectory, with its numbers surging dramatically over the last three decades [46,54], resulting in severe ecological and economic impacts and leading the Greek authorities to categorize the species as an agricultural pest [46,55].

While this complexity underscores the challenges inherent in balancing conservation priorities with agricultural interests and highlights the complexity of managing *O. cuniculus* on Lemnos, effective decision-making necessitates a contemporary, spatially explicit understanding of the species' distribution. Previous efforts to model the species distribution on the island in 2008 [46] offered valuable baseline data, but the continuous assessment and monitoring of its populations through time are necessary. Over the past 16 years, significant changes in the socioecological [56], and climatic conditions [57] have likely reshaped the species' distribution patterns, habitat preferences, and population densities. These dynamic shifts, compounded by the absence of current fine-scale spatial data, including the identification of the species' hotspots and localized areas of concern, challenge our ability to formulate targeted management strategies that effectively balance conservation priorities with the imperative of mitigating agricultural impacts. Therefore, the identification of the species' spatial patterns is vital for determining the regions that require urgent intervention, whether to mitigate crop damage, prevent habitat degradation, or protect native species. Without such information, conservation measures risk being overly generalized, potentially overlooking localized impacts or misallocating resources, thus diminishing their effectiveness.

To address these challenges, our study focuses on Lemnos Island, Greece, aiming to (a) identify the key abiotic, biotic, and anthropogenic factors influencing the presence of *O. cuniculus*; (b) analyze its spatial distribution patterns to detect emerging hotspots, clusters, and spatial outliers, thereby providing essential information to guide targeted conservation strategies aimed at mitigating the impacts of the species on vulnerable habitats and native

species; and (c) determine the underlying drivers of these observed spatial patterns, with a specific focus on the intensity of hotspots and the dynamics of spatial clustering.

2. Materials and Methods

2.1. Study Area

Lemnos Island, located in the northeastern Aegean Sea, is the eighth largest island of Greece, covering an area of 476.6 km^2 (Figure 1). Positioned approximately 30 km from the nearest island and 60 km from the continental coast, Lemnos exhibits a pronounced insular character due to its geographic isolation. Unlike most Aegean islands, which are typically characterized by rugged coastlines and steep mountainous terrain, Lemnos features a predominantly low-relief landscape, with its highest point reaching 430 m a.s.l. [58]. The western and northern regions are dominated by phrygana and short grasslands, which are often subject to intensive sheep and goat grazing [59]. In contrast, the central and eastern plains are fertile and support extensive agricultural mosaics, including vineyards, cereal fields, and livestock pastures [60].

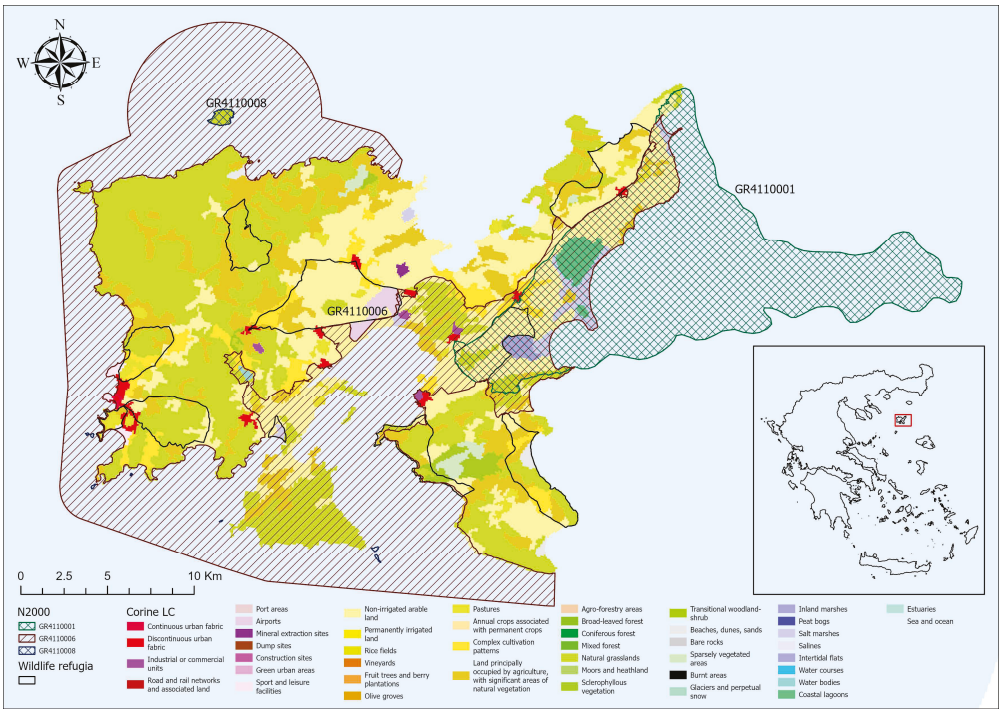

Figure 1. Distribution map of the main land habitats across the island of Lemnos. Key geographical features, including Natura 2000 sites and wildlife refugia, are highlighted.

The island also hosts semi-natural habitats with high ecological value, such as wetlands, lagoons, sand dunes, remnants of oak forests, and scrublands, which collectively enhance the biodiversity richness [58,61]. Approximately 26.5% of Lemnos' terrestrial area (126.6 km^2) is included in the Natura 2000 network (sites GR4110001, GR4110006, and GR4110008) [62], encompassing a range of critical habitats such as coastal marshes, brackish lakes, seasonal wetlands, and sand dune systems [63]. Additionally, 11 designated wildlife refugia, covering approximately 109 km^2, contribute further to habitat conservation. While some of these wildlife refugia overlap partially with Natura 2000 sites (Figure 1), they collectively form a conservation network essential for preserving the island's unique ecological features.

The climate of Lemnos is classified as meso-Mediterranean, characterized by hot, dry summers and mild, wet winters, with an average annual temperature of 16.1 °C and an average annual precipitation of 502.7 mm/year [64].

2.2. Field Surveys

To assess the spatial distribution of *O. cuniculus*, we employed a randomized grid-based approach to ensure comprehensive and unbiased coverage of the island's landscape. Using ArcGIS 10.7 software (ESRI Inc., Redlands, CA, USA), we divided the island into 165 grid cells (2 × 2 km), with most measuring 4 km^2, while some were smaller due to adjustments being required to align with the island's irregular geographical boundaries.

We conducted a total of 40 field surveys over a 10-week period from mid-June to late August 2023, covering the entire island. This period was strategically chosen based on previous studies [46] to coincide with post-harvest conditions, which improved visibility and access to agricultural fields. Additionally, this period followed the breeding season and preceded the hunting season, reducing anthropogenic disturbances and maximizing the likelihood of detecting the species. Each cell was visited once, and the order of visitation was randomized to minimize biases associated with habitat type, terrain accessibility, or temporal variation in the species' activity. To achieve this, each grid cell was assigned a unique identifier, and the visitation sequence was pre-generated in a random manner before fieldwork commenced. The randomization process was executed by generating a shuffled list of grid cell identifiers, ensuring that each grid cell had an equal probability of selection.

Randomization was also applied across days and survey routes, ensuring an even distribution of survey effort by avoiding the over-representation of specific habitats. Survey days were randomly assigned by selecting grid cells from the pre-generated list without imposing any spatial constraints, meaning that adjacent grid cells could be visited sequentially if selected by chance. Additionally, the survey routes were determined each day by randomly selecting an access point and a travel direction within each grid cell to further minimize observer biases.

The surveys were conducted four days per week during the early morning (thirty minutes before sunrise to approximately four hours after sunrise) and the late afternoon to early night (two hours before sunset to three hours after sunset). These time windows align with the crepuscular–nocturnal activity patterns of *O. cuniculus* [65–67], when its movement peaks and environmental conditions are most favorable for visual detection [68]. Midday surveys, when high temperatures typically suppress the species' activity, as well as days with adverse weather conditions (e.g., heavy rain, strong winds) were avoided to maintain observation reliability.

We employed a combination of vehicle-based and on-foot surveys to adapt to varying habitat types, terrain accessibility, and visibility conditions across grid cells. Vehicle-based surveys were conducted along accessible primary and secondary road networks at a controlled speed of 10–20 km/h. During these surveys, a team of two researchers used binoculars for daytime observations and spotlights at night. In contrast, on-foot surveys were conducted in areas inaccessible by vehicles, following natural trails while scanning the surrounding habitat for the species' presence. Observer interference was minimized by adhering to standardized survey protocols, including maintaining a consistent low survey speed, reducing noise levels during on-foot surveys, and using unobtrusive observation methods (binoculars and spotlights) to reduce direct human influence on rabbit behavior.

Throughout all surveys, only direct sightings of *O. cuniculus* were recorded to ensure methodological consistency and avoid potential biases associated with indirect signs such as tracks, droppings, or burrows. Each observation was georeferenced using handheld GPS

devices and accompanied by metadata, including time of observation, habitat type, and weather conditions.

2.3. Abiotic, Biotic, and Anthropogenic Metrics

To identify the key abiotic, biotic, and anthropogenic factors influencing the presence of *O. cuniculus* on the island, we extracted and analyzed a set of 31 spatial variables using ArcGIS 10.7 (ESRI Inc., Redlands, CA, USA). For this, we created circular plots with a radius of 50 m centered on each recorded presence point, representing an ecologically relevant spatial scale based on the average home range estimates from previous studies [68–70]. Within each 50 m circular plot, we initially extracted key abiotic metrics which are known to influence burrow construction, predator avoidance, and thermoregulation [71]. These included elevation (minimum, maximum, mean) and slope (minimum, maximum, mean) which were derived from a 10 × 10 m Digital Elevation Model (DEM) provided by the Biodiversity Conservation Laboratory, University of the Aegean. Additionally, we included soil depth, an essential factor affecting burrow stability and vegetation productivity, which was sourced from the ISRIC SoilGrids dataset with a 250 × 250 m spatial resolution [72].

Thereafter, we extracted key biotic metrics to characterize vegetation structure, productivity, and land cover composition, all of which are essential determinants of the *O. cuniculus* habitat preferences. Vegetation productivity (mean, minimum, maximum) was assessed for the months corresponding to the survey period using high-resolution total productivity data from the Copernicus Vegetation Productivity Dataset [73]. To capture the fine-scale vegetation structure, we included grasslands and small woody feature cover, derived from Copernicus High-Resolution Layers [74] at a 10 × 10 m resolution. Grasslands are known to be a primary foraging habitat for *O. cuniculus*, while small woody features provide shelter and aid in predator avoidance [34]. Additionally, non-tree areas, derived from the Copernicus Forest Type Dataset [75], were included to represent open habitats favored by rabbit populations due to the improved visibility and reduced predation risk [44]. Land-use composition was quantified using datasets from CORINE Land Cover [76] and the Greek Payment Authority of Common Agricultural Policy Aid Schemes (OPEKEPE) [77]. From these datasets, we distinguished two key variables: the total agricultural cover derived from the CLC dataset, which reflects broad European land-use classifications, and the agricultural land cover from the OPEKEPE dataset, which provides more localized and detailed information on actively managed agricultural areas in Greece. We also extracted the percentage cover for arable land, pastures, vineyards, and olive groves, and we calculated the extent of both agricultural land meeting the European Union's sustainability standards (good agricultural and environmental conditions—GAEC), and agricultural landscapes receiving support measures through state-backed programs (state-supported agricultural areas).

To evaluate the potential influence of human activities on the species' habitat preferences, we extracted and calculated several anthropogenic factors, including proximity to roads and settlements. Furthermore, to incorporate the potential regulatory and protective influence of conservation areas, we also included the proximity to Natura 2000 sites and to designated wildlife refugia as key metrics. In addition, we recorded whether each circular plot overlapped with the boundaries of Natura 2000 sites (GR4110001 and GR4110006) or wildlife refugia, and we calculated the percentage of each circular plot falling within these conservation areas. These datasets were sourced from the open platform of geospatial data and services for Greece (geodata.gov.gr (accessed on 15 November 2024)).

2.4. Spatial Analysis

To investigate the spatial distribution patterns of *O. cuniculus* and to explore both large-scale patterns and localized anomalies, we applied a suite of spatial techniques designed to identify density trends, emerging hotspots, spatial clusters, and potential outliers. We initially used the Kernel Density Estimation (KDE), a non-parametric statistical technique widely used for estimating the probability density function of spatial point patterns [78,79], to estimate the underlying probability density of the species across the island. We employed a kernel function that weighted the surrounding points based on their proximity to confirmed records to account for spatial clustering and ensure unbiased density estimates. Moreover, the bandwidth was set at 50 m based on ecological considerations to represent the average home range radius of *O. cuniculus* [68–70].

Next, in order to detect regions of clustering, hotspots, and spatial outliers, we applied two complementary spatial statistical techniques: Getis-Ord Gi * and Anselin Local Moran's I. The term "hotspot" has been widely used across disciplines to describe areas exhibiting relatively higher values compared to their surroundings [80–84]. Here, we define hotspots as locations demonstrating statistically significant clustering (local autocorrelation) in the spatial distribution of *O. cuniculus* presence records.

We applied the Getis-Ord Gi * statistic [85,86] to identify regions where the observed spatial clustering deviates significantly from a random spatial distribution, determining whether high or low species presence records are spatially concentrated beyond what would be expected by chance. We used a fixed distance band of 50 m for calculating neighborhood statistics; this distance band resulted in high z-score values as an indication of the clustering patterns in the presence data. This analysis generates z-scores (GiZscore) and *p*-values, which are essential for interpreting the statistical significance and direction of clustering [87]. Positive z-scores with significant *p*-values represent hotspots (intense clustering of high values), indicating areas where rabbit presence is significantly higher than expected, while negative z-scores with significant *p*-values represent coldspots (intense clustering of low values), reflecting areas where rabbit presence is significantly lower than expected.

To complement the hotspot analysis and examine the localized spatial relationships, we applied Anselin Local Moran's I statistic [86]. This method identifies spatial clusters of similar values (High–High or Low–Low) as well as spatial outliers (High–Low or Low–High) through the calculation of Local Moran's I z-scores (LMiZscores), providing a finer scale perspective on the spatial arrangement of *O. cuniculus* presence records.

2.5. Statistical Analysis

To enable a robust comparison between locations with and without *O. cuniculus*, we randomly generated pseudo-absences using ArcGIS 10.7 (ESRI Inc., Redlands, CA, USA). The use of pseudo-absences was essential due to the inherent challenges of confidently identifying true absences for such a mobile species, particularly in habitats where detectability is influenced by vegetation density, terrain complexity, or limited accessibility. To ensure the ecological plausibility of the pseudo-absence locations, each point was randomly placed across the study area while maintaining a minimum distance of 100 m from any recorded presence point. This distance, double the 50 m radius used for the presence point circular plots, minimized the risk of spatial overlap and reduced the potential biases stemming from proximity to known presence records. The number of pseudo-absences was set to match the number of presences [88], ensuring a balanced dataset for subsequent statistical analysis. For each pseudo-absence, we mirrored the same extraction and calculation procedures applied to the presences, quantifying abiotic, biotic, and anthropogenic metrics within

a 50 m circular plot centered on each pseudo-absence. To standardize the datasets for statistical analysis, all extracted continuous variables were transformed into z-scores.

We then applied a principal component analysis (PCA) in R statistical environment (v. 4.4.2, R Core Team, Vienna, Austria) [89] to address multicollinearity among the variables and reduce dimensionality [90]. Before performing the PCA, we ensured dataset suitability by examining the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and performing Bartlett's test of sphericity. A KMO value between 0.5 and 1.0 confirmed that sampling adequacy was sufficient for reliable component extraction, while Bartlett's test ensured that the dataset displayed significant correlations suitable for PCA. Variables with low communality values (less than 0.6), indicating a limited contribution to the retained components, were excluded to improve the analytical robustness. Principal components with eigenvalues greater than 1.0 were retained using the Varimax rotation method, enhancing interpretability by maximizing the variance explained by each extracted component. The PCA was performed using the "FactoMineR" package in R.

The extracted principal components were subsequently used as predictor variables in a binary logistic regression (BLR) model conducted in R (v. 4.4.2, R Core Team, Vienna, Austria) using the "glm" function with a logit link function, where the presence–absence dataset served as the dependent variable. Model performance was assessed using Nagelkerke's R^2 [91], while goodness-of-fit was evaluated through the Hosmer–Lemeshow test. The model's discrimination ability was analyzed using a classification table comparing observed versus predicted values. Additionally, the area under the Receiver Operating Characteristic (ROC) curve was employed to quantify the model's predictive accuracy and identify the optimal probability threshold for classifying presence and absence records [92]. The "pROC" package in R was used to compute AUC values and visualize model performance. To further interpret the contribution of each predictor variable, we conducted Relative Importance Analysis (RIA), which quantified the proportional contribution of each principal component to the overall predictive performance of the BLR model. This analysis, based on standardized coefficients and likelihood-based metrics, ranked the predictors in terms of their relative influence, providing insights into which factors most strongly impacted *O. cuniculus* presence. To complement these analyses, we generated effect plots for each principal component using the "effects" package in R to visualize their influence on the probability of *O. cuniculus* presence.

Finally, to evaluate whether the principal components derived from the PCA could explain the spatial distribution patterns of *O. cuniculus*, specifically (a) the intensity of hotspot formation and (b) the clustering dynamics, controlling for the spatial autocorrelation inherent in the data, we employed a spatial lag model (SLM) using GeoDa software (v. 1.22.0.10) [93]. The SLM is a spatial regression approach commonly used to analyze spatial patterns in ecological datasets [94–97] by accounting for spatial autocorrelation through the inclusion of a spatial lag coefficient (Rho). This spatial autoregressive parameter allows the dependent variable at each location to be influenced not only by the independent variables at that location but also by the dependent variable values of neighboring locations, thus addressing potential spatial spillover effects. In this context, spatial spillover effects refer to the phenomenon where the spatial patterns observed in one location extend beyond their immediate boundaries, influencing adjacent areas through ecological and landscape-level interactions. In the SLM framework, this effect is statistically captured when the Rho coefficient is significant, indicating that variations in the dependent variable are not solely driven by local predictors but also by interactions across spatially adjacent locations [93,96]. The Rho coefficient quantifies the degree of spatial dependence in the model by measuring the extent to which neighboring observations influence local outcomes. A high and statistically significant Rho value indicates that species

distributions are not spatially independent but instead follow a structured pattern shaped by spatial dependencies with adjacent areas.

The Getis-Ord Gi z-scores (GiZscore) and Local Moran's I z-scores (LMiZscore) were used as dependent variables to assess hotspot intensity and clustering dynamics, respectively. The extracted principal components served as independent predictor variables, while the inclusion of a spatial lag coefficient accounted for the influence of neighboring clustering patterns.

3. Results

3.1. Spatial Distribution of Oryctolagus cuniculus on Lemnos Island

We documented a total of 1534 *O. cuniculus* presence records across Lemnos Island, providing a comprehensive dataset to evaluate the species' habitat preferences and spatial patterns (Figure 2). A higher concentration of the species was documented in the central and eastern regions, where fertile agricultural plains dominate. In contrast, fewer records were documented in the western and northern areas, and they were characterized by phrygana and natural grasslands. Similarly, a few records were noted in the southern and southeastern coastal zones.

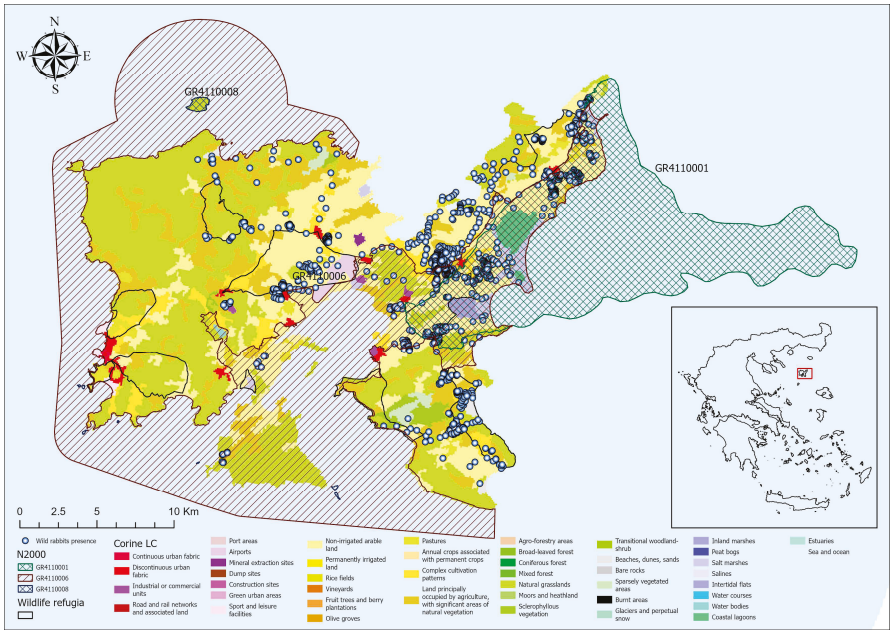

Figure 2. Spatial distribution of *Oryctolagus cuniculus* presence records across Lemnos Island, Greece. The map highlights land cover categories based on CORINE Land Cover (CLC) classification and includes key geographical features such as Natura 2000 sites (GR4110001, GR4110006) and designated wildlife refugia.

The species predominantly occupied low-elevation areas (30.3 ± 24.3 m) with shallow gradients, reflected in a mean slope of 2.3 ± 2.2% and soils with substantial depth (199.9 ± 1.2 cm). Proximity to anthropogenic features varied, with *O. cuniculus* exhibiting a mean distance of 77.2 ± 69.2 m to roads and 1067.6 ± 568.4 m to settlements.

In terms of habitat usage, the majority of records were in non-irrigated arable land, which accounted for 57.4% of the total records, followed by complex cultivation patterns, representing 18.8% of the records. Natural grasslands and phrygana accounted for only 3.2% and 0.2% of records, respectively, while a substantial proportion of them overlapped with conservation areas. Specifically, 738 records (48.1%) were within Natura 2000 sites, while 322 (21%) were found within designated wildlife refugia.

3.2. Principal Components of Abiotic, Biotic, and Anthropogenic Metrics

The PCA identified five significant components with eigenvalues exceeding 1, cumulatively explaining 87.59% of the total variance in the dataset (Table 1). The first component (PC_1—elevation gradient), with an eigenvalue of 4.687, explained 36.05% of the total variance and was strongly associated with elevation metrics (minimum, maximum, and mean elevation). The second component (PC_2—arable and subsidized agriculture), with an initial eigenvalue of 2.391, accounted for 18.39% of the variance and was driven by cereal crop cover, agricultural land cover, and state-supported agricultural area. The third component (PC_3—productivity potential), with an initial eigenvalue of 2.122, contributed 16.32% of the variance and was dominated by productivity metrics. The fourth component (PC_4—grazing-supporting landscapes), with an initial eigenvalue of 1.119, explained 8.61% of the total variance, with total agricultural cover and pasture cover as the dominant variables. Finally, the fifth component (PC_5—soil and field conditions), with an initial eigenvalue of 1.068, accounted for 8.22% of the variance, being strongly associated with good agricultural and environmental conditions (GAEC) and soil depth.

Table 1. The PCA loadings and communality values of the five rotated principal components (PCs).

Principal Components	Variable	Principal Components Loadings				
		PC_1	**PC_2**	**PC_3**	**PC_4**	**PC_5**
PC_1—Elevation gradient	Minimum elevation	0.967	-	-	-	-
	Maximum elevation	0.958	-	-	-	-
	Mean Elevation	0.970	-	-	-	-
PC_2—Arable and subsidized agriculture	Cereal crop cover	-	0.939	-	-	-
	Agricultural land cover	-	0.929	-	-	-
	State-supported agricultural area	-	0.871	-	-	-
PC_3—Productivity potential	Minimum productivity	-	-	0.792	-	-
	Maximum productivity	-	-	0.870	-	-
	Mean productivity	-	-	0.969	-	-
PC_4—Grazing-supporting landscapes	Total agricultural cover	-	-	-	0.884	-
	Pasture cover	-	-	-	0.751	-
PC_5—Soil and field conditions	GAEC	-	-	-	-	0.933
	Soil depth	-	-	-	-	0.651

3.3. Modeling the Presence of Oryctolagus cuniculus on Lemnos Island

The BLR model retained all five PCs identified through PCA as significant predictors of the presence of *Oryctolagus cuniculus* on Lemnos Island. The model exhibited a robust statistical performance, (χ^2 (5, N = 3068) = 2850.50, $p < 0.001$; Table 2), confirming the collective influence of these predictors. The Nagelkerke R^2 indicated that the model explained 81.3% of the variance in rabbit presence, while the Hosmer–Lemeshow test showed that the model's goodness of fit can be accepted (Hosmer–Lemeshow = 674.98, $p > 0.05$) due to the absence of Chi-square significance.

Table 2. The binary logistic regression model which illustrates the presence of *Oryctolagus cuniculus* on Lemnos Island. B = logistic coefficient; S.E. = standard error of estimate; Wald = Wald Chi-square; df = degree of freedom; *p*-value = significance.

Predictor	B	S.E.	Wald's χ^2	df	*p*-Value
PC_1—Elevation gradient	−2.123	0.145	213.673	1	<0.001
PC_2—Arable and subsidized agriculture	1.497	0.098	233.958	1	<0.001
PC_3—Productivity potential	1.023	0.088	135.888	1	<0.001

Table 2. *Cont.*

Predictor	B	S.E.	Wald's χ^2	df	*p*-Value
PC_4—Grazing-supporting landscapes	−2.519	0.152	275.567	1	<0.001
PC_5—Soil and field conditions	4.404	0.176	624.502	1	<0.001
Constant	−0.380	0.111	11.723	1	<0.001

The area under the ROC curve (AUC = 0.967, S.E. = 0.001, 95% CI 0.961–0.974, $p < 0.001$), as a metric of the sensitivity values for all possible values of specificity, with a threshold (0.699) resulting from Youden's index [98] and achieving an overall classification accuracy of 94.3%, correctly identified 95.3% of absences and 93.3% of presences, illustrating its high discriminative ability.

Soil and field conditions (PC_5) emerged as the most influential variable, with a strong positive association with rabbit presence (B = 4.404, $p < 0.001$). Similarly, arable and subsidized agricultural areas (PC_2) and productivity potential (PC_3) positively influenced rabbit presence (PC_2: B = 1.497, $p < 0.001$; PC_3: B = 1.023, $p < 0.001$). In contrast, elevation gradient (PC_1: B = −2.123, $p < 0.001$) and grazing-supporting landscapes (PC_4: B = −2.519, $p < 0.001$) had a significant negative impact on rabbit presence.

The Relative Importance Analysis (RIA) highlighted the varying contributions of each predictor variable in the BLR model. Among the variables, soil and field conditions (PC_5) emerged as the most influential predictor, accounting for 57.84% of the model's relative importance (z = 12.49, $p < 0.001$). The grazing-supporting landscapes (PC_4) contributed the most substantial negative effect, representing −18.92% of the relative importance (z = −8.29, $p < 0.001$), followed by the elevation gradient (PC_1) with −13.43% (z = −7.30, $p < 0.001$). Positive contributions were observed for arable and subsidized agricultural areas (PC_2) at 6.68% (z = 7.64, $p < 0.001$) and productivity potential (PC_3) at 3.12% (z = 5.82, $p < 0.001$).

To complement these findings, effect plots were utilized to visually illustrate the impact of the most influential variables on *O. cuniculus* presence. These plots provide a clear representation of how changes in predictor variables influence the probability of the species occurrence. These visualizations (Figure 3) complement the RIA, enabling a comprehensive understanding of the direction and magnitude of each variable's effect.

3.4. Spatial Patterns and Hotspot Areas of Oryctolagus cuniculus

The KDE analysis provided a spatially detailed representation of the distinct distribution patterns of *O. cuniculus*, emphasizing the high-density areas with concentrated presence records across Lemnos Island (Figure 4). These high-density zones, primarily localized in the central and northeastern region, indicate key habitats where environmental and anthropogenic factors align to support robust rabbit populations.

The Getis-Ord Gi * analysis revealed the significant spatial clustering of *O. cuniculus* presence across Lemnos Island, categorized into hotspots, coldspots, and non-significant areas based on confidence levels (Figure 5). Significant hotspot areas, defined by z-scores, were predominantly concentrated in the central and northeastern regions of the island. A total of 72 hotspots were identified at the 99% confidence level (z-score > 2.58), encompassing 460 records, with z-scores ranging from 2.75 to 8.33 (mean = 5.16, SD = 1.37). Of these, 43 hotspots were located within Natura 2000 sites.

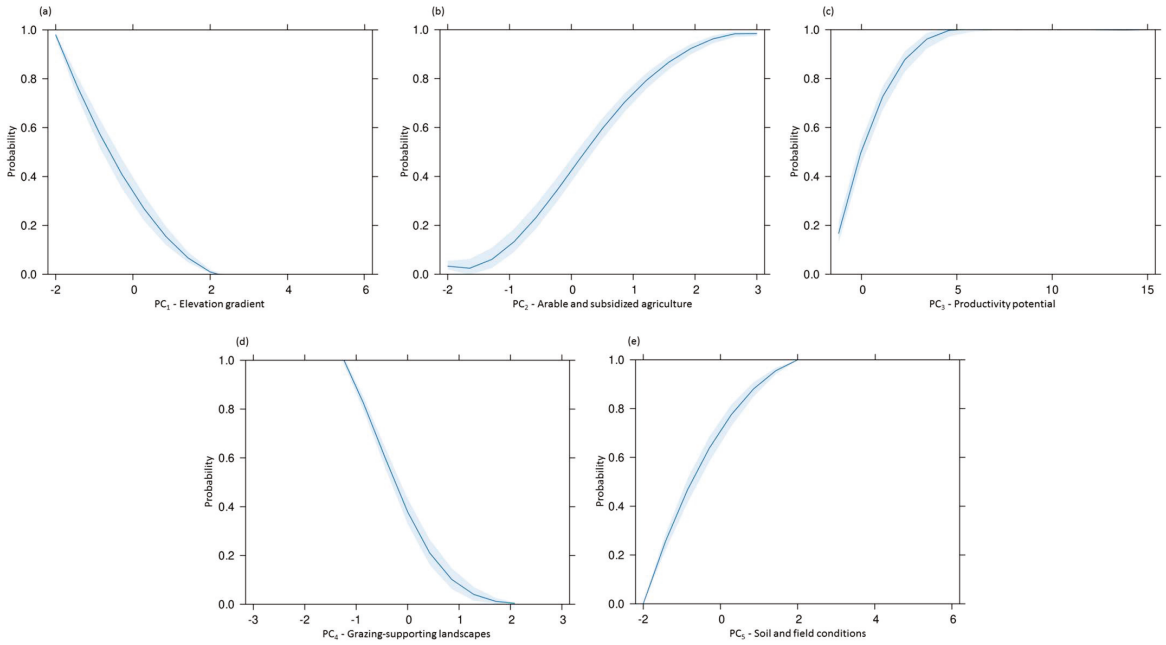

Figure 3. Effect plots of the impact of (**a**) PC_1—elevation gradient, (**b**) PC_2—arable and subsidized agriculture, (**c**) PC_3—productivity potential, (**d**) PC_4—grazing-supporting landscapes, and (**e**) PC_5—soil and field conditions, on the presence of *Oryctolagus cuniculus* on Lemnos Island. The x-axis represents the PCs, while the y-axis displays the corresponding probability.

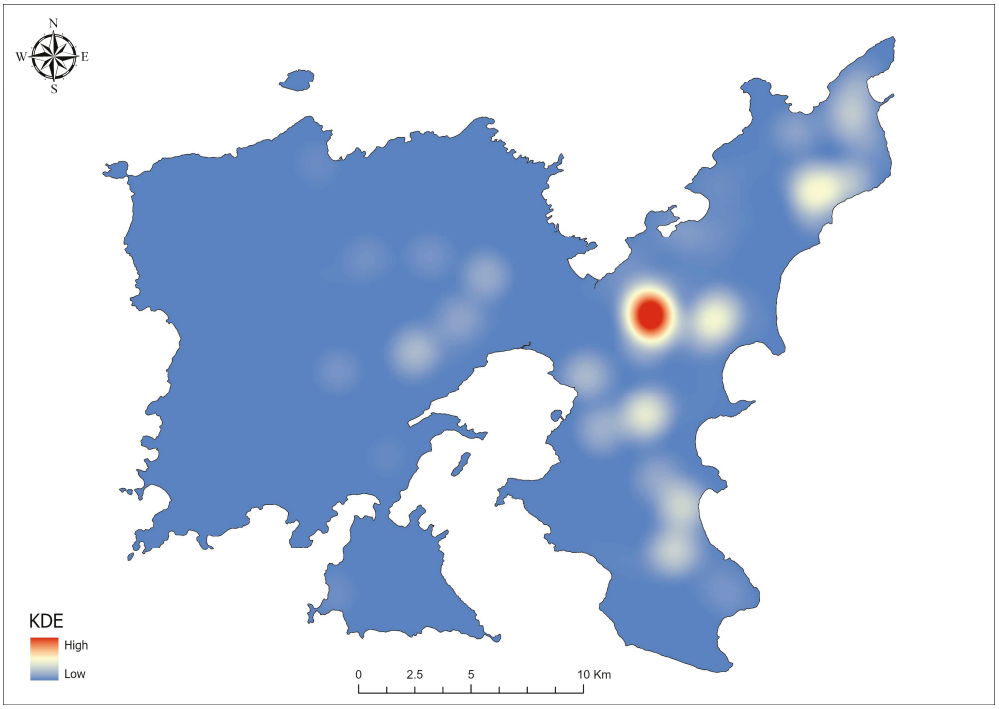

Figure 4. Kernel Density Estimation (KDE) map illustrating the spatial distribution of *Oryctolagus cuniculus* presence across Lemnos Island. High-density areas (red) indicate regions with concentrated presences, while low-density areas (blue) represent regions with fewer records.

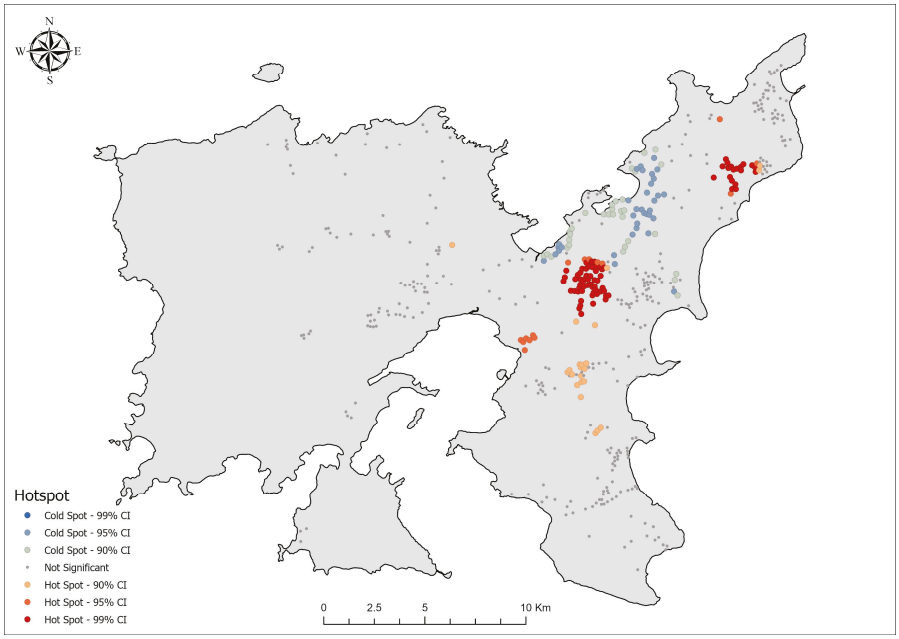

Figure 5. Hotspot analysis map of *Oryctolagus cuniculus* presence on Lemnos Island using the Getis-Ord Gi * statistic. Hotspot regions are categorized by confidence levels (99%, 95%, and 90%) and displayed in red gradients, while coldspot regions are similarly categorized and displayed in blue gradients. Non-significant areas are shown in gray.

At the 95% confidence level ($1.96 < \text{z-score} \leq 2.58$), fifteen hotspots were detected, nine of which were situated within Natura 2000 sites, containing a total of 74 records. The z-scores for these hotspots ranged from 2.01 to 2.52 (mean = 2.19, SD = 0.08). Additionally, 22 moderate hotspots at the 90% confidence level ($1.65 < \text{z-score} \leq 1.96$) were identified, accounting for 89 records. These hotspots exhibited z-scores ranging from 1.68 to 1.90 (mean = 1.78, SD = 0.06).

In contrast, coldspots, representing areas with significantly low concentrations of *O. cuniculus*, were predominantly observed in the northeastern parts of the island, between the main hotspot areas. At the 90% confidence level ($-1.96 \leq \text{z-score} < -1.65$), 32 moderate coldspots were detected, containing 37 records, with z-scores ranging from -1.95 to -1.68 (mean = -1.84, SD = 0.09). Similarly, 34 coldspots were identified at the 95% confidence level ($-2.58 \leq \text{z-score} < -1.96$), encompassing 34 records, with z-scores ranging from -2.43 to -1.96 (mean = -2.12, SD = 0.10). Among these, seven coldspots were located within Natura 2000 sites.

Non-significant areas, representing neutral spatial patterns with no significant clustering, covered a large portion of the island. These areas comprised 328 neutral spots, containing a total of 840 records. The z-scores in these regions ranged from -1.65 to 1.65 (mean = 0.27, SD = 0.87).

3.5. Identification of Significant Clustering Patterns of Oryctolagus cuniculus Distribution

Anselin Local Moran's I analysis revealed the distinct spatial clustering patterns of *O. cuniculus* presence on Lemnos Island, identifying High–High (HH), Low–Low (LL), and Low–High (LH) clusters (Figure 6). High–High (HH) clusters, representing areas where high presence values are surrounded by other high values, emerged as the most prominent spatial pattern. A total of 37 HH clusters were identified, encompassing 416 records. Of these, 25 clusters were located within Natura 2000 sites. Local Moran's I z-scores for HH

clusters ranged from 1.93 to 8.46, with a mean of 5.21 (SD = 2.06), indicating the significant aggregation of records in the central and northeastern parts of the island.

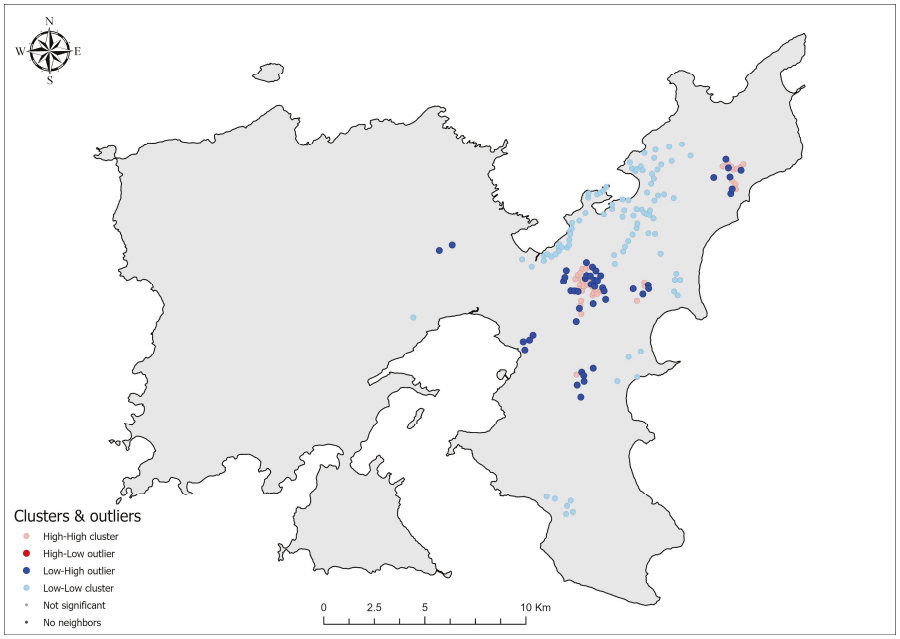

Figure 6. Anselin Local Moran's I analysis of *Oryctolagus cuniculus* on Lemnos Island. The map categorizes clusters into High–High (HH) clusters, where high presence values are surrounded by other high values; Low–Low (LL) clusters, where low values are surrounded by other low values; and Low–High (LH) outliers, where low values are surrounded by high values.

Low–Low (LL) clusters, indicative of areas where low presence values are surrounded by other low values, were also significant. A total of 91 LL clusters were detected, containing 102 records, with 19 clusters situated within Natura 2000 sites. Local Moran's I z-scores for LL clusters ranged from 0.98 to 2.25 (mean = 1.51, SD = 0.24). These clusters were primarily located in the southern and northeastern regions of the island (Figure 6).

Low–High (LH) clusters, representing spatial outliers where low values are surrounded by high values, were less frequent but notable. A total of 46 LH clusters were identified, encompassing 78 records. Of these, 31 clusters were located within Natura 2000 sites. Local Moran's I z-scores for LH clusters ranged from −7.89 to −1.99, with a mean of −3.82 (SD = 1.79), highlighting isolated low-density areas amidst high-density regions.

3.6. Factors Influencing Hotspot Intensity and Clustering Dynamics

The spatial lag model (SLM) identified significant spatial dependence, highlighting the influence of neighboring areas in shaping the intensity and distribution of *O. cuniculus* hotspots and clustering dynamics on Lemnos Island. The spatial lag coefficient (Rho = 0.6396, $p < 0.001$) revealed that hotspot intensity at a given location is statistically associated with clustering patterns in adjacent areas. The model exhibited robust performance, as indicated by the Akaike Information Criterion (AIC = 5237.42), Schwarz Criterion (SC = 5269.40), and a Likelihood Ratio Test ($p < 0.001$), confirming the presence of significant spatial dependence.

The SLM achieved a high explanatory power, accounting for 83.3% of the total variance ($R^2 = 0.833$). Notably, 40.9% of the explained variance was attributed to the spatial lag coefficient, emphasizing the critical role of spatial dependence, while the remaining 42.4% was explained by environmental predictors (PC_2–PC_5). Among these, PC_2 (arable and subsidized agriculture) emerged as the most influential predictor, showing a positive

relationship with hotspot intensity (B = 0.154, $p < 0.001$) and contributing 12.5% to the total variance. In contrast, PC_4 (grazing-supporting landscapes) exhibited the strongest negative effect (B = −0.212, $p < 0.001$), accounting for 17.3% of the variance. PC_3 (productivity potential) also negatively influenced hotspot intensity (B = −0.082, $p < 0.001$), explaining 6.6% of the variance, whereas PC_5 (soil and field conditions) contributed positively (B = 0.073, $p = 0.014$), explaining 6.0% of the variance (Table 3a).

Table 3. Spatial lag model (SLM) analysis for the hotspot intensity and clustering dynamics of *Oryctolagus cuniculus* on Lemnos Island. Significant predictors are shown with their coefficients, standard errors (S.E.), z-values, and *p*-values. PC_1 (elevation gradient) was not significant in the initial model and was excluded from the analysis.

Variable	B	S.E.	z-Value	*p*-Value
(a) SL-Hotspot Intensity Model				
$W_{ij}GiZscore_j$ (Spatial lag)	0.639	0.009	80.837	<0.001
Constant	0.344	0.044	7.770	<0.001
PC_2—Arable and subsidized agriculture	0.154	0.034	4.497	<0.001
PC_3—Productivity potential	−0.082	0.024	−3.404	<0.001
PC_4—Grazing-supporting landscapes	−0.212	0.048	−4.367	<0.001
PC_5—Soil and field conditions	0.073	0.029	2.463	<0.013
(b) SL-Clustering Dynamics Model				
$W_{ij}LMiZscore_j$ (Spatial lag)	0.667	0.008	92.391	<0.001
Constant	0.246	0.045	5.434	<0.001
PC_2—Arable and subsidized agriculture	0.080	0.036	2.354	<0.01
PC_3—Productivity potential	−0.073	0.025	−2.931	<0.01
PC_4—Grazing-supporting landscapes	−0.076	0.051	−1.513	<0.05
PC_5—Soil and field conditions	0.081	0.031	2.600	<0.001

When assessing the clustering dynamics based on Local Moran's I z-scores, the SLM similarly revealed a significant spatial dependence (Rho = 0.6673, $p < 0.001$), further supporting the role of neighboring spatial patterns in shaping clustering dynamics.

Diagnostic metrics, including an AIC of 5475.47 and SC of 5507.45, confirmed the strong performance of the model, with the Likelihood Ratio Test ($p < 0.001$) validating the significant spatial dependence. The model explained 85.17% of the total variance in Local Moran's I z-scores. Of this, 44.5% was attributed to the spatial lag coefficient, indicating the pervasive influence of spatial dependence. The remaining 40.67% of the variance was explained by environmental predictors (PC_2–PC_5). Consistent with the analysis of hotspot intensity, PC_2 (arable and subsidized agriculture) emerged as the most significant predictor, positively influencing clustering intensity (B = 0.084, $p < 0.01$) and contributing 10.8% to the total variance. PC_5 (soil and field conditions) also demonstrated a positive effect (B = 0.081, $p < 0.001$), explaining 10.5% of the variance. Conversely, PC_3 (productivity potential) had a moderately negative impact (B = −0.073, $p < 0.01$), contributing 9.5% to the variance, while PC_4 (grazing-supporting landscapes) showed a weaker negative effect (B = −0.076, $p < 0.05$), accounting for 9.8% of the variance (Table 3b).

4. Discussion

In this study, we presented a holistic and spatially multidimensional approach to investigate the spatial distribution, clustering dynamics, and environmental determinants influencing the presence of *Oryctolagus cuniculus* on Lemnos Island. By combining advanced spatial techniques with a randomized grid-based field survey design, we identified the spatial patterns of the species and elucidated the key habitat associations, while explicitly accounting for the spatial dependencies that influence hotspot intensity and clustering dynamics.

In parallel, the explicit integration of conservation areas into the analysis provided valuable perspectives into the complexities of managing an introduced species that has established extensive populations and poses significant challenges for agricultural and ecological management on Lemnos. This complexity is starkly highlighted by the finding that nearly 60% of the most significant hotspots (99% confidence level) overlap with Natura 2000 sites, as well as by the economic burden caused by the species' presence on the island. After all, from 2007 to 2022, the Hellenic Organization of Agricultural Insurances (ELGA) [99] has disbursed over 7 million euros in compensation for crop yield losses caused by *O. cuniculus* on the island, averaging more than 450,000 euros annually [99]. These figures underscore the pressing need for spatially informed, ecologically sensitive strategies that address the species' impacts on natural and agricultural landscapes on the island.

Exacerbating this dynamic are the unique socioecological conditions of Lemnos, shaped largely by its geographic isolation and the abandonment of agricultural land since the 1980s. Situated far from the continental coast and neighboring Aegean islands, Lemnos stands as a continental-shelf island that was once connected to the mainland and other adjacent landmasses during the Late Pleistocene, when sea levels were much lower [100,101]. This historical connection allowed it to retain a diverse array of species, including those with limited dispersal capabilities [102]. However, unlike many other continental-shelf islands, Lemnos exhibits significant ecological gaps, most notably the complete absence of terrestrial predators [46]. Although a relative predation pressure exists from birds of prey and domestic cats (pers. obs.), their impacts are generally limited due to the low predator density, non-specialized hunting behaviors, and the ability of rabbits to mitigate predation through behavioral adaptations [103,104] and high reproductive rates [37].

This predator-relaxed environment has profound implications for the island's ecological dynamics, differentiating Lemnos from both the native range of *O. cuniculus* [105], and regions where it has been introduced, including the UK [34], Australia [106], New Zealand [107,108], and Chile [109]. In these areas, *O. cuniculus* serves as a critical prey item for terrestrial predators, which exert regulatory pressures on its population. In contrast, the absence of such predators on the island has facilitated unrestrained population growth, mirroring the ecological disruptions observed on isolated oceanic islands where introduced mammals frequently disturb local ecosystems [29,40]. This ecological imbalance has been further exacerbated by human-induced changes, including relatively recent demographic shifts [60] that led to the abandonment of agricultural land and the transition from traditional to modern farming practices.

Consequently, these interconnected factors have created a highly favorable environment for the expansion of *O. cuniculus* on Lemnos Island. This is exemplified by the high number of records obtained during our field surveys, representing the largest and most detailed spatial dataset of the species, not only for the island but also for Greece. Each record represents a distinct instance of direct observation; however, it is important to note that our survey design does not account for factors such as individual movement behaviors [110], seasonal variations [66], or overlapping home ranges [111], which could influence the distribution patterns presented in this study. Moreover, the inherent challenges posed by the biology of the species [37,110,112–114], coupled with their crepuscular–nocturnal activity, likely resulted in a reduced detection probability during our field surveys.

However, our decision to focus exclusively on crepuscular windows rather than extending into nocturnal time windows was informed by established methods in the Mediterranean region, where dusk and dawn counts are considered highly effective for estimating the species abundance [115–117], as more than half of the individuals are simultaneously aboveground during these periods, making them particularly suitable for monitoring [118,119]. Moreover, even

during the hours before or after these time windows, the detectability of the species remained quite satisfactory due to the absence of predation, allowing rabbits to spend extended periods outside their burrows, engage in reduced anti-predator behavior [120,121], and exhibit a weaker midday resting phase (pers. obs.). This aligns with broader findings on mammal responses to predation risk, which can drive behavioral adaptations under selective pressure [122], mirroring the patterns observed in urban environments [123,124] and in other mammalian species, which often exploit predator-free niches [79].

These behavioral adaptations reflect how ecological conditions influence habitat use, creating a framework for understanding the environmental and anthropogenic drivers analyzed through PCA. The resulting components highlight the intricate interplay of topography, human-modified landscapes, vegetation productivity, and soil conditions in shaping *O. cuniculus* habitat preferences on Lemnos Island.

In this light, the BLR model allowed us to quantify the influence of the identified components, enabling a detailed evaluation of the probability of rabbit presence and the relative importance of each component in explaining this likelihood. The positive association and strong contribution of PC_5 (soil and field conditions) emphasizes the role of underlying abiotic factors in shaping rabbit presence (Table 2, Figure 3). The deep alluvial soils found in the relatively flat central and eastern regions of Lemnos not only facilitate burrowing, but also support the island's agricultural productivity. Soil depth, in particular, is a key determinant of warren suitability, as deeper soils are easier to excavate [125–127] and offer greater permeability to water [128]. Such soil characteristics enhance both the structural integrity and the microclimatic stability of warrens, making these areas highly favorable for rabbits [126]. Similarly, PC_2 (arable and subsidized agriculture) and PC_3 (productivity potential) exhibited strong positive associations with rabbit presence, highlighting the critical role of human-modified landscapes and vegetation productivity in shaping habitat preferences. PC_2 reflects the influence of arable land and state-supported agricultural areas, where government subsidies or incentives promote the cultivation of crops like cereals and legumes [129,130]. Complementing this, PC_3 emphasizes the significance of vegetation growth and habitat productivity. Areas with high productivity potential offer ample forage opportunities and support the nutritional needs of the species [131].

Conversely, PC_4 (grazing-supporting landscapes) and PC_1 (elevation gradient) exerted significant negative effects on the species presence. The negative association with PC_4 can be attributed to land-use practices associated with semi-natural grasslands and grazing [60], including pastures where livestock and wild herbivores, such as the fallow deer (*Dama dama*), graze freely (pers. obs.). These practices encompass the early-season grazing of cereal fields and late-season grazing of stubble fields [60], leading to a reduction in available shelter and food resources, making these areas less suitable for *O. cuniculus*. In parallel, the seasonal movement of grazing animals across different fields and pastures further contributes to the diminishing quality of *O. cuniculus* habitats, thereby impacting its presence. The negative influence of PC_1 reflects the topographical challenges in the northwestern region of Lemnos, primarily linked to the physical and ecological constraints of the terrain rather than the weather conditions. These areas often feature steeper slopes, reduced soil depth, and lower soil quality, all of which limit the suitable sites for burrow construction [47,127].

The spatial distribution patterns of *O. cuniculus* as extracted by KDE, the Getis-Ord Gi *, and Anselin Local Moran's I provided important information into the hotspots' intensity and clustering dynamics across the island. The density surfaces generated through KDE offered a foundational representation of the spatial distribution of *O. cuniculus*, with distinct peaks indicating areas of high rabbit presence. While these patterns revealed notable clusters, including a primary concentration in the central part of the island and 19 less

dense regions across the study area (Figure 4), they did not conclusively determine whether these clusters were the result of randomness or underlying ecological processes [132].

To address this limitation, the Getis-Ord Gi * analysis not only validated the patterns observed in KDE but also provided robust statistical evidence, offering a more precise understanding of whether the observed clustering was statistically significant or the result of random variation [85]. The identified hotspots, supported by statistically validated clustering patterns, emphasized the central and northeastern parts of the island (Figure 5) as critical areas necessitating urgent conservation and management measures. The central hotspots align with fertile lowland plains characterized by a mosaic of cultivated and abandoned fields, which are interspersed with a few intermittently flowing streams bordered by fragmented riparian vegetation (pers. obs.). These areas provide abundant food sources and shelter for rabbits, making them highly suitable habitats. Approximately 30% of the land in this region remains uncultivated and unmanaged, primarily due to recurrent crop losses attributed to rabbit herbivory (pers. obs.). This land abandonment further enhances habitat suitability for *O. cuniculus*, as fallow fields promote the growth of dense vegetation, offering both forage and cover. Similarly, the northeastern hotspots are associated with cereal crop cultivation in flat, soft, and coastal terrain, which are highly favorable for burrowing [133]. The absence of significant slopes and the prevalence of cereal farming create productive environments that align with the species' habitat preferences, contributing to the hotspots observed in this region.

In contrast, the coldspots in the central and northeastern parts of the island are located on highly eroded, shallow soils dominated by phrygana and maquis vegetation [59], as well as small forest patches of *Quercus ithaburensis* [58]. Although these areas exhibit some ecological similarities to the central hotspots, they are characterized by a lower agricultural intensity. The juxtaposition of these coldspots with the adjacent hotspots emphasizes the influence of microhabitat variability [134] and land-use practices on the spatial distribution of *O. cuniculus* [135].

The clustering patterns derived from Anselin Local Moran's I analysis provide a granular understanding of the spatial distribution of *O. cuniculus* (Figure 6), offering a further refinement of the insights gained through KDE and Getis-Ord Gi *. The significant overlap of High–High (HH) clusters with conservation areas underscores the ecological importance of these regions, suggesting that conservation priorities must carefully balance habitat preservation with the need to manage rabbit populations, particularly to mitigate conflicts with agricultural activities. Moreover, the presence of Low–High (LH) clusters likely represents transitional zones or edge areas where habitat conditions are suboptimal for supporting high-density populations. These zones may also act as ecological buffers, reflecting gradients of habitat quality influenced by factors such as edge effects or varying land-use intensity.

Interestingly, no High–Low (HL) clusters were detected, which would represent isolated high-density areas surrounded by low-density areas. This absence suggests that high-density rabbit populations are embedded within larger, continuous clusters, highlighting the importance of spatial connectivity in sustaining robust populations of the species. The reliance of *O. cuniculus* on interconnected habitats underscores the role of landscape-scale factors, where environmental and anthropogenic influences converge to create favorable conditions. This spatial continuity, as evidenced by the lack of HL clusters, further emphasizes the need for integrated management strategies that maintain habitat quality across broader ecological networks, while addressing the processes driving population dynamics.

Expanding on this need for a landscape-scale perspective, the spatial lag model (SLM) offers deeper insights into the factors shaping hotspot intensity and clustering dynamics, emphasizing the interconnected nature of *O. cuniculus* populations on Lemnos Island. By accounting for spatial spillover effects, through the Rho coefficient, the SLM reveals how

clustering patterns and hotspot intensities at one location are significantly influenced by the conditions in neighboring areas, with the spatial lag coefficient explaining a substantial portion of the variance (40.9% for hotspot intensity and 44.5% for clustering dynamics). This suggests that clustering patterns extend beyond localized environmental factors and reflects the presence of spatial spillover effects, as observed in studies applying spatial autoregressive models to investigate species distribution patterns, where the clustering patterns in one area influence those in adjacent locations [79,96,136,137]. This spatial dependency echoes the clustering patterns revealed by Anselin Local Moran's I analysis, where High–High (HH) clusters demonstrated significant overlap with critical conservation areas. The observed spatial dependence does not imply direct species dispersal but rather reflects the spatial autocorrelation detected through the SLM, indicating that variations in hotspots and clustering dynamics are influenced by the spatial configuration of adjacent areas, suggesting the existence of underlying landscape-level processes due to shared environmental gradients and habitat connectivity [95,96]. This interconnectedness underscores the importance of viewing the species distribution not as isolated occurrences but as part of a broader spatial network, where habitat conditions and population density in adjacent areas collectively shape the species' spatial structure across the island.

A particularly intriguing result is the negative relationship of PC_3 (productivity potential) with both hotspot intensity and clustering dynamics in the SLM (Table 3), contrasting its positive association in the BLR model. This divergence likely reflects the scale-dependent complexity of ecological processes. At a local scale, as captured by the BLR model, increased productivity enhances habitat quality by providing abundant forage, thereby supporting the rabbit's presence. However, at a broader spatial scale, as analyzed in the SLM, areas of excessive productivity may develop dense vegetation that limits movement, visibility, and the ability to construct burrows. This duality underscores the need to interpret productivity's role within the specific spatial context in which it operates, as its effects can vary depending on whether the focus is on local habitat selection or broader landscape dynamics. Therefore, these findings reinforce the importance of adopting landscape-scale management strategies that integrate spatial spillover effects into conservation and mitigation planning. Effective management should prioritize targeted population control in high-density hotspots, particularly in agricultural areas, through well-documented methods such as culling [51,138], warren destruction [139], and exclusion fencing to protect crops [140], which have demonstrated success in reducing rabbit populations when combined with habitat management. Furthermore, the establishment of buffer zones around cultivated areas serves as an effective barrier to rabbit movement, mitigating human–wildlife conflicts [141].

However, approximately 60% of identified rabbit hotspots overlap with Natura 2000 conservation areas, necessitating an evaluation of the ecological trade-offs associated with these interventions. Culling within protected areas may disturb native fauna [142] through increased human presence and noise [143], disrupt predator–prey dynamics [144], and inadvertently impact non-target species [145]. Similarly, warren destruction could compromise soil integrity, disrupt plant communities, and affect species that are reliant on undisturbed habitats. Exclusion fencing, while non-lethal, may fragment habitats and alter wildlife movement patterns [146].

To mitigate these challenges, enhancing the populations of natural predators, particularly avian species such as buzzards, kestrels, and owls, presents a sustainable biocontrol option. This can be achieved through the creation of nesting sites in agricultural landscapes [147], providing safe breeding habitats that encourage predator presence. Additionally, integrating remote sensing technologies into management frameworks ensures the real-time monitoring of rabbit populations, enabling adaptive responses based on popu-

lation dynamics [54]. Moreover, successful rabbit management on Lemnos Island hinges on collaborative efforts with local stakeholders, particularly farmers, whose participation ensures that management actions are not only scientifically sound but also socially supported and economically viable. By addressing the interconnectedness of habitats across Lemnos Island, such strategies can more effectively balance the needs of agricultural landscapes with ecological processes driving *O. cuniculus* distribution, ensuring the sustainable management of both rabbit populations and their habitats.

5. Conclusions

Our study provides the first spatially explicit baseline dataset for *Oryctolagus cuniculus* on Lemnos Island, contributing to a better understanding of the environmental and anthropogenic factors influencing its distribution. By integrating spatial methods, such as hotspot analysis, clustering metrics, and spatial lag modeling, we identified the complex interplay between environmental factors and spatial dependencies that influence the species distribution patterns.

Our results underscore the need for spatially informed management strategies that focus on mitigating *O. cuniculus* impacts in high-density areas while preserving broader landscape connectivity. The identified hotspots, which predominantly overlap with fertile lowland plains and conservation zones, highlight areas requiring immediate attention, and guiding measures such as habitat restoration, exclusion zones, and integrated pest management. Engaging local stakeholders, particularly farmers, in these strategies will ensure their long-term success and sustainability. By providing detailed spatial data and identifying areas of concern, we offer a foundation for informed decision-making to manage rabbit populations while balancing conservation and agricultural priorities. These insights may also inform strategies for similar conflicts in other insular ecosystems.

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Insights into Population Status and Habitat Patches of Conservation Concern for the Endangered Indian Pangolin (*Manis crassicaudata*) in Nowshera District, Northwestern Pakistan

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Simple Summary: The Indian pangolin, an endangered species found in Pakistan, was studied in the Nowshera district to assess its population and suitable habitat. We used the line transect method and recorded 56 signs of pangolins, including burrows and digging, over a 156 km^2 area. The estimated population was very low, with only 29 individuals and a density of 0.013 individuals/km^2. The MaxEnt model, which predicts habitat suitability, showed that 12.01% of the area was highly suitable and 13.61% was moderately suitable for the pangolin. To protect this fragile population and available habitat patches, the results obtained in the current study recommend stronger law enforcement and conservation efforts.

Abstract: The Indian pangolin (*Manis crassicaudata*) stands out among the four surviving species of Asian pangolins, being the sole species present in Pakistan and listed as endangered owing to trafficking and illicit commerce. In the present study, we explored the population status of the Indian pangolin and the existing suitable habitats in Nowshera district, Pakistan. We employed the line transect method to confirm the species presence and subsequent population estimation. In a survey effort of 156 km^2, a total of 56 signs of Indian pangolin were recorded within the research area. Amongst the 56 signs, 46 were burrows (living burrows (53.57%) and feeding burrows (28.57%)). Digging was observed nine (16.07%) times, along with one direct sighting (1.7%). Our results revealed a population estimate of only 29 pangolins in the Nowshera district, with a population density of 0.013 individuals/km^2. Later, MaxEnt was applied to the species' presence points, along with climatic and topographical variables. The MaxEnt model accuracy was good (AUC = 0.811). Of the total area studied, 210 km^2 (12.01%) were highly suitable and 238 km^2 (13.61%) were moderately suitable habitat for the Indian pangolin. To safeguard the fragile population and habitat of the Indian pangolin, we highly suggest strengthening watch and ward and law enforcement in the study area. By adopting a comprehensive approach that addresses both the direct threats to Indian pangolins and the underlying factors driving their decline, we can effectively protect this endangered species and ensure the preservation of its essential habitats for robust conservation.

Keywords: species distribution modeling; sign survey; living burrows; Nizampur National Park; *Manis crassicaudata*; threatened species

1. Introduction

Population monitoring is one of the basic tenets of wildlife conservation, and is essential for improving knowledge of population dynamics and wildlife management [1,2]. Since many species' population sizes are crucial markers for conservation and central to wildlife management goals, wildlife managers often assess management procedures by measuring these population sizes. Many species' population sizes can serve as crucial markers for their conservation [3–5]. Conservation biologists investigate population sizes and trends by estimating the abundance of any target species [6,7]. Understanding and assessment of global or national level species extinction risks heavily depends on reliable population estimates [8]. Therefore, it is thought that one of the main concerns in conservation biology is to look into accurate population sizes, particularly for species that appear to be at risk of extinction or in danger of collapsing [9].

For propagation and completing life cycles, species require suitable surroundings called "habitats" [10]. Habitats are critical to a species' survival because they provide the resources needed for it to persist [11,12]. Wild species tends to select the best habitats amongst the available habitats by complex behavioral processes, which ensure the fitness and survival of their populations [13]. However, natural wild habitats have been largely damaged by human activities, and this has put many species in danger of going extinct. Because of the terrible consequences of human activities, researchers are now trying to protect endangered species by maintaining their natural habitats [14]. Therefore, habitat research is vital in protecting and managing wildlife, as it offers a scientific justification for strengthening conservation laws [15,16].

It is possible to look at how wildlife interacts with its ecosystem and find possible habitats for endangered species by using ecological models like habitat suitability models (HSMs) [17]. HSMs aids in assessing a particular environment and identifies suitable substitute habitats for conservation-related initiatives [18]. The use of HSMs to map possible habitats for numerous species is growing [19]. The distribution, abundance, and habitat selection of species are all influenced by a number of anthropogenic and environmental factors, which ultimately affect species survival [20]. HSMs integrate data on species occurrence with climatic and other characteristics to estimate the environmental appropriateness of a species over a particular spatial range [21,22].

Small and medium-sized mammals play a variety of important roles in ecosystems, including soil aeration and seed and fungal dispersal [23,24]. They eat insects and other invertebrates and act as simultaneous food for several predators, preserving the diversity, distribution, and population dynamics of other animal species [25,26]. Several small and medium-sized mammal species have experienced sharp population declines due to human activities and environmental shifts, rendering them endangered [27,28], and the Indian pangolin is one of them [29].

The Indian pangolin is one of the four pangolin species found in Asia [30]. It is primarily myrmecophagous—having remarkable anatomical and behavioral characteristics to eat termites and ants [11]. The Indian pangolin, the only species of pangolin found in Pakistan, is arguably the least studied of all the Asian pangolin species [31]. Throughout its geographic range, the Indian pangolin lives in a variety of habitats, such as plains, farmlands, plateaus, and woods [30,32]. It occurs throughout Pakistan, with exception in the northern parts of Giglit-Baltistan (GB) and Khyber Pakhtunkhwa province [33].

The largest threat to this species is the illicit trade in its body scales, which are shipped to China and Vietnam from other range countries, where they are utilized in traditional remedies [34]. Due to the threat mentioned above, the Indian pangolin is also listed in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES Appendix-I) [34]. Given the conservation status of the Indian pangolin, it is crucial to regularly assess its population across its entire range and identify priority landscapes for conservation. Therefore, the current study was designed with the aims to (1) investigate the population status of the Indian pangolin and the carrying capacity of the study area,

and (2) to identify its suitable habitats in the Nowshera district of Khyber Pakhtunkhwa (KP) Province.

2. Materials and Methods

2.1. Study Area

The current study was carried out in Khyber Pakhtunkhwa Province's Nowshera district, which is located at 34°005500 N and 71°5802900 E. The area consists of lowlands, hills, barren ground, agricultural land, urbanized townships, shrublands, and grasslands. This district is 1748 km^2 in size and has an undulating terrain, with elevations between 234 and 1534 m above sea level (a.s.l). The district is divided into 47 union councils (UCs) for administrative purposes; Nizampur is the largest UC—now declared a sub-division—and Pabbi is the smallest. The average annual temperature of 24.4 °C and the average annual rainfall of 532 mm characterize the semiarid climate of our study area. The winter temperature falls below 0 °C in areas with high elevations (1534 m a.s.l), and it sporadically receives moderate snow. This region is dominated by scrub forests, which provide refuge for over 40 birds and 21 mammal species [35,36]. The Nowshera district comprises protected areas (PAs) i.e., game reserves and wildlife parks, as designated by the government [35,37].

2.2. Study Methods

2.2.1. Sign Survey

A sign survey was carried out between July–September 2022 and November 2022–January 2023. Before the survey commencement, we performed a reconnaissance survey in the area along with wildlife department staff to mark potential habitats. We broadly divided the study area into two zones, namely, zone 1 and zone 2. Zone 1 included UCs of Dak Ismail Khel, Jaroba, Chapri, Spinkhak, Jallozai, Saleh Khana, Shahkot, Jabba, Bakhtai, and some portions of Azakhel. Zone 2 encompassed Nizampur sub-division, including Nizampur National Park (NNP). Later on, the aforementioned zones were divided into 5×5 km^2 grids. To differentiate pangolin burrows and related signs from other species, we employed a mixed approach that combined local knowledge with verifiable evidence to identify pangolin burrows. Before starting the transect surveys, we interviewed wildlife rangers to identify the burrowing ranges of Indian pangolins and gathered local knowledge on distinguishing pangolin burrows from those of other animals in the forest and surrounding habitats. We learned that footprints, claw marks, fecal samples, scratch marks, and the shape of the burrow entrance were key indicators for accurately identifying pangolin burrows [30]. In order to confirm the species presence within each surveyed grid, we selected 10 random sampling points with a radius not less than 100 m. Sampling points were thoroughly searched for species signs, including burrows (living or feeding), digging (presumably digging referred to any non-burrow-related excavation by pangolins), scats, claw marks, or any direct sighting. A global positioning system (GPS) (Maverick v2.8) was used to record species presence locations [33,37,38].

2.2.2. Population Estimation

To estimate the population size of the species, we employed the line transect method [39]. The length and width of each transect were approximately 4 km and 1 km, respectively. A total of 18 transects were established in zone 1 and 21 transects in zone 2. In order to apply the active burrow count method to determine population, transects were created in each of the selected sites [40]. The following formula was used for population density estimation [41].

$$D = n/A \quad (1)$$

where "D" is population density, "n" is the number of active living burrows, and "A" is the total area sampled. To estimate total population of Indian pangolins in the Nowshera district, we extrapolated the density estimates of each zone over the whole district as follows:

$$N = D \times \text{total area of Nowshera district} \quad (2)$$

where "N" is the total pangolin population and "D" is the estimated population density.

Since pangolins are solitary animals, active burrows were assumed to have one individual. For estimating the carrying capacity of the area, we used the home range size of pangolins as reported by Gray et al. [42] (1 pangolin/1.58 km^2) in relation to the highly suitable areas.

2.3. Habitat Suitability Modeling

To predict suitable habitats for Indian pangolin in the study area, we analyzed and processed the species presence data (obtained in the sign survey) by using maximum entropy modeling (MaxEnt) 3.3.3k [43]. MaxEnt's precise simulation prediction skills and additional descriptive qualities make it a popular choice for habitat suitability modeling [10,44]. MaxEnt's predictive accuracy has allowed it to outperform other species distribution models, even with presence-only and small datasets [37,38].

2.3.1. Selecting Environmental Variables and Presence Data

Each environmental layer was converted to the same size and resolution, i.e., 1 × 1 km. ArcGIS 10.8 was used to convert Indian pangolin presence points into a vector file. In order to eliminate spatially correlated data points (those located within two kilometers), Indian pangolin presence records were checked for spatial autocorrelation using average nearest neighbor analysis in ArcGIS 10.8 (SDM toolkit) [42] and to guarantee individuality [45]. In the research area, 28 different locations generated habitat suitability models for Indian pangolins after spatial autocorrelation. A set of 26 environmental variables were first taken into consideration (Table S1). Nineteen of the 26 variables were bioclimatic, obtained from the WorldClim database (http://worldclim.org/, accessed on 3 September 2023), a collection of global climate layers collected from more than 4000 weather stations and including information on seasonality, extreme or limiting temperatures, precipitation, and annual averages that may have an impact on the distribution of species [46]. There were five topographic variables: soil, land cover, rivers, slope, and elevation. One variable human population density was anthropogenic. The Normalized Difference Vegetation Index (NDVI) was the vegetation-related variable. The topographic and bioclimatic variables had a spatial resolution of one kilometer. The bilinear resampling method was used to convert the resolution of the other variables to 1 km in 10.8 ArcGIS [38,47]. To eliminate strongly linked variables from the analysis, we used the Pearson correlation matrix in the R program (version 3.6.2) [48]. Ten non-correlated variables ($r < 0.7$) were kept after this procedure [37,38,47].

2.3.2. Model Simulation and Evaluation

Data on the presence of Indian pangolins and a few other variables were changed to fit the MaxEnt software's format (v 3.3.3k) [43]. Five repetitions were run using the typeset as a sub-sample, and we retained 5% of the data for random tests. We also utilized a random seed option. The other parameters, which included a regularization multiplier of one, a maximum of 10,000 randomly generated background points, and 500 maximum iterations with a 0.00001 convergence threshold, were left at their default values. The significance and contribution of each variable were ascertained using a jackknife estimator [49]. Sensitivity analysis was performed for each variable with a logistic output format. For every variable, sensitivity analysis was carried out using a logistic output format. Receiver operating characteristic (ROC) values were used to confirm the MaxEnt model's success (Monterroso et al., 2009; Bai et al., 2018): 0.5–0.6 for rejected, 0.6–0.7 for poor, 0.7–0.8 for average, 0.8–0.9 for good, and 0.9–1.0 for exceptional ROC values [44,50]. The distribution of the appropriate habitat

for Indian pangolins was reclassified using the output results. In order to create a habitat suitability map, the ASCII output format file was loaded into ArcGIS 10.8 and converted into raster data. Reclassification of raster data was used to determine the suitable area [37,38].

3. Results

3.1. Species Presence Records

In the current study, a total of 56 signs of Indian pangolins were observed in the study area. Amongst the 56 signs, 46 were burrows (living burrows (53.57%) and feeding burrows (28.57%)). Digging was observed nine (16.07%) times, along with one direct sighting (1.7%).

3.2. Estimated Population

A total of 39 transects were walked in both zones, yielding a sampling effort of 156 km^2. The single direct sighting during the transect survey was counted as an active living burrow for population estimation. Results obtained in the current study revealed an estimated population of 23 individuals in the Nowshera district, with a population density of 0.013 individuals per km^2 (Table 1).

Table 1. Estimated population density of Indian pangolin in the Nowshera district.

Site Name	Number of Living Burrows of Species		Area Sampled (km^2)	D=N/A per 4 km^2	Total Number of Pangolins Extrapolated throughout the District
	Active Living Burrow	Inactive Living Burrow			
Zone 1	5	13	72	0.069	23
Zone 2	3	9	84	0.035	
Total	8	22	156	0.052 per 4 km^2	
Mean ± SE	4 ± 0.82			0.013 per km^2	

* *The total area of Nowshera District is 1748 km^2.*

3.3. Habitat Suitability

3.3.1. MaxEnt Prediction Evaluation

Twenty-eight sites were utilized to create the current species distribution model (SDM) of the Indian pangolin in the research area after the spatially correlated locations were excluded. Based on the values of the area under the curve, our study produced a reliable and practical model (AUC). With an average AUC value of 0.811, the ROC findings (Figure 1) demonstrated the quality of the MaxEnt model's predictions.

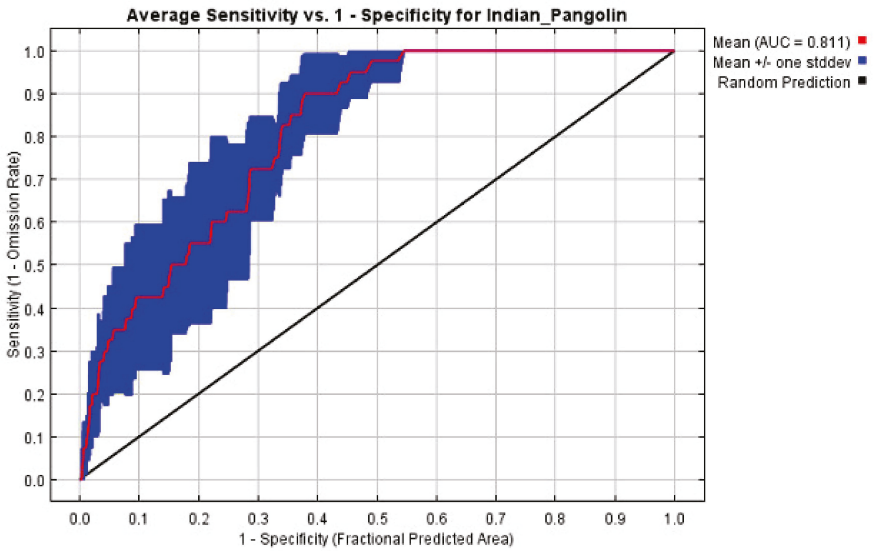

Figure 1. The ROC verification of distribution of suitable Indian pangolin habitat in the current study area.

3.3.2. Influential Factors Determining Habitat Suitability

The contribution of each model to the current SDM of the Indian pangolin was determined by MaxEnt (Table 2, Figure 2). Results reveal that the digital elevation model (DEM) contributed 45.5% to the habitat selection of Indian pangolins. Other variables with maximum contribution in habitat selection of Indian pangolins were bio16 (17.7%), slope (14.3%), and bio13 (7%). The variables with the lowest contribution rates were bio8 (1.3%) and bio 14 (0.7%).

Table 2. Variables' contribution to determining suitable habitats for Indian pangolins in the study area.

S.No.	Variable	Percent Contribution	Permutation Importance
1	DEM (Digital Elevation Model)	45.5	4.1
2	bio_16 (precipitation of wettest quarter)	17.7	21.9
3	Slope	14.3	4.4
4	bio_13 (precipitation of wettest month)	7	0.4
5	pak_pd_2020 (human population density)	5.4	52.7
6	bio_19 (precipitation of coldest quarter)	3.5	2.3
7	GLOBCOVER (land cover)	2.8	3.8
8	bio_9 (mean temprature of driest quarter)	1.8	4.4
9	bio_8 (mean temprature of wettest quarter)	1.3	4.5
10	bio_14 (precipitation of driest month)	0.7	1.6

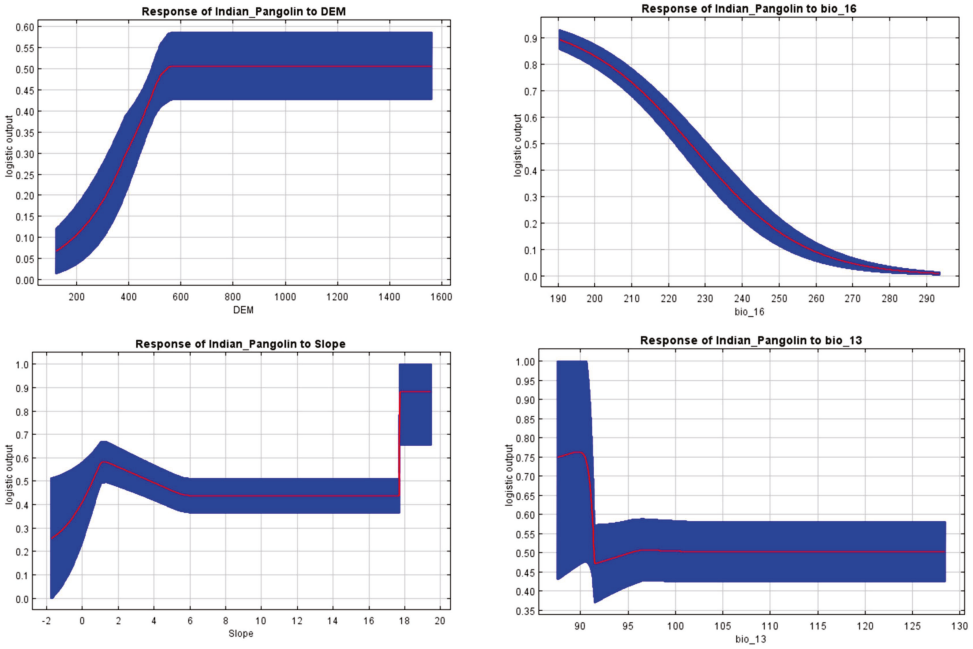

Figure 2. Response curves of the predictors for the presence of Indian pangolins in the research region. Note: The blue curves (two shades for categorical variables) reflect the mean +/− one standard deviation, whereas the red curves show the mean response of the five replicate MaxEnt runs. The Y-axis displays the predicted habitat suitability value (logistic output), and the X-axis displays the range of the environmental predictors.

The jackknife test results show that the DEM provided the most valuable information on its own, yielding the highest gain when used independently. Conversely, when pak_pd_2020 was removed, the gain dropped the most, indicating that this variable contained unique data not captured by the others. The displayed values represent averages across multiple runs (Figure 3).

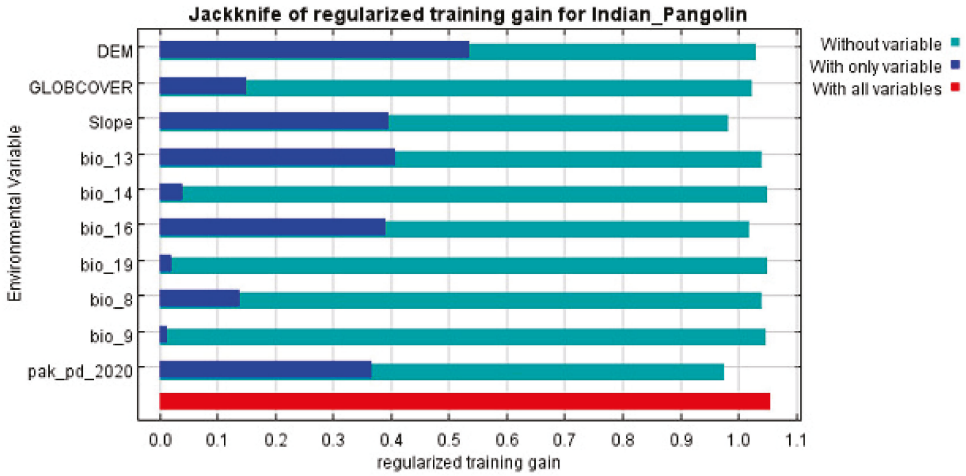

Figure 3. The Indian pangolin habitat suitability model's regularized training gain of the variables tested.

3.3.3. Distribution of Indian Pangolin Suitable Habitats

The habitat suitability map produced through MaxEnt modeling showed that highly suitable habitats of Indian pangolins are present in the southern and southwestern parts of the study area. These southwestern patches of highly suitable areas are located in the buffer zones of Cherat Wildlife Park. The southern patches exclusively fall within Nizampur National Park (NNP). Most of the moderately suitable habitats of Indian pangolins are located in and around protected areas encompassing southern, southeastern, and some central parts of the district. In addition, small patches of moderately suitable habitats were also identified in the western and northwestern sides towards Peshawar and Charsada districts, respectively. The districts mentioned above lie within the IUCN-declared range for the Indian pangolin. Unsuitable habitats were mostly restricted to western, northern, and eastern parts of the study area, which are the most heavily human-dominated areas, including city infrastructure, roads, and highways, along with railway networks and so on.

3.3.4. Numerical Classification of Suitable Habitats

Based on the thresholds, the habitat suitability maps were divided into three groups: places that were highly suitable (0.46–0.92), moderately suitable (0.20–0.45), and unsuitable (0.00–0.19). The reclassified map was processed, and the results reveal that the study area's inappropriate habitats made up 1299.9 km^2 (74.36%), moderately suitable habitats 238 km^2 (13.61%), and highly suitable habitats 210 km^2 (12.01%).

3.3.5. Carrying Capacity of the Study Area

Based on pangolin home range size, our results reveal a carrying capacity of 132 pangolins for the highly suitable areas in the Nowshera district.

4. Discussion

There is a paucity of information on the population dynamics of the pangolin species throughout its four range countries [41]. As far as we are aware, this is the first comprehensive attempt to determine the appropriate habitats and population status of Indian pangolins in this region. Keeping in view the estimated carrying capacity (132 pangolins), results obtained in this study reveal alarmingly low numbers (n = 23) of Indian pangolins, with a population density of 0.013 individuals/km^2 in the study area. Other studies con-

ducted in different parts of Pakistan reported comparatively higher population densities of the Indian pangolin. A recent study conducted in the Kohat district of KP province revealed a population density of 0.29 pangolins/km^2 [41]. Extrapolating these results to the whole Kohat district yielded population estimates many folds higher (n = 867) compared to our findings. In the Chakwal district, which lies in the southern zone of Potohar Plateau, a population density of 1.0 individuals/km^2 was reported [31]. A population density of 0.36 individuals/km^2 was reported from Margalla Hills National Park, Islamabad [51]. Similarly, a population density of 0.77 individuals/km^2 was reported from Azad Jammu and Kashmir (in and around Pir Lasura National Park) [52]. From the Mansehra district in KP, a population density of 0.28 individuals/km^2 was reported [53].

Likewise, another recent study conducted in the adjacent Mardan district reported a population density of 0.09 individuals/km^2, with an estimated population of 147 pangolins throughout the district [34]. On the other hand a very low population (n = 29) was reported for the southern belt (Bannu, Lakki Marwat, Tank, and Dera Ismail Khan districts) of KP province [54]. Compared to the findings of the aforementioned studies, we presume that the very low numbers of Indian pangolins in the Nowshera district and in the southern belt of the province can be attributed to several factors responsible for the low numbers, as described by Roberts [32] and Aisher [55]. In Pakistan, pangolins are being poached in several parts of the country, with the highest number of 275 pangolins in the Chakwal district between 2013–2018 [56]. According to the wildlife officials of the study area, several live pangolins and a potential quantity of scales has been seized in last few years from the study area (personal communication with Range Officer Wildlife, Nowshera Division). However, we believe that further in-depth research is needed to dig out the further causes, like prey base availability, etc., which, in association with poaching, could possibly contribute to such low numbers of Indian pangolins in these areas. Low population densities have been also reported from other parts of the country, as evident from the findings by Irshad et al. [40]. It is believed that low population densities indeed increase the risks of localized extinctions [55].

Our study revealed and highlighted the existing suitable habitats available for Indian pangolins in the Nowshera district. Our results show that a number of parameters affected habitat suitability, with the Digital Elevation Model having the greatest influence (45.5%), followed by bio16 (precipitation of wettest quarter) (17.7%) and slope (14.3%). Our study area elevation ranged between 200 and 1500 m a.s.l. Results suggest that Indian pangolin habitat suitability peaked at 200–500 m a.s.l, yet maintaining a constant probability across higher elevations. The Indian pangolin is mostly restricted in its distribution below 762 m a.s.l, suggesting a specific altitudinal range within which this species is predominantly found [32]. This altitude restriction may be due to various ecological factors, such as temperature, humidity, vegetation type, and prey availability. Yet in several Asian countries, the same species (*Manis crassicaudata*) can be found at elevations just lower than 1524 m [57]. Likewise, for other species of pangolin, i.e., the Chinese pangolin (*Manis pentadactyla*), it has been reported that their distribution is confined to areas where the elevation is either 1500 m a.s.l or just below [57]. In our study area, the most suitable habitats lay between 300 and 700 m a.s.l. However, we presume that in the Nowshera district, Indian pangolins may avoid higher elevations (900 m and above) because of the rocky terrain, which is hard to dig [34].

Our results also show that bio16 (17.7%) was the second most important factor. The response curve gradually decreased from 190 to 290 mm, indicating that the likelihood of occurrence or suitability increased with decreasing precipitation. The Indian pangolin is an insectivore that consumes termites and ants [11]. Indian pangolin prey species depend on wet environments to thrive and procreate. However, seasonal fluctuations and increases in precipitation seasonality decrease their numbers, which has an adverse effect on the pangolin [58,59].

Slopes less than 50° are preferred by pangolins [60]. Our results reveal an increasing trend along with the slope. At 6 degrees, the curve maintained a constant lateral line until

it peaked at 18°. Findings by Shrestha et al. [61] and Khattak et al. [33] strongly validate our results. In the current study, we found that most of the suitable habitats lay in areas with slopes of 5–55°. Pangolins often favor mild slopes over vertical ones [62]. It is believed that the species' avoidance of steep slopes may make it easier for them to move around and to limit high energy expenditures in daily excursions [33,63]. Our findings, however, go counter to those of Tamang et al. [64], who found that Chinese pangolins preferred much sharper slopes.

Our results show that the highly suitable habitats (210 km^2) for the Indian pangolin are mostly located in and around buffer zones of the protected areas, including Nizampur National Park and Cherat Wildlife Park. Based on our findings, we presume that the available habitats for the Indian pangolin are possibly shrunken due to the rapid conversion of natural habitats into human settlements and infrastructures [65,66]. The highly suitable habitats in our study area harbor sub-tropical broad-leaved evergreen forests, along with several species of shrubs and tall grasses [35,67]. The Indian pangolin is highly adaptable to undulating terrains with sub-tropical thorn forests [32], which is a prominent geographical feature of the Nowshera district. The distribution of highly suitable habitats in areas with forests and thick vegetation revealed that the Indian pangolin prefers forests to agricultural lands or grasslands. Our findings are in agreement with those of Waseem et al. [65]. This preference suggests that pangolins may have access to more cover for shelter and protection compared to grasslands and agricultural areas [65].

5. Conclusions

Our research sheds light on the Indian pangolin population status and habitat appropriateness in the Nowshera district. According to the results of the current study, the Nowshera district still has a tiny, vulnerable population of Indian pangolins that require urgent care. Furthermore, the research region still has very favorable habitats. Nevertheless, given the current population condition, we strongly advise stepping up surveillance and monitoring procedures in the study area to save the ideal habitats that are still there. Every year, a close examination of the current population is necessary to evaluate population trends. Finally, we emphatically urge carrying out in-depth research to determine the fundamental causes—presumably poaching and food availability—possibly contributing to the low population of the Indian pangolin in the Nowshera district.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology13090727/s1, Table S1: List of variables used in modeling potential habitats for Indian pangolin.

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Forecasting Suitable Habitats of the Clouded Leopard (*Neofelis nebulosa*) in Asia: Insights into the Present and Future Climate Projections Within and Beyond Extant Boundaries

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Simple Summary: The clouded leopard (*Neofelis nebulosa*) is listed as "vulnerable" due to significant population declines across mainland Asia. Despite existing ecological research, the understanding of the species' habitat suitability, fragmentation, and corridor connectivity remains limited. The present investigation recognizes that, in addition to habitat suitability within its current extent, the species' historical ranges also encompass a significant proportion of suitable habitats. Climate change is expected to exacerbate habitat loss, intensifying fragmentation and reducing the number of viable habitat patches. This study highlights the importance of several transboundary biological corridors, with Southeast Asia predicted to experience the most pronounced connectivity declines, while Bhutan, Nepal, and India are projected to retain more robust ecological networks. To address these challenges, a coordinated conservation strategy is crucial, focusing on mitigating habitat loss and fragmentation as well as addressing the species' shrinking range and increased vulnerability to inbreeding. This study provides critical insights into potential translocation and reintroduction sites, laying the groundwork for a targeted conservation plan designed to protect the clouded leopard across South and Southeast Asia in response to climate change.

Abstract: Terrestrial carnivores, such as the clouded leopard (*Neofelis nebulosa*), are 'vulnerable' and experiencing significant population declines in mainland Asia. Considering the assessed threats, the IUCN has repetitively revised the range of this felid and now characterized it into four distinct categories (extant, possibly extant, possibly extinct, and extinct). Although several ecological works have been accomplished on this enigmatic carnivore, the overall view of its habitat suitability, fragmentation, and corridor connectivity in both present and historical ranges is lacking. Thus, achieving this ecological information under present and future climate scenarios is crucial. The model identified merely 44,033 sq. km falling within the extant range (representing 31.66%) and 20,034 sq. km (8.13%) in the possibly extant range. Fascinatingly, within the historical ranges, an additional 15,264 sq. km (6.58%) has been identified as suitable habitat in the possibly extinct range and 14,022 sq. km (2.38%) in the extinct range. Notably, a total of 25,614 sq. km of suitable habitat is found within designated protected areas across the entire range. Nevertheless, climate change is expected to drive habitat loss of up to 41% (overall IUCN range) for *N. nebulosa* in both present and historical extent, with habitat patches becoming increasingly fragmented. This is reflected in a projected decline in the number of viable habitat patches (NP) by up to 23.29% in the future. This study also identified 18 transboundary biological corridors for *N. nebulosa*, with Southeast Asian countries expected to experience the most significant declines in corridor connectivity. In contrast, the South Asian countries (Bhutan, Nepal, and India) are projected to maintain relatively higher

connectivity in the future. Nevertheless, a substantial decline in overall mean corridor connectivity is projected in the near future due to the impacts of climate change. This study underscores the urgent need for a coordinated and multifaceted conservation strategy for *N. nebulosa*, focusing on mitigating habitat loss and fragmentation. Practical measures must be implemented to protect the species' shrinking range, considering its declining corridor networks and heightened vulnerability to inbreeding depression. Moreover, the assessment of habitat suitability both within and beyond the extant range, alongside corridor connectivity measures, provides valuable insights into potential translocation and reintroduction sites for this species. These findings provide a critical foundation for developing a strategic conservation plan tailored to the specific needs of this felid species across South and Southeast Asia, ensuring enhanced climate resilience and mitigating associated threats.

Keywords: carnivore; feline; predator; species distribution model; corridor; conservation

1. Introduction

Biodiversity is a critical component of Earth's ecosystems, contributing a wide array of resources and playing an essential role in maintaining ecosystem stability and functionality [1]. However, it is increasingly under threat from a variety of anthropogenic drivers, such as deforestation, expansion of agriculture, urbanization, overexploitation of resources, etc. [2]. Besides these, climate change has also become a major factor exacerbating biodiversity loss, intensifying the impacts of ongoing human-driven land use changes and their associated risks [3]. The alterations in climate have led to significant reductions in habitat availability and increased landscape fragmentation, both of which negatively affect biodiversity at multiple scales [4]. These processes have contributed to a large-scale decline in species richness and ecosystem complexity, with numerous species facing habitat loss and intensified extinction risks, particularly those with small or fragmented populations [5,6].

As environmental conditions shift, many species have begun to migrate in an effort to track suitable ecological niches, a trend that is projected to accelerate in response to continued climate change [7,8]. Furthermore, the species that are unable to relocate or adapt to these rapid environmental changes are at an increased risk of local or global extinction [9]. As a result, the fragmentation of habitats is further disrupting ecological connectivity and increasing population isolation [4]. This connectivity is essential for enhancing the long-term resilience of species, as it facilitates gene flow and demographic stability, both of which are critical for maintaining population viability over extended periods [10,11]. Therefore, ensuring ecological connectivity is critical not only for the local conservation of species but also for supporting range shifts and adaptations in response to future threats such as land use changes and further climate disruptions [12,13].

This scenario of global biodiversity loss is particularly alarming for terrestrial carnivores, which have experienced significant population declines [14,15]. These species are especially sensitive to habitat fragmentation due to their large home ranges and typically low population densities [16–18]. Moreover, their vulnerability is further amplified by small population sizes, slow reproductive rates, extensive space requirements, dependence on stable prey populations, and frequent conflicts with humans [19,20]. Furthermore, this is especially concerning as terrestrial carnivores play vital roles in maintaining ecosystem balance, promoting overall ecosystem health, and regulating populations at lower trophic levels [21,22]. Despite their ecological importance and central role in food webs, terrestrial carnivores have experienced significant declines in both population size and geographic range over the past century, often leading to cascading effects such as decreased biodiversity and reduced species richness within ecosystems [23,24]. Given the mounting pressures from anthropogenic disturbances, habitat loss, fragmentation, low reproductive rates, and climate change, these species are ideal focal points for conservation efforts, as their populations have plummeted significantly [25–28].

The clouded leopard (*Neofelis nebulosa*) is a medium-sized felid and remains the least studied among the larger wildcats, largely due to its elusive nature, nocturnal habits, and low population density [29]. Morphologically, this species has a dark gray or ochreous coat with distinctive large, irregular dark blotches, forming a cloud-like pattern [30]. The clouded leopard exhibits a strong arboreal tendency and is predominantly associated with forest habitats, particularly primary evergreen tropical rainforests, while also being recorded in deciduous and mixed forest ecosystems [31]. Additionally, the species has been recorded at elevations exceeding 3500 m in the Himalayas [32]. Furthermore, this species serves as an umbrella species for the Asian forest ecosystem, with a distribution that extends from the foothills of the Himalayas in Nepal, Bhutan, and India, across South China, and down to Peninsular Malaysia [33,34]. However, *N. nebulosa* is now extinct on the island of Taiwan, and populations in Vietnam, China, and Bangladesh are critically low, with some areas facing the risk of extirpation [31,35]. Furthermore, in its 2021 assessment, the IUCN Red List classified *N. nebulosa* as 'vulnerable,' a status that reflects a significant decline in populations across much of its range. This decline is attributed to multiple factors, including habitat destruction, direct exploitation, targeted hunting, and incidental mortality from snares intended for other wildlife [31]. The clouded leopard is listed under CITES Appendix I and is protected by national wildlife laws across its range in various countries. Despite legal protections, body parts of the *N. nebulosa* continue to appear in illegal trade, with evidence indicating that both targeted poaching and incidental capture in snares contribute to population declines. Moreover, it is estimated that populations of *N. nebulosa* have decreased by over 30% between 1999 and 2019, particularly in Myanmar, Cambodia, Laos, Vietnam, Bangladesh, and China. However, these countries likely harbored substantial populations in previous decades, but recent biodiversity loss in the region has significantly impacted these species and adversely affected their numbers. Furthermore, population trends in other range countries, such as Nepal, Bhutan, India, Malaysia, and Thailand, remain inadequately documented, underscoring the need for further research and targeted conservation strategies to ensure the survival of this elusive felid [31].

Prior to this study, most ecological research on *N. nebulosa* centered on its occupancy, habitat use, range, and behavior within fragmented areas of South and Southeast Asia [27,31,36–38]. However, key issues such as the potential impacts of climate change, habitat fragmentation, and the importance of corridor connectivity have yet to be thoroughly explored for this elusive carnivore. Moreover, the diet of this species includes a broad spectrum of prey, such as primates, small mammals, ungulates, and Galliformes. However, studies have highlighted concerns that the distribution of these sympatric prey species may shift due to habitat loss driven by climate change, which could significantly impact the *N. nebulosa* [39–41]. Thus, understanding habitat suitability and connectivity within the range of *N. nebulosa* is essential for effective management, particularly given the increasing anthropogenic pressures on natural habitats and the looming threats posed by climate change [42–45]. Consequently, many studies have utilized species distribution models (SDMs) to assess habitat suitability and connectivity for various Felidae species [46–48]. These models quantify the relationships between species and their environments to determine habitat suitability and can aid in monitoring population dynamics, community ecology, and spatial management planning [49–51]. Furthermore, ensemble modeling has emerged as a more robust and reliable method for predicting species distribution compared to individual SDM approaches [52–54]. This methodology enhances predictive accuracy by integrating outputs from multiple models, thereby reducing reliance on any single model that may not be optimal for addressing specific research objectives [55]. These approaches yield essential insights into the species' habitat within and beyond the IUCN-defined extant range, thereby enhancing the conservation strategy not only for its current distribution but also for potential habitats outside this range. Consequently, the current study utilizes SDM within the categorical IUCN range of *N. nebulosa*, focusing on both the existing (extant and possibly extant) and the likely historical ranges (possibly extinct and extinct) in present and future climatic scenarios to (a) identify suitable habitat extent and its dynamics; (b) assess

the fragmentation of suitable habitats; and (c) evaluate corridor connectivity within and beyond its present extent. These approaches will facilitate the conservation of this species within its existing ranges and support the assessment and development of management plans for habitats resembling historical ranges, thereby enhancing the protection of the species in the wild.

2. Materials and Methods

2.1. Study Area and Species Occurrence Records

The clouded leopard is distributed from the Himalayan foothills in Nepal through mainland Southeast Asia into China [56] (Figure 1). However, the species is extinct on the island of Taiwan, and populations are critically low, with near-extirpation in Vietnam, China, and Bangladesh [35]. As a result, the IUCN range is divided into four categories: extant, possibly extant, extinct, and possibly extinct [31]. The extant range covers much of Bhutan, Northeast India, Myanmar, Thailand, Cambodia, Laos, and the Malay Peninsula, with small fragments in Nepal and southern Bangladesh. In contrast, China, despite having the largest area within the species' range, falls under the historical ranges and is not part of the extant range (Figure 1). Similarly, Vietnam is classified under both the extinct and possibly extinct ranges for this species. The criteria for dividing the species' entire range into four distinct categories were based on the population decline rate and detection frequency in each region. For example, in countries like Vietnam, Laos, and China, populations had already decreased by over 60% by 1999, and recent assessments of 2021 assumed that these areas may no longer support any viable populations. Additionally, some regions reported extremely low population numbers and detection rates, which is considered a 'possibly extinct' category. However, some regions lacked population estimates, and the species has not been reported for a long time, classifying these areas as 'extinct' range. Interestingly, in certain regions, sporadic sightings from camera traps or studies on other large carnivores suggest that the species may still persist there, warranting their classification as 'possibly extant' range. Additionally, areas with recent and regular sightings were categorized as 'extant'. This study utilized a dataset consisting of 159 location points, of which nine were obtained from primary field visits, while 151 were sourced from secondary data via the GeoCat website (accessed on 16 August 2024), aggregating information from GBIF and iNaturalist [57]. Additionally, in order to ensure the accuracy and ecological relevance of the dataset, records related to preserved specimens and individuals held in captivity were deliberately excluded from the analysis. The location points from primary studies were collected during opportunistic visits to forests of northeast India, specifically from the Mathanguri area of Manas National Park (n = 2), Jeypore rainforest (n = 3), and Soraipung (n = 2) in Dihing Patkai National Park in Assam state. Additionally, sightings were recorded in Namdapha National Park near the Deban area (n = 2) in Arunachal Pradesh. The spatial correlation was evaluated and constrained through the application of the spatial rarefaction function in SDM Toolbox v2.4, utilizing a resolution of 4.5 km to correspond with the raster data pixel size [58]. This approach reduced the potential for model overfitting and enhanced the robustness and accuracy of the analysis [59]. As a result, following the removal of spatially correlated occurrences, 148 unique location points were included in the analysis. Furthermore, to evaluate the extent of protected areas within this landscape, data were obtained from the Protected Planet website (https://www.protectedplanet.net/en) (accessed on 22 August 2024) [60].

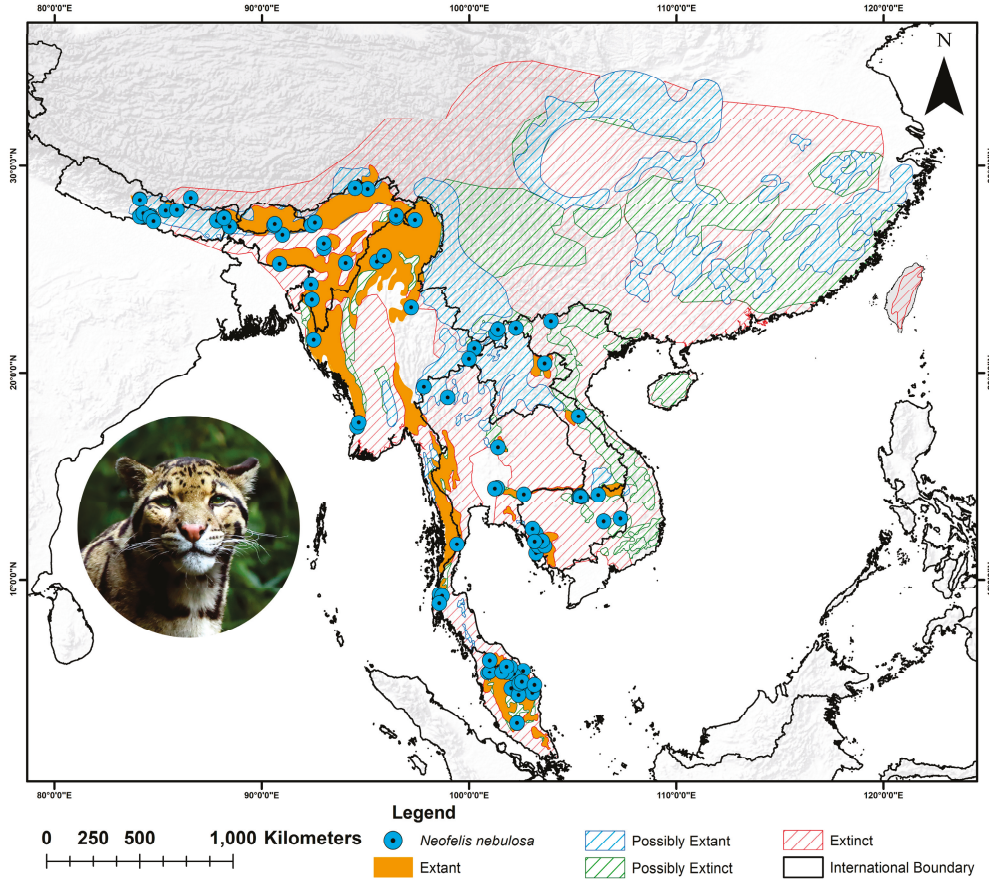

Figure 1. The figure illustrates the entire IUCN extant range of the clouded leopard (*Neofelis nebulosa*), divided into four distinct ranges across South and Southeast Asia, with presence points gathered through primary field surveys and secondary sources. The photograph of the clouded leopard was retrieved from the web.

2.2. Distribution Predictors for the Clouded Leopard

This study employed a comprehensive set of covariates to model habitat suitability for *N. nebulosa*, incorporating bioclimatic, topographic, habitat, and anthropogenic factors. A total of 19 bioclimatic variables, including temperature, precipitation, and other climate-related factors that influence species distribution, were sourced from WorldClim (https://www.worldclim.org/) (accessed on 22 August 2024) [61]. The topographic variables, such as elevation and slope, were obtained from NASA's Shuttle Radar Topographic Mission (SRTM) with a spatial resolution of 90 m (http://srtm.csi.cgiar.org/srtmdata/) (accessed on 22 August 2024). Furthermore, the habitat variables were selected based on the IUCN Red List, focusing on evergreen and mixed-deciduous forests, which represent key environments that meet the ecological needs of the species. Additionally, to assess the influence of human activity, anthropogenic variables such as built-up/urban areas and cropland were included. The habitat variables were derived from the Copernicus Global Land Service land use and land cover (LULC) categorical raster and transformed into continuous raster datasets using Euclidean distance in ArcGIS, enabling detailed analysis of species' responses to habitat proximity [62,63]. Furthermore, all variables were resampled to a uniform resolution of 2.5 min (approximately 4.5 km^2) using ArcGIS 10.6 to ensure consistency across datasets and improve the accuracy of the analysis. Moreover, to address potential multicollinearity, the SAHM (Software for Assisted Habitat Modeling) package in VisTrails was used [64]. The variables with a correlation coefficient (r) greater than 0.8 were excluded to avoid redundancy [65] (Figure S1). After this process, 12 variables were retained for the final model, ensuring robust and accurate predictions of habitat suitability.

2.3. Assessment of Future Climate Change Projections

In order to evaluate the potential impacts of climate change under two distinct shared socioeconomic pathways (SSPs)—SSP245 and SSP585—this study utilized future climate projections for the periods 2041–2060 and 2061–2080. These SSPs serve as frameworks for examining various trajectories of socioeconomic development and their corresponding effects on greenhouse gas emissions and climate change dynamics. The SSP245 scenario projects a future characterized by moderate mitigation efforts, including balanced environmental and social policies, moderate population growth, and advancements in technology, whereas the SSP585 scenario delineates a high-emissions trajectory characterized by minimal adaptation measures, rapid population growth, elevated energy demands, and insufficient environmental regulation, resulting in continued emissions throughout the century [66,67]. The Hadley Centre Global Environment Model, Global Coupled Configuration 3.1 (HadGEM3-GC31 LL), part of the sixth Coupled Model Intercomparison Project (CMIP6), was chosen for its strong performance in modeling climate variability and temperature distribution in South and Southeast Asia [68,69]. To isolate the effects of climate change in future projections, non-climatic variables—including habitat types such as evergreen and mixed forests, urban areas, and cropland, as well as elevation and slope—were held constant that restricted species distribution probabilities to ecologically relevant areas for this species [70,71]. A comprehensive national-scale assessment of habitat suitability was conducted, which integrated the legal frameworks of each country to inform targeted conservation strategies, assessed utilizing the zonal statistics function in ArcGIS v.10.6 to systematically evaluate suitable habitats within the species' range across multiple countries.

2.4. Species Distribution Model for Clouded Leopard

The habitat suitability modeling in this study employed an ensemble approach, integrating multiple algorithms to create the final model for the species utilizing five distinct algorithms: Boosted Regression Tree (BRT), Generalized Linear Model (GLM), Multivariate Adaptive Regression Splines (MARS), Maximum Entropy (MaxEnt), and Random Forest (RF) [72–74]. The selection of these models captures various aspects of species–environment relationships. Specifically, MaxEnt handles presence-only data, BRT and RF manage nonlinear interactions with high accuracy, while GLM and MARS offer interpretability and flexibility, creating a comprehensive modeling approach. These algorithms were implemented through the SAHM package in VisTrails software, resulting in probability maps that ranged from 0 (least suitable) to 1 (most suitable), and binary maps were subsequently generated using the minimum training presence as a threshold [64,75]. The evaluation of the models was based on an Area Under the Curve (AUC) threshold of 0.75, which served as the primary criterion for model selection [76]. Moreover, to evaluate habitat configurations, an ensemble count map was generated with a scale from 0 to 5, where each pixel indicated the level of agreement among the models, with a value of 5 signifying complete accord across all models. Additionally, several performance metrics were calculated for both the training and cross-validation datasets (n = 10) to further evaluate model performance. Furthermore, metrics such as AUC, True Skill Statistic (TSS), Cohen's Kappa, Proportion Correctly Classified (PCC), specificity, and sensitivity further ensured a robust and reliable model for predicting the species' distribution [77–80].

2.5. Assessment of Habitat Quality and Shape Geometry

To assess the qualitative and geometric characteristics of suitable habitat patches in both current and projected future scenarios, this study employed a range of class-level metrics using FRAGSTATS version 4.2.1 [81]. This specialized software is well-established in the fields of landscape ecology and environmental management, facilitating the analysis of spatial patterns within ecosystems and offering an extensive array of metrics and indices that thoroughly assess and clarify the structure and composition of landscapes [82]. The metrics utilized in this analysis included the number of patches

(NP), aggregate index (AI), patch density (PD), largest patch index (LPI), total edge (TE), and landscape shape index (LSI). The metrics such as NP, PD, TE, and LPI offer detailed insights into the geometry of habitat patches, including their size, edge characteristics, and density within a specific area. In contrast, the LSI metric assesses the complexity of patch geometries, indicating the level of irregularity or convolution present in their shapes, whereas the AI quantifies the closeness or clustering of patches, reflecting the extent to which they are grouped or spread out across the landscape. These metrics hold biological significance, as they provide insights into ecological processes within habitats and elucidate the effects of changes in suitable areas on landscape dynamics [83]. This methodology allows for a deeper understanding of landscape characteristics and facilitates a comprehensive analysis across the species' distribution range. Consequently, these metrics were applied to evaluate habitat features and levels of fragmentation within the modeled area under various scenarios, including current conditions and future climate change projections [62].

2.6. Assessment of Biological Corridor Connectivity

Recognizing the importance of enhancing habitat connectivity as a vital conservation strategy for species preservation, it was essential to evaluate the biological connectivity between habitat patches [84]. Hence, to accomplish this, the circuit model—frequently used for designing animal corridors—was employed. Specifically, the Circuitscape toolbox for ArcGIS 10.6 was utilized to simulate these corridors in present and future climatic scenarios [85–87]. This toolbox allows for the simulation of ecological corridors by modeling species movement across heterogeneous landscapes. In this framework, pairwise source/ground mode settings were applied using the probability maps generated by the model as conductance rasters. The specified location points served as focal nodes within the pairwise configuration. The output comprised current maps, which facilitated a more in-depth assessment and analysis of connectivity. This simulation of corridors was executed for both contemporary conditions and projected future climatic scenarios.

3. Results

3.1. Ecological Niche Modelling and Predictor Importance

The models' performance for this species was consistently outstanding across all algorithms, as evidenced by results from both training and cross-validation datasets. Among the five models employed, MaxEnt utilized the complete set of variables during replication, whereas the BRT model incorporated only six out of the twelve available variables, highlighting their contrasting abilities to assimilate data for habitat prediction. Moreover, all algorithms with an AUC score above 0.75 were included in the final ensemble map, with AUC values ranging from 0.972 to 0.804 during training and 0.909 to 0.804 during cross-validation, indicating high predictive accuracy (Figure 2, Figures S2 and S3). The Generalized Linear Model (GLM) had the smallest ΔAUC (0.093), while the Boosted Regression Tree (BRT) showed the largest deviation (ΔAUC up to 0.125), highlighting model sensitivity. Additionally, evaluation metrics such as True Skill Statistic (TSS), Proportion Correctly Classified (PCC), Cohen's Kappa, sensitivity, and specificity further validated the models' high performance (Table 1).

The ensemble model analysis indicated that on average (μ) across the five models, temperature annual range (bio_7) was the most influential predictor of species distribution, contributing 37.37% to overall model performance (Table 2). This variable, representing the difference between the highest temperature in the warmest month and the lowest in the coldest, serves as a key measure of temperature variability and seasonal extremes affecting species distribution and habitat suitability. Among habitat variables, Evergreen Forest had the highest influence, contributing 9.74%, while the anthropogenic variable Cropland contributed 8.19% to the model. Among the topographic variables, slope had the highest contribution, accounting for 2.35%. Conversely, the habitat variable Mixed/Deciduous Forest (mixed_for) had the lowest contribution, at 2.10% (Figure 2 and Figure S4, Table 2).

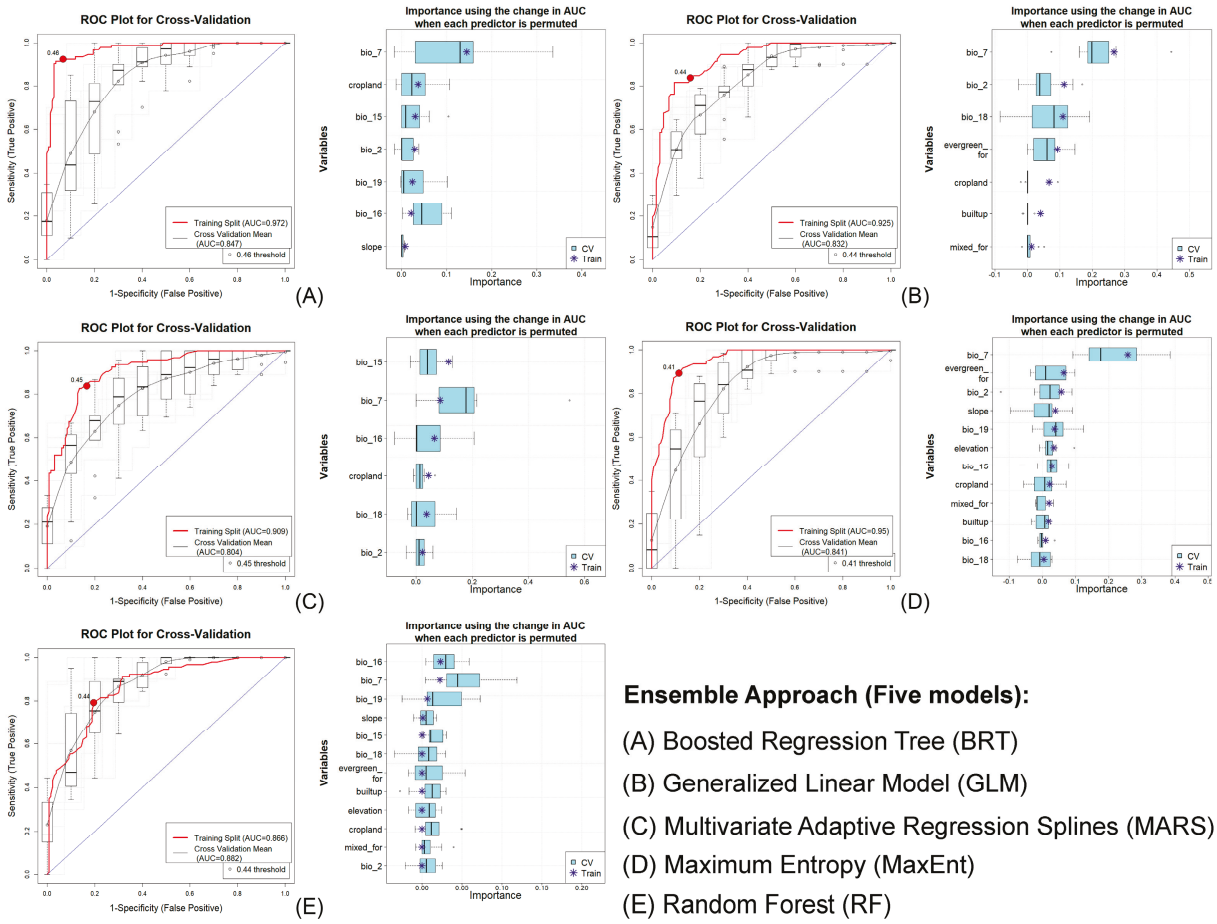

Figure 2. Model evaluation plot showing the average training ROC of both training and cross-validation (CV) and the predictors chosen by the model for the replicate runs under five models.

Table 1. Model fit metrics for each of the participating modeling methods and for the final ensemble model for estimation of habitat suitability of clouded leopard. A total of five model algorithms, i.e., Maximum Entropy (MaxEnt), Boosted Regression Tree (BRT), Generalized Linear Model (GLM), Multivariate Adaptive Regression Splines (MARS), and Random Forest (RF). AUC: Area under Curve, ΔAUC: Change in Area under Curve (Training Cross Validation), PCC: Proportion Correctly Classified, TSS: True Skill Statistic.

Model	Dataset	AUC	ΔAUC	PCC	TSS	Kappa	Specificity	Sensitivity
BRT	Train	0.972	0.125	92.9	0.856	0.853	0.932	0.924
	CV	0.847		75.7	0.51	0.505	0.777	0.733
GLM	Train	0.925	0.093	84	0.679	0.672	0.842	0.837
	CV	0.832		74.2	0.477	0.474	0.775	0.702
MARS	Train	0.909	0.105	83.6	0.672	0.664	0.835	0.837
	CV	0.804		72.8	0.465	0.452	0.73	0.735
MaxEnt	Train	0.95	0.109	88.8	0.778	0.771	0.886	0.891
	CV	0.841		78.4	0.565	0.558	0.809	0.755
RF	Train	0.866	0.016	80	0.598	0.591	0.805	0.793
	CV	0.882		77	0.531	0.526	0.797	0.733

Table 2. The mean percentage contribution of the covariates generated from the final ensemble model for the clouded leopard.

Predictor	Abbreviations	BRT	GLM	MARS	MAXENT	RF	Mean	Mean %
Precipitation Seasonality (Coefficient of Variation)	bio_15	0.030	0.000	0.116	0.029	0.000	0.035	8.43
Precipitation of Wettest Quarter	bio_16	0.021	0.000	0.065	0.010	0.023	0.024	5.75
Precipitation of Warmest Quarter	bio_18	0.000	0.110	0.037	0.005	0.000	0.030	7.33
Precipitation of Coldest Quarter	bio_19	0.024	0.000	0.000	0.037	0.007	0.014	3.28
Mean Diurnal Range (Mean of monthly (max temp—min temp))	bio_2	0.029	0.113	0.023	0.058	0.000	0.045	10.70
Temperature Annual Range	bio_7	0.145	0.267	0.085	0.258	0.023	0.155	37.37
Built-up	builtup	0.000	0.040	0.000	0.019	0.000	0.012	2.87
Cropland	cropland	0.037	0.067	0.044	0.023	0.000	0.034	8.19
Elevation	elevation	0.000	0.000	0.000	0.039	0.000	0.008	1.87
Evergreen Forests	evergreen_for	0.000	0.092	0.000	0.070	0.040	0.041	9.74
Mixed/Deciduous Forest	mixed_for	0.000	0.013	0.000	0.021	0.010	0.009	2.10
Slope	slope	0.008	0.000	0.000	0.040	0.001	0.010	2.35

3.2. Habitat Suitability in Present and Historical Range

The total IUCN range for the species, encompassing all four categories, spans approximately 1,201,540 sq. km across South and Southeast Asia, of which only 93,353 sq. km, or 7.76%, was identified as suitable habitat in the current scenario (Figure 3, Table 3). Furthermore, the model identified 44,033 sq. km suitable within the extant range, representing 31.66% of the area, whereas in the possibly extant range, only 20,034 sq. km, or 8.13%, was found to be suitable. Additionally, in the extinct range, only 14,022 sq. km, or 2.38%, still exhibited suitability, while in the possibly extinct range, 15,264 sq. km, or 6.58%, was identified as suitable habitat. Nevertheless, only 25,614 sq. km of suitable habitat within the entire range was found to be under protected areas (Table 3).

Table 3. This table represents the suitable habitat extent (in sq. km) for the clouded leopard within its IUCN range under present and future climatic scenarios.

Scenario	Overall Range	Extant	Possibly Extant	Extinct	Possibly Extinct	Protected Areas
Present	93,353	44,033	20,034	14,022	15,264	25,614
SSP 245 (2041–2060)	68,171	37,706	7349	11,008	12,110	24,327
SSP 245 (2061–2080)	61,146	33,804	6107	10,198	11,038	22,352
SSP 585 (2041–2060)	61,666	36,200	4599	10,245	10,623	23,576
SSP 585 (2061–2080)	54,968	31,830	3841	9732	9565	21,217

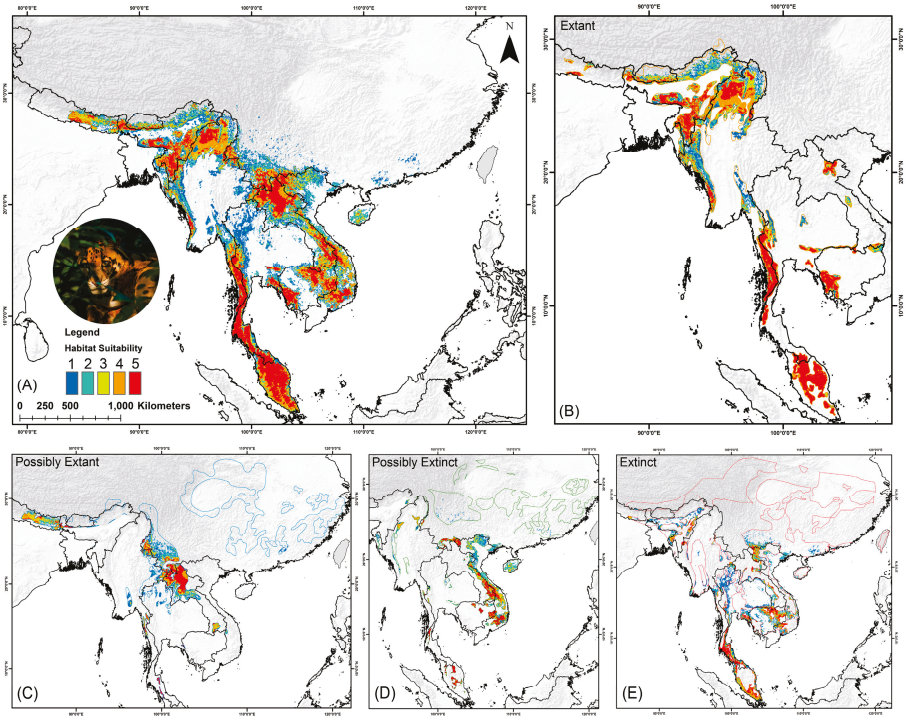

Figure 3. This figure displays the present suitable habitats for the clouded leopard in the study area. The five classes (1–5) on the map represent the model agreements used in the study, while Class "0" (no suitability and zero model agreement) is not shown. The map sections are as follows: (**A**) Habitat suitability across the entire IUCN range, (**B**) extant range, (**C**) possible extant range, (**D**) possibly extinct range, and (**E**) extinct range. The photograph of the clouded leopard was taken by Dhritiman Mukherjee.

In future scenarios, the suitable habitat is projected to decline across all ranges due to climatic changes. Overall, the entire IUCN range is expected to experience a decline of over 26.97% from present conditions (Figure 4). Specifically, in the extant range, a reduction of 14% to 27% is expected, while the possibly extant range is projected to experience a substantial decline of up to 80.83%. Similarly, the extinct range is forecast to undergo a reduction of over 21%, and the possibly extinct range is expected to decrease by up to 37.34% (Figure 4). Moreover, suitable areas within PAs are projected to decrease by approximately 17.17% due to climate change impacts (Table 3).

3.3. Country-Level Mean Habitat Suitability

The analysis of mean habitat suitability across the species' range nations indicates that Malaysia currently exhibits the highest mean suitability (0.743), followed by Laos (0.493), India (0.488), and Cambodia (0.410) (Table 4). In contrast, China shows the lowest suitability (0.098), with no suitable habitat identified in Taiwan. As climatic changes are projected to reduce suitable habitat extent, these shifts are expected to have a substantial impact on the overall quality of habitat throughout the species' range. The most significant decline is forecasted for India, where mean suitability may decrease by as much as 56.12% from present levels, followed by Myanmar, where reductions of 32% to 41% are anticipated (Table 4). Moreover, the mean habitat suitability across all nations within the *N. nebulosa* range is anticipated to decline in the future due to climate change. However, among the nations currently deemed suitable, Malaysia stands out with the smallest expected decrease, facing a reduction of only 12.59% in the future. In comparison, China is predicted to experience the least overall decline, with suitability dropping by 5% to 15% across different future scenarios.

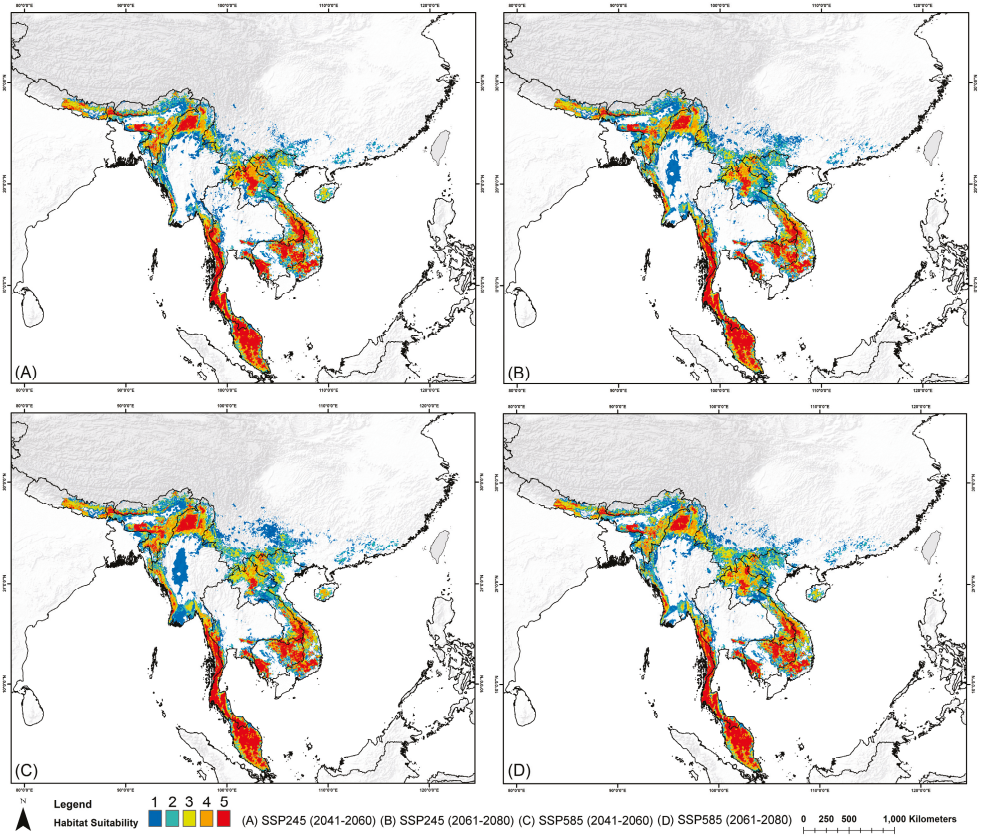

Figure 4. Map illustrating habitat suitability for the clouded leopard across the entire IUCN range under future climate change scenarios. The subfigures represent different scenarios and timeframes.

Table 4. The mean habitat suitability for clouded leopard in both current and future scenarios within different countries (descending order) in the entire IUCN extant. The gain is represented by "+", whereas the loss is represented by "−". GR: growth rate.

Country	Present	SSP 245 (2041–2060)	GR of SSP 245 (2041–2060) from Present	SSP 245 (2061–2080)	GR of SSP 245 (2061–2080) from Present	SSP 585 (2041–2060)	GR of SSP 585 (2041–2060) from Present	SSP 585 (2061–2080)	GR of SSP 585 (2061–2080) from Present
Malaysia	+0.743	+0.672	−9.54	+0.662	−10.88	+0.659	−11.25	+0.649	−12.59
Laos	+0.493	+0.352	−28.62	+0.342	−30.68	+0.323	−34.47	+0.315	−36.10
India	+0.488	+0.261	−46.43	+0.251	−48.53	+0.234	−52.02	+0.214	−56.12
Cambodia	+0.410	+0.330	−19.44	+0.321	−21.70	+0.291	−29.03	+0.276	−32.69
Nepal	+0.404	+0.340	−15.83	+0.330	−18.31	+0.311	−23.07	+0.299	−26.04
Vietnam	+0.365	+0.279	−23.44	+0.268	−26.46	+0.259	−29.05	+0.243	−33.43
Myanmar	+0.343	+0.232	−32.42	+0.222	−35.35	+0.201	−41.33	+0.199	−41.91
Thailand	+0.317	+0.259	−18.51	+0.249	−21.66	+0.241	−24.05	+0.226	−28.78
Bhutan	+0.273	+0.232	−14.93	+0.222	−18.59	+0.210	−23.14	+0.189	−30.83
Bangladesh	+0.242	+0.215	−11.35	+0.205	−15.48	+0.195	−19.46	+0.188	−22.35
China	+0.098	+0.093	−5.39	+0.092	−6.42	+0.089	−9.15	+0.083	−15.27

3.4. Habitat Quality and Shape Geometry

The analysis of habitat shape geometry indicates significant fragmentation driven by future climate changes. A high degree of disintegration is observed as habitat patches become fragmented, with some viable patches completely lost (Table 5). This is reflected in a decline in the number of patches (NP) by up to 23.29% in the future from the present scenario. Furthermore, the reduction in patch density (PD), which has decreased by

over 8.10%, underscores the extent of this fragmentation. In addition to the loss of patches, the remaining patches are now smaller in size, as evidenced by a substantial decline of 24.50% in the LPI. Consequently, the edge area is also diminished, with total edge (TE) declining by more than 18.01%. Moreover, the remaining patches exhibit simpler shapes, as indicated by a decrease of up to 13.22% in the LSI (Table 5). The spatial arrangement of these patches has also changed, with increased distances between them, as demonstrated by a decline of over 14% in the AI. Collectively, these metrics comprehensively illustrate and elucidate the fragmentation of suitable habitat patches induced by climate change.

Table 5. Assessment of habitat shape geometry of clouded leopard in present and future scenarios. NP: no. of patches, PD: patch density, LPI: largest patch index, TE: total edge, LSI: landscape shape index, AI: aggregation index.

Scenario	NP	PD	LPI	TE	LSI	AI
Present	468	993,511	2.767	640.794	26.4291	82.1631
SSP 245 (2041–2060)	429	913,023	2.420	525.378	24.5273	70.399
SSP 245 (2061–2080)	385	819,379	2.200	477.288	23.4691	66.2725
SSP 585 (2041–2060)	378	804,481	2.349	506.31	23.8346	62.7976

3.5. Biological Corridor Connectivity

The assessment of biological corridor connectivity revealed 18 transboundary corridors for this species in its IUCN range (Figure 5). The highest mean corridor connectivity was observed between Bhutan and India, with a connectivity score of 2.441 in the current scenario (Table 6). This is followed by the Nepal–India corridor (2.114), Myanmar–China (1.140), and Nepal–China (1.044). The lowest connectivity was recorded between Thailand and Malaysia, with a mean score of 0.253 (Table 6). However, these transboundary corridors are projected to be significantly affected by climate change in the future (Figure 5). The most substantial decline in mean corridor connectivity is anticipated between Bhutan and China, with reductions of up to 61% in the future from the present scenario. However, other high-connectivity corridors are also expected to experience decreases, with the Bhutan–India corridor projected to decline by 15% to 31% and the Nepal–India corridor by up to 29.53% from the present scenario. The highest decline in the highest mean corridor connectivity is demonstrated by the Southeast Asian countries, while South Asian nations such as Bhutan, Nepal, and India are expected to maintain relatively higher connectivity compared to their counterparts in the future (Figure 5). Nonetheless, an overall loss of mean corridor connectivity for this species is anticipated, primarily driven by the impacts of climate change.

Table 6. Assessment of corridor connectivity of clouded leopard within the entire IUCN range in both the current and future climate change scenarios. The gain is represented by "+", whereas the loss is represented by "−". CAM: Cambodia; LAO: Laos; BHU: Bhutan; IND: India; MYA: Myanmar; BAN: Bangladesh; CHI: China; VIET: Vietnam; THA: Thailand; MAL: Malaysia; GR: Growth Rate; SSP: Shared Socioeconomic Pathways.

Corridors	Present	SSP 245 (2041–2060)	GR of SSP 245 (2041–2060) from Present	SSP 245 (2061–2080)	GR of SSP 245 (2061–2080) from Present	SSP 585 (2041–2060)	GR of SSP 585 (2041–2060) from Present	SSP 585 (2061–2080)	GR of SSP 585 (2061–2080) from Present
BHU_IND	+2.441	+2.063	−15.48	+1.901	−22.10	+1.802	−26.16	+1.662	−31.89
NEP_IND	+2.114	+1.901	−10.09	+1.719	−18.67	+1.600	−24.33	+1.490	−29.53
MYA_CHI	+1.140	+0.996	−12.60	+0.886	−22.27	+0.825	−27.62	+0.801	−29.73
NEP_CHI	+1.044	+0.767	−26.48	+0.657	−37.02	+0.644	−38.30	+0.621	−40.51

Table 6. *Cont.*

Corridors	Present	SSP 245 (2041–2060)	GR of SSP 245 (2041–2060) from Present	SSP 245 (2061–2080)	GR of SSP 245 (2061–2080) from Present	SSP 585 (2041–2060)	GR of SSP 585 (2041–2060) from Present	SSP 585 (2061–2080)	GR of SSP 585 (2061–2080) from Present
IND_BAN	+1.041	+0.909	−12.76	+0.709	−31.96	+0.689	−33.88	+0.656	−37.01
CHI_LAO	+0.938	+0.797	−15.01	+0.697	−25.67	+0.657	−29.97	+0.622	−33.70
MYA_LAO	+0.881	+0.548	−37.88	+0.491	−44.35	+0.455	−48.37	+0.423	−52.00
MYA_THA	+0.754	+0.593	−21.32	+0.489	−35.16	+0.421	−44.18	+0.409	−45.77
BHU_CHI	+0.614	+0.375	−39.00	+0.289	−52.93	+0.269	−56.19	+0.235	−61.73
LAO_VIET	+0.612	+0.420	−31.43	+0.400	−34.64	+0.370	−39.54	+0.343	−43.95
BAN_MYA	+0.545	+0.397	−27.16	+0.376	−31.05	+0.343	−37.10	+0.321	−41.14
LAO_CAM	+0.446	+0.356	−20.15	+0.333	−25.31	+0.314	−29.57	+0.301	−32.49
CHI_VIET	+0.347	+0.275	−20.77	+0.234	−32.51	+0.212	−38.86	+0.198	−42.90
THA_LAO	+0.343	+0.231	−32.59	+0.211	−38.53	+0.199	−42.02	+0.177	−48.43
IND_CHI	+0.339	+0.233	−31.25	+0.209	−38.26	+0.189	−44.17	+0.156	−53.92
LAO_VIET	+0.302	+0.209	−30.82	+0.190	−37.10	+0.170	−43.72	+0.140	−53.65
THA_CAM	+0.266	+0.170	−36.04	+0.160	−39.76	+0.140	−47.29	+0.120	−54.82
THA_MAL	+0.253	+0.176	−30.31	+0.157	−37.89	+0.136	−46.20	+0.122	−51.74

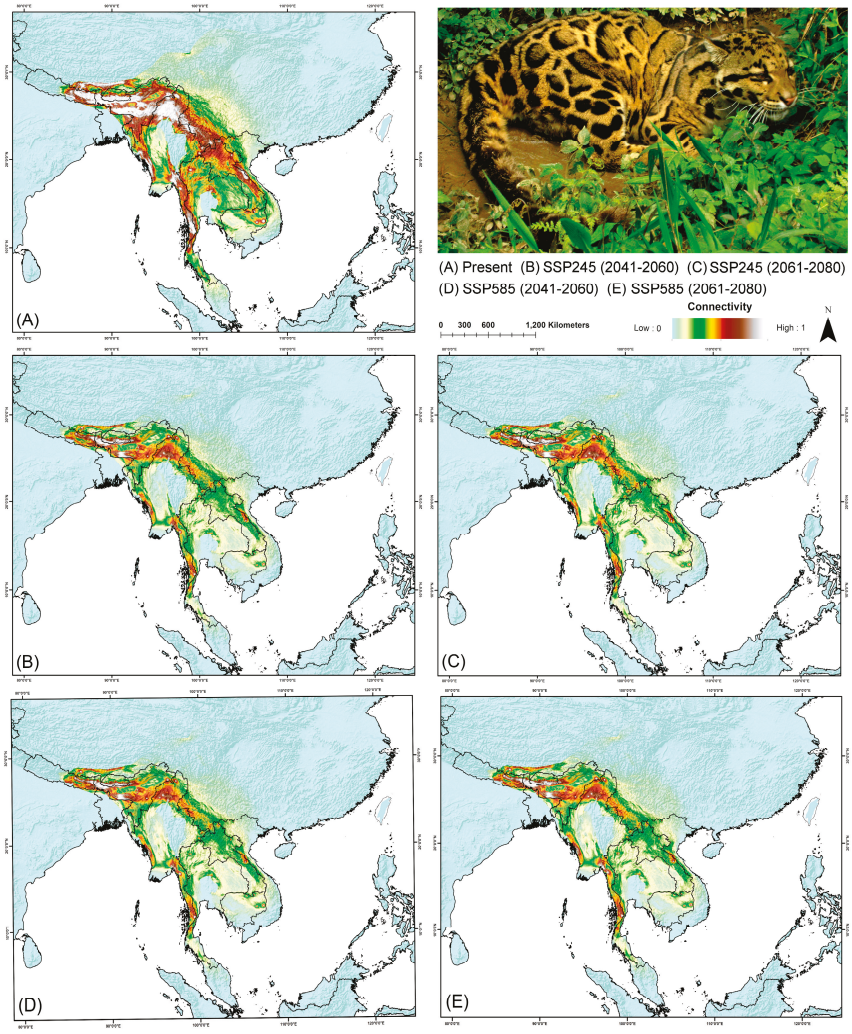

Figure 5. Map depicting (**A**–**E**) the connectivity of the clouded leopard across its entire IUCN range in Southeast Asia under current and future climatic scenarios. The photograph of the clouded leopard was taken by the first author (I.A.).

4. Discussion

Given the ongoing global biodiversity loss, terrestrial predator populations are increasingly impacted by both environmental changes and direct and indirect anthropogenic pressures [15]. In this context, identifying the extent of suitable habitat for carnivore species—considering bioclimatic, ecological, and human-induced factors—becomes essential for species conservation [27]. This study represents the first attempt to assess the impact of climate change on the habitat and corridor connectivity of *N. nebulosa* across its present and historical range. The findings of this study will contribute to the development of more effective and comprehensive management plans for the species, addressing the threats it faces in the future.

The forests of South and Southeast Asia are undergoing significant deforestation, and this ongoing habitat loss, combined with climate change, poses a substantial threat to the survival of *N. nebulosa* [88]. The model identified a strong affinity of this species for evergreen forest, which contributed 9.74%, making it the highest contributing habitat variable. This result corroborates previous field studies indicating that *N. nebulosa* predominantly inhabits evergreen forests over other vegetation types [33,89]. In contrast, the contribution of mixed/deciduous forest habitats was relatively low, accounting for only 2.10%, highlighting the species' specific habitat preferences. This finding underscores the species' distinct habitat preferences and aligns with previous research, which identified it as primarily an evergreen forest dweller [31]. Moreover, the low contribution may reflect the fact that sightings in deciduous or mixed forests are very rare and occasional, which further reinforces the conclusion that the species is largely confined to evergreen forest ecosystems. Among the bioclimatic variables, "Temperature Annual Range" (bio_7) was identified as the most significant factor, accounting for 37.37% of the model's contribution. This aligns with prior research highlighting the critical importance of this variable for medium-sized felids [47,48]. Additionally, previous studies have underscored the importance of primary forest habitats and topographic variables, such as slope, which also demonstrated a relatively high contribution in the assessed model. Additionally, elevation is another significant variable, as *N. nebulosa* is known to utilize higher altitudes to evade larger predators [90–92]. Hence, the present study meticulously revealed the importance of each variable in the distribution of this species in its range.

Among the four distinct categories in the IUCN designated ranges, the majority of this suitable habitat is concentrated within the extant (44,033 sq. km) and possibly extant ranges (20,034 sq. km). However, it is noteworthy that substantial portions of suitable habitat were also found within regions classified as possibly extinct (15,264 sq. km) and extinct (14,022 sq. km). This finding is particularly intriguing, as these areas may still hold ecological value and should be prioritized for field validation. Although these regions may not currently support any individuals of *N. nebulosa*, there remains the possibility that they offer the necessary habitat conditions. As a result, these areas could be considered potential sites for species reintroduction or relocation efforts, offering an opportunity to expand the species' range and ensure its long-term persistence. Furthermore, a significant portion of the identified suitable habitat falls within PAs, particularly under the extant range, emphasizing the critical role these areas play as key refuges for the species. The presence of suitable habitats within PAs highlights their importance for ongoing conservation efforts, as they provide secure environments free from human disturbance. However, this study also reveals that climate-induced habitat loss poses a severe threat to the species across all its ranges. The entire species range is expected to experience a considerable decline in suitable habitat due to future climate scenarios. Despite this, the extant range is projected to undergo the least severe reduction, with a decline of up to 27.71%. This emphasizes the urgent need to focus conservation efforts on these crucial habitats, as they represent the last strongholds for the species. Therefore, any additional human-induced impacts, such as deforestation or habitat degradation, could further deteriorate the precarious status of species, making immediate protection and conservation of these areas' paramount. These findings are consistent with previous research that has documented the significant decline in suitable

habitats and species ranges due to the ongoing loss of tropical forests in the region [93–95]. The countries that currently exhibit relatively high levels of habitat suitability for the species, such as Malaysia, Laos, and India, should be prioritized for conservation, as they represent critical areas for the species' survival in the future. However, these nations are also projected to experience substantial losses in habitat quality due to deforestation, land-use change, and climate change. The continued fragmentation of tropical forests in the region further complicates this scenario, as it isolates suitable habitats and increases the risk of population decline [96].

N. nebulosa is projected to face significant habitat fragmentation across its entire range, largely driven by climate-induced changes. This is evidenced by the anticipated decline in the number of viable habitat patches, as indicated by the reduction in NP in future scenarios. Furthermore, these patches are expected to decrease in size, as reflected by the declining LPI, and the density of occurrences will be much lower, as shown by the reduced PD. In addition to these changes, the patches will exhibit simpler shapes with less edge complexity, as indicated by decreases in TE and LSI. The fragmentation is further aggravated by the increased proximity of these patches to one another, as revealed by the decline in AI. Overall, the habitat patches are predicted to become smaller, more simplified, and more isolated, a concerning trend supported by previous field studies [97]. This increasing habitat fragmentation is likely to severely impact corridor connectivity for the species, limiting its ability to move between habitat patches and threatening its long-term survival within its range.

The present study identified 18 transboundary biological corridors for this species within its present and historical extent. Notably, the findings indicate that the highest mean corridor connectivity occurs between Bhutan and India, followed closely by the Nepal–India corridor. This elevated connectivity, particularly in the foothills of Bhutan, can be attributed to the presence of extensive, contiguous forested areas, many of which are protected under the administrations of both India and Bhutan. Specifically, areas such as Manas National Park, Royal Manas National Park, Phibsoo Wildlife Sanctuary, Raimona National Park, and Buxa Tiger Reserve, extending continuously toward Nepal, contribute significantly to this high connectivity, providing critical habitat for the species. In contrast, this study revealed that the lowest connectivity was found between Thailand and the Malay Peninsula. This may be largely due to physical barriers, such as the Isthmus of Kra, which interrupts habitat continuity in the region. Furthermore, it is important to highlight that these biological corridors are increasingly vulnerable to the impacts of climate change, habitat loss, and fragmentation. The loss of connectivity poses a substantial risk to this predator, which requires large, uninterrupted movement ranges for ecological activities [98]. Moreover, a significant portion of this decline in corridor connectivity is occurring in Southeast Asian countries, a region that is particularly susceptible to the combined effects of rapid climate shifts and escalating anthropogenic pressures [99,100]. Thus, this study underscores the urgent need for targeted conservation strategies in Southeast Asian countries to preserve these vital corridors in the face of increasing environmental threats.

Given the escalating threats facing *N. nebulosa* under both current and projected future conditions, there is an urgent need to implement a comprehensive, proactive, and landscape-level management plan to ensure the long-term conservation of this species and other colligated felids inhabiting its range [33,101]. In response, the present study advocates for the establishment of a detailed protection action plan at the national level, with an emphasis on strengthening transboundary cooperation. Such a collaborative approach is essential to effectively safeguarding this species across its range, particularly in regions where habitat and corridors span multiple countries, demanding coordinated conservation efforts between neighboring nations. A key aspect of this strategy involves expanding the network of PAs within the species' distribution. Moreover, various transboundary initiatives have already been implemented across this region for different wildlife species. These initiatives encompass a range of coordinated actions, including joint patrols, increased

community involvement, anti-poaching operations, intelligence-sharing mechanisms, and concerted efforts to maintain forest connectivity and reduce habitat fragmentation. Such integrated approaches have led to significant progress in reducing illegal activities such as logging and poaching while also facilitating the restoration of essential habitats and ecological corridors, which have, in turn, contributed to a notable recovery in wildlife populations. Some of the notable transboundary efforts in this specific region include the India–Nepal Terai Arc Landscape (TAL), the India–Bhutan Manas Tiger Reserve, and the Thailand–Myanmar Tenasserim Range. Hence, it is critical that potential habitats within regions classified as extinct or possibly extinct are ground-truthed through field validation to assess their suitability as future refuges for the species. This approach would not only help identify new conservation opportunities but also ensure that areas with high potential for recolonization or reintroduction are preserved. Furthermore, this study also highlights the alarming loss of corridor connectivity, which poses a significant threat to the species' genetic diversity. Moreover, recent research has highlighted that reduced genetic diversity, combined with the accumulation of harmful genetic mutations, is strongly associated with a decline in reproductive success [102]. Therefore, protecting and maintaining the biological corridors identified in this study is crucial for mitigating genetic isolation and ensuring the species' long-term viability. The preservation of these corridors would allow for the natural movement of individuals, which is essential for gene flow and the maintenance of genetically healthy populations. Furthermore, it is imperative to conduct ground-level assessments based on the results of the corridor connectivity between protected areas (PAs) and suitable patches to gain a clearer understanding of the movement pathways utilized by this species. The connectivity of these corridors may be disrupted by various anthropogenic factors such as urban development, agricultural activities, and the construction of roads, all of which require thorough evaluation to formulate site-specific conservation measures. Moreover, ensuring the protection of these critical movement corridors and initiating restoration projects where necessary is vital to maintaining habitat connectivity. Additionally, expanding and reinforcing these corridors by creating buffer zones can enhance the species' mobility, especially in the event of habitat interruptions, and improve the corridors' resilience to climate change. Such measures must consider climatic, geographical, and ecological aspects to ensure long-term effectiveness. A transboundary corridor management plan is also essential to maintain ecological continuity across international borders, allowing the species to migrate and adapt to shifting prey availability and changing climate conditions. Hence, the lessons learned from the successful implementation of similar strategies for other species (Yellowstone to Yukon Conservation Initiative in North America and Canada, the Tiger Corridor Project in India, and the Jaguar Corridor Initiative in South America) can provide valuable insights and inform conservation efforts for this species as well. In addition to the protection of corridors, safeguarding large, continuous forested areas is critical. The increasing prevalence of commercial logging in these regions is a serious concern, as it contributes to the fragmentation of critical habitats. Moreover, forest loss may drive the species out of its natural habitat, exposing it to higher risks of poaching or human–wildlife conflict. Therefore, efforts must be made to protect these forested areas from further degradation. Moreover, phylogeographic studies across various regions are crucial to assessing the genetic diversity of the species, ensuring the identification of distinct populations that can be strategically selected for breeding programs. Such assessments are essential for reintroduction efforts, as they will enhance genetic diversity and viability, thereby expanding the gene pool and improving the long-term resilience of the population. This genetic insight will aid in maintaining healthy, genetically diverse populations, which is vital for the species' adaptive potential and overall survival, especially in the context of habitat fragmentation and changing environmental conditions. Alongside these conservation efforts, it is equally crucial to actively involve local communities residing near *N. nebulosa* habitats through awareness campaigns aimed at reducing hunting and the illegal trade of this species. These programs should not only highlight the importance of biodiversity conservation but also promote alternative livelihoods, addressing the un-

derlying socio-economic factors that drive wildlife exploitation. The illegal trade of *N. nebulosa* body parts continues to be a major threat, with numerous records of confiscations. This underscores the need for increased vigilance by law enforcement agencies, particularly in monitoring and disrupting trade routes known to facilitate wildlife trafficking. Strengthened enforcement of wildlife protection laws, along with cross-border cooperation, is vital for combating illegal trade. Further, designating *N. nebulosa* as a flagship species could further enhance its conservation efforts by raising its profile and rallying public and governmental support. As a flagship species, *N. nebulosa* would not only benefit from increased attention and resources but also serve as an umbrella species, helping to protect other sympatric felids that share its habitat. This approach has been found effective in other cases where the designation of a flagship species has facilitated the conservation of entire ecosystems. Accordingly, there is an urgent need to identify a flagship species for evergreen forests, which could be served by designating the *N. nebulosa* as the flagship, which would lead to conservation efforts that also benefit sympatric species within the same habitat, fostering broader ecosystem protection. Therefore, prioritizing and implementing these measures could significantly enhance the conservation of this species within its range and help ensure its survival in the evergreen forests of South and Southeast Asia.

5. Conclusions

This study presents a thorough evaluation of the threats confronting *N. nebulosa* across its existing and historical ranges while also identifying potential suitable habitats within these areas. The research highlights significant challenges to the species' survival, including habitat loss, fragmentation, and reduced corridor connectivity, which are increasingly intensified by the effects of climate change. These issues are especially alarming in light of the escalating threats posed by biodiversity loss across the region, which not only jeopardize the survival of the clouded leopard but also imperil other sympatric felid species. Therefore, the findings underscore the urgent need for a coordinated and multifaceted conservation strategy to ensure the long-term protection of *N. nebulosa*. Furthermore, this study emphasizes the necessity of implementing practical measures to address the species' shrinking range, particularly considering its declining genetic diversity and increased vulnerability to inbreeding depression. Moreover, protecting viable forest patches alongside the corridors that maintain connectivity between them is critical to mitigating these threats. The findings and recommendations of this research offer essential resources for scientists and conservationists to implement targeted conservation strategies in the suitable areas delineated by this study, considering field validation across South and Southeast Asia. Moreover, this research establishes a robust foundation for developing a comprehensive conservation and management plan tailored to the unique needs of *N. nebulosa* populations throughout the region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology13110902/s1, Figure S1. Figure showing the correlation between the covariates chosen for the final model for clouded leopard; Figure S2. Evaluation Matrix performance across model runs for clouded leopard. Brown—represents the correlation coefficient among the five different models. Yellow—represents the proportion of deviance explained; Green—represents the proportion of correctly classified; Blue—represents the area under curve (AUC); and Pink—represents true skill statistics; Figure S3. Confusion matrixes, model calibration plots, and residual plots for clouded leopard. Row 1 represents a spatial pattern of residuals, where the color ramp indicates the magnitude of deviance and size represents the quantity. Row 2 represents the model calibration plot across all five different models for cross-validation split. Row 3 represents the confusion matrix for all five models, plotted by observed vs. predicted, where the color ramp from the lowest value of 0% (white) to 100% (red) indicates the quantification of particular pair types. Column a. represents plots for BRT, Column b. represents plots for GLM, Column c. represents plots for MARS, and Column d. represents plots for MaxEnt; Figure S4. The response curves of the covariates selected by each of the participating ensemble model for clouded leopard. (A) BRT, (B) GLM, (C) MARS, (D) MaxEnt, and (E) RF.

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Responses of Fish Zeta Diversity (ζ) to Human Pressure and Cumulative Effects: A Feasibility Study of Fishing Ban Measures in the Pearl River Basin, China

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Simple Summary: This study analyzed nearly four decades of fish data from China's Pearl and Yangtze Rivers to develop a new method for identifying areas that need conservation or restoration due to declining freshwater fish diversity. Researchers created a novel indicator that measures the combined impacts of human activities (fishing, agriculture, and urban development) on fish communities by examining how different species are distributed across the river basin and respond to various pressures. They found that fish communities in low-elevation areas are more severely affected by human activities than those in mountainous regions, with some areas maintaining unique fish communities that require immediate protection while others have become homogenized and need restoration efforts. This research provides a scientific framework for decision-makers to prioritize conservation areas, implement fishing restrictions where needed, and guide restoration efforts in degraded regions, offering an approach that can be applied to other river systems worldwide facing similar challenges from human development and declining fish populations.

Abstract: Amid declining fish diversity and human pressures in freshwater ecosystems, robust basin-scale assessments are vital for effective fisheries management. This study collated nearly four decades of fishery yields from the Pearl and Yangtze Rivers to identify conservation priorities in the Pearl River Basin. It introduced a novel cumulative effect indicator based on zeta diversity—a biodiversity pattern metric—integrated with cumulative effects analysis for management decision-making. The research employed a multi-site generalized dissimilarity model to examine the non-linear relationships between fish species composition (ζ_n) and human pressures, environmental factors, and geospatial variations across elevation gradients. The cumulative effect indicator, reflecting responses to anthropogenic stress when assessing ζ_2 (related to β diversity), helped evaluate basins for conservation or restoration needs based on their unique or homogenized biotic communities. The results suggest that ζ diversity in low-elevation sub-basins has a stronger filtering effect on ζ by human pressures than in mid- to high-elevation sub-basins, where community aggregation is more random. The impact varied with diversity aspects (nestedness vs. turnover) and zeta order. A negative correlation between cumulative effects and community uniqueness validated the novel cumulative effect indicator's effectiveness for guiding restoration in the Pearl River Delta, potential fishing bans, and karst conservation.

This approach offers a theoretical basis for prioritizing areas for freshwater fish diversity conservation and fishing restrictions in the Pearl River Basin.

Keywords: zeta diversity; beta diversity; cumulative effects; freshwater fish; human pressures; the Pearl River Basin; fishing ban measures

1. Introduction

Fish biodiversity is fundamental to human well-being [1]. More than half of the global GDP depends on the natural environment, undertaking the risk of disruption and devaluation [2]. Of these, fisheries, as a typical representative, cannot be neglected due to their contribution to global economic development [3]. Anthropogenic biodiversity decline has become a sequential focus of government and the public. Many of the multiple major drivers of fish species extinction and ecosystem dysfunction have become more prominent alongside economic development [4]. Fortunately, awareness of the conservation of natural systems and participation in conservation activities have been strengthened in past decades [5,6]. Notable initiatives include the establishment of over 18,000 marine protected areas globally, covering approximately 8% of ocean areas, the implementation of sustainable fisheries certification programs, such as MSC, which has certified over 400 fisheries worldwide, and international frameworks like the Convention on Biological Diversity that has mobilized conservation actions across 196 countries with measurable biodiversity targets. Indisputably, however, there is a massive divide between people's expectations for action to ameliorate fish biodiversity decline and the decision-making framework in the world, especially in developing countries [2,7].

Compared to developed countries, maintaining a balance between economic development and ecological protection is extremely difficult in developing countries when compensation and employment subsidies are absent [8–13]. Fishing management, even as a widely accepted policy worldwide, has triggered conflicts between ecological and economic performance in underdeveloped areas. Following the fishing management law, fishermen could not obtain alternative incomes [14,15]. The Pearl River Basin, similar to the Yangtze River Basin, has experienced extremely heavy perturbations and human stress from anthropogenic activities during the past decades as a consequence of the development of the regional economy [16]. These anthropogenic stressors include intensive industrial development (particularly manufacturing and petrochemical industries, contributing >60% of regional GDP), rapid urbanization, with population density exceeding 500 people/km^2 in delta regions, extensive infrastructure construction, including dam building (over 100 large dams constructed since 1980s), agricultural intensification, with 45% land conversion to cultivated areas, and sand mining activities that have extracted >1.5 billion tons annually, collectively contributing to habitat fragmentation, water quality degradation, and species displacement, with fishing pressure accounting for approximately 15–20% of the total biodiversity impact. Therefore, the Pearl River Basin, one of China's significantly and quickly developing economic zones, provides the possibility to apply fishing management or bans for sustainable development of fisheries and conservation of fish biodiversity.

The 10-year fishing ban was implemented in the Yangtze River Basin in 2021 [17]. Meanwhile, the Yangtze River Protection Law was enacted in 2021 to restore the river further [18]. These are crucial for the government and the public in protecting fish biodiversity and environmental protection, and a concession of economic development to natural restoration. Notably, as an excellent fish biodiversity basin in China, sustainable fisheries

and fish biodiversity security in the Pearl River Basin have undertaken the sustained stress from overfishing and anthropogenic perturbations [7,19]. Owing to the lack of effective fisheries management policies and enforcement measures, the fishing ban policy in the Pearl River Basin remains insufficient and stagnant. One of the most important reasons for the underdeveloped policy is that it is not supported by adequate scientific investigation. The potential policies and Pearl River Protection law being proposed by the Chinese government would be the first significant step to achieving major sustainability objectives of target ten of the Kunming-Montreal Global Biodiversity Framework and the goal of the Sustainable Development Goals (SDGs) of the United Nations [20–23].

To address these challenges, several analytical approaches are essential for assessing biodiversity changes and informing conservation priorities in river basins. Beta diversity assessment, particularly through Local Contributions to Beta Diversity (LCBD), provides crucial metrics for identifying sites with unique species compositions that require conservation attention [24,25]. Sites with high LCBD values indicate either unique ecological conditions worth preserving or degraded areas experiencing distinct species loss patterns, thus serving as early warning indicators for ecosystem health decline [24]. For instance, a river reach with elevated LCBD may signal localized pollution impacts or habitat fragmentation that has altered its aquatic communities' assemblage compared to neighboring sites [26,27]. Advanced methods, such as multi-site generalized dissimilarity models (MS-GDMs), enhance our understanding of how environmental and human pressures influence species turnover across various sites by utilizing zeta diversity (ζ_n) metrics [28,29]. MS-GDM provides a robust theoretical framework for spatial inference by capturing non-linear responses of species compositional turnover to environmental gradients, while zeta diversity quantifies the shared species patterns across multiple sites simultaneously, making their combination particularly suitable for identifying biodiversity hotspots and cumulative impact zones across heterogeneous landscapes. This synergy enables spatially explicit conservation prioritization by linking mechanistic understanding of species responses with multi-site community patterns [27].

These approaches help distinguish between the impacts on common species that support ecosystem functions and rare species that face greater extinction risks. Recent international studies have demonstrated the effectiveness of zeta diversity in watershed conservation prioritization, from identifying critical wetland complexes that promote biodiversity [30] to revealing metacommunity assembly processes in freshwater systems through functional zeta diversity [31]. Additionally, cumulative effect assessments that quantify the combined impact of multiple human pressures are increasingly vital for effective ecosystem management [32,33]. Unlike traditional indices (α or β diversity) that focus on single-site patterns or pairwise comparisons, our cumulative effect indicator uniquely integrates non-linear responses of species turnover to multiple stressors through I-spline transformations, enabling managers to identify areas requiring conservation versus restoration based on their vulnerability to anthropogenic impacts [27]. By integrating MS-GDMs with LCBD, we can develop metrics based on species turnover that offer novel perspectives for managing biodiversity under multiple stressors [27,34,35].

In this study, nearly 40 years of freshwater fisheries production in the Pearl River are collected, fish species turnover is assessed by MS-GDM in response to human pressures and environmental and geospatial differences between sub-basins, and cumulative effect indicators are developed based on the sum of responses to human pressure variables. Therefore, the main aims of this study are to (1) investigate the situation of fishery productively during the past 40 years and to measure the difference in productivity between the Pearl River and Yangtze River Basin, (2) focus on the impacts of different human pressures on species com-

positional turnover in the Pearl River Basin through the quantification of fish community responses, and (3) compare LCBD of the Pearl River Basin and cumulative effect indicators, combining with a non-linear response, to determine the correlation between a particular species composition and cumulative effect. The present study systematically gathers data on fisheries economics and fish biodiversity in the Pearl River Basin over the past 39 years, which provides a better understanding of the practical and theoretical basis for fisheries' resilient and sustainable development.

2. Methods

2.1. Study Area

The Pearl River Basin is located at 102°15′~115°35′ E, 21°50′~26°48′ N, with an area of 45.4×10^4 km^2, annual average temperature of 14 °C~22 °C, annual average precipitation of 1200 mm~2000 mm, and annual average runoff of 0.338×10^{12} m^3. The annual runoff is more than 3.49×10^{12} m^3, ranking second in China's river system [36]. The unique natural conditions of the Pearl River Basin (karst landform), including extensive underground river systems, varied water chemistry with high calcium carbonate content, diverse microhabitats, such as caves and sinkholes, and fragmented hydrological connectivity that creates isolated aquatic environments favoring speciation and endemism, breed a wealth of freshwater fish whose diversity, endemic species, and endangered species rank first in China [19]. Since 2017, the fishing ban has been extended in the Pearl River Basin, and the fishing ban has been implemented from 1 March to 30 June every year [14]. Despite this, frequent utilization of hydraulic resources, thousands of years of agricultural development history, and high modern population density have caused a substantial disturbance of the freshwater fish ecosystem in the Pearl River Basin [37,38].

The sub-basins' division was screened according to the following process: First, the "Near Analysis" toolbox in ArcGIS10.2 was used to correct the occurrence locations of all species to the water area. Next, a buffer was established for each occurrence point for all species [39,40]. Buffer distances for each occurrence point for each species were estimated based on fish dispersal capacity. Functional characteristics (i.e., full length and caudal fin aspect ratio) related to dispersal ability were extracted from the FISHMORPH database [41] and FishBase [42] for each species. These specific traits were selected as dispersal indicators because body size (full length) is strongly correlated with swimming endurance and active dispersal capacity in freshwater fish, while the caudal fin aspect ratio determines swimming efficiency and burst speed, which are critical for upstream migration and lateral movement across different habitat types [40]. The river grade at each occurrence point was also extracted from HydroRIVERS [43]. The buffer distance of each occurrence point was estimated with the R package 'fishmove' [40]. The estimated diffusion distance was used as the radius to construct a buffer for each occurrence point. Then, the buffer zone was superimposed with the sub-basins of level 7 (the average area was 3.3×10^3 km^2) in the HydroBASINS [43]. Level 7 was used because coverage at the point of occurrence was estimated to be lower at finer scales, namely levels 8–12, according to the compiled database. Moreover, based on estimates of dispersal capacity and existing studies, fish community composition varied considerably at higher levels (i.e., levels 1–7) [39,40]. However, larger sub-basins, such as level 6, may include more complex water body types, making it difficult to distinguish the effect of receiving water body types on the cumulative human pressure. For sub-basin clipping, we took the sub-basins where the occurrence point existed as the distribution range of the species, and those upstream or lower sub-basins of the occurrence point overlaps with the buffer zone were also listed as its distribution sub-basins. The ζ diversity of freshwater fish sub-basins (n = 137) of the Pearl River Basin was then assessed (Figure 1).

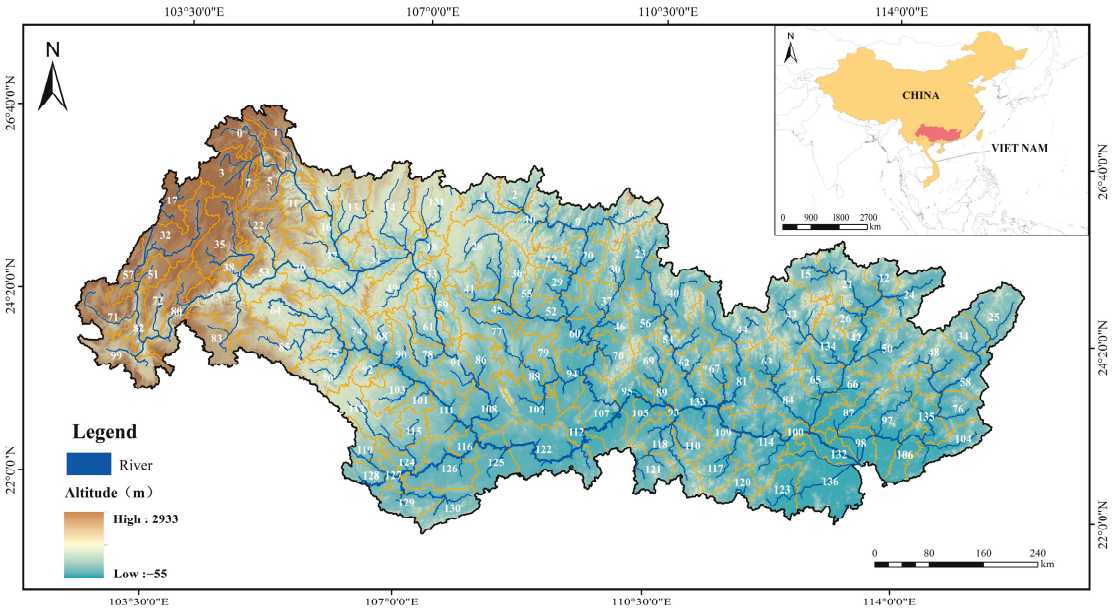

Figure 1. The sub-basin division map of the Pearl River Basin. The numbers (0~136) represent the identification numbers of different sub-basins, and the red area is the study area of this paper.

2.2. Fish Occurrence Records

A comprehensive review of scholarly sources was meticulously carried out across several repositories, encompassing Web of Science, Scopus, and CNKI, with an emphasis on monographs, digital repositories, such as FishBase and GBIF, alongside grey literature. Emphasis was placed on Chinese publications to encapsulate the full spectrum of native ichthyofauna in the Pearl River Basin, enhancing the breadth of the investigation. The initial inquiry was launched in October of the year prior to last (2022), with a subsequent update in the spring of the following year (May 2023). Adherence to the PRISMA guidelines ensured a structured and transparent selection process [44]. The resulting dataset underwent rigorous validation by two seasoned ichthyologists, with any dubious entries being expunged. This inquiry also gauged sample sufficiency across each sub-basin to pinpoint data-deficient regions and standardize inter-basin comparisons. Sub-basins with only one survey location or one species recorded were first removed, and sample coverage based on species richness ($q = 0$) was calculated on raw incidence data with extrapolation to two times the sample size. Only sub-basins with at least 90% coverage were retained for analyses, as this threshold ensures adequate representativeness of species communities while maintaining sufficient statistical power [27], yielding a final set of 14,308 with native fish occurrence records. Temporal observations within these basins spanned from 1984 to the present year. The study encompassed 579 fish taxa, of which 537 were native (both freshwater and diadromous), with the IUCN recognizing 200 as threatened (VU, EN, and CR) within the basin. Moreover, 78 taxa have been accorded national protection status under Classes I and II. The remaining taxa consisted of 35 non-native species to the Pearl River ecosystem and 7 species classified as extinct (EX) by IUCN assessment—these were consequently excluded from the species pool in the subsequent analyses. The prevalence of non-native species served as a surrogate for anthropogenic pressure, aiding in the examination of native biodiversity patterns.

In addition, this study collected fishery statistics data from the provinces (autonomous regions or municipalities directly under the Central Government) in the China Fishery Statistical Yearbook (1981–2022). According to the proportion of the Pearl River Basin in

the waters of the provinces, the freshwater fishing output, fishery labor force, the number and engine capacity of fishing boats, the intensity of fishery supervision (measured by the number of workers and employees of fishery administration units and the number of fishery administration units), and the annual per capita income of fishermen in the Pearl River Basin were calculated. At the same time, the catch fishery and fish diversity in the Yangtze River Basin were compared with that in the Pearl River Basin, and the changes in the catch fishery in the Pearl River Basin in the past 40 years were analyzed. Based on the changes, we determined whether the Pearl River Basin has priority in the conservation of freshwater fish species among major river basins in China.

2.3. Variable Collection and Processing

In the pursuit of comprehending the spatial distribution of freshwater ichthyofauna, this investigation incorporated a multifaceted environmental data compendium. The environmental dataset included 19 environmental layers (raster data), including forest coverage, normalized vegetation index (NDVI), enhanced vegetation index (EVI), average light intensity, NPP vegetation primary productivity, evapotranspiration, and leaf area index. Human stress variables (GDP, fishing pressure, population density, mean fluctuation, road network density, water network density, arable area share, grassland area share, building area share, unused land area, and riparian disturbance (as a percentage of total land cover change within the 30 m riparian zone)), the number of large power stations, and 19 bioclimatic factors were extracted from Chelsa-climate (http://chelsa-climate.org, spatial resolution of 30 arc-seconds, accessed on 29 June 2023). Average annual fishing pressure data (t/km^2) were obtained from the local fishery statistics yearbooks of each province and city for the last five years, weighted by the area of freshwater in each sub-basin per unit basin area. Prior to analysis, all environmental and climatic raster layers were standardized to a 1000 m resolution through cropping, resampling, and averaging processes, aligned within each sub-basin to ensure analytical precision. Moreover, spatial delineations of hydrological confines and geospatial attributes were assimilated, bolstering the predictive capacity of the model and facilitating a comparative assessment of these determinants against anthropogenic stressors, as proposed by Iacarella [27].

Recent research has elucidated that climatic gradients and elevational variance significantly influence beta diversity among freshwater fish across extensive spatial dimensions [45]. Ecological predictors, such as arboreal density, foliar biomass indices, and spectral vegetation signatures (NDVI and EVI), along with hydric flux and primary productivity metrics, have been validated as reliable proxies for forecasting ζ diversity in both flora and fauna [35,46]. In the context of the Pearl River Basin, anthropogenic disturbance metrics have demonstrated a robust correlation with species richness, echoing findings that underscore the pivotal role these stressors play in modulating species turnover within aquatic habitats [27,47]. Direct quantification of anthropogenic impacts on fish communities, such as those arising from increased fishing effort and fluvial habitat disruption, remains a formidable challenge in landscape ecology, particularly over expansive geographical scales [27]. Consequently, surrogate landscape indices of human disturbance are frequently employed as indicators of the cumulative effects on ichthyological communities and their habitats. These indices have proven instrumental in delineating patterns of fish diversity [27,35]. For this study, an evaluation of 19 anthropogenic pressure variables was conducted. Only those exhibiting weak correlation ($|r| < 0.6$) were retained for further analysis. The selected variables encompassed fishing pressure, GDP, surface temperature, leaf area index, proportion of arable land area, riparian disturbance, and number of power stations with generation capacity > 50 MW. Additionally, the prevalence of alien species

was considered a distinct variable indicative of human-induced stress, given the Pearl River Basin's status as a hotspot for ichthyological invasions, which significantly influences the distribution of endemic fish species [47,48]. Euclidean distances between sub-basins were computed using spatial coordinates to assess the influence of geographical separation on community heterogeneity. The extent of geographical dispersion was found to correlate with community divergence. Utilizing QGIS3.8, the area and centroid coordinates of each sub-basin were calculated, with subsequent logarithmic transformation to meet assumptions of normality. A principal component analysis (PCA) was then applied to 19 bioclimatic predictors to distill the environmental variables, considering their inherent collinearity (refer to Figure S1 for the correlation matrix). PCA facilitated a reduction in environmental predictors, thereby sharpening the focus on anthropogenic stressors. The first two principal components accounted for 88.7% of the variance, with the mean temperature of the warmest quarter ($|r| = 0.97$) and annual temperature range ($|r| = 0.95$) exhibiting high correlation. Consequently, these temperature metrics were utilized in the diversity analysis. Ultimately, the selected variables representing human pressure, environmental conditions, and geographical space were confirmed to be independent ($|r| < 0.6$), as this threshold ensured effective multicollinearity reduction while preserving ecological information content.

2.4. Sub-Basin Level Analyses

Elevational gradients have been identified as pivotal determinants of ζ diversity within lotic ichthyological communities [27]. To elucidate this relationship, our analysis stratified sub-basins into elevation-based cohorts, encompassing the entire spectrum of basins. Employing a k-means clustering approach, we delineated sub-basins into three to five clusters, achieving homogeneity in elevation without succumbing to model overfitting, as indicated by the high proportion of variance explained (91.4–96.6%; refer to Figure S2). The selection of an optimal cluster count was informed by the Ward's minimum variance method, applied within a hierarchical clustering on principal components framework ('FactoMineR' package in R; [49]), which substantiated the superiority of a tripartite division. Consequently, three elevation-based clusters were constituted to streamline the complexity of downstream analyses. These clusters were demarcated into high (1458–2116 m, $n = 19$), intermediate (711–1302 m, $n = 23$), and low elevation (4–691 m, $n = 95$) bands, with each band's human stress factors, environmental variables, and geospatial characteristics being evaluated within its respective altitudinal context (as depicted in Figure S3). Despite the notable difference in sample sizes across elevation bands, particularly the smaller number of high-elevation sub-basins ($n = 19$) compared to low-elevation sub-basins ($n = 95$), statistical validation through bootstrap resampling confirmed the robustness of our results, indicating that this sample size disparity did not significantly compromise the credibility of our findings.

Disaggregating ζ diversity, inclusive of its β-diversity component (ζ_2), into facets of species turnover and nestedness offers critical insights for biodiversity management strategizing. Species turnover encapsulates the concept of distinct species assemblages between habitats, whereas nestedness implies that species-poor communities represent subsets of more diverse ones along a biotic gradient [50]. Understanding the proportional influence of these components on overall zeta diversity is instrumental for prioritizing conservation efforts, whether safeguarding ecologically unique locales or bolstering sites of pronounced local species richness to preserve regional biotic diversity [50,51]. The initial step involved quantifying total zeta diversity via the Sorensen index, subsequently partitioning it into components of species turnover (measured by Simpson's index) and

nestedness (as derived from the Sorensen–Simpson relationship), to appraise their relative significance both basin-wide and within specific elevational strata ('adespatial' package in R; [50,52]). The analytic continuum integrated both Sorensen and Simpson indices to encapsulate total ζ diversity and its turnover aspect. To elucidate the variance in ζ diversity of lotic fish fauna across the Pearl River Basin as a function of environmental and geographical heterogeneity among sub-basins, a multi-site generalized dissimilarity modeling approach was employed ('zetadiv' package in R; [53]). The characteristic decay of zeta diversity with ascending zeta orders offers insights into community assembly processes—whether stochasticity (exemplified by an exponential decay) or environmental filtering (indicated by a power-law relationship) predominates, with these dynamics potentially shifting across different orders [54,55]. Thus, the MS-GDM framework was harnessed to compute zeta diversity across various orders for all sub-basins and within each elevational tier. Commencing with the average zeta values for consecutive orders, we then applied linear regression fitting and power-law modeling to the logarithmically transformed data, selecting the most parsimonious model for the decline in zeta diversity with increasing order based on the Akaike information criterion (AIC).

In this study, ecological modeling was conducted utilizing Sorensen and Simpson diversity indices, taking into account a suite of 12 environmental predictors across the examined basins. For the Sorensen index, ζ diversity was normalized through the ratio of shared species across n sub-basins to the mean species richness within these sub-basins (ζ_n/ζ_1, with ζ_1 representing mean species richness per sub-basin). Conversely, the Simpson index was normalized against the minimum species richness encountered in the sub-basins ($\zeta_n/\min(\zeta_1)$) [56]. The multi-site generalized dissimilarity modeling (MS-GDM) employed generated non-linear, monotonic interpolations, specifically I-splines, normalized between 0 and 1 for each variable after adjustment (normalization achieved by subtracting the minimum and scaling to the range). Each I-spline curve delineated a cumulative impact metric for the sub-basins, aggregating all human pressure indices into a weighted sum, wherein the weights were informed by the non-linear impact of anthropogenic stress on ichthyofaunal compositional turnover [27]. These cumulative impact metrics served as indicators to pinpoint fish communities less influenced by human activities, potentially flagging areas of significant conservation interest, as well as those more heavily impacted, thus highlighting regions where restoration should be prioritized [23,57]. The apex of an I-spline curve signifies the overarching relative importance of a variable, while the slope variations within the curve indicate the range over which the variable most significantly impacts ζ diversity. To ensure robustness, each model iteration was conducted 30 times, with MS-GDM randomly sampling 1000 combinations of sub-basins for each iteration. This randomization allowed for consistent sub-basin sample sizes across both elevational clusters and zeta orders. Model performance was evaluated by the Pearson *r*, comparing observed to predicted values, to quantify the explanatory power of the MS-GDM [28,54,56].

The utility of the I-spline curve's cumulative effect diminished when the synthesized zeta values across multiple sites exhibited minimal variability or when the model's incorporated pressure variables failed to capture such variability, thus undermining the effective delineation of areas necessitating management intervention [27]. Within the lower-altitude sub-basins, the high explanatory power of the model variables and the pronounced fluctuation of zeta values between orders 2 and 3 (see Figure S4) guided the selection of Simpson diversity ζ_2 for juxtaposition with paired Local Contribution to Beta Diversity (LCBD) indices. This approach facilitated a comparative analysis of anthropogenic impact with sub-basins' compositional uniqueness to evaluate the appropriateness of these metrics for guiding management priorities. The distinctiveness of sub-basins' composition within each

elevational band was quantified using the Local Contributions to Beta Diversity based on Simpson's diversity (utilizing the 'adespatial' package in R; [52]). Subsequently, for each sub-basin situated at lower elevations, cumulative effect estimates were juxtaposed with LCBD values to unravel the interplay between these two metrics. In this analysis, LCBD values were treated as the dependent variable, conforming to a normal distribution, while the cumulative effect served as the independent variable in a linear regression model to determine their relationship. All statistical procedures were conducted using R version 4.3.0, with analyses employing packages including fishmove, zetadiv, adespatial, FactoMineR, and base R functions. Independent sample *t*-tests were used to compare means between river basins after confirming normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) assumptions.

3. Results

The number of fish and threatened species in the Pearl River Basin was twice that in the Yangtze River Basin (Figure 2a). Although the fishery labor force per unit (Figure 2b) and the number of motor fishing vessels per unit (Figure 2c) were much lower than those in the Yangtze River Basin, the fishery output per unit of the freshwater fishery (Figure 2a) and the intensity of fishery supervision (Figure 2d) had no significant difference from those in the Yangtze River Basin through the independent sample *t*-test ($p > 0.05$, Cohen's d < 0.2, indicating negligible effect sizes). In addition, the annual per capita income of fishermen has been gradually lower than the national per capita income since the beginning of the 21st century (Figure 2e), which indicates that the freshwater fishery status of the Pearl River Basin and the Yangtze River Basin are also facing severe threats from fishing. Because of the diversity of freshwater fish and the particular freshwater environment, it is urgent to take further protective measures.

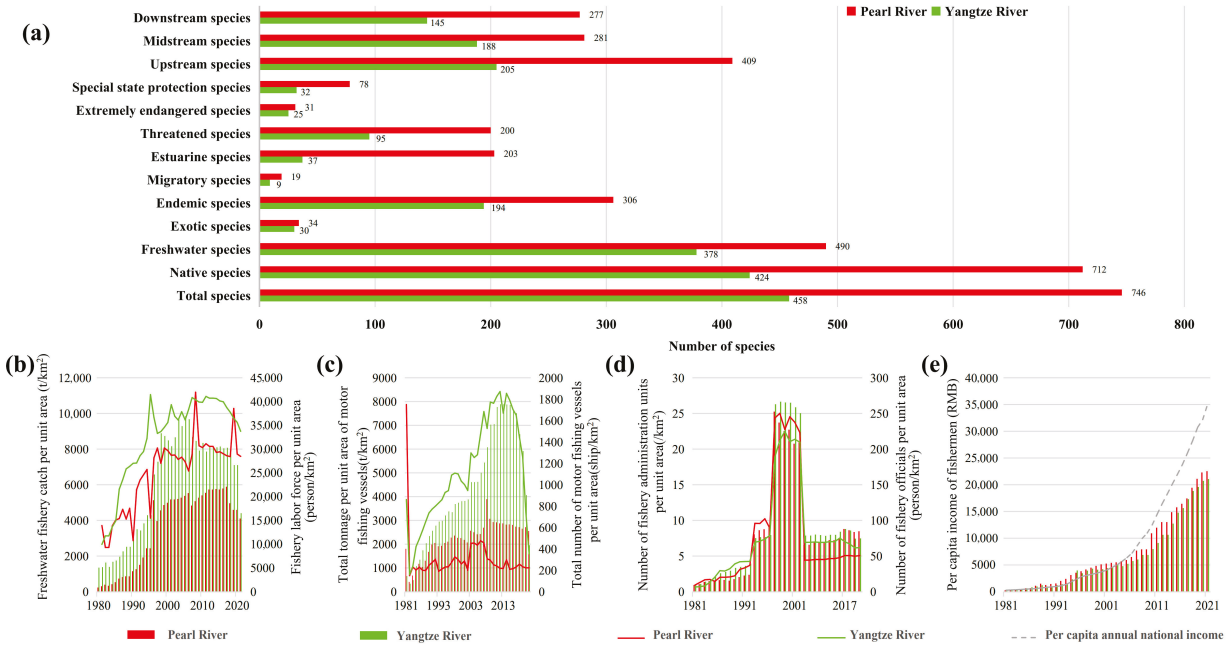

Figure 2. Comparison of freshwater fishery status in the Pearl River and Yangtze River Basin in the last 40 years. (**a**) The number of fish species in the basin. (**b**) Unit catch yield of freshwater fisheries and fishery labor force. (**c**) The number and tonnage of motor fishing vessels per unit. (**d**) The number of staff of fishery administration units and the number of fishery administration units. (**e**) Annual per capita income of fishermen and national per capita income. The legend on the left *Y*-axis is the polyline, and the legend on the right *Y*-axis is the column.

According to the classification of β diversity, the contribution of species turnover to the total β diversity was much greater than that of nestedness (turnover accounted for 78.32%, Sorensen = 0.36). High-altitude and middle-altitude species turnover accounted for 88.51% (Sorensen = 0.42) and 79.27% (Sorensen = 0.32), respectively, while low-altitude species turnover (47.21%) and nestedness (52.79%) contributed equally to β diversity (Sorensen = 0.31).

Analysis of zeta diversity metrics revealed a notable contraction in the number of shared species across sub-basins to fewer than 60 when the comparison extended beyond 2 sub-basins (zeta order ≥ 3), encompassing the entire basin, as well as within mid- and high-elevation sub-basin groupings (refer to Supplementary Table S1 and Figure S4). In contrast, low-elevation sub-basins exhibited a higher degree of species overlap, with a minimum of 60 species commonly found in comparisons involving up to 14 sub-basins. Furthermore, the observed patterns of zeta diversity reduction across the gradient from high to low elevations suggested an increasing influence of environmental filtering on the structuring of aquatic communities (as shown in Table 1). Across the entire basin and within individual elevational basins, both exponential and power-law models were fitted to the data, with the power-law model demonstrating superior fit. This pattern indicates that community assembly was predominantly influenced by environmental filtering, as evidenced consistently across the first three zeta orders.

Results from the model showed an increase in the percentage variance of variable explanations from high- to low-altitude sub-basins, consistent with an increase in filtering in low-altitude environments, as shown by the decline in zeta diversity (Tables 1 and 2). The variance rate of model interpretation for low-altitude sub-basins increased with the increase in the number of basins at orders 2–4, Sorensen and Simpson diversity begin to decline at orders 6 and 5, respectively, and then the sets became more random (only the results of orders 2–4 are provided for the sake of simplicity in the subsequent analysis). The variance explained by Sorensen was also generally higher than Simpson's diversity (Table 2). The contrasting patterns between exponential and power-law fits across elevation gradients (Table 1) combined with variable explanatory capacity (Table 2) highlight the differential sensitivity of Sorensen and Simpson indices. The higher explanatory power observed for Sorensen diversity (e.g., 65.40% for all sub-basins vs. 62.19% for Simpson) reflects its greater sensitivity to species richness differences, making it more effective for detecting comprehensive biodiversity patterns across environmental gradients. Conversely, Simpson diversity's relatively stable performance across elevation bands (ranging from 50.98% to 56.16%) indicates its reduced sensitivity to richness variations, allowing it to better capture true compositional turnover patterns for targeted restoration strategies in degraded areas.

Mean I-splines of MS-GDMs were shown for Simpson diversity for all sub-basin groups (Figures 3 and 4), as well as for Sorensen diversity for low-elevation watersheds (Figure 4), as beta diversity partitioning indicated an equivalent influence of turnover and nestedness only at low elevations. Among the components and orders of zeta diversity, climate factor, human pressure, and geospatial variables all ranked at the top of the maximum I-spline value, indicating that the above variables had relatively important influences on zeta diversity (Figures 3 and 4). For all sub-basins, the largest driver of species turnover was the mean altitude, followed by riparian disturbance, geographic distance, annual temperature range, and proportion of cultivated land area. In contrast, the fishing pressure contributed minimally to the turnover component of zeta diversity (Figure 3a). In high-elevation sub-basins, geographical distance and the number of alien fish were the main drivers of turnover, which had a greater effect on composition, mainly

when rescaled variables were low (<0.5), followed by water network density and fishing pressure at medium–high values (Figure 3b). The proportion of cultivated land area and water network density greatly affected the middle-elevation sub-basins (Figure 3c). In addition to geographical distance, the most significant driving factors in the low-altitude sub-basins were the fishing pressure, annual temperature range, and the number of alien fish (Figure 3d). The total zeta diversity at low altitudes varied greatly among different basins for orders 2–4, such as the fishing pressure, geographical distance, number of alien fish, annual temperature range, and mean annual runoff (Figure 4a,c). These variables were also crucial for the turnover of low-altitude basins. This suggests that the fishing pressure, geographical distance, the number of alien fish, annual temperature range, and the mean annual runoff mainly influenced the turnover component of the total zeta diversity (Figure 4a,c). The LCBD, composed of unique species, quantified important sub-basins within each elevation (high, middle, and low) of the Pearl River Basin (Figure 5). In addition, the lower cumulative environmental effect measured the higher LCBD and the fewer unique communities of the basin with a greater cumulative effect on beta diversity ($r = 0.88$, $t = -5.058$, $p < 0.01$; Figures 5 and 6).

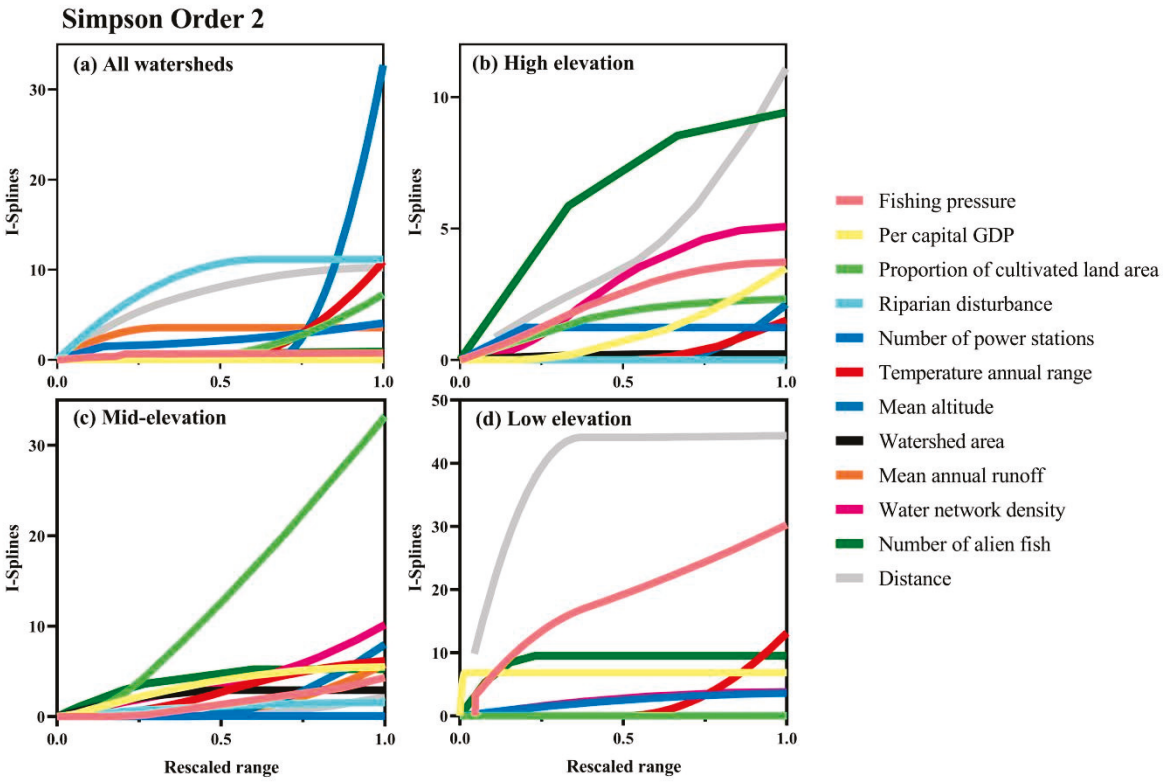

Figure 3. Depicts the average I-splines derived from MS-GDM analyses to illustrate the influence of anthropogenic pressures, environmental conditions, and geospatial attributes on species turnover, as assessed by Simpson zeta diversity among paired sub-basins ('order 2'). This is presented for (**a**) the collective set of sub-basins, as well as segregated by (**b**) high-, (**c**) medium-, and (**d**) low-elevation sub-basins. The apex of the I-spline curve signifies the relative significance of each variable in elucidating zeta diversity, while the slope's fluctuation reflects the changing pace of community dissimilarity across the variable gradient. The x-axes represent the normalized range of variables from 0 to 1. Notably, elevation was exclusively incorporated in the models for the comprehensive sub-basins analysis (**a**).

Table 1. The fitting of zeta diversity declines using exponential and power-law models across ascending zeta orders for both the collective sub-basins and those categorized by elevation. The model providing a superior fit was identified by an Akaike information criterion (AIC) value that was at least 2 units lower (indicated in bold). Instances where a shift in the fitting model occurred across different orders are highlighted. An exponential fit is suggestive of stochastic processes governing community assembly, while a power-law fit implies a deterministic community structuring influenced by environmental filtering mechanisms.

Sub-Basins Group	Order of Zeta	Exponential Coefficient			Power-Law Coefficient		
		b	*p*	AIC	*c*	*p*	AIC
All	1–3	−0.11	0.15	−8.16	−0.49	0.06	**−13.98**
High	1–3	−0.08	0.22	−8.66	−0.34	0.11	**−12.44**
	1–4	−0.05	0.09	−11.18	−0.29	0.03	**−15.90**
Mid	1–3	−0.11	0.13	−9.22	−0.47	0.04	**−16.98**
Low	1–3	−0.09	0.16	−9.31	−0.38	0.06	−14.71
	1–4	−0.07	0.06	−11.65	−0.35	0.01	**−18.49**

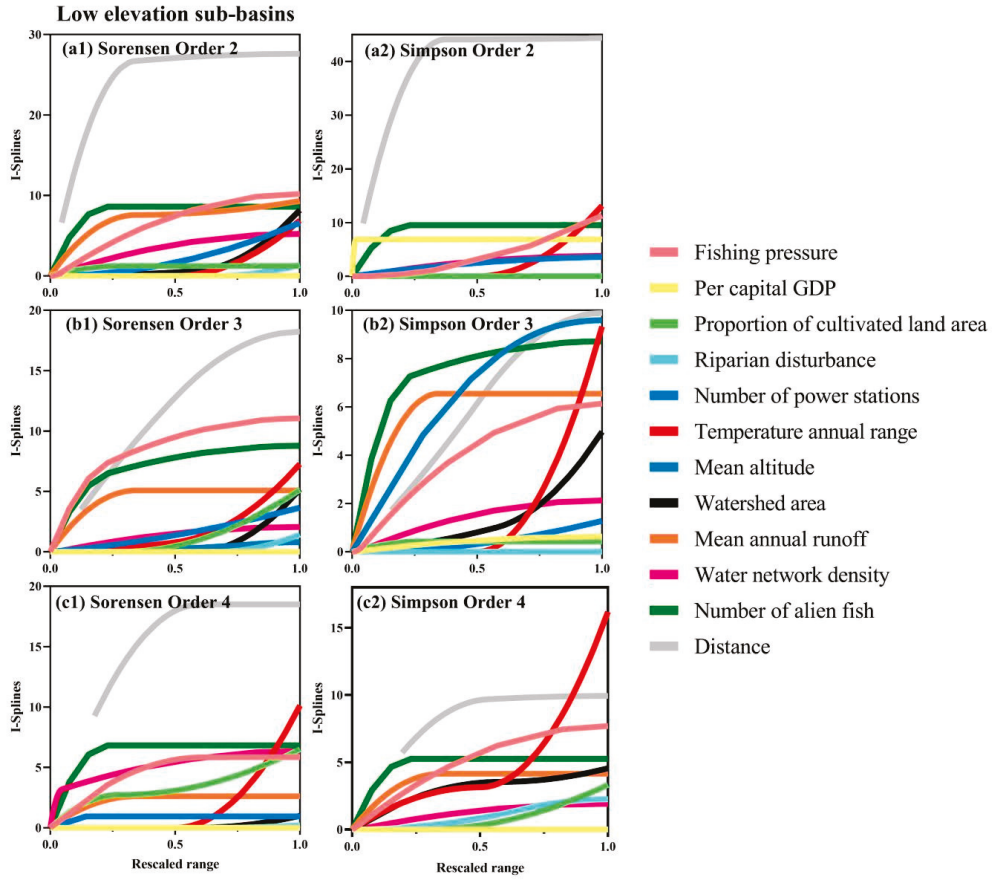

Figure 4. This figure illustrates the mean I-splines generated through MS-GDM, which quantify the impacts of anthropogenic, environmental, and geospatial factors on (**1**) the aggregate zeta diversity ('Sorensen') and (**2**) the species turnover ('Simpson') within low-elevation sub-basins across zeta orders (**a**) 2, (**b**) 3, and (**c**) 4. The peak value of each I-spline curve denotes the comparative contribution of the variable to the explanation of zeta diversity, while alterations in the slope's trajectory reveal shifts in the rate of community dissimilarity across the variable's spectrum. Pronounced I-splines for 'Sorensen' without corresponding 'Simpson' effects highlight influences on the nestedness aspect of zeta diversity. In contrast, I-splines associated with 'Simpson' delineate impacts on the turnover facet. The x-axes present the variables normalized from 0 to 1.

Table 2. The proportion of variance elucidated (as indicated by the squared Pearson correlation coefficient, r), attributed to anthropogenic pressures, environmental factors, and geospatial determinants, as computed by MS-GDM. This encompasses the comprehensive zeta diversity ('Sorensen') and species turnover dynamics ('Simpson') for the second zeta order across all sub-basin clusters, as well as for ascending orders within low-elevation sub-basin groups. The reported values represent the mean ±1 standard deviation derived from 30 iterations of the model.

Sub-Basin Groups	Order of Zeta	Sorensen, *r*	Simpson, *r*
All	2	65.40% ± 5.95%	62.19% ± 6.67%
High	2	57.18% ± 8.04%	56.16% ± 5.14%
Mid	2	55.87% ± 5.25%	50.98% ± 5.61%
Low	2	55.56% ± 9.64%	54.79% ± 7.36%
	3	63.19% ± 6.63%	63.14% ± 6.12%
	4	66.99% ± 5.99%	65.61% ± 9.49%
	5	68.18% ± 7.86%	64.86% ± 6.62%
	6	67.17% ± 5.97%	63.39% ± 5.96%

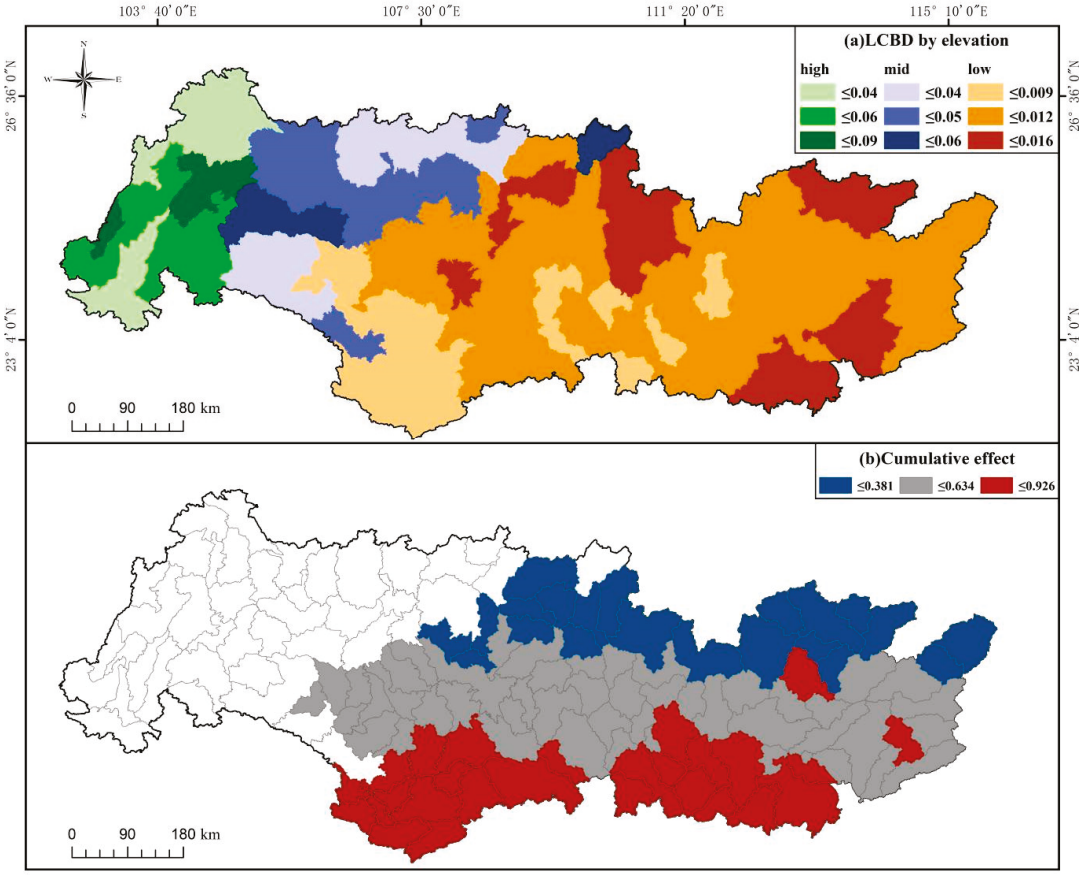

Figure 5. Presents the prioritization strategies for freshwater biodiversity conservation within the Peal River Basin, pinpointed through (**a**) the distinctiveness of ichthyofaunal assemblages, evaluated via Local Contributions to Beta Diversity (LCBD) for Simpson diversity across varying elevations—high (depicted in green), medium (blue), and low (orange)—and (**b**) the aggregated impact of anthropogenic stressors on sub-basins at lower elevations.

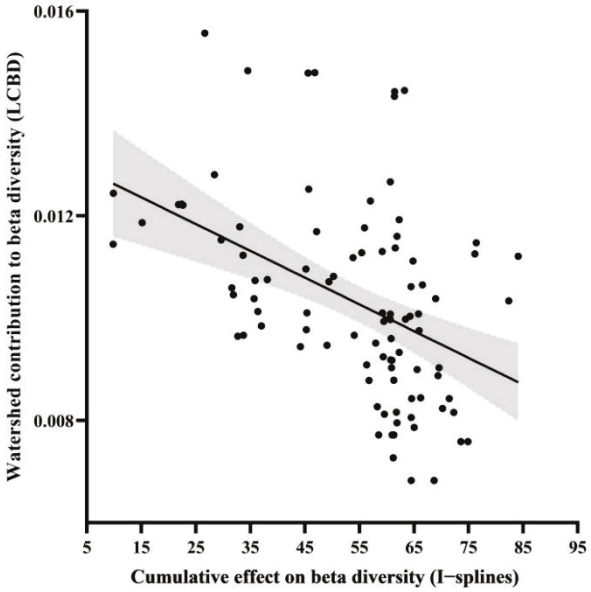

Figure 6. Delineates the inverse relationship between the distinctiveness of fish communities, quantified by Local Contributions to Beta Diversity (LCBD) for Simpson diversity, and the compounded anthropogenic disturbances within low-elevation sub-basins. These cumulative effects were computed as the aggregate of non-linear reactions of ichthyofaunal community turnover, ascertained through I-splines from multi-site generalized dissimilarity models. The graph includes shading that represents 95% confidence intervals obtained from a linear regression analysis ($p < 0.001$).

4. Discussion

The Pearl River Basin faces urgent conservation challenges, as fishing incomes remain below national averages, while per-unit freshwater fishery harvests exceed those of the Yangtze River, where a decade-long moratorium has been implemented. Fishermen's dependency on freshwater fish for livelihoods drives overexploitation, with nearly all aquatic species targeted commercially after the brief four-month fishing hiatus, directly impacting fish abundance and diversity [58]. Despite harboring China's highest freshwater fish species richness and endemism, the Pearl River Basin receives insufficient conservation attention compared to the Yangtze River, partly due to the need for conservation strategies informed by both large-scale patterns and localized distributions [19,59].

This study addressed critical gaps in evaluating freshwater fish responses to complex anthropogenic stressors by establishing a novel linkage between beta diversity and cumulative impacts. Our enhanced methodology incorporated qualitative vulnerability weights derived from species' responses to stressors, using zeta diversity and multi-site generalized dissimilarity modeling (MS-GDM) to highlight relative stressor importance. By connecting cumulative impact assessments to species assemblage distinctiveness across sub-basins (LCBD), we identified priority conservation areas. This integrated approach provided actionable insights for fishery managers by strategically focusing conservation and management initiatives based on regional distinctions, species-specific responses, and anthropogenic pressures [24,27].

Within the Pearl River Basin, freshwater fish zeta diversity showed disparate responses to anthropogenic stressors, including fishing pressure, riparian disturbance, alien fish numbers, cultivated land proportion, and water network density. Many significant shifts occurred at lower impact thresholds, plateauing beyond higher levels. Fish community composition changed markedly with minimal urban and agricultural development, while species richness showed reduced sensitivity [47]. Therefore, biodiversity assess-

ments should emphasize species turnover over richness, and cumulative effect evaluations must highlight non-linear diversity–stress dynamics [27]. Concentrating human activities within smaller sub-basins appears more effective than dispersing low-impact activities throughout entire regions. The MS-GDM's explanatory capacity aligns with other ecosystem studies [27,35], with fishing pressure and GDP differentials significantly influencing species turnover at lower elevations, while having minimal effect at higher elevations.

This investigation compiled ichthyofaunal distribution, environmental variables, and anthropogenic pressures within Pearl River Basin sub-basins. The inverse relationship between cumulative anthropogenic stress (MS-GDM) and fish species composition distinctiveness (LCBD values) supported these metrics for conservation prioritization. Areas with low cumulative effects and high LCBD scores are prime candidates for Kunming-Montreal Global Biodiversity Framework objectives [23,27]. Karstic middle-to-high-elevation sub-basins harbor numerous endangered cave-dwelling endemic species requiring urgent protection due to inadequate survey data [60]. Conversely, sub-basins with elevated cumulative impacts and reduced LCBD may undergo biotic homogenization, necessitating targeted restoration. The Pearl River Delta contains 131 rare or absent aquatic species (one-third of total ichthyofauna), including *Acipenser sinensis* and *Tanichthys albonubes*. Overfishing, compromised water quality, and habitat conversion for economic development severely threaten delta fish populations [37,38].

Historically, the Pearl River and Yangtze River basins shared analogous environmental challenges, where brief annual moratoria on fishing failed to yield the anticipated conservation outcomes due to pervasive overexploitation of fisheries, aquatic contamination, coastal land reclamation, and rampant sand extraction activities [14,16]. The absence of a 'fishing flood' phenomenon in the Pearl River Basin, coupled with a persistent downtrend in biodiversity metrics, underscores the stark reality of an ecosystem under siege [61]. A 'fishing flood' refers to the seasonal surge in fish catches that typically follows spawning periods when restrictions are lifted, indicating healthy fish population recovery. Its absence signals the failure of current short-term fishing bans to restore fish stocks. Temporal or localized prohibitions on fishing are insufficient to stem the ecological crisis in the basin, given the resumption of unregulated harvests post-spawning season and the confluence of multiple, intensifying anthropogenic stressors [14,58]. The integration of Local Contribution to Beta Diversity (LCBD) indices with assessments of cumulative impacts offered considerable insight for prioritizing biodiversity conservation and identifying requisite rehabilitative measures. A blanket fishing prohibition across the entire Pearl River Basin is not advocated, as the fishing pressure minimally impacted the turnover component of zeta diversity within all sub-basins. Moreover, alien fish species significantly affected the zeta diversity of fish assemblages across various elevations. Consequently, fishing restrictions aimed at protecting native species might inadvertently provide reproductive opportunities for these invasive groups [14,48].

Therefore, this study proposes the following:

(i) Effective restoration of aquatic life and fisheries may be achieved through the strategic adjustment of human-induced pressures and stressors, in conjunction with stringent fishery regulations [14]. This approach necessitates the establishment of an extensive monitoring framework for the Pearl River ecosystem, the enhancement of current fishing restrictions in both duration and coverage, rigorous enforcement of said regulations, and the initiation of large-scale ecological rehabilitation initiatives. Such measures are crucial for the revival of the Pearl River Basin, home to China's richest freshwater fish diversity and fisheries. This model is also pertinent to developing regions in Africa, Southeast Asia, and South America, where similar patterns of

economic advancement and population growth are accompanied by the ecological deterioration of aquatic habitats (Chen et al., 2020) [14].

(ii) To promote the recovery and replenishment of aquatic species, a 10-year moratorium on fishing is proposed to be implemented in the waters of Guangdong–Hong Kong–Macao (GH-MO) Greater Bay Area (Pearl River Delta) in view of the high cumulative effects of human pressures (e.g., fishing pressure) and species rarity in the low-elevation Pearl River Delta. This conservation strategy, which is inextricably linked to the National Development Agenda, aims to raise the ecological quality and governance of the Guangdong–Hong Kong–Macao Greater Bay Area to global standards and implement the most stringent environmental protection protocols.

(iii) A compensatory framework is suggested for the fishing prohibition in the Guangdong–Hong Kong–Macao Greater Bay Area, which would steer the Pearl River Basin Delta's fishing community toward alternative livelihoods, expediting their shift from fishing to other forms of production. This transition should be supported by comprehensive policies addressing employment and social security for the fishermen transitioning out of the industry.

(iv) Special consideration is warranted for the conservation of aquatic ecosystems, including groundwater, within the karst regions of the Pearl River Basin. Intensified monitoring and protection of freshwater fish biodiversity in these areas are imperative. A substantial body of fundamental research is required to ascertain the endangered status and categorize the risk levels of fish species inhabiting these karst environments.

(v) By implementing successful fisheries regulation and mitigating anthropogenic impacts, China can enhance freshwater management while leading by example for developing nations. Future management should integrate advanced technologies, including remote sensing, environmental DNA (eDNA) monitoring, and real-time water quality sensors, for dynamic, adaptive management. Satellite-based habitat monitoring combined with machine learning algorithms can provide early warning systems for biodiversity loss. Establishing international collaborative platforms for sharing monitoring data, management protocols, and technological innovations will enhance the global freshwater biodiversity conservation capacity, representing next-generation conservation strategies that move beyond static protection toward responsive ecosystem management.

5. Conclusions

This study reveals fish zeta diversity responses to human pressures in the Pearl River Basin, showing that cumulative pressure effects in low-elevation areas significantly exceed those in mid- to high-elevation regions and negatively correlate with community uniqueness. We recommend implementing long-term fishing bans in high-pressure areas like the Pearl River Delta while strengthening protection in mid- to high-elevation watersheds. Integrating zeta diversity analysis with cumulative effects assessment provides a novel framework for watershed ecosystem management and freshwater fish conservation, offering important guidance for sustainable fisheries management in developing countries.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology14070796/s1, Figure S1. Correlation plot for all human pressure and bioclimatic (CHELSA01-19) variables; Figure S2. K-means within cluster sum of squares across different numbers of cluster groups based on elevation; Figure S3. Range of values for human pressure, environmental, and geospatial variables and across watershed elevations as grouped by high, mid-, and low elevation clusters; Figure S4. Zeta decline for high, mid-, and low elevation

watersheds shows the rate at which the number of shared species ('zeta values') decreases as more watersheds are compared at once ('zeta order'); Table S1. Zeta values (number of shared species) for increasing orders of zeta (number of watersheds compared at once).

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Animal Research and Ethics Committees of Guilin University of Technology and followed the animal experiments guidelines of Guilin University of Technology (Approval Code: GUT-2023-16; Approval Date: 20 June 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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Stock Status of Noncommercial Fish Species in Aras Dam Reservoir: Mismanagement Endangers Sustainable Fisheries

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Simple Summary: This study addressed the critical issue of unsustainable fisheries in the Aras Dam reservoir, a vital source of fish in northwest Iran, where fish populations are threatened by mismanagement and outdated information. Our goal was to assess the current health of four important noncommercial fish species: the silver bream, common bream, common roach, and freshwater bream. Using advanced computer models that can work with limited data, we analyzed historical catches and fish measurements. Our results showed that the silver bream population is healthy and could potentially support more fishing. However, the populations of common bream, common roach, and freshwater bream are overfished or severely depleted, meaning current fishing levels are not sustainable. These findings are essential for local fishery managers. They provide clear guidance to implement measures like limiting overall catches, reducing fishing pressure, and stopping illegal fishing. This will help these fish populations recover and ensure that the Aras Dam reservoir remains a valuable resource for local communities for generations to come.

Abstract: Aras Dam reservoir, a key inland fishery in northwest Iran, faces increasing socioecological threats that jeopardize sustainable resource use. To provide updated information on stock conditions under limited data availability, this study applied the catch maximum sustainable yield (CMSY^{++}) model using biometric measurements and catch records from 2013 to 2022. Four noncommercial species commonly harvested in the reservoir—silver bream (*Blicca bjoerkna*), common bream (*Abramis brama*), common roach (*Rutilus rutilus*), and freshwater bream (*Carassius auratus*)—were assessed. Stock status was evaluated through biomass relative to reference points (B/B_{MSY}) and fishing mortality (F/F_{MSY}). Results revealed unsustainable conditions for common bream, common roach, and freshwater bream, while silver bream remained in a healthy state. These findings suggest that maintaining current fishing levels may intensify pressure and risk stock depletion for several species. The CMSY^{++} model demonstrated efficiency in providing rapid assessments in data-limited situations, offering insights for inland fishery management. To ensure sustainable fisheries in the Aras Dam reservoir, managers should implement stricter measures to control illegal fishing, reduce excessive effort, and promote recovery of depleted stocks.

Keywords: Aras Dam Reservoir; fishery management; sustainable fisheries; data-limited method; stock status

1. Introduction

The number of overfished stocks has increased worldwide, leading to a decline in fish abundance and marine ecosystems [1]. Thus, stock management strategies should be applied to ensure sustainable fisheries worldwide. All stocks that are being exploited should be monitored and managed [2]. However, limited data and expertise for doing so render the monitoring and management of fish stocks challenging. In recent years, data-limited methods such as the catch maximum sustainable yield (CMSY) model have been used to estimate reference points for fisheries and provide reasonable predictions of relative biomass and exploitation rates [3,4]; such predictions can provide a comprehensive understanding of the stock status of fish species and the amount of overfishing by local fishermen.

Data-limited methods have been widely used, often in combination with other parallel methods, to evaluate the stocks of different fish species, including approximately 400 European stocks [5], >1300 exploited marine fish and invertebrate species [6–10]. However, the CMSY model is associated with uncertainty and errors that must be addressed [11,12]. Nevertheless, CMSY is one of the most rapid methods for evaluating the stock of data-poor fisheries, providing timely insights that can guide the formulation of policies for sustainable fishery management. While other rapid assessment approaches, such as depletion-based or length-based methods, are available, CMSY was chosen for this study due to its proven applicability in data-limited contexts and its suitability for the inland fish stocks of the Aras reservoir.

The Aras River is an internationally important river, with a length of 1072 km, average discharge of 5323×10^6 m^3/year, and abundance of biogenic compounds and exhibits seasonal temperature fluctuations and is one of the most important feeders of the Caspian Sea. It originates in Turkiye and crosses Armenia, Iran, and Azerbaijan, and it has varying hydro-political interactions across these regions and economic, social, and ecological consequences [13,14]. In northwest Iran, the Aras Dam reservoir is one of the main sources of inland fisheries. Notably, $>3.7 \times 10^3$ mt [15] of more than 12 of the 27 species in the Aras River ecosystem are harvested legally and illegally from the Aras Dam reservoir each year [16]. This reservoir is economically crucial to local fishermen. Notably, illegal fishing in the Aras Dam reservoir has increased, threatening local legal fishery companies by causing low fish production and high stock pressure; these threats have led to a decrease in the stocks of commercial species and an increase in the stocks of noncommercial species in the reservoir.

Exploited noncommercial fish species in the Aras Dam reservoir include silver bream (*Blicca bjoerkna*), common bream (*Abramis brama*), common roach *(Rutilus rutilus*), and freshwater bream (*Carassius auratus*), which are mainly used in fish powder factories. The four species selected account for over 90% of the total catches in the Aras Dam reservoir. Although they are mostly noncommercial, their dominance in terms of abundance and biomass makes them representative indicators for assessing the overall stock status of the reservoir. Notably, the most recent study regarding the fish fauna and stock status of Aras Dam reservoir was that by Abbasi and Sarpanah [16], who conducted research from 1994 to 1995. No contemporary study has investigated the stock status for this crucial ecosystem. Any threat to the fish stock in the Aras Dam reservoir due to mismanagement would have natural, economic, and social consequences.

2. Materials and Methods

2.1. Study Area and Fishery Data

The Aras Dam was established over the Aras River in 1970; it has a height of 40 m (130 ft). The Aras River forms a section of Iran's international boundaries with Armenia, Azerbaijan, and Turkey, and it is one of the most crucial and largest rivers in northwest Iran (Figure 1). The river has a length of 1072 km and flows into the Caspian Sea after joining the Kura stream [17]. The Aras River has a unique ecosystem in terms of fish species diversity; 27 species have been detected in the river [16].

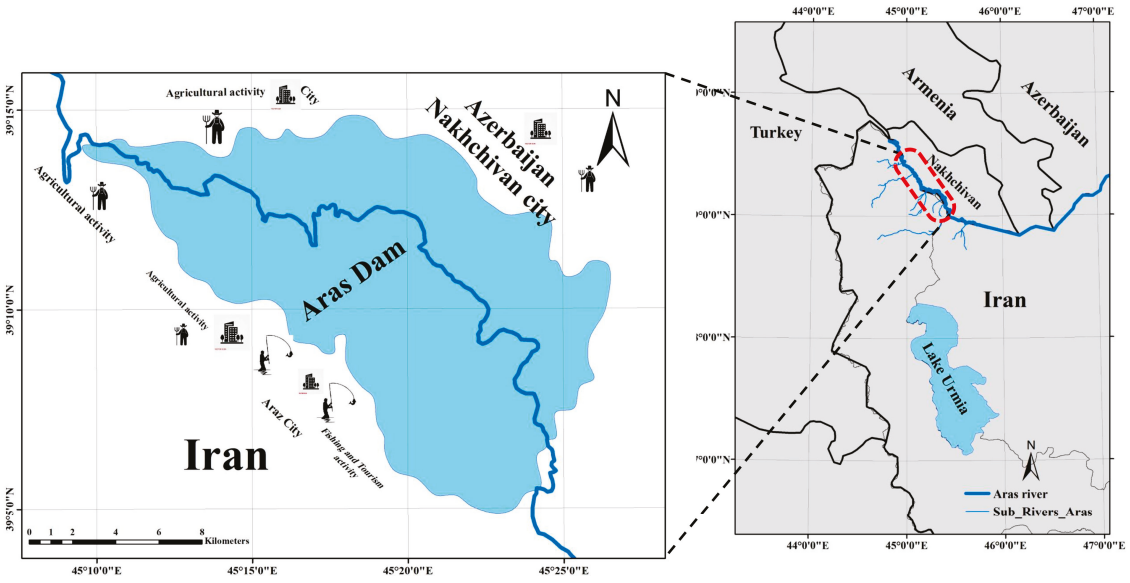

Figure 1. Aras Dam reservoir and fishing locations in northwest Iran.

The current study obtained time-series catch and fishing effort data from Beach seins net fishing (950 m length, 18 m height, and 35 mm mesh size in the central part of the net) to calculate catch per unit effort (CPUE) from 2013 to 2022 that were collected from west Azerbaijan fisheries officials in Iran; these data were input into the CMSY^{++} model. Legal fishing by fishing companies is conducted from late September to early April because of the fishing ban policy of west Azerbaijan fisheries. In this study, illegal fishing activity was estimated using the daily fish input data of fish powder factories located in the Aras Dam area. Estimates of illegal catches derived from fish supplied to the factory were cross-checked against official fisheries landing reports and independent local market surveys.

2.2. CMSY^{++} and BSM Methods

CMSY^{++} is an advanced state-space Bayesian method for stock assessment that estimates reference points for fisheries (MSY, F_{MSY}, and B_{MSY}). It also estimates status or relative stock size (B/B_{MSY}) and fishing pressure or exploitation (F/F_{MSY}) from catch and (optionally) abundance data, a prior for resilience or productivity (r), and broad priors for the ratio of biomass to unfished biomass (B/k) at the beginning of the year, midyear, and the end of the time series. This model is a more advanced version of the Catch-MSY model developed by Martell and Froese [4]. The basis of both these models is the modified Schaefer surplus production model, which enables fitting of abundance indicators, if available.

The CMSY^{++} model is a further advancement of the CMSY method developed by Froese et al. [3]. CMSY^{++} overcomes several of the limitations of the CMSY method. A

major improvement in the CMSY method and Bayesian state-space implementation of the Schaefer model (BSM) is the introduction of multivariate normal priors for r and k in log-space, replacing the previous uniform prior distributions. This enables a simplified means of determining the "best" r–k pair in CMSY and achieves faster run times. The CMSY^{++} model is based on the first derivative of the logistic curve of population growth; the numbers of individuals are replaced by the sum of their body weights [18]. The CMSY^{++} model is represented as Equation (1):

$$B_{t+1} = \{B_t + r(1 - B_t / k) - C_t e^{\varepsilon_t}\} e^{\eta_t} \quad (1)$$

where B_t is the biomass, C_t is the catch in metric tons (t) in year t, r (year^{-1}) is the intrinsic rate of population growth, and k is the carrying capacity of the environment for this population in metric tons (t). ε_t is the normally distributed observation error of catches, and ηt is the process error. They are implemented as lognormal error terms. These lognormal error terms are omitted in subsequent equations. Thus, if reasonable estimates of the start biomass and k are available for quantifying the unexploited and initial stock sizes and if a reasonable estimate of r can be inferred from life-history traits [12], a time series of biomass can be projected on the basis of the time series of catches, with the maximum sustainable fishing mortality calculated as $F_{MSY} = r/2$ and the minimum biomass that can produce maximum sustainable yield (MSY) calculated as $B_{MSY} = k/2$. This approach is referred to as stochastic reduction analysis [19,20].

If stock biomass is severely depleted (<0.25 k), recruitment is reduced. This is expressed by Equation (2), which is a slight modification of Equation (1):

$$B_{t+1} = B_t + \left(\frac{4B_t}{k} r\right)\left(1 - \frac{B_t}{k}\right) B_t - C_t \left| \frac{B_t}{k} < 0.25 \right. \quad (2)$$

where $(4B_t/k)$ results in a linear decline of r if the biomass is less than $k/4$, which accounts for reduced recruitment and thus decreased productivity at a low population size. Half of B_{MSY}, which is $k/4$ in the Schaefer model, is typically employed as a proxy for the biomass threshold, below which recruitment may be reduced.

The Schaefer model can be expressed as a function of r and MSY, without k, as indicated in Equation (3). However, this does not change the dynamics of the model, and the surplus production seems to be less intuitive than that in Equation (1):

$$B_{t+1} = B_t + r \cdot B_t - \frac{(rB_t)^2}{4MSY} - C_t \quad (3)$$

To retain the original form of the CMSY base model, as expressed in Equation (2), with parameters r and k, the within-stock correlation between r and k is accounted for in a multivariate lognormal (MVLN) distribution. This distribution is implemented by drawing a large sample (n = 10,000) of independent $\log(\widetilde{r})$ and $\log(\widetilde{MSY})$ from their prior distributions, computing the corresponding $\log(\widetilde{k}) = \log(4) + \log(\widetilde{MSY}) - \log(\widetilde{r})$, and computing the means and covariance of $\log(\widetilde{r})$ and $\log(\widetilde{k})$, which are then passed on as covariance matrix for the r–k ~ MVLN prior in the CMSY^{++} model and BSM formulations.

A feed-forward artificial neural network (ANN) is selected for classifying the stock status as being above or below the MSY level, as expressed in Equation (4):

$$(B/k)_{tprior} = \frac{1 + A_t \sqrt{1 - C_t / MSY_{prior}}}{2} \quad (4)$$

This equation only provides real-number solutions if $C_t \leq MSY_{prior}$. Therefore, its application is restricted to cases where $C_t < 0.99\ MSY_{prior}$.

This equation presents the derivation of a point estimate of relative equilibrium biomass (B/k) from catch data relative to MSY. Catch and biomass are rarely in equilibrium in real-world stocks, and the width and shape of uncertainty vary with the position of the equilibrium point estimate in the B_t/k–C_t/MSY space.

The equilibrium curve for the interplay between relative biomass (B/k) and relative catch (C_t/MSY) in the modified Schaefer model is derived in Equation (5):

$$\frac{C}{MSY} = (4\frac{B}{k} - (2\frac{B}{k})^2)\cdot RC \tag{5}$$

where RC denotes recruitment correction; $RC = 4\ B/k$ if $B/k < 0.25$ and $RC = 1$ otherwise, as is true for same as in Equation (2).

The equilibrium curve for the model developed by Fox (1970) is derived from Equation (6):

$$\frac{C}{MSY} = e\frac{B}{k}(1 - \log\left(e\cdot\frac{B}{k}\right)) \tag{6}$$

where e represents Euler's number 2.718.

For verification, the predictions of the CMSY^{++} method are compared with simulated data, where the "true" values of parameters and biomass data are known. For evaluation against real-world fishery data, the predictions of the CMSY^{++} method are compared against corresponding parameters and abundance estimates derived from fully or partly assessed stocks, for which biomass or CPUE data are available in addition to catch data. For this purpose, BSM is developed, where r, k, and MSY are predicted from catch and abundance data. The basic biomass dynamics are governed by Equation (7):

$$B_{t+1} = B_t + r(1 - B_t/k)B_t - C_t \tag{7}$$

where B_t is the biomass in year t, B_{t+1} is the exploited biomass in year $t + 1$, r is the intrinsic rate of population increase, k is the carrying capacity (i.e., mean unexploited stock size), and C_t is the catch data in year t.

If stock biomass is severely depleted and is <0.25 k, recruitment is reduced. This is expressed by Equation (8), which is a slight modification of Equation (7):

$$B_{t+1} = B_t + \left(\frac{4B_t}{k}r\right)\left(1 - \frac{B_t}{k}\right)B_t - C_t \left| \frac{B_t}{k} < 0.25 \right. \tag{8}$$

where the term $4rB_t/k$ denotes the assumption that the intrinsic rate of population growth declines linearly with biomass, which is less than half the biomass associated with MSY. Resilience data in FishBase and SeaLifeBase are translated into the prior of r-ranges.

The prior of k is computed using the following equations:

$$k_{low} = \frac{max(C)}{r_{high}};\ k_{high} = \frac{4max(C)}{r_{low}} \tag{9}$$

$$k_{low} = \frac{2max(C)}{r_{high}};\ k_{high} = \frac{12max(C)}{r_{low}} \tag{10}$$

where r_{low} and r_{high} are, respectively, the lower and higher bounds of the range of r-values explored using the CMSY method, k_{low} and k_{high} are, respectively, the lower and higher limits of k, and $max(C)$ is the maximum catch in the available data.

The BSM method (i.e., including relative abundance data) is used to calculate the standard deviation of r, which falls between 0.001 and 0.02 *irf*.

$$irf = 3/\left(r_{high} - r_{low}\right) \tag{11}$$

where *irf* is a factor applied to estimate r and r_{high} and r_{low} are ranges suggested in FishBase (www.fishbase.org).

The Schaefer model is used to transform the CPUE into biomass on the basis of the catchability coefficient q and is presented in Equation (12):

$$CPUE_t = q \cdot B_t \tag{12}$$

where $CPUE_t$ and B_t are the mean CPUE and biomass in year t, respectively, and q is the catchability coefficient.

The population dynamic can be expressed by relative abundance (CPUE) using Equation (13):

$$CPUE_t + 1 = CPUE_t + r(1 - CPUE_t/qk)CPUE_t - qC_t \tag{13}$$

where the variables and parameters are defined as in Equations (7) and (12). The prior q is derived from Equation (14):

$$Y = rB(1 - B/k) \tag{14}$$

where Y is the equilibrium yield for B and the other parameters are defined as in Equation (7).

BSM can be used to estimate q. For stocks with high recent biomass, the lower and higher priors for q are calculated using Equations (15) and (16) as follows:

$$q_{low} = 0.25 r_{pgm} CPUE_{mean}/C_{mean} \tag{15}$$

$$q_{high} = 0.5 r_{high} CPUE_{mean}/C_{mean} \tag{16}$$

where q_{low} and q_{high} are the lower and upper prior catchability coefficients for stocks with high recent biomass, respectively, r_{pgm} is the geometric mean of r, r_{high} is the upper prior range for r, $CPUE_{mean}$ is the mean CPUE over the last 5 or 10 years, and C_{mean} is the mean catch over the same period.

B_{start}/k and B_{end}/k are the priors of relative biomass at the start and the end of each time series, and their ranges are estimated on the basis of the assumed depletion level [3].

All available r values are assigned to bins in log-space with equal widths; the most probable r value is derived from the 75th percentile of the mid-values of the occupied bins. If the r value is higher than the 50th percentile of the mid-values of the occupied bins, the most probable k value is derived through linear regression:

$$MSY = rk/4 \rightarrow log(k) = log(4MSY) + (-1)log(r) \tag{17}$$

The relative biomass (B/B_{MSY}) in the final year, which represents the status of a stock, can be estimated using both CMSY and BSM. In addition, Kobe plots, which are generated using relative fishing mortality coefficients and the ratio of fishing mortality to maximum sustainable fishing mortality (B/B_{SMY} and F/F_{MSY}, respectively), are used to simultaneously assess the status of all stocks in the most recent year. To ensure consistency in interpretation, stock status categories were defined following Froese et al. [21]. Specifically,

stocks with $B/B_{MSY} < 0.5$ were classified as considerably depleted, $0.5 \leq B/B_{MSY} < 0.8$ as moderately depleted, and $B/B_{MSY} \geq 0.8$ as healthy.

2.3. Biometric Data and Length–Weight Relationship

To determine the length–weight relationship, this study analyzed 1206 fish specimens, including 463 Silver bream, 391 Common roach, and 352 Freshwater bream, collected from fishing cooperative positions using Beach seins net fishing (950 m length, 18 m height, and 35 mm mesh size in the central part of the net) once a week for 6 months (October 2023–March 2024). This study did not investigate the length–weight relationship for Common bream because this species was not present in the beach sein net and, consequently, biometric data could not be obtained for the species. For each specimen, the total length (L) was measured to the nearest 0.1 cm, and total weight (W) was measured to the nearest 0.1 g by using a measuring board and a digital scale, respectively. The parameters of the length–weight relationship were calculated for noncommercial species by fitting the power function to the length and weight data and applying Equation (18).

$$W = a \cdot L^b \tag{18}$$

where W is the wet body weight (g), TL is the total length (cm), a is the intercept, and b is the allometric coefficient. Pauly's t test [22] was used to determine if the slope of relationships was significantly different from the value of 3.

2.4. Statistical Analysis

Statistical analyses were performed using SPSS (version 26) with R Studio (version 1.1.446) software. The R studio software program, which implements the CMSY^{++} method [21], was applied to assess the status of fishery stocks for the four noncommercial species considered in this study in the Aras Dam reservoir. In addition, BSM, which is part of the CMSY^{++}, was applied to account for variability in both population dynamics (process error) and measurement and sampling (observation error) [3,23,24]. At the end of the time series, CMSY^{++} provided routine reference points for fisheries to enable exploration of the stock status of selected species. All data files and R-code of the CMSY^{++} method are provided in the supplement in Froese et al. [21].

3. Results

3.1. CMSY^{++} Model and BSM

This study estimated the main parameters for obtaining reference points for the fisheries of noncommercial species in the Aras Dam reservoir (Tables 1 and 2) by using the CMSY^{++} model and BSM. According to the results obtained using the CMSY^{++} model and BSM, the estimated value of B/B_{MSY} for *B. bjoerkna* was >1.0, and the value of F/F_{MSY} was <1.0 (Table 1), indicating the Aras Dam reservoir has a healthy stock of this species (Kobe plot, Figure 2A,B and Figure 3A,B). The estimates obtained using the two models indicated an increase in fishing activity from 2013 to 2022, and the catch amount for the aforementioned species was lower than the MSY in all years. Consequently, during these years, the size (biomass) of the stock increased (Figures 2C and 3C). The obtained reference points for fisheries were used to estimate the MSY for *B. bjoerkna*, which was 769 mt when the CMSY^{++} model was applied and 750 mt when the BSM was; the catch amount never reached these points or surpassed the sustainable level. Hence, the catch amount for this species can be slightly increased.

Table 1. CMSY^{++} model and BSM results for predicting key reference point indicators for fisheries of *Blicca bjoerkna* in the Aras Dam reservoir (values in parenthesis represent 2.5th and 97.5th percentiles). Indicators are based on 1000 metric tons (t).

Parameter	Estimated Values	
	CMSY^{++}	**BSM**
Biomass (B)	5.98 (3.59–9.63)	5.91 (3.45–9.38)
MSY	0.769 (0.537–1.21)	0.75 (0.537–1.19)
B_{MSY}	4.82 (3.47–7.96)	4.73 (3.46–7.96)
F_{MSY}	0.16 (0.11–0.209)	0.159 (0.11–0.207)
B/B_{MSY}	1.12 (0.795–1.44)	1.11 (0.772–1.44)
Exploitation F/F_{MSY}	0.557 (0.294–1.07)	0.556 (0.301–1.1)
Fishing mortality (F)	0.0821 (0.0464–0.146)	0.0825 (0.0474–0.153)
Relative biomass in last year	0.559 k (0.397–0.718)	0.553 k (0.386–0.72)
K	9.64 (6.93–15.9)	9.46 (6.91–15.9)
R	0.319 (0.22–0.418)	0.317 (0.219–0.414)
B_{MSY}/K	0.62	0.62
Depletion level	Low	Low
Trophic level	3.2	

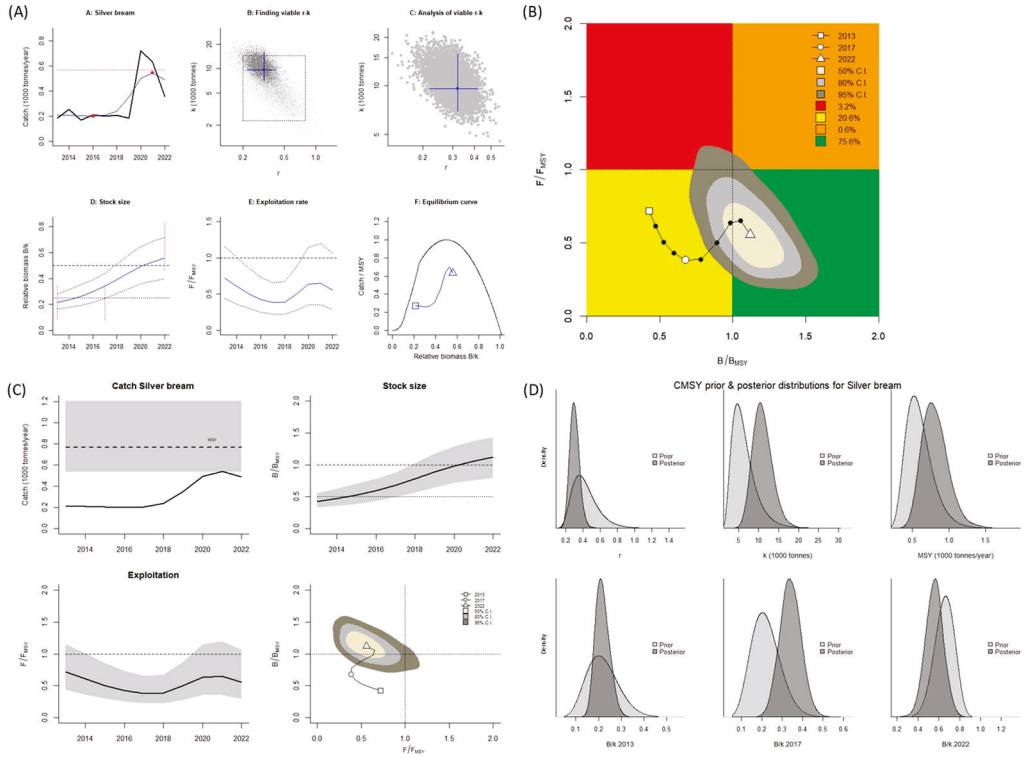

Figure 2. CMSY^{++} model results for *Blicca bjoerkna* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

Table 2. CMSY^{++} model and BSM results for predicting key reference point indicators for fisheries of *Abramis brama* in the Aras Dam reservoir (values in parenthesis represent 2.5th and 97.5th percentiles). Indicators are based on 1000 metric tons (t).

Parameter	Estimated Values	
	CMSY^{++}	**BSM**
Biomass (B)	3.76 (2.05–6.38)	0.817 (0.109–1.89)
MSY	0.168 (0.109–0.258)	0.137 (0.0906–0.261)
B_{MSY}	4.59 (3.28–8.26)	2.13 (1.03–8.33)
F_{MSY}	0.0365 (0.0196–0.0534)	0.0644 (0.02–0.116)
B/B_{MSY}	0.708 (0.498–0.945)	0.167 (0.083–0.277)
Exploitation F/F_{MSY}	0.62 (0.325–1.23)	8.05 (2.48–45.2)
Fishing mortality (F)	0.0197 (0.104–0.0373)	0.0887 (0.0367–0.671)
Relative biomass in last year	0.354 k (0.244–0.473)	0.0836 k (0.0415–0.139)
K	9.17 (6.55–16.5)	4.25 (2.07–16.7)
r	0.0731 (0.0392–0.107)	0.129 (0.0399–0.232)
B_{MSY}/K	0.41	0.19
Depletion level	Medium	Very Strong
Trophic level	3.1	

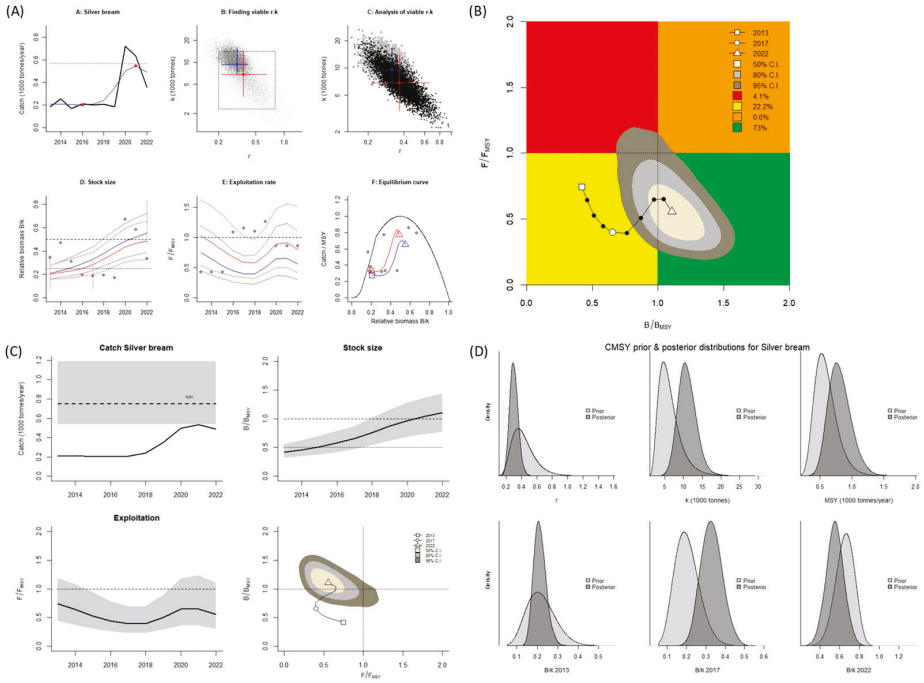

Figure 3. BSM results for *Blicca bjoerkna* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

For *A. brama*, the estimated values for B/B_{MSY} and F/F_{MSY} when the CMSY^{++} model was used were both <1.0. When the BSM was used, the B/B_{MSY} value was <1.0, and the F/F_{MSY} value was >1.0 (Table 2). According to the values calculated using the CMSY^{++} model, the stock is overfished (Kobe plot, Figure 4A,B). According to the values calculated using BSM, overfishing of this species occurs, and the stock is in an overfished state (Kobe plot, Figure 5A,B). A graph was created of the predicted total catch versus the MSY obtained using the two models; it indicated a decreasing trend of fishing from 2013 to

2022 (Figures 4 and 5C). According to the reference points for fisheries obtained using the CMSY^{++} model, the MSY for *A. brama* was nearly 168 mt; in 2013 and 2014, the catch amount was higher than the MSY was. This led to a decrease in the stock size (biomass).

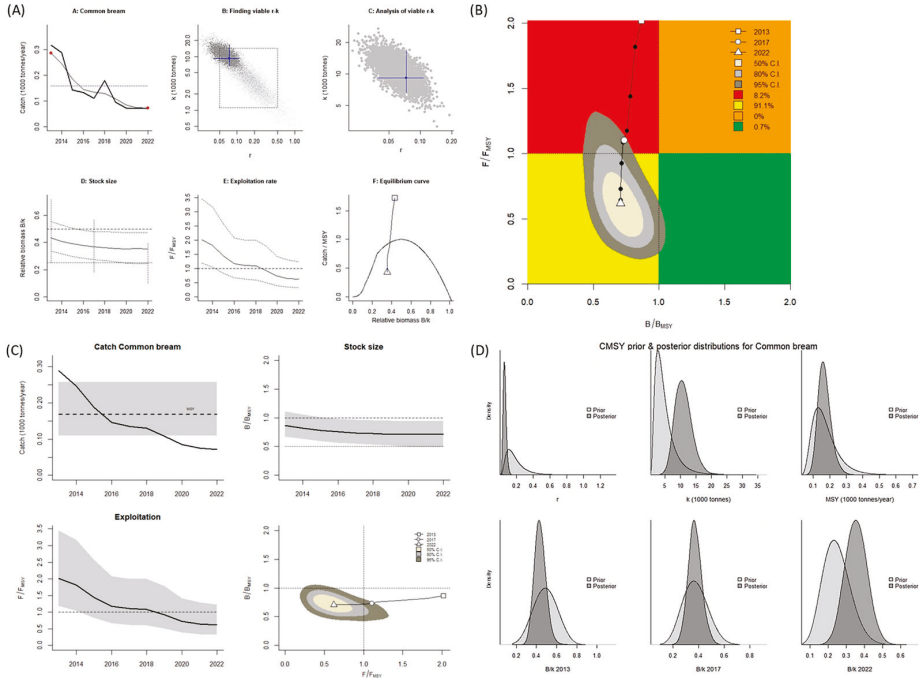

Figure 4. CMSY^{++} model results for *Abramis brama* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

Fishing pressure in the years following 2014 led to the catch amount consistently being lower than the MSY, and the high pressure led to stock depletion (Figure 4C), with the stock for this species being overfished. According to the BSM, the estimated MSY was lower (137 mt), and the stock status was similar to those calculated using the CMSY^{++} model. However, the catch amount was higher than the MSY in 2013 and 2015. Fishing pressure in the years following 2015 led to the catch amount consistently being lower than the MSY, and the high pressure led to stock depletion (Figure 5C), indicating this stock has been overfished and is in an overfished state. Because of its stock status and the absence of this species in the beach sein net, biometrics was not calculated during the sampling season for this species.

For *R. rutilus*, the CMSY^{++} model results revealed that the biological reference point of B/B_{MSY} was mostly <1.0, whereas F/F_{MSY} was mostly >1.0. According to these values, this stock is overfished and in an overfished state (Kobe plot, Figure 6A,B). A graph of the predicted total catch versus the MSY, calculated using the CMSY^{++} model, indicates an increasing trend of fishing from 2013 to 2022. The catch amount was lower than the MSY until 2020, after which it was higher. This indicates that the stock size (biomass) decreased because exploitation of this species surpassed the sustainable level (Figure 6C). For *R. rutilus*, the MSY was estimated to be 724 mt when the CMSY^{++} model was used.

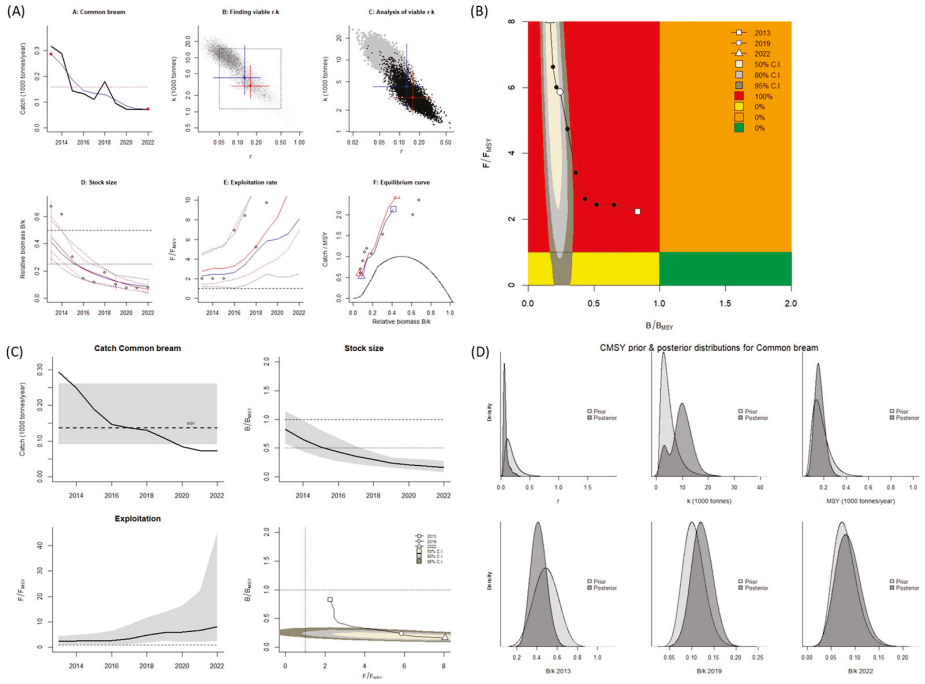

Figure 5. BSM results for *Abramis brama* in the Aras Dam reservoir from 2013 to 2022. (**A**): shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**): is a Kobe phase plot; (**C**): is the stock size and exploitation trend; (**D**): the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

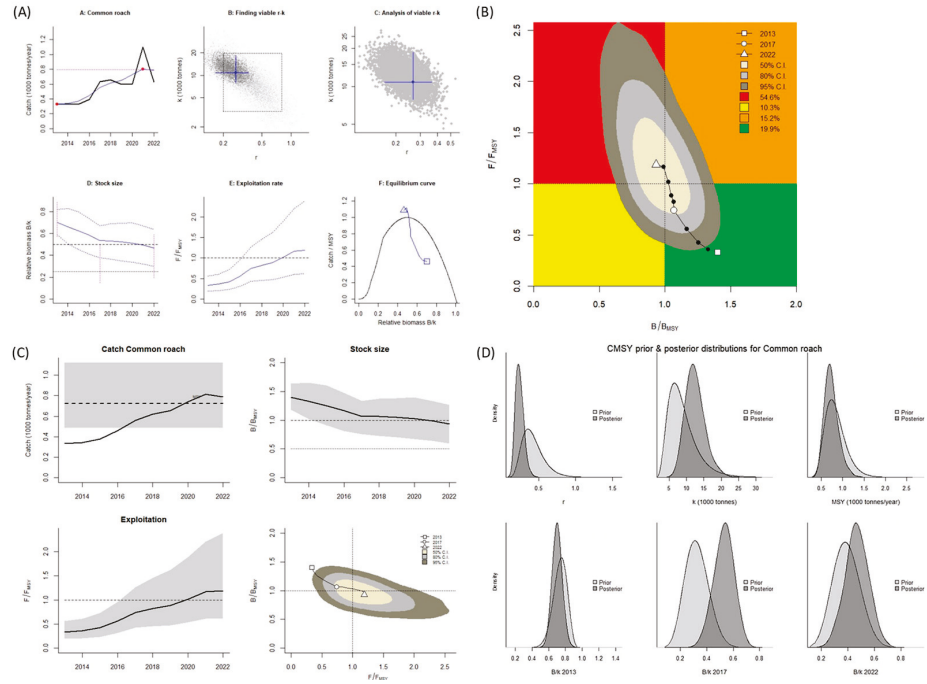

Figure 6. CMSY^{++} model results for *Rutilus rutilus* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

When the BSM was used, the B/B_{MSY} and F/F_{MSY} were both > 1.0. These values indicate the species is in an overfished state (Kobe plot, Figure 7A,B). A graph of the predicted total catch versus the MSY, as calculated using BSM, reveals an increasing trend of fishing from 2013 to 2022. However, up until 2018, the catch amount remained lower than the MSY. After 2018, the catch amount exceeded the MSY, which led to a slight decrease in the stock size (biomass; Figure 7C). When the BSM was used, the MSY was 717 mt. In the years after 2019, the catch amount exceeded the MSY, indicating the stock of this species was overfished and in an overfished state.

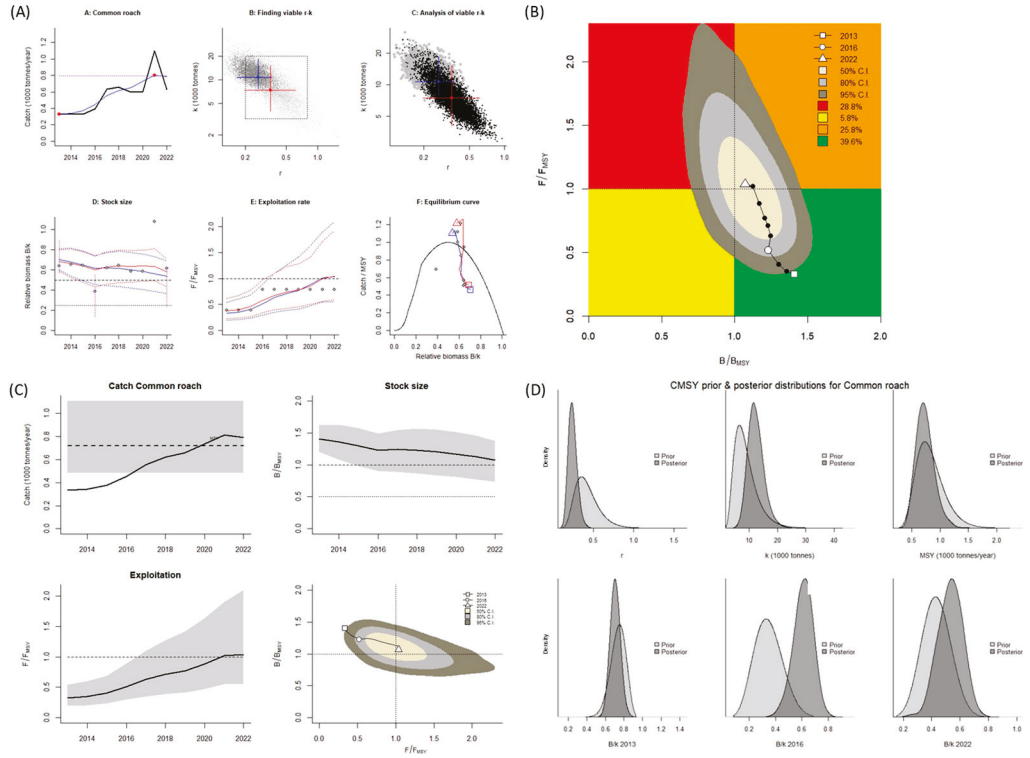

Figure 7. BSM results for *Rutilus rutilus* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

For *C. auratus*, when the CMSY^{++} model was used, the biological reference point of B/B_{MSY} was >1.0, and that of F/F_{MSY} was <1.0. These values indicate that exploitation of the stock of this species exceeded the limits of reference points in the Aras Dam reservoir (Kobe plot, Figure 8A,B). When the BSM was used, the B/B_{MSY} and F/F_{MSY} values were both <1.0, indicating the stock of this species is recovering (Kobe plot, Figure 9A,B). A graph of the predicted total catch versus MSY obtained using the two models indicated an increasing trend in total fishing from 2013 to 2018, followed by a decline in fishing in the subsequent years. In all years, the catch amount consistently remained lower than the MSY. Consequently, according to the values obtained using the CMSY^{++} model, the stock size (biomass) considerably increased (Figure 8C). According to the values obtained using the BSM, the stock size increased slightly (Figure 9C). When the CMSY^{++} model was used, the MSY for *C. auratus* was 1050 mt, and when the BSM was used, it was 770 mt. These values indicate an increase in the catch amount for this species in the Aras Dam reservoir.

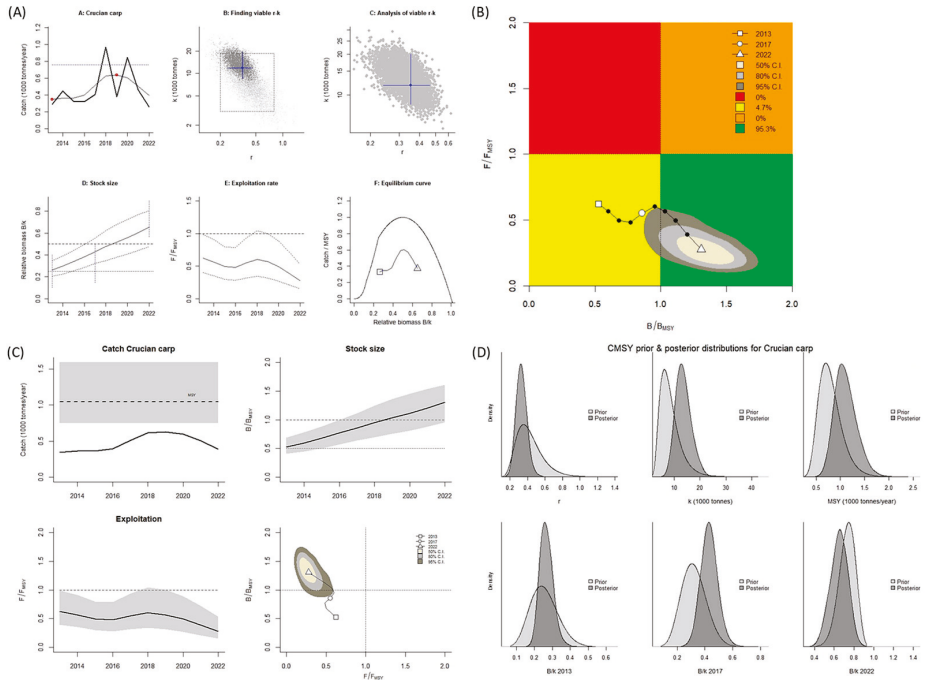

Figure 8. CMSY^{++} model results for *Carassius auratus* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

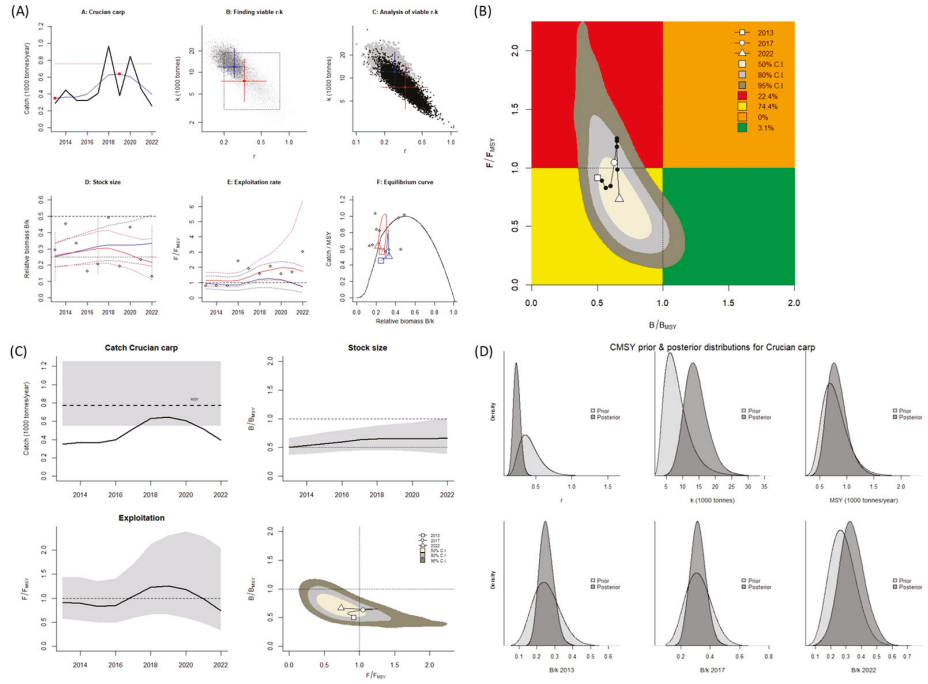

Figure 9. BSM results for *Carassius auratus* in the Aras Dam reservoir from 2013 to 2022. (**A**) shows the time series of catches and smoothed data as used in the estimation of prior biomass by the default rules; (**B**) is a Kobe phase plot; (**C**) is the stock size and exploitation trend; (**D**) the priors (light grey), and posterior understanding (dark grey) of the stock. The colors and symbols are standard use for Kobe phase plot.

3.2. Length–Weight Relationship

Figure 10 presents the results regarding the length–weight relationships. The coefficient of determination (R^2) ranged between 0.783 (for *B. bjoerkna*) and 0.967 (for *R. rutilus*). In addition, the R^2 values were >0.90 for *R. rutilus* and *C. auratus* and >0.70 for *B. bjoerkna*. The b values ranged from 2.955 (for *B. bjoerkna*) to 3.265 (for *R. rutilus*). Regarding growth type, positive allometries (b > 3) were found for *R. rutilus* and *C. auratus*, and isometries (b = 3) were found for *B. bjoerkna*.

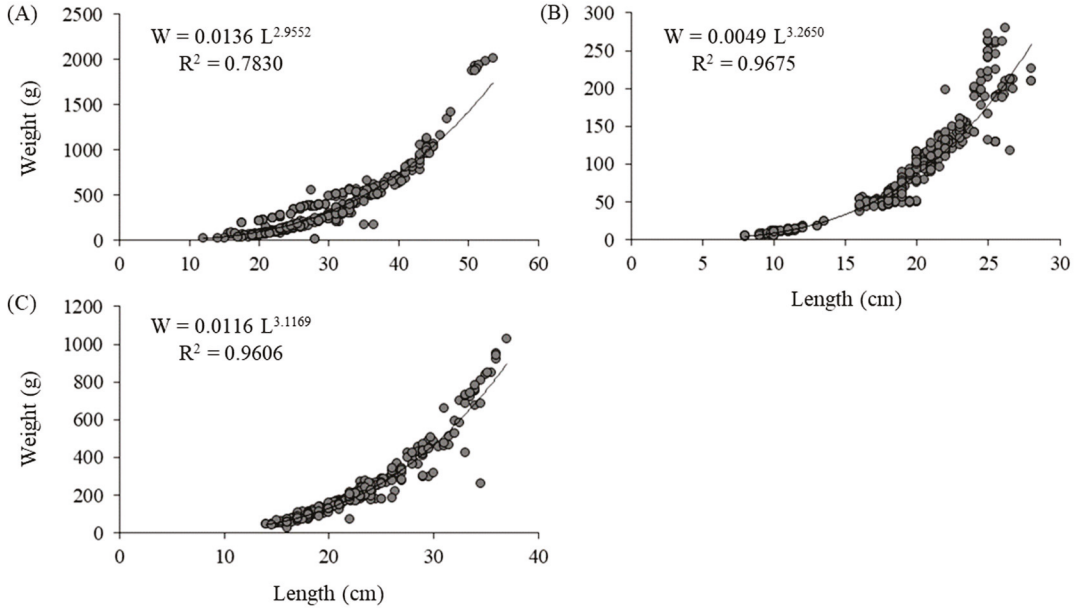

Figure 10. Length–weight (L-W) relationships of noncommercial species in the Aras Dam reservoir for (**A**) *Blicca bjoerkna*, (**B**) *Rutilus rutilus*, and (**C**) *Carassius auratus.* The coefficient of determination (R^2) is shown in each panel.

4. Discussion

The Aras Dam reservoir is a unique ecosystem that supports economic and social activities. It provides many benefits for people residing in that area. In addition, many businesses depend on the reservoir, particularly those related to fisheries, aquaculture, local food markets, and small-scale trade. The fisheries in the reservoir are crucial to the economies in numerous cities in north and west Iran, and they also contribute to livelihoods through employment in fishing cooperatives, transportation, and related services. However, little research has been conducted to determine the sustainability and trends of these fisheries, which are difficult to manage. This study is the first to examine the stock status of several fish species in the area by using new data-limited methods and species biometric data.

This study addresses a critical knowledge gap by providing stock status assessments for key species in the Aras Dam reservoir, which were previously unavailable. Using CMSY^{++} combined with species-specific biometric data, our work offers practical insights for fishery managers and policymakers. While this approach improves data confidence, potential biases such as underreporting or species misidentification cannot be ruled out. Future field-based surveys would further strengthen the validation of these estimates. The results of this study can help local fishery managers formulate appropriate fishing strategies for this crucial border ecosystem.

Although stock assessment models are associated with some uncertainties and bias related to overestimation [11,12], they enable rapid stock monitoring. While data on temporal water level changes in the Aras Dam were not available, fluctuations in reservoir hydrology could influence habitat availability, recruitment, and catchability and should be considered in future assessments. Nevertheless, the accuracy of these models may be influenced by specific environmental conditions, the quality of data, and prior parameter settings [25,26]. Because of this, researchers and decision-makers have increasingly used data-limited methods for stock assessment [7,9,10]. The CMSY^{++} model is sensitive to data quality and life-history parameters, but cross-checked catch data and the Bayesian state-space framework help mitigate uncertainties. In addition, research has revealed good agreement between estimates of population parameters obtained using CMSY and those obtained using Schaefer and Fox models [27], as those shown in a stock assessment study using the CMSY model [28]. Thus, the CMSY^{++} method may enable reliable assessment in data-limited fisheries, including assessment of inland stocks such as those in the Aras Dam.

The related BSMs input with additional information (e.g., CPUE data) produce more accurate confidence intervals [29]. In the present study, the CMSY^{++} method was used in conjunction with the BSM to provide a more detailed assessment of the stock status of four species in the Aras Dam reservoir. With the exception of *B. bjoerkna*, which had a healthy status, all species were considerably or moderately depleted, indicating exploitation of these species must be adjusted. This result also indicates that *r*-selected species are capable of resisting strong fishing pressure [10], although lower fecundity may reduce the recovery potential of the stock. Research indicates that adjusting the mesh sizes of gear enables more juveniles to escape capture, which can contribute to stock recovery [10].

For *B. bjoerkna*, this study identified no signs of overfishing or of overfished stock. This indicates that the catch amount for this species can be moderately increased to improve profits and provide economic benefits to fishermen. By contrast, this study indicated that *A. brama* and *C. auratus* stocks were considerably depleted and *R. rutilus* stocks were moderately depleted, indicating these stocks have been overfished. Various measures should be implemented to improve management of the fisheries in the Aras Dam, especially for the species concerned. Studies have recommended several strategies for preserving fish stocks, including imposing total allowable catches, fishing capacity limitations, and size, gear, and season limitations [30,31].

Studies have also reported that climate-driven environmental variability [32,33] and overexploitation [34] play key roles in population fluctuations, and studies have indicated that effective fishery management is the main driver of the recovery of fish stocks [35,36]. Therefore, strict control measures, such as those related to mesh size and season limitations implemented in China [37], especially for illegal fishing, are recommended to achieve balanced stocks and sustainable fisheries. In China, strategies have been implemented to control excessive fishing, strengthening this study's importance and serving as scientific evidence supporting fishing reduction efforts. In the future, joint international efforts should be expended to manage fishery stocks in the boundary ecosystem of the Aras Dam.

Data on the functional length–weight relationship are crucial to fish stock assessment and management [38]. Length and weight data are required for estimating growth rates and age structure [39] and for calculating standing stock biomass [40], condition indices such as those based on length and weight information [41], and several aspects of fish population dynamics [42]. The best fit values of the parameters for the length–weight relationships were those for *R. rutilus* and *C. auratus* (R^2 = 0.967 and 0.960, respectively). The results indicate highly significant relationships for *R. rutilus* and *C. auratus* and less significant relationships for *B. bjoerkna* (R^2 = 0.783). Significant positive allometric growth

was noted for *R. rutilus* and *C. auratus*, with a slope of b > 3. This finding indicates that fish become more rotund as the total length increases [43,44].

The findings also reveal isometric growth for *B. bjoerkna*, with b approximating 3. This indicates that the species exhibits identical length and weight growth rates. The values of parameter b in the present study were within the expected range of 2.5–3.5, but they can vary between 2 and 4 [45]. Yılmaz et al. [46] demonstrated positive allometric growth for this species in Lake Ladik, Turkiye. In addition, positive allometric growth has been reported for *R. rutilus* in other regions [47,48].

In general, the length–weight relationship of fish is affected by factors such as season, habitat, gonad maturity, sex, diet, stomach fullness, health, preservation techniques, and annual differences in environmental conditions [45,49]. Differences in b values can be ascribed to one or a combination of factors, including differences in the number of specimens examined, effects of the area/season, the observed length ranges of the specimens caught, and the duration of sample collection [50].

5. Conclusions

Different policies can be implemented for border ecosystems such as the Aras Dam. As such an ecosystem, the Aras Dam supports economic and social activities, including fishery and tourism activities. Unmanaged fisheries may lead to a collapse of stocks, and recovery following such a collapse can be time consuming and have social consequences. This study applied the CMSY^{++} model and BSM and considered length–weight relationships to rapidly assess fisheries in the Aras Dam reservoir. The results indicate that the stocks of two species in this reservoir are considerably depleted, and the stocks of the other species are moderately depleted or low. The results were also verified by fishermen through the low fishing activities in the area. To achieve sustainable fisheries in the Aras Dam reservoir, fishery managers can boost stock recovery by adjusting annual catch amounts, implementing mesh size and seasonal limitations, and enforcing strict regulations to control illegal fishing. Our results highlight the need for catch limits aligned with CMSY^{++} reference points and temporary closures during spawning. Declines in fish stocks could also affect local livelihoods, particularly fish powder factories and small-scale trade, so management must balance ecological and economic aspects.

Author Contributions: Conceptualization, A.H.V. and M.G.; methodology and software, A.H.V.; validation, M.G. and N.-J.S.; writing—original draft preparation, A.H.V. and M.G.; writing—review and editing, N.-J.S.; visualization, A.H.V.; supervision, N.-J.S.; funding acquisition, N.-J.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Conservation Measures and Future Perspectives for Europe's Most Threatened Frog: The Action Plan for Karpathos Water Frog (*Pelophylax cerigensis*)

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Simple Summary

The Karpathos water frog (*Pelophylax cerigensis*) was until recently known to occur only on the island of Karpathos, Greece, and is classified as Endangered (EN) by the IUCN. Its survival is seriously threatened by the lack of freshwater habitats in the southern Aegean, a problem that has worsened due to reduced rainfall and higher summer temperatures. Population studies over the last decade show a strong decline in numbers. During the dry summer months, the remaining natural ponds are often shared with the Levantine freshwater crab, which increases frog mortality through predation. Despite these challenges, some positive developments have occurred, including the construction of a dam in southern Karpathos and the recognition of the Rhodes water frog population as the same species. To address the species' critical status, the Hellenic Herpetological Society developed a National Action Plan. This plan includes creating artificial ponds, conducting hydrological studies, and implementing education and awareness programs to support the frog's long-term conservation.

Abstract

Until recently, the Karpathos water frog (*Pelophylax cerigensis*) was considered endemic to Karpathos Island (Greece) and has recently been reclassified by the IUCN as Endangered (EN), having been previously assessed as Critically Endangered (CR). The species faces severe threats primarily associated with the scarcity of freshwater bodies in the southern Aegean Sea. Over the past decade, demographic assessments have revealed a marked population decline, driven by the intensifying effects of climate change, including reduced rainfall, and increasing summer temperatures. In addition, the few natural ponds that persist during the dry summer months are often shared with the Levantine freshwater crab (*Potamon potamios*), resulting in increased frog mortality due to predation. Despite these challenges, recent developments provide cautious optimism. These include the construction of a dam in southern Karpathos and the taxonomic reassessment of the water frog population on the neighboring island of Rhodes as conspecific with *P. cerigensis*. In response to the species' precarious status, the Hellenic Herpetological Society designed and implemented a National Action Plan aimed at the protection and conservation of the Karpathos water frog. The Action Plan includes a series of targeted mitigation measures, such as the construction of artificial ponds to retain water during the summer, as well

as a hydrological study addressing the seasonal drying of the ecologically important Eleimonitria spring. A key component of the Action Plan involves education and outreach initiatives targeting primary school students, local residents, and visitors, highlighting the frog's ecological importance and conservation needs. Informational brochures will be distributed across the island to raise awareness of the species' conservation status and the importance of safeguarding its habitat. The implementation of this Action Plan aims to secure the long-term survival of the Karpathos water frog and to strengthen integrated conservation efforts across its extremely limited range.

Keywords: Aegean Archipelago; anuran amphibians; ecological observations; endemic species; Greece; Karpathos Island; water scarcity

1. Introduction

Amphibian survival is strongly dependent on high levels of environmental humidity [1–3]. This physiological dependence is reflected in the disproportionately high number of amphibian species threatened with extinction worldwide, which currently reaches approximately 41% according to the IUCN [4]. Among the primary drivers of this decline is the increasing scarcity of freshwater bodies, a process that has been exacerbated by global climate warming [5,6]. In addition to climate-related pressures, amphibian populations are affected by a suite of anthropogenic and natural stressors, including pollution [7–9], infectious diseases [10–12], habitat degradation and fragmentation [13–15], invasive species [16,17], and road mortality [18–20]. Collectively, these pressures have rendered amphibians one of the most vulnerable vertebrate groups globally.

Climate warming represents one of the most severe environmental challenges of the 21st century [21]. Rising global temperatures are disrupting ecosystems worldwide, with profound consequences for biodiversity and ecosystem functioning [22,23]. Beyond direct physiological effects on individual species, climate change alters ecological interactions and destabilizes community dynamics, leading to cascading impacts across trophic levels [24]. Furthermore, climate warming increases the frequency and intensity of extreme weather events, such as droughts and heatwaves, which impose additional stress on already vulnerable species and habitats [25].

Amphibians are particularly sensitive to climate change due to their biphasic life cycle and dependence on both aquatic and terrestrial environments [1,26,27]. Rising temperatures can disrupt breeding phenology, as many amphibian species require narrow thermal windows for successful reproduction [28,29]. Altered precipitation regimes, including prolonged droughts, can lead to the desiccation of breeding sites such as ponds, springs, and temporary water bodies [30]. Climate change may also facilitate the spread of pathogens, such as chytrid fungi, while simultaneously reducing immune competence in amphibians [31,32]. In addition, changes in temperature and water availability influence dispersal capacity, prey availability, and predator–prey interactions, ultimately reshaping amphibian communities [1]. The combined effects of these processes can drive local population declines and, in extreme cases, extinction. Consequently, habitat conservation and targeted mitigation measures aimed at buffering the impacts of climate change are urgently required [33].

In response to the global amphibian crisis, species-specific action plans and conservation programs have been developed to safeguard threatened taxa and their habitats [34–38]. Such initiatives typically combine population monitoring, habitat restoration and management, environmental assessment, and public awareness and engagement [39].

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The Karpathos water frog (*Pelophylax cerigensis* Beerli, Hotz, Tunner, Heppich & Uzzell, 1994) is widely regarded as Europe's most endangered anuran amphibian, with its total population estimated at approximately 500–600 individuals [40]. The species was considered endemic to Karpathos Island, where freshwater habitats are extremely scarce, but it has recently been confirmed on Rhodes, another island in the southern Aegean Sea (Greece). One of the main pressures affecting the species is the pronounced reduction in rainfall over the past three decades [41,42], which has drastically limited the availability of suitable aquatic habitats. As a result, only a small number of ponds and rivulets persist during the prolonged dry period on Karpathos, which typically extends from late March to early November [40,43].

To address the rapid decline of *P. cerigensis*, the National and Kapodistrian University of Athens, in collaboration with WWF Greece, developed a National Action Plan for the species. The plan was implemented by the Hellenic Herpetological Society between 2020 and 2026. In the present study, we outline the key components of this Action Plan, describe the challenges encountered during its implementation, present the results achieved to date, and discuss the progress made towards securing the long-term conservation of the Karpathos water frog.

2. Materials and Methods

2.1. Study System

Karpathos Island is the second largest island of the Dodecanese complex, with a total area of 324.7 km^2 and a maximum elevation of 1215 m at Kali Limni Mountain [44]. Karpathos began to form geologically about 12 million years ago with the opening of the Mid-Aegean Trench [45]. It was initially isolated around 8 million years ago, while its final separation from Rhodes occurred approximately 3.5 million years ago [46,47]. This long-term isolation has resulted in high levels of biodiversity, including numerous endemic taxa and largely intact natural landscapes [48].

The climate of Karpathos is typically Mediterranean, characterized by hot, dry summers and mild, relatively humid winters. However, the northern part of the island experiences distinct microclimatic conditions, with increased exposure to strong winds and generally drier and cooler conditions compared to the rest of the island [49]. Due to its ecological importance, a substantial proportion of Karpathos is included in the Natura 2000 network, with two designated protected areas (GR4210002 and GR4210003).

The Karpathos water frog (*Pelophylax cerigensis*) is a medium-sized anuran, with a snout–vent length (SVL) ranging from approximately 5 to 7 cm. Dorsal coloration varies from brown and brown-grey to olive-brown, typically with numerous small dark spots and blotches (Figure 1). In some individuals, a light yellow-green or cream-colored vertebral stripe is present [41,50]. The species is primarily diurnal and remains active throughout the year, although during the summer months, activity shifts towards dusk. Breeding takes place in spring [41]. Feeding is opportunistic and largely dependent on prey availability, with Coleoptera, Hymenoptera, and spiders constituting the main prey groups [43].

Until recently, *P. cerigensis* was considered endemic exclusively to Karpathos Island. However, recent phylogenetic and molecular evidence has demonstrated that the water frog population on the neighboring island of Rhodes also belongs to this species [51,52]. The Karpathos water frog, which until recently was classified as Critically Endangered (CR), is now listed as Endangered (EN) on both the IUCN Red List [53] and the Greek Red List [54].

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Figure 1. An adult Karpathos water frog in the Argoni area (Photo by A.C.).

2.2. Study Area

The Action Plan primarily focused on the northern part of Karpathos Island, where the main extant populations of the species occur. The principal distribution areas include the rivulets of Olympos and Achamantia, as well as the Argoni and Nati areas (Figure 2). These freshwater systems constitute the core habitats supporting the remaining populations of *P. cerigensis* on the island.



Figure 2. Map of Karpathos Island showing the distribution areas of the Karpathos water frog (red dots); (1) Panagia Eleimonitria Spring, (2) Olympos stream system, (3) Nati rivulet, (4) Argoni rivulet, (5) Achamantia area, and (6) Schina Dam. Triangles indicate the locations where new ponds were constructed to enhance the species' habitat.

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Vegetation surrounding the rivulets is dominated by phrygana and sparse shrub formations. Characteristic plant species include thyme (*Thymbra capitata*), thorny burnet (*Sarcopoterium spinosum*), lentisk (*Pistacia lentiscus*), and, in some areas, open stands of eastern Mediterranean pine (*Pinus brutia*). In several locations along the rivulets, thermo-Mediterranean riparian galleries are present, with dominant vegetation consisting of oleander (*Nerium oleander*) and spiny rush (*Juncus articulatus*) [43].

In addition to natural water bodies, small frog populations were also recorded in traditional artificial structures, such as concrete water tanks and livestock watering troughs, which locally function as alternative refugia during dry periods.

2.3. Actions and Methods

The overarching aim of the Action Plan was to secure the long-term survival of the Karpathos water frog and to promote integrated conservation efforts across its limited range. To achieve this goal, a set of priority actions and mitigation measures was identified and implemented, structured around four main objectives.

2.3.1. Halting and Reversing Population Decline

To mitigate the effects of prolonged drought and habitat loss, new artificial ponds were constructed to retain water during dry periods and function as refugia for the species. In parallel, a hydrogeological study was conducted at the Panagia Eleimonitria Spring, followed by targeted technical interventions aimed at restoring water availability. The reactivation of this spring had a positive effect on the frog population inhabiting the Olympos rivulets.

2.3.2. Improvement of Habitat Quality

Efforts were made to enhance the capacity of existing habitats to support viable frog populations. This included the regular maintenance of natural and artificial water bodies, as well as the removal of waste, debris, and sediment from all known sites occupied by the species.

2.3.3. Enhancement of Biological and Ecological Knowledge

A key priority was the investigation of the taxonomic status and genetic structure of the Rhodes Island populations, following earlier suggestions that they might belong to the same species as the Karpathos population. Molecular analyses were conducted, and genetic diversity was assessed at the population level [51,55,56]. To assess the full extent of the species' distribution on Karpathos Island, extensive field surveys were carried out across a large number of water bodies. A total of 219 sites were surveyed, including concrete water tanks (n = 152), springs (n = 27), streams and rivulets (n = 15), livestock watering troughs (n = 8), cisterns (n = 7), temporary ponds (n = 5), wells (n = 3), a marsh (n = 1), and a dam (n = 1) (Figure 3a–h). In addition, frog populations were systematically monitored throughout the duration of the project, with surveys conducted twice annually using line transects and point counts. Further emphasis was placed on documenting the species' ecology through systematic field observations.

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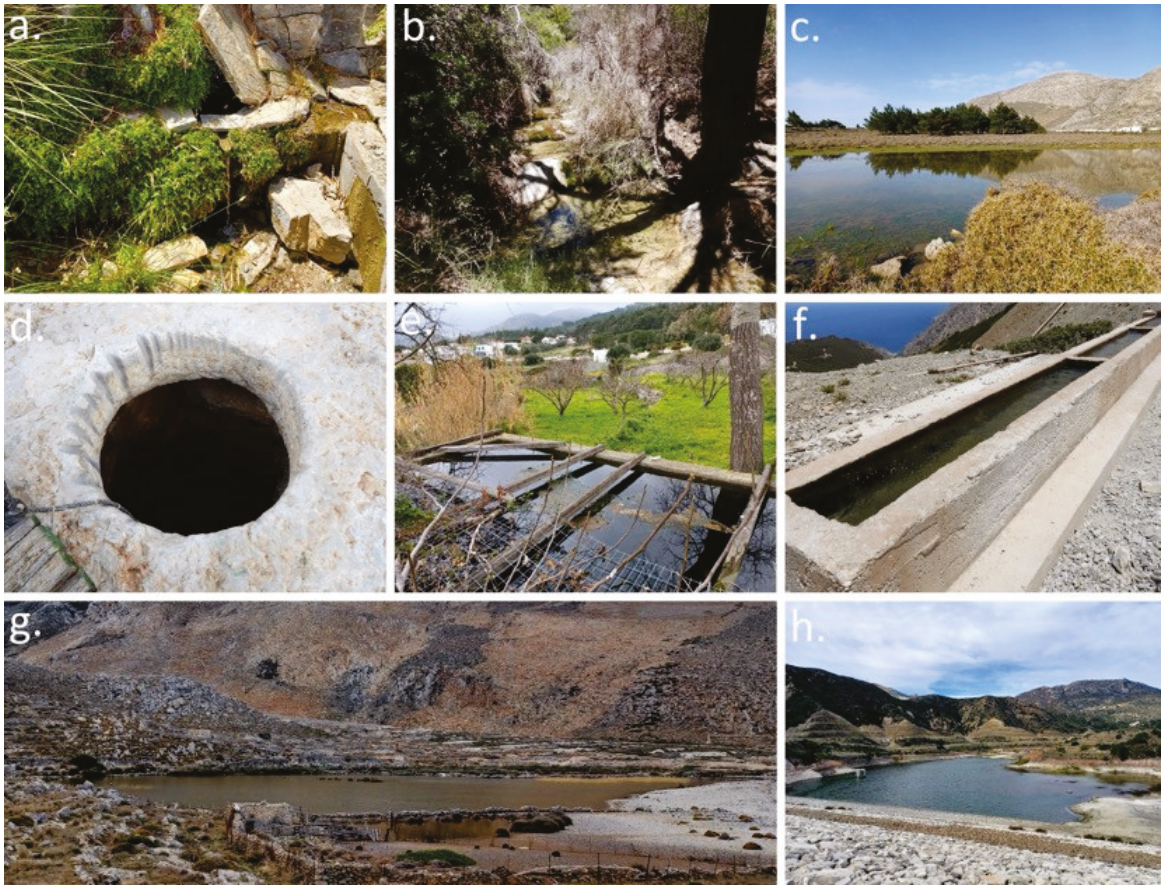

Figure 3. (**a**) The Ligo Nero Spring located on the east side of central Karpathos. (**b**) The rivulet Forokli at Panagia Prastiotissa area. (**c**) Temporal pond in Lastos Plateau. (**d**) Well in the Achordaea area. (**e**) Concrete water tank in the settlement of Stes. (**f**) Livestock watering trough in northern Karpathos. (**g**) The marsh of Tristomo area. (**h**) Schina Dam at the area of Pigadia (Photos by A.C.).

2.3.4. Public Awareness and Stakeholder Engagement

To foster local support for conservation actions, a targeted educational program was developed for primary school students on Karpathos. In addition, an informational brochure, available in both Greek and English, was produced to communicate the ecological importance of the species and the conservation measures implemented. The brochure was distributed to local residents and visitors to the island.

3. Results

3.1. Pond Construction

Small artificial ponds were constructed as part of the Action Plan to mitigate the effects of summer drought and the drying of natural water bodies. Their construction was approved by the local Management Unit of Protected Areas of the Natural Environment and Climate Change Agency (N.E.C.C.A.) and licensed by the Dodecanese Department of Forest Administration (Approval No. Α.Π. 58826). Summers on Karpathos are characterized by prolonged aridity, during which many rivulets inhabited by the species dry out completely (Figure 4). The newly constructed ponds were therefore designed to provide permanent or semi-permanent refugia for frogs during dry periods.

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Figure 4. Water scarcity in the Nati rivulet during spring (Photo by P.P.).

In total, five water tanks were constructed following similar design specifications: approximately 2 m in length, 1.5 m in width, and 1 m in depth, with walls approximately 40 cm thick. Site selection was based on three main criteria: accessibility for construction machinery, availability of adequate space, and proximity to a spring capable of supplying water to the tanks. The ponds were built using local stone to ensure visual and ecological compatibility with the surrounding landscape, while the interior surfaces were coated with cement mortar to ensure waterproofing. Small ramps were incorporated both inside and outside the tanks to facilitate frog movement in and out of the water. In locations adjacent to slopes, low retaining walls (30–50 cm high) were constructed to prevent sediment and eroded material from entering the tanks. Water was supplied to the tanks via polyvinyl chloride (PVC) hoses connected to nearby springs (Figure 5a,b).



Figure 5. (**a**) Two of the newly built water tanks in the area of Achamantia. (**b**) An older water tank connected with the new water tanks in the area of Argoni (Photos by A.C.).

3.2. Panagia Eleimonitria Spring Study

The Panagia Eleimonitria Spring, located in the Olympos area, historically supported a section of a rivulet inhabited by a small frog population. In recent years, however, water discharge from the spring has declined substantially and, during certain visits both before and during the implementation of the Action Plan, it ceased entirely, primarily in the

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summer and autumn months. During the early years of the project, both adult frogs and tadpoles were recorded at the spring, whereas following periods of water depletion, only few adult individuals, or none at all, were observed (see Table 1), indicating a marked reduction in local frog presence. To address this issue, a hydrological study was commissioned to investigate the spring's hydrogeological characteristics and identify potential measures to secure a more stable water supply. The study found that the reduced yield of the Eleimonitria Spring is mainly due to natural hydrogeological factors, including a 33% decrease in rainfall over the past 20–25 years, particularly summer droughts, and the local geology, while human activities mainly affect the movement of water after it leaves the spring toward the rivulet [42].

Table 1. Population monitoring of Karpathos water frog across the island (2021–2025). Annual counts of adults, juveniles, and tadpoles across all known distribution areas, providing demographic data and site-specific population dynamics. The Nati rivulet is not included, as the species has not been recorded there since 2020.

Location	Year	Adults	Youngs	Tadpoles
Argoni Rivulet	2021	72	18	338
	2022	75	60	345
	2023	94	11	173
	2024	75	7	220
	2025	90	27	318
Eleimonitria Spring	2021	2	0	10
	2022	1	0	0
	2023	0	2	0
	2024	0	0	0
	2025	0	0	0
Olympos Rivulet, N–NNE	2021	54	10	34
	2022	15	3	10
	2023	16	9	32
	2024	14	1	70
	2025	21	6	75
Olympos Rivulet, E	2021	22	0	0
	2022	30	7	15
	2023	50	0	10
	2024	17	4	45
	2025	24	14	35
Achamantia area	2021	1	0	0
	2022	7	0	0
	2023	1	0	0
	2024	3	0	10
	2025	4	5	1
Schina Dam (Pigadia)	2021	0	0	0
	2022	0	0	0
	2023	0	0	0
	2024	15	0	0
	2025	2	0	0

Based on the findings of this study, mild technical interventions were implemented. These included the construction of an artificial pond fed by an existing upstream tank supplied by the spring. Overflow from both the new and the pre-existing tanks was directed into the rivulet, thereby ensuring a more continuous flow of water. As the summer refugia previously used by frogs in this rivulet were drying out completely, the intervention is expected to maintain water availability throughout the year, provided that the spring continues to discharge. The construction was approved by the Directorate for the Management

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of Protected Areas (Sector B) of N.E.C.C.A. and licensed by the Dodecanese Department of Forest Administration (Approval No. Α.Π. 212954).

3.3. Maintenance of Existing Water Bodies

At the onset of the Action Plan, surveys were conducted at all known frog habitats in northern Karpathos, including both natural and artificial water bodies, in order to assess their condition. Overall, most rivulets and water tanks were found to be in good condition, with minimal evidence of human disturbance, littering, or infilling by debris. In a limited number of cases, however, water tanks contained accumulated debris or small amounts of discarded waste were observed within rivulets, specifically in a few rivulet puddles of the Olympus stream system located near a residential area, while the more remote streams showed no signs of littering or infilling.

All foreign materials were removed from affected sites to prevent further degradation and reduce the risk of complete infilling by soil and stones. These maintenance actions contributed to preserving the functionality of existing water bodies as suitable frog habitats.

3.4. Molecular Analyses

Molecular analyses were conducted on samples collected from Karpathos and Rhodes during the first year of the project. The results confirmed that frog populations from both islands belong to the same species, forming a well-supported monophyletic clade with low genetic divergence between populations [52].

3.5. Surveys of Water Bodies on Karpathos

Most of the surveyed areas across the island did not reveal new frog populations. However, a small population was newly discovered during this project at Schina Dam, near the island's capital, Pigadia, in southern Karpathos. The dam, completed in 2020–2021, covers an area of approximately 5.42 ha. The presence of frogs at this site is presumed to be the result of accidental human-mediated translocation.

3.6. General Ecological Observations

Field surveys revealed that frogs actively migrate over short distances within rivulets and streams, typically several tens of meters, among nearby puddles in response to declining water availability. These movements were occasional and involved individual frogs rather than large groups. As shallow pools dry out, individuals move upstream to sections where small puddles retain water, even during the driest periods of the year. Consequently, large aggregations of frogs (up to 37 individuals) were observed within single puddles during summer and autumn, irrespective of puddle size (Figure 6). In contrast, during winter and spring, when water availability increases, frogs were more evenly distributed across multiple pools, typically with 1–5 individuals per puddle depending on size.

While adult frogs are capable of relocating between water bodies, tadpoles are highly vulnerable to desiccation. On multiple occasions, large numbers of tadpoles that had not yet completed metamorphosis were found dead in puddles that had dried out completely during July and September.

Predation pressure increased markedly in the few remaining water bodies during drought conditions. The most frequently observed predator was the Karpathos freshwater crab (*Potamon karpathos*) (Figure 7). Although the two species have coexisted historically, the scarcity of suitable habitats forces both to occupy the same limited water bodies for extended periods, leading to repeated predation events [57]. In addition, six species of migratory batrachophagous birds (*Ardea cinerea*, *Ardea purpurea*, *Egretta garzetta*, *Ardeola ralloides*, *Nycticorax nycticorax*, and *Circus aeruginosus*) were observed feeding in habitats occupied by the frog. Direct predation on tadpoles or juveniles was confirmed for *E. garzetta*,

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A. ralloides, and *N. nycticorax*. A single record of frog remains in beech marten (*Martes foina*) droppings suggests occasional predation by mammals, although further study is required to assess its significance.

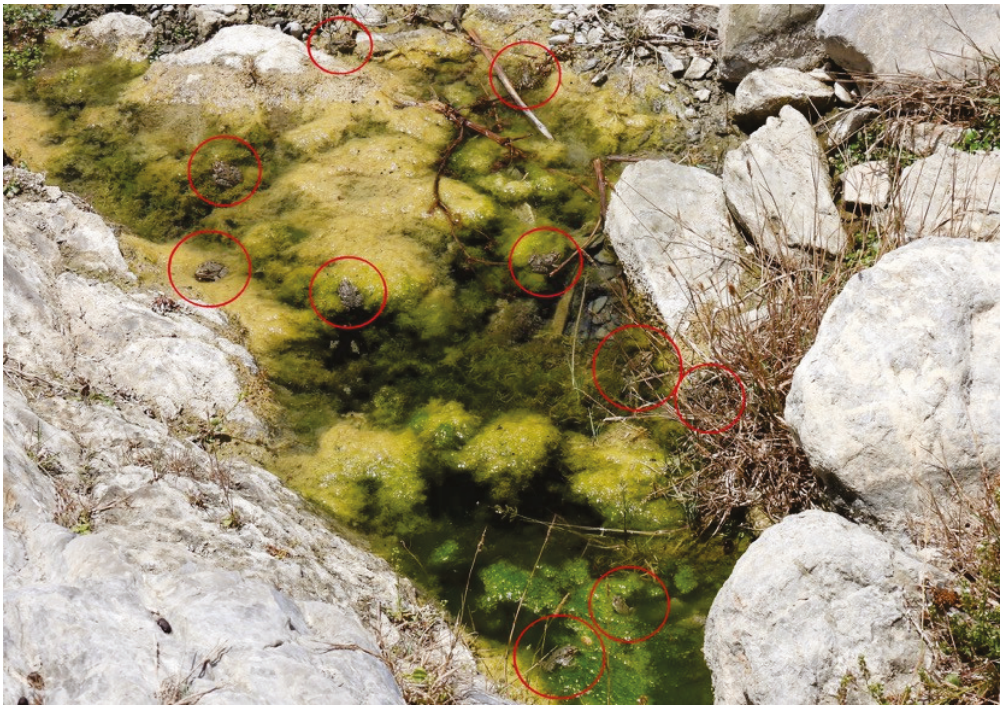

Figure 6. Seasonal frog aggregation in a remaining puddle of a rivulet in the Olympos area during summer drought, when most of the streambed dries out. Red circles indicate the position of individual frogs (Photo by A.C.).



Figure 7. An incident of predation of a Karpathos water frog by the Karpathos freshwater crab in shallow waters in Argoni rivulet during drought conditions (Photo by A.C.).

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3.7. Population Monitoring and Colonization of Newly Constructed Ponds

Continuous population monitoring was conducted across all study sites to evaluate demographic structure, temporal trends, and site-specific population dynamics. The number of frogs recorded was systematically documented each year, with individuals categorized as adults, juveniles, and tadpoles, providing insights into overall population fluctuations and long-term trends. These data allowed for an assessment of population health and the identification of sites with higher or lower densities, contributing to a broader understanding of species status on the island. The results of population monitoring are presented in Table 1.

One of the central objectives of the Action Plan was the creation of artificial ponds to expand available habitat. The newly constructed ponds were systematically monitored to assess their use by the species. Results were encouraging; despite their very recent construction, field observations indicated early colonization by the target species, with adult frogs present and breeding observed in Argoni ponds during the first year of their construction, and a small number of tadpoles recorded in Achamantia ponds during the second year, indicating successful use of these artificial habitats for both refuge and reproduction. These observations should be regarded as indicative rather than conclusive, and a systematic, long-term monitoring scheme is required to reliably assess the effectiveness of the constructed ponds in supporting amphibian populations under prolonged drought conditions. In Table 2, yearly counts of individuals have been included to provide a more detailed picture of colonization success.

Table 2. Colonization of newly constructed ponds by Karpathos water frog (2023–2025). Yearly records of adults, juveniles, and tadpoles in artificial ponds in the Argoni and Achamantia areas, providing demographic data and indicating early colonization and reproductive activity. The newly constructed ponds in Nati and Eleimonitria are not included, as no frogs were recorded there during monitoring.

Location	Year	Adults	Youngs	Tadpoles
Argoni pond 1	2023	1	0	0
	2024	2	0	2
	2025	2	0	0
Argoni pond 2	2023	2	0	4
	2024	6	1	7
	2025	1	2	2
Achamantia pond 1	2023	0	0	0
	2024	0	0	1
	2025	0	0	0
Achamantia pond 2	2023	0	0	0
	2024	0	0	10
	2025	0	1	1

3.8. Education and Awareness

A six-lesson educational package was developed specifically for primary school students. Each lesson targeted defined learning outcomes and combined classroom-based activities with field exercises, allowing students to directly observe the frog and its habitat. Through these activities, students explored local environmental conditions, temporal and spatial changes, and the ecological role of the species, fostering awareness of the relationship between biodiversity and human well-being.

In addition, two outreach actions targeted the wider public. Presentations on the biology and conservation of the Karpathos water frog were delivered in all primary schools on the island. Furthermore, an informational brochure, produced in both Greek and English,

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was published and will be distributed to residents and visitors, highlighting the species' conservation status and the measures implemented under the Action Plan.

4. Discussion

The conservation actions implemented for the Karpathos water frog (*P. cerigensis*) represent a targeted response to the severe and ongoing pressures faced by one of Europe's most threatened amphibian species. The results of the Action Plan demonstrate that carefully designed habitat-based interventions, combined with systematic monitoring and public engagement, can mitigate some of the immediate effects of drought and habitat loss in insular environments characterized by extreme water scarcity.

The construction of artificial ponds proved to be one of the most effective measures implemented under the Action Plan. Prolonged summer droughts on Karpathos frequently result in the complete desiccation of natural rivulets and puddles, leading to high mortality rates, particularly among tadpoles that are unable to relocate. The rapid colonization of newly constructed ponds, including the presence of both adult frogs and tadpoles during the year of construction and the subsequent year, indicates that these artificial water bodies function successfully as refugia and breeding sites. Similar findings have been reported in other amphibian conservation and monitoring programs, where artificial or restored aquatic habitats have partially compensated for the loss of natural water bodies under changing climatic conditions [30,58–60]. In the context of Karpathos, where freshwater availability is extremely limited, such interventions are likely to play a critical role in sustaining local populations, particularly in areas such as Achamantia, where population size is currently very low.

The hydrological interventions at the Panagia Eleimonitria Spring further highlight the importance of maintaining year-round water availability in key breeding and refuge sites. The decline in water discharge from the spring had previously resulted in the near-collapse of the local frog population. The redirection and stabilization of water flow through mild technical measures are expected to improve habitat stability and reduce seasonal bottlenecks. Although the long-term effectiveness of this intervention will depend on future precipitation patterns and the persistence of spring discharge, the approach demonstrates how site-specific hydrological management can contribute meaningfully to amphibian conservation in water-limited landscapes.

Field observations revealed pronounced seasonal shifts in frog distribution, driven primarily by water availability. During dry periods, frogs congregated in a small number of remaining puddles, often at high densities, whereas in wetter seasons, individuals were more evenly distributed across available habitats. This behavioral response increases vulnerability to predation and density-dependent mortality during droughts. The repeated observation of predation by the Karpathos freshwater crab (*P. karpathos*), as well as by migratory birds, underscores the indirect effects of habitat contraction. While predation is a natural ecological process, the forced co-occurrence of predators and prey in a limited number of shrinking water bodies likely amplifies its impact beyond natural levels. Similar interactions have been documented in other drought-affected amphibian systems, where reduced habitat availability intensifies predator–prey encounters and accelerates population decline [1,61,62].

Tadpole mortality emerged as a critical bottleneck for population persistence. The repeated loss of entire cohorts due to puddle desiccation highlights the vulnerability of early life stages to climatic extremes. This pattern is consistent with global evidence indicating that larval mortality driven by drought is a major contributor to amphibian population declines, particularly in species with restricted distributions and limited dispersal capacity [30,58]. The presence of tadpoles in newly constructed ponds suggests that artificial

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water bodies may partially alleviate this bottleneck by extending hydroperiods during the breeding season.

The molecular confirmation that frog populations on Karpathos and Rhodes belong to the same species has important conservation implications. The species' persistence is supported by the presence of geographically isolated populations, which reduces the risk of total extinction if one population is lost, and emphasizes the importance of island-specific management strategies. Each population is exposed to distinct environmental pressures, and conservation actions should aim to preserve local genetic and ecological characteristics rather than treating the species as a single homogeneous unit.

The unexpected discovery of a small population at the Schina Dam highlights both the species' capacity to exploit newly available aquatic habitats and the potential role of human-mediated translocation. Although the long-term viability of this population remains uncertain, large artificial reservoirs may offer relatively stable aquatic environments in otherwise arid landscapes. Nevertheless, reliance on such habitats should be approached cautiously, as they may introduce new threats, including altered predator communities and water management practices beyond conservation control.

Beyond direct habitat interventions, education and public awareness emerged as essential components of the Action Plan. Engaging local communities, particularly younger generations, fosters long-term stewardship and reduces the likelihood of habitat degradation. The generally high environmental awareness reported among residents of northern Karpathos suggests that conservation initiatives can build upon existing positive attitudes, reinforcing local involvement in species protection.

Overall, the results of this study indicate that while the Karpathos water frog remains highly vulnerable to climate change, drought, and habitat limitation, targeted and adaptive management actions can slow or partially reverse local declines. Continued long-term monitoring will be essential to evaluate population trends, assess the durability of implemented measures, and refine conservation strategies in response to ongoing environmental change. The Action Plan for *Pelophylax cerigensis* provides a valuable framework that may be applicable to other insular amphibian species facing similar challenges under increasingly arid climatic conditions.

5. Conclusions

Amphibians are the most threatened vertebrate group globally, and the Karpathos water frog (*Pelophylax cerigensis*) exemplifies the vulnerability of insular species exposed to increasing climatic stress and habitat limitation. The implementation of the National Action Plan has produced encouraging outcomes, demonstrating that targeted, science-based interventions can mitigate key threats acting on critically endangered amphibians.

Habitat restoration through artificial pond construction, hydrological management of critical springs, systematic population monitoring, and genetic assessment have collectively improved habitat availability and supported population persistence. The rapid use of newly created water bodies by adults and tadpoles highlights their effectiveness in buffering drought impacts and reducing larval mortality. Confirmation that the Rhodes and Karpathos populations belong to the same species further refines the conservation framework for *P. cerigensis*.

Nevertheless, significant challenges remain. Climate change-driven reductions in rainfall, rising temperatures, and increased predation pressure in shrinking habitats continue to threaten long-term viability. Sustained maintenance and expansion of artificial and semi-natural refugia, combined with adaptive management, will be essential in this water-limited island system.

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Community engagement and education are equally crucial. Raising awareness among residents and visitors fosters local stewardship and strengthens long-term conservation success. In conclusion, although full recovery remains uncertain, the actions implemented to date provide a strong foundation for the long-term conservation of the Karpathos water frog. This Action Plan offers a valuable model for the conservation of insular amphibians facing similar challenges under ongoing environmental change.

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