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HeRA: A New Tool for Assessing the Invasiveness Potential of Non-Native Species

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Abstract

With over 140 species of primary freshwater fish, including 73 that are endemic, Greece is recognized as a biodiversity hotspot in Europe. However, like freshwater systems worldwide, these ecosystems face serious threats, particularly from the introduction of non-native species. This ongoing increase in invasive species has heightened scientific and policy-maker awareness, as such introductions can lead to population declines and even extinctions of native fish. In response to this growing concern, various risk assessment tools have been developed to evaluate the potential hazards posed by non-native species, both those already established and those likely to be introduced. These tools are critical for informing policy decisions and managing biological invasions effectively. In the current study, a new tool, the Hellenic Risk Assessment (HeRA), is proposed. Unlike its predecessors, HeRA places greater emphasis on assessing the biological traits of introduced species and incorporates region-specific considerations tailored to the Mediterranean basin. Its scoring system evaluates both the likelihood of a species establishing itself and its potential environmental impact, making it a valuable resource for stakeholders in prioritizing management actions and making informed decisions regarding the import of live fish species.

Keywords: risk assessment; HeRA; alien species; invasive; endemic; freshwater fish

1. Introduction

Freshwater ecosystems, though covering only about 0.8% of Earth's surface, support approximately 12% of the world's known animal species, making them vital biodiversity reservoirs [1,2]. Despite their limited spatial coverage, freshwater habitats such as rivers, lakes, and wetlands sustain a disproportionately high number of species relative to their size [1,2]. Among the taxa inhabiting these ecosystems, freshwater fish are particularly vulnerable, representing more than 51% of all known fish species [2–4]. Approximately 30.1% of the freshwater fish species assessed by the International Union for Conservation of Nature (IUCN) are classified as threatened with extinction—Critically Endangered (CR), Endangered (EN), or Vulnerable (VU) [4].

Globally, native freshwater fish face multiple anthropogenic threats including water abstraction, habitat fragmentation, pollution, hydrological changes, climate change, and especially the introduction of non-native species [5–12]. These pressures often act synergistically, exacerbating ecosystem degradation. For example, hydrological changes, such



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as flow alteration from dams, can concentrate pollutants, increasing local contaminant loads and amplifying water quality issues [6,10–12]. Simultaneously, climate change shifts species' distributions and phenology, altering life-history timing and metabolic rates, often disrupting ecological interactions and leading to broader community disassembly [13]. Consequently, many freshwater species experience population declines or local extinctions at alarming rates.

Among freshwater taxa, fish are especially vulnerable: over 30% of assessed species are considered threatened according to the IUCN Red List [4]. A principal driver of biodiversity loss in freshwater systems is the introduction of non-native species, either intentionally (e.g., for aquaculture or fisheries enhancement) or unintentionally (e.g., via ballast water or aquarium releases) [7,8]. The establishment of invasive species contributes to biotic homogenization, wherein regionally distinct assemblages are replaced by wide-spread cosmopolitan species, leading to increased similarity across regions and a simultaneous decline in biodiversity [8,14,15]. Studies have demonstrated that non-native species can drive native and endemic species toward extinction through mechanisms such as predation, food competition, genetic pollution, and the spread of novel pathogens [16–18], ultimately altering community structures and ecosystem functions [18].

Invasive species are introduced through both deliberate and unintentional pathways. These include aquaculture (e.g., common carp, rainbow trout), ornamental fish release (e.g., goldfish), biological control (e.g., mosquitofish for mosquito control, bighead and silver carp for water quality and weed management), and accidental introductions (e.g., via ballast water or fish farm escapes) [12]. The Mediterranean basin is one of the most prominent global biodiversity hotspots, particularly vulnerable to biological invasions due to its climatic uniqueness, high endemism, and dense human activity [12,14]. The region's heterogeneous landscape, combined with a long history of human settlement and trade, has facilitated the introduction and establishment of numerous non-native species. The Mediterranean's warm, seasonal climate and variable hydrological regimes further influence invasion dynamics, often favoring species with broad environmental tolerances and high reproductive capacity [15,16].

Greece, specifically, hosts 177 species in inland waters—of which 143 are strictly freshwater; 73 (51%) are endemic, 37 (26%) are alien, and 33 (23%) are cosmopolitan (Table S1) [19–21]. Greece likely hosts the highest percentage of freshwater fish endemism in Europe [22], highlighting its status as a biodiversity hotspot [23–28].

One of the first recorded introductions of non-indigenous fish species in Greek waters dates to Roman times, with the introduction of *Cyprinus carpio* [21,28]. Later introductions include *C. carpio* and *Tinca tinca* into Lake Pamvotis in 1929 [20]. The most significant wave of deliberate introductions occurred during the 1980s for aquaculture (e.g., sturgeons, rainbow trout), recreational fisheries, and ecosystem management (e.g., grass carp to address eutrophication). Other introductions occurred inadvertently or through deliberate releases by hobbyists (e.g., goldfish, sailfin molly) [9,15–18,20,22,23].

Identifying the primary drivers of non-native species establishment and their impact on endemic fish remains a critical challenge in conservation biogeography [12,28,29]. Risk assessments are essential in determining which invasive species should be prioritized for control to prevent further ecological damage [30–32]. Risk assessment has been recognized as a key component of policymaking by the Convention on Biological Diversity (CBD) and the European Strategy for Invasive Alien Species [33]. While risk assessments have been widely applied in the United States [34,35], European freshwater fish remain understudied [33,36–40].

Several tools have been developed to assess species invasiveness. Notably, the Fish Invasiveness Screening Kit (FISK) [36,37] and the Fish Risk Assessment Model (FRAM) [41]

utilize questionnaire-based frameworks to evaluate Establishment Risk and Environmental Impact Risk. Generic models, while useful, may not capture the unique interactions and species traits relevant to the Mediterranean environment, potentially leading to under- or overestimation of risks [37,39]. Thus, there is a pressing need for customized tools that incorporate region-specific data and expertise.

To address this limitation, the present study introduces the Hellenic Risk Assessment (HeRA), a novel tool designed to assess the invasiveness of non-native freshwater fish in Greece. HeRA focuses on the species' biological and ecological characteristics, recognizing that establishment success is closely linked to these traits. By incorporating local ecological data and pressures specific to the Mediterranean basin, HeRA enhances the accuracy of risk screening and supports a trait-based approach to risk evaluation [41–43].

This paper describes the structure and rationale of HeRA, applies the tool to 37 alien freshwater species recorded in Greek inland waters, and validates its scoring system against existing literature. By enhancing the resolution and relevance of invasion risk assessments, HeRA offers a valuable framework for policymakers, researchers, and conservation practitioners aiming to safeguard freshwater biodiversity in the Mediterranean region.

2. Materials and Methods

Risk Assessment

The Hellenic Risk Assessment Tool (HeRA) is an answering protocol based on the main idea of relevant tools, i.e., FISK, FRAM. However, HeRA is a more biologically oriented assessment tool since it describes the biological aspects of the invasive species. HeRA consists of 68 questions based on 12 traits, grouped into two sets: part A, assessing the “Risk of Establishment” and part B, assessing the “Risk of Environmental Impact”. For each question, there are three possible answers (No/Yes/Unsure), and for each possible response there is a numerical score; its range depends on the assessed trait (from −1 to 0, from −4 to 1, etc., see Tables S3 and S4 for further information on the scoring scheme). The inclusion of negative scores was intended to account for biological or management-related characteristics that reduce invasion success. Unlike traditional additive scoring systems that only reward invasion-promoting traits, HeRA also incorporates traits associated with reduced establishment probability or reduced environmental impact. For example, highly specialized reproductive requirements, intensive exploitation by fisheries, the presence of natural predators, delayed maturation, or slow population growth may substantially limit the capacity of a species to establish viable populations. Negative scores therefore function as corrective factors that reduce the overall invasion risk estimate when evidence suggests biological constraints on establishment or spread. The result of HeRA for each species under investigation is a set of two scores being calculated by adding the individual score for each question, one for the “Risk of Establishment” and one for the “Risk of Environmental Impact”, respectively. The highest score in both parts of the questionnaire represents the highest invasion potential.

According to Leonardos [21], 37 non-native species have been recorded in Greek freshwater systems. These 37 species were selected since their natural distribution is delimited to another country or even continent, and they were introduced in the Greek freshwater systems either intentionally (for aquaculture, pest control, etc.) or accidentally (transferred accidentally with other species used for aquaculture purposes). These non-native species were used for the tool trial assessment (Tables S3 and S4). All species were evaluated for both their Risk of Establishment (Table S3—part A) and their Risk of Environmental Impact (Table S4—part B) according to Jenkins [3].

As was mentioned before, the assessment tool presented here consisted of two parts. In part A there are 18 questions in total, organized in four sections: (1) History of

spread/climate match, (2) Ability to reproduce outside their native distribution, (3) Vectors and propagule pressure and (4) Factors that control the establishment (Table S3).

The purpose of this section was to determine whether the new environment favored or not the establishment and survival of the non-indigenous species. This could be determined by taking into consideration the historical data of the species' establishment in other ecosystems, within or out of its natural range and by comparing the environmental characteristics of these areas with those prevailing on the Greek freshwater systems. The ability of the species to establish a viable population was also evaluated by its ability to achieve successful reproduction. Successful reproduction indicates the acclimatization of the species in new conditions and that all requirements to complete its life cycle have been met. Within the reproduction component, negative scores are assigned to species requiring highly specific spawning habitats or environmental conditions that are absent or uncommon in Greece. Such constraints reduce the likelihood of successful reproduction and therefore lower establishment probability despite the species being present in the environment. Thus, the higher the score, the higher the probability that the species can establish populations in new areas. The HeRA questionnaire captures information on commercial value through Question 4.1 in Part A ("Is this species exploited?"), where a positive response (Yes) receives a negative score (−1), reflecting the reduced establishment risk associated with harvesting pressure. Exploitation was defined broadly to include commercial fisheries, recreational angling, and aquaculture harvesting, based on information from FishBase [44] and national fisheries reports. This scoring approach assumes that species with higher commercial value experience greater fishing mortality, which may limit population growth and reduce establishment success in the wild.

Regarding part B of the protocol, there are 49 questions grouped into 8 traits (Table S2): (1) Reported impacts within its natural range in other countries, (2) Feeding and competition/Feeding behavior, (3) Reproductive tactics, (4) Growth and population factors, (5) Dispersal mechanisms, (6) Physical and chemical tolerances, (7) Invasive relatives and special quarantine requirements and (8) Undesirable traits. The purpose of the second part of the protocol was to determine the level of risk that a non-native species poses on the native species or even on humans by examining their spawning strategy, their behavior against native species (feeding competition or competition for domination over a habitat by altering its quality) and by investigating if they are poisonous or can reduce the amount of water available for human consumption (lowering water quality). Similarly, in the population growth component, negative scores are associated with slow growth rates, delayed maturity, long generation times, and low population turnover. These traits generally reduce the rate at which populations can expand after introduction and consequently lower invasion success. As in part A, the highest score achieved by a species, the highest degree of impact imposed by the species.

Several life-history parameters commonly used in fisheries science were incorporated into HeRA because they are directly associated with establishment success and invasiveness. The von Bertalanffy growth coefficient (k) describes the rate at which a species approaches its maximum body size. Species with high k values generally grow rapidly and can reach reproductive maturity sooner, facilitating rapid population establishment. Age at first maturity (t_m) represents the age at which individuals first reproduce. Species maturing at younger ages often exhibit greater invasive potential because population growth can begin shortly after introduction. Maximum age (t_{max}) represents the longest recorded lifespan of a species. The ratio t_m/t_{max} provides an estimate of the proportion of a species' lifespan required before reproduction begins. Lower values indicate earlier maturation relative to lifespan and are generally associated with higher population growth potential. Population doubling time describes the period required for a population to double in size

under favorable conditions. Species exhibiting short doubling times may rapidly increase abundance following introduction and therefore present a greater establishment risk.

The traits of non-native freshwater fishes distributed in the Greek systems were identified and recorded through the most extensive literature survey conducted to date, involving bibliographical sources (i.e., Fishbase, scientific papers, books, action plans, technical reports, Master and PhD theses). In the data employed in this study, the taxonomy and nomenclature provided by Kottelat & Freyhof [43] and Froese & Pauly [44] were used; sub-species were not considered.

The assessment of the tool was performed by three scientists (the authors of the current study), who used the tool independently for all species. In this way, three assessments for each species were performed and compared with their classification according to the literature. In case there was a differentiation in the assessments of the assessors, i.e., there was a 20% variation in the final scores, all the answers were compared. The questions that presented the highest differentiation were rephrased to give a precise definition of the described trait and thus to avoid the confusion of the assessors. To minimize potential biases associated with expert elicitation, each assessor completed the questionnaire independently and without consultation with the other assessors. Only after the completion of all evaluations were the results compared and discussed. This approach follows recommendations aimed at reducing social influence and preventing premature consensus among experts, which may otherwise lead to overconfidence or convergence on inaccurate assessments [45].

Following the completion of the HeRA questionnaire for all 37 species and the resolution of any scoring discrepancies among the three assessors, the individual scores for “Risk of Establishment” (Part A) and “Risk of Environmental Impact” (Part B) were calculated for each species by summing the numerical scores assigned to each question (see Tables S3 and S4 for the complete scoring scheme).

To visualize the combined risk profile of each species and explore the relationship between the two risk components, a simple scatter plot was generated using Microsoft Excel 365. In this plot, each species is represented by a single point, with its Establishment Risk score plotted on the x-axis and its Environmental Impact Risk score plotted on the y-axis. This graphical approach allowed for the simultaneous examination of both dimensions of invasion risk and facilitated the identification of patterns and clusters among the assessed species.

Subsequently, to create a practical and user-friendly classification system that could assist stakeholders in prioritizing management actions, the species were grouped into risk categories based on their Establishment Risk scores. The threshold values for these categories were determined empirically by examining the distribution of the Establishment Risk scores obtained from the dataset. This approach, which involves identifying natural breaks or clusters in the data to define risk categories, is consistent with methodologies commonly used in the development and calibration of other aquatic invasive species screening tools [46]. Specifically, the scores revealed three distinct groupings of species that also corresponded to differences in key biological and ecological characteristics (e.g., thermal tolerance, reproductive strategy, and commercial value). Based on this analysis, species with an Establishment Risk score of less than 10 were classified as “Low Risk”, species with scores greater than 10 and up to 20 were classified as “Medium Risk”, and species with scores greater than 20 were classified as “High Risk”. These thresholds were selected as they effectively separated species into groups with distinctly different biological and ecological profiles, as detailed in the Section 3.

The scores obtained for Establishment Risk and Environmental Impact were subsequently analyzed using Correspondence Analysis to investigate relationships between

invasiveness patterns and selected biological characteristics of the assessed species, including origin, spawning strategy, thermal tolerance, and commercial value.

It must be mentioned that during the development of HeRA, Correspondence Analysis was applied for the purpose of providing insights into the relationships/correlations among species traits such as temperature tolerance and spawning strategy and the environmental impact or the establishment risk with the origin of the species. All statistical analyses were performed using SPSS V22.

3. Results

The scores obtained regarding the environmental impact and the risk of establishment were plotted to assess the combined effect for each species and explore the relationship of specific characteristics (spawning strategy, origin, temperature tolerance) of the species under investigation with their degree of invasiveness. In general terms, the classification of the species obtained by HeRA was consistent with previously reported invasiveness classifications available in the scientific literature and in FishBase [45]. For comparison purposes, the literature-based classification was derived from published information regarding the invasiveness status of each species, including evidence of establishment success outside the native range, documented ecological impacts on recipient ecosystems, dispersal ability, and previous categorization in published risk assessment studies. Based on this information, species were comparatively assigned into relative invasion-risk categories (low, medium, or high), which served as a reference framework for evaluating the consistency and predictive performance of HeRA. This first observation was an indication of the ability of the tool to identify the alien species and the risk they pose for other species and the environment based on the biological characteristics/strategies of the species.

The assessed non-native species were categorized into three groups (Figure 1). The first group achieved an Establishment Risk value higher than 20 (>20). This group included eight species with high potential of Establishment Risk, i.e., *Carassius gibelio*, *Lepomis gibbosus*, *C. carpio*, *Gambusia holbrooki*, *Oreochromis niloticus*, *Carassius auratus*, *Neogobius fluviatilis*, *C. langsdorfii*, *C. carassius* (Table S2, Figure 1). The species included in this first group are mainly eurytherm species (*C. carpio*, *C. gibelio*, *O. niloticus*), following special spawning strategies (*G. holbrooki*: viviparous, *L. gibbosus*, *O. niloticus* and *N. fluviatilis*: parental care, *C. gibelio* and *C. langsdorfii* sperm dependent parthenogenesis).

The second group of medium risk species included 13 species (*Pseudorasbora parva*, *T. tinca*, *Ctenopharyngodon idella*, *Parabramis pekinensis*, *Pygocentrus nattereri*, *Hypophthalmichthys molitrix*, *Clarias gariepinus*, *Poecilia cf latipinna*, *Planiliza haematocheilus*, *Hypophthalmichthys nobilis*, *Salmo letnica*, *Ictalurus punctatus*) achieving a minimum Establishment Risk value greater than 10 (>10) and a maximum value equal to or less than 20 (≤ 20) (Table S2, Figure 1). The species in this group are characterized by an intermediate temperature range and can reproduce in the natural environment.

The third group consisted of 16 species with an Establishment Risk value equal to or less than 10 (≤ 10) (Table S2, Figure 1). This group included species belonging to Acipenseridae, Salmonidae and Polyodontidae families, which are characterized as stenothermal and psychrophilic species, since they prefer habitats with cold, well-oxygenated water. Most of these species cannot reproduce in the natural environment and finally, the populations of these species are conserved after artificial propagation of genitors.

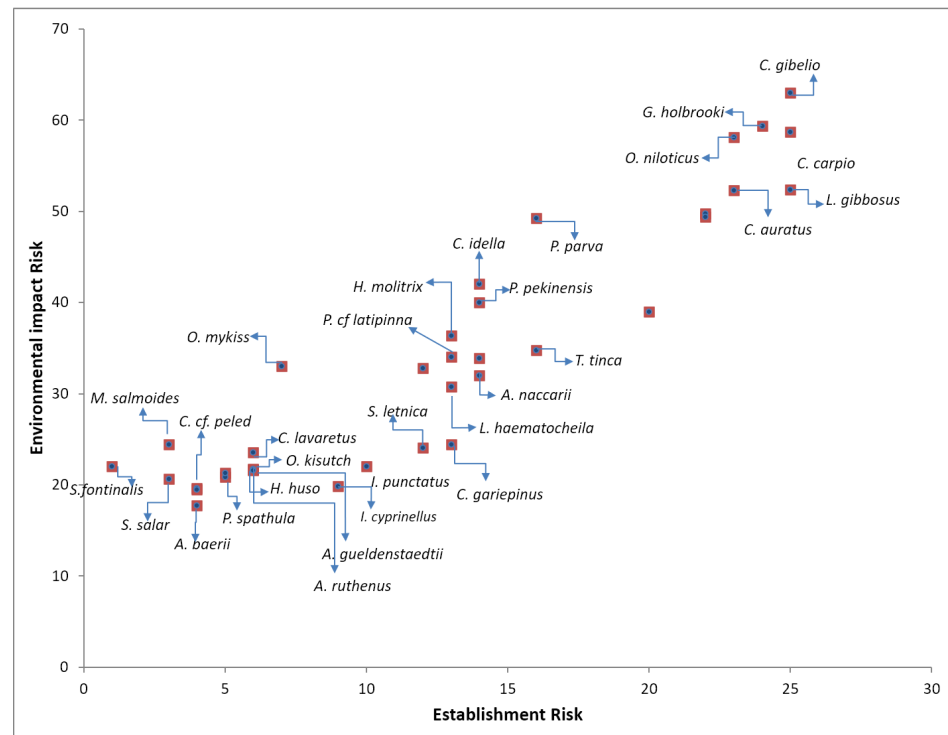


Figure 1. Relationship between the Environmental Impact Risk and the Establishment Risk, according to the results of the HeRA. The vertical lines at 10 and 20 set the limits between the three groups: Low Establishment Risk (<10), Medium Risk (>10 and ≤20) and High Risk (>20).

The minimum and maximum values identified for each species' group regarding Establishment Risk are the thresholds that could be used to categorize a species in the Low, Medium or High risk group. Consequently, low-risk species achieved an Establishment Risk value lower than 10, medium-risk species obtained a value between 10 and 20, while the high-risk species achieved values greater than 20.

Moreover, considering the origin of the species, species with natural distribution in South America and China exhibited “medium” to “very high” establishment potential (Figure 2). On the other hand, species with a natural distribution in North Europe have a “very low” Establishment Risk (Figure 2), while species from Central Europe, North Asia and North America pose a “low” Establishment Risk.

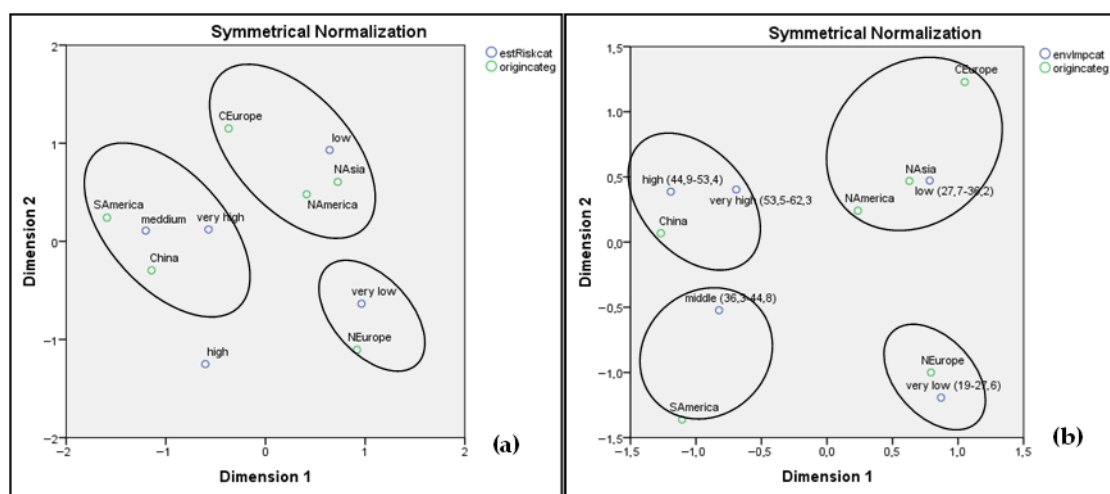


Figure 2. Graphical depiction of the Correspondence analysis results, presenting the relationship between the (a) Establishment Risk and (b) the Environmental Impact with the Origin of species.

Furthermore, correspondence analysis separated species into four groups based on their Environmental Impact. More specifically, species originating from China obtain a “High” and “Very High” Risk of Establishment value (Figure 2a). At the same time, species from Central Europe, North Asia and North America present “low” Environmental Impact (Figure 2b). On the other hand, species originating from South America are characterized by “Middle” Environmental Impact Risk, while species with a North European origin by “Very low” Environmental Impact Risk (Figure 2a).

The HeRA protocol incorporates commercial exploitation as a factor that may reduce establishment probability through Question 4.1 in Part A (“Is this species exploited?”), where a positive response receives a negative score (−1). Based on this scoring, species were categorized according to their exploitation status, with information on commercial value derived from FishBase [44] and national fisheries reports. As visualized in Figure 3, species with “high” and “very high” commercial value (i.e., those targeted by commercial fisheries or aquaculture) tended to achieve lower Establishment Risk scores compared to species with low or no commercial value. This inverse relationship suggests that harvesting pressure may act as a limiting factor on population establishment, reducing the likelihood of successful invasion for commercially valuable species. However, this observation is descriptive in nature and should be interpreted cautiously, as other biological traits (e.g., reproductive strategy, environmental tolerance) may also contribute to the observed pattern.

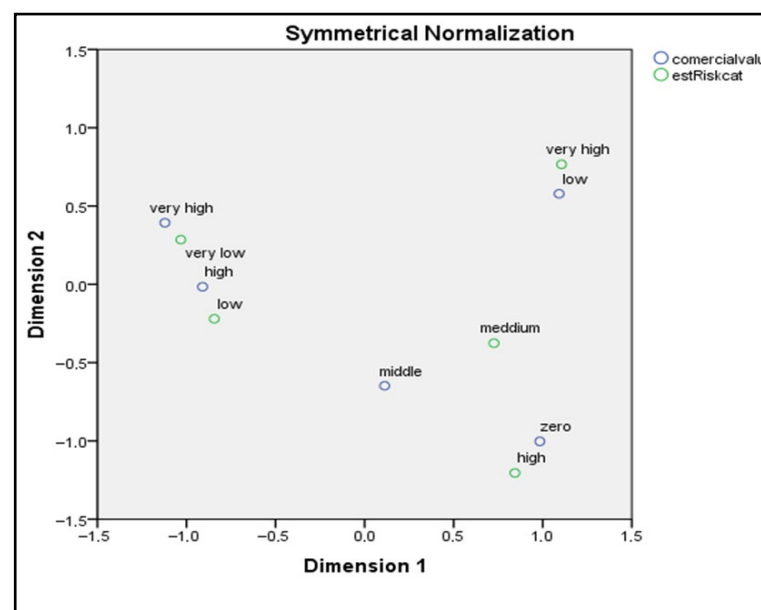


Figure 3. Graphical depiction of the Correspondence analysis results, presenting the relationship between the Establishment Risk and the commercial value of the invasive species.

4. Discussion

The present study developed and evaluated the Hellenic Risk Assessment (HeRA), a trait-based tool designed to assess the establishment probability and environmental impact of non-native freshwater fish species in Mediterranean ecosystems. The results demonstrate that HeRA successfully differentiated species according to their invasiveness potential and identified species already recognized as successful invaders in Greek and Mediterranean freshwaters [39,47]. Therefore, the results support the initial hypothesis that species-specific biological and ecological traits can be used to estimate both establishment success and potential environmental impact of introduced freshwater fishes.

One of the principal strengths of HeRA is its emphasis on biological traits directly associated with invasion success. Species classified as high risk, including *C. gibelio*, *L.*

gibbosus, *G. holbrooki* and *O. niloticus*, share characteristics frequently associated with successful biological invasions, including broad environmental tolerance, flexible reproductive strategies, rapid population growth and high dispersal capacity [15–17]. These findings are consistent with previous studies indicating that life-history traits represent reliable predictors of establishment success in freshwater ecosystems [12,15,16].

Such a tool is also presented in the current study, the Hellenic Risk Assessment or HeRA, named after the Greek Goddess, Hera. In Greek Mythology, Hera was the wife and one of the three sisters of Zeus [48], being famous for her jealousy against Zeus' mortal lovers and offsprings, especially Hercules. Through this short flashback on Mythology, Hera was against the mortals and tried to punish them. In a parallelism, "HeRA" is a tool to identify the non-native (mortal) species and categorize them according to their potential invasiveness, to assist stakeholders in implementing measures to reduce their range and the risk they pose as they establish viable populations.

Determining the main factors that drive the establishment of non-native species, as well as understanding their effects on endemic fish populations, continues to be a major challenge in conservation biogeography [12,22]. Risk analysis is essential in determining which invasive species should be prioritized for control to prevent further ecological damage [30–32]. Furthermore, risk assessment has been acknowledged as an essential element in policymaking under both the Convention on Biological Diversity (CBD) and the European Strategy for Invasive Alien Species [33]. While risk assessments have been widely applied in the United States [34,35], European freshwater fish remain understudied [33,36–39].

To address this limitation, HeRA was developed as a question-based, trait-oriented assessment tool focused on species' biological and ecological characteristics. All the necessary information can be sourced from published studies or databases such as FishBase [44]. HeRA assesses biological parameters of introduced species and incorporates region-specific traits including climate sensitivity, commercial exploitation, reproductive biology, and local ecological factors, thus enhancing accuracy for Mediterranean contexts.

The HeRA framework is composed of two complementary assessment modules. The first module evaluates the probability of establishment of an introduced species based on climate matching, habitat availability, propagule pressure, reproductive requirements, dispersal ability, environmental tolerance, and factors limiting establishment. The second module assesses the potential environmental impact of the species by considering trophic interactions, competition with native species, habitat modification, reproductive traits, population growth characteristics, and dispersal mechanisms. Species are scored according to predefined criteria, and the resulting scores are used to classify species into relative invasion-risk categories ranging from low to high risk. The complete questionnaire and scoring structure are provided in Supplementary Tables S3 and S4.

The increased global transport of live fish—via aquaculture, trade, or recreation—has exacerbated the risk of biological invasions. Existing tools like FISK and FRAM are not fully adapted to Mediterranean complexity [37,39]. For instance, the application of FISK in Greece required specific threshold calibration to reliably assess invasion risks in the region [39]. HeRA introduces region-specific criteria such as climate sensitivity, commercial exploitation, and reproductive biology. It is also adaptable to other regional applications [49].

Although HeRA was specifically designed for Mediterranean freshwater ecosystems, most of its assessment criteria are based on biological and ecological traits that are universally associated with invasion success, including reproductive capacity, dispersal potential, environmental tolerance, and habitat suitability. Consequently, the framework can be adapted for use in other geographical regions by modifying region-specific parameters,

such as climatic matching, habitat characteristics, and introduction pathways, while retaining the core structure of the assessment.

The results indicate that commercial value correlates inversely with invasiveness potential due to harvesting pressure. This supports tailored management approaches, emphasizing prevention and monitoring for low-value, high-risk species.

Comparison with existing protocols further demonstrates the potential value of HeRA (Table 1). Assessment tools such as FISK and FRAM have proven effective in identifying potentially invasive fish species [36,40]. According to Copp et al. [49], FISK is not a decision-making tool but a tool to assist stakeholders in proposing management plans and strategies to diminish the establishment risk of non-native species. According to Wilding and Rowe [40], the difference between these tools is that FISK identifies the impact of species that could invade other ecosystems, while FRAM focuses on those likely to become invasive and cause severe environmental impact. The major difference between these protocols and HeRA is that FRAM and FISK were designed for broader geographical applications and do not specifically address ecological conditions characteristic of Mediterranean freshwater ecosystems [34,37,38,41]. On the contrary, HeRA includes more specialized questions designed for freshwater ecosystems within the European Mediterranean biogeographic region. HeRA incorporates region-specific considerations, including climate suitability, reproductive habitat availability, species exploitation rates and environmental constraints relevant to Greek inland waters. This regional focus may improve assessment accuracy and provide management authorities with information that is directly applicable to Mediterranean freshwater conservation.

Table 1. Comparison Table: HeRA vs. Other Risk Assessment Tools.

Feature/Tool	FISK	FRAM	AS-ISK	HeRA (Proposed)
Geographic Adaptation	Global (generic)	New Zealand focus	Global (customizable)	Mediterranean-specific
Trait-Based Scoring	Yes	Yes	Yes	Yes
Number of Questions	~49	~40	~55	68
Establishment & Impact Split	Yes	Yes	Yes	Yes
Weighted Trait Scoring	No	No	Partially	No
Uncertainty Quantification	Confidence ratings	No	Confidence scores	No
Socio-Economic Assessment	No	Limited	Limited	No
Climate Sensitivity	Generalized	Generalized	Customizable	Mediterranean focus
Validation with Empirical Data	Moderate	Limited	High	Partial
Application Platform	Excel/Paper	Excel/Paper	Online/Spreadsheet	Excel/Paper
Cross-Taxa Utility	No (fish-specific)	No (fish-specific)	Yes	No (Fish-specific)

The weighting of biological traits within HeRA was developed to reflect their relative contribution to invasion success. HeRA does not assign equal importance to all biological traits, but rather is directly linked to establishment success, including climate matching, reproductive capability, environmental tolerance, and dispersal capacity, receive higher scores because previous invasion biology studies have consistently identified them as primary predictors of invasion success [15,16,32]. Conversely, traits exerting indirect effects or acting primarily as modifiers of invasion risk receive lower scores. This semi-weighted approach was developed to better reflect the relative contribution of different biological mechanisms to invasion success in Mediterranean freshwater ecosystems.

During trial assessments, HeRA successfully categorized non-native species in Greek freshwater systems into three groups based on Establishment Risk and Environmental Impact. High-risk species identified by HeRA matched findings from other Mediterranean

countries [50] and previous assessments using FISK v2 in Greek freshwaters [39]. Correspondence analysis showed that HeRA grouped species based on invasiveness, origin, thermal tolerance, and commercial value. Commercial value appeared inversely correlated with invasiveness: higher demand leads to increased fishing effort, reducing environmental impact. In their natural range, endemic fish species employ survival strategies to counter predators such as birds, mammals, or other fish. For instance, *G. holbrooki* is viviparous with high reproductive rates [51], and female *O. niloticus* carry up to 200 fertilized eggs in their mouth until larvae hatch [4]. *L. gibbosus* and *N. fluviatilis* build nests and guard eggs and juveniles. *C. gibelio*, a “sperm parasite,” uses sperm of other species for reproduction, bypassing the need for males of its own species [41]. These strategies, combined with the absence of predators and wide thermal tolerance (eurythermy), lead to rapid population establishment.

Conversely, species scoring <30 (Table S2) were unlikely to spawn naturally and require artificial reproduction, thus posing low establishment risks [38]. However, poor containment measures result in annual escapes of thousands of salmonid fries into rivers, increasing pressure on wild populations. Acipenseridae species ranked lowest (Table S2), as they reproduce after 10–20 years and are overexploited for caviar [42]. Consequently, their probability of establishing viable populations is extremely low, though individual escapes—such as a *Huso huso* caught in northern Greece in 2005—have been recorded [43,52].

The Hellenic Risk Assessment (HeRA) framework was developed in accordance with widely accepted principles used in invasive species risk assessment and incorporates biological, ecological, and environmental criteria commonly employed in established tools. Future developments could further enhance its robustness through additional calibration, uncertainty quantification, and validation procedures consistent with internationally recognized best practices for invasive species risk assessment [33,46]. Building upon methodologies such as FISK, FRAM, and AS-ISK, HeRA could incorporate a quantitative validation framework using tools like Receiver Operating Characteristic (ROC) curves or Cohen’s Kappa to assess predictive accuracy. Weighted trait scoring could enhance the tool’s sensitivity to key biological factors such as reproductive strategy and thermal tolerance [32,34], while integrating uncertainty analyses would align it with frameworks like AS-ISK that emphasize assessor confidence. Additionally, HeRA would benefit from including a socio-economic risk module that evaluates commercial impacts, aligning with calls for integrated ecological and economic assessments [53]. By adopting these best practices, HeRA could not only improve its precision and credibility but also enhance its utility for policy implementation under EU Regulation 1143/2014 and the Convention on Biological Diversity [41].

Although HeRA was evaluated by experienced assessors, expert-based approaches inevitably involve some degree of subjectivity. Roy et al. [45] highlighted that social influence among experts can result in overconfidence and convergence toward inaccurate outcomes if assessments are conducted collectively. In the present study, this limitation was reduced by requiring independent assessments prior to discussion of the results. Nevertheless, future applications of HeRA could benefit from formal uncertainty analyses, confidence scoring, or Delphi-type procedures to further quantify assessor agreement and reduce potential biases.

To further contextualize HeRA’s development, it is essential to recognize the broader scientific foundation underlying effective risk assessment practices. As Roy et al. [41] outlined, minimum standards for risk assessments include transparency, repeatability, and rigorous expert validation, which HeRA aims to meet. Vilizzi et al. [46] emphasized the need for regional calibration of tools like FISK, a principle central to HeRA’s design for Mediterranean conditions. Roy et al. [41] provided key insights into the predictive power of biological traits such as fecundity, thermal tolerance, and dispersal capacity—traits directly

incorporated into HeRA's structure. Moreover, integrating socio-economic dimensions of biological invasions has been recommended by Gallardo et al. [42], reflecting a holistic approach that HeRA aspires to adopt. These foundational studies not only validate HeRA's design but also suggest pathways for future enhancements through empirical calibration, uncertainty quantification, and interdisciplinary integration.

The exact consequences of invasive species establishment remain incompletely understood and often controversial. While some emphasize increased fishery yields, others highlight serious environmental threats [21]. Recent studies underline the dangers posed by invasive species to freshwater quality and native populations [21,53]. Therefore, effective management plans are essential, and HeRA provides a much-needed tool for stakeholders to identify, assess, and mitigate the risks of non-native species.

Overall, the results indicate that HeRA provides a practical and biologically informed framework for evaluating the invasiveness potential of non-native freshwater fishes. By integrating species traits, ecological constraints and region-specific environmental characteristics, the tool offers a complementary approach to existing risk assessment methodologies. Future validation, uncertainty quantification and incorporation of socio-economic indicators will further improve its robustness and increase its value for conservation planning, fisheries management and implementation of invasive species policies in Greece and across the Mediterranean region.

5. Conclusions

In conclusion, HeRA is a scientifically grounded, practical, and effective tool for assessing the invasiveness potential of introduced species by evaluating their biological traits and environmental needs. Designed primarily for non-native freshwater fish in Mediterranean environments, HeRA enables early detection, prioritization, and informed policymaking aligned with EU biodiversity strategies [41,54]. It is applicable beyond Greece and scalable for use across similar ecological contexts, making it valuable for managing live fish importation for aquaculture or aquarium purposes. By leveraging publicly available data sources such as FishBase [44], stakeholders can make informed decisions, support integrated biodiversity conservation planning, and better protect freshwater ecosystems from invasive threats.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app16136428/s1>. Table S1: Invasive species introduced in the Greek freshwater ecosystems, their origin, the biogeographical areas that were introduced and the main purpose for the introduction [8,28,43,44]; Table S2: The invasive species assessed and the scores that each species achieved according to HeRA; Table S3: The first part of the HeRA tool assessing the Establishment Risk of invasive species in the Greek systems; Table S4: The second part of the HeRA tool regarding the Environmental Impact Risk of invasive species in the Greek systems.

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