water

Effects of Species Introduction on Aquatic Communities

Edited by

Frédéric Santoul and Stéphanie Boulêtreau

Printed Edition of the Special Issue Published in *Water*

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Frédéric Santoul
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Editors

Frédéric Santoul	Stéphanie Boulêtreau
University Toulouse III— Paul Sabatier	University Toulouse III— Paul Sabatier
France	France

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Water* (ISSN 2073-4441) (available at: https://www.mdpi.com/journal/water/special_issues/Species_Aquatic).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-3651-4 (Hbk)
ISBN 978-3-0365-3652-1 (PDF)

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Preface to ”Effects of Species Introduction on Aquatic Communities”

Dear Readers,

It is well-known that aquatic ecosystems, particularly freshwater ecosystems are particularly sensitive to species introductions. This Special Issue provides five typical examples of the consequences of the introduction of recent or well-established species. These studies cover a large geographical area, from the Iberian Peninsula to the state of Florida through the Czech Republic, and look at different aquatic species from animal groups of mollusk, crayfish or fish. The methods used also vary, from stomach content and isotopic analyses in trophic ecology, radio tracking, and long-term temporal series analyses to combined approaches, including genetic and morphometric makeup analyses. All results suggest that concrete action is needed, as well as continued investigations into species introductions in aquatic ecosystems.

If you want to know more about the recent invasion of the Pikeperch in Portugal, the genetic diversity of the Asian clam in Ireland, how the Asp has adapted to river fragmentation in the Lipno reservoir, the economic impact of the suckermouth armored catfish in the commercial haul seine fishery in Florida and how the signal crayfish competes with the native fish community in Iberian rivers, read on!

Happy reading to all,

Frédéric Santoul, Stéphanie Boulêtreau
Editors

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Article

Behavioral Interactions and Trophic Overlap between Invasive Signal Crayfish *Pacifastacus leniusculus* (Decapoda, Astacidae) and Native Fishes in Iberian Rivers

Iván Vedia [1], David Almeida [2], Amaia A. Rodeles [1], Pedro M. Leunda [1], Enrique Baquero [1], David Galicia [1], Javier Oscoz [1], David Elustondo [3], Jesús M. Santamaría [3] and Rafael Miranda [1,*]

[1] Department of Environmental Biology, School of Sciences, University of Navarra, C/ Irunlarrea 1, E-31080 Pamplona, Spain; ivedia@alumni.unav.es (I.V.); aangulo@alumni.unav.es (A.A.R.); pleuurr@alumni.unav.es (P.M.L.); ebaquero@unav.es (E.B.); dgalicia@unav.es (D.G.); joscoz@alumni.unav.es (J.O.)

[2] Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Campus de Montepríncipe, E-28925 Alcorcón, Madrid, Spain; david.almeidareal@ceu.es

[3] University of Navarra, LICA, C/ Irunlarrea 1, E-31080 Pamplona, Spain; delusto@unav.es (D.E.); chusmi@unav.es (J.M.S.)

* Correspondence: rmiranda@unav.es

Received: 24 January 2019; Accepted: 27 February 2019; Published: 5 March 2019

Abstract: The signal crayfish *Pacifastacus leniusculus* Dana, 1852 is a successful invasive species in the Iberian Peninsula. This is of particular conservation concern, as fish fauna is highly endemic and also threatened within this ecoregion. The aim of this study was to analyze behavioral interactions and trophic overlap between signal crayfish and native fishes in Iberian rivers (northern Spain). Video cameras were used to record fish "dominance/evasion" after spatial encounters with crayfish. Diet composition and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) were compared to evaluate the niche overlap. Fish were dominant in 25% of the encounters with juvenile crayfish, whereas this percentage was only 4% with adult crayfish. Observations showed a high fish "evasion" response for Pyrenean stone loach *Barbatula quignardi* (Băcescu-Meşter, 1967) (>30%). Dietary results showed a high trophic overlap between signal crayfish with the pelagic Pyrenean minnow *Phoxinus bigerri* Kottelat, 2007 and the benthic loach. However, the isotopic niche overlap was low, with brown trout *Salmo trutta* L., 1758 showing the highest area (only 0.1 ‰^{2}). Overall, our findings suggest that interferences may occur with native species for food (i.e., benthic invertebrates). Consequently, measures should be applied to control invasive crayfish in Iberian rivers.

Keywords: aquatic communities; direct/indirect interactions; ecological impacts; species introduction; stable isotope analysis; trophic levels

1. Introduction

Biological invasions are one of the most important causes of biodiversity loss at the global scale [1–3]. This conservation concern is particularly relevant across inland waters of the Mediterranean region in Europe [4], where endemic freshwater fish are highly threatened by invasive species [5]. As an example, >50% of the native fish species are unique to the Iberian Peninsula (i.e., Spain and Portugal) [6,7]. Amongst the variety of aquatic introductions, non-native crayfishes are common freshwater invaders worldwide, with these being keystone species because of their significant ecological and socioeconomic impacts [4]. The signal crayfish *Pacifastacus leniusculus* Dana, 1852 is native to North America and was introduced into the Iberian Peninsula from Sweden in 1974 for the purpose of expanding aquaculture [8]. In this region, signal crayfish were first introduced into the North of Ebro Basin >40 years ago, where it spread hundreds of km in a few years [8]. This crayfish species is currently very common across central and northern Iberian waters, where it is a strong competitor to native fauna and is a vector for the crayfish plague *Aphanomyces astaci* Schikora, 1906 [8]. Indeed, signal crayfish is partly responsible for the recent extirpation of the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) from many Iberian streams [8].

The mechanisms by which fish and crayfish interact may be indirect through habitat alteration [9] or direct by means of predation, behavioral interference (e.g., aggression), and competition for food/space [10]. In field studies, Guan and Wiles [11] found an inverse relationship between the abundances of the introduced signal crayfish and benthic fishes, such as bullhead *Cottus gobio* L., 1758 and stone loach *Barbatula barbatula* (L., 1758), inhabiting a British lowland river. Peay et al. [12] also observed a negative association between densities of signal crayfish and juvenile brown trout *Salmo trutta* L., 1758 in a British stream. These findings are based on traditional comparisons of the abundances of crayfish and fish populations. Consequently, this "indirect" design does not elucidate the particular effects of invasive crayfish on native fish. To provide a better understanding of the mechanisms of interaction, observations have been carried out on signal crayfish and a variety of fish species under laboratory conditions. For example, Bubb et al. [13] assessed behavioral interactions and competition for shelter between bullhead and signal crayfish in experimental aquaria. These authors observed that signal crayfish were dominant over bullhead through aggressive approaches. In addition, a negative relationship between the densities of signal crayfish and bullhead was observed. Also, under controlled conditions, Griffiths et al. [14] observed that the proportion of Atlantic salmon *Salmo salar* L., 1758 sheltering was significantly lower in the presence of aggressive signal crayfish when refuge was limiting. In contrast, these authors showed that the proportion of signal crayfish sheltering was independent of the presence of Atlantic salmon.

Despite the wide field of research on crayfish ecology under captive conditions, laboratory experiments have restrictions concerning their applicability to natural ecosystems due to the limitation of space and trophic resources [15]. Particularly for crayfish, Bergman and Moore [16] found that agonistic interactions among two species of genus Orconectes (currently Faxonius, see Crandall and De Grave [17]) were shorter, less intense and less likely to end with a tail flip in nature than in the laboratory facilities. Nonetheless, very few attempts have been made to evaluate the real effects of introduced crayfish species on native fishes under natural conditions. In this respect, Almeida et al. [18] highlighted the usefulness of observations in the wild for assessing the effects of invasive species on aquatic communities. Despite the conservation concern posed by non-native crayfish, to our knowledge, no previous study has assessed the behavioral interactions between introduced crayfish and Iberian fishes under natural conditions.

As mentioned above, competition for food is another potential mechanism of interaction between invasive crayfish and fish communities [10]. Quantifying the trophic ecology in animals has traditionally focused on direct analysis (e.g., stomach content), although these methods do not adequately reflect the quantity of nutrients assimilated into tissues [19]. In recent years, ecologists have successfully employed stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) to define the trophic niche of species [20], as this technique can reveal the consumer's long-term feeding habits [21,22]. Specifically, the ratios of carbon and nitrogen isotopes provide a time- and space-integrated representation of the food source and trophic level, respectively (e.g., [23]). In addition, isotopic biplots of ^{13}C and ^{15}N are useful to assess the extent of isotopic niche overlap between species, which can show the potential for coexistence or competitive exclusion posed by invasive crayfish [24,25].

In light of the dearth of information on these biotic relationships in the Iberian Peninsula, the aim of this study was to analyze behavioral interactions and trophic overlap between signal crayfish and native fishes within this ecoregion. Specifically, we recorded the behavior after spatial encounters between signal crayfish and native fishes in Iberian rivers (northern Spain) and also compared diet composition and isotopic "signatures" between these taxa. We hypothesized that (i) signal crayfish will physically displace native fishes, especially benthic species, as previous aquarium experiments have shown (e.g., [13]) and (ii) both dietary and isotopic data will reveal a high trophic overlap between signal crayfish and native fishes (e.g., [24]).

2. Materials and Methods

2.1. Study Area

The upper and middle reaches were surveyed in the main rivers of the Navarra region (northern Spain). Sampling sites (n = 20) were located in water courses that drain to the Ebro River, which is one of the main Iberian basins, with a catchment area of 85,362 km^2 (Figure 1). Specifically, the following rivers were sampled (from West to East Navarra); Ega, Urederra, Arakil, Lizarrusti, Basaburua, Larraun, Ulzama, Arga, Erro, Urrobi, Irati, Areta, and Salazar (Figure 1). Flow regime is pluvio-nival in the study rivers and the period of greatest discharge lasts from November until April [26]. Riparian vegetation is abundant in all the rivers. In upper reaches, livestock is common in grasslands, while cereal fields are dominant in middle reaches.

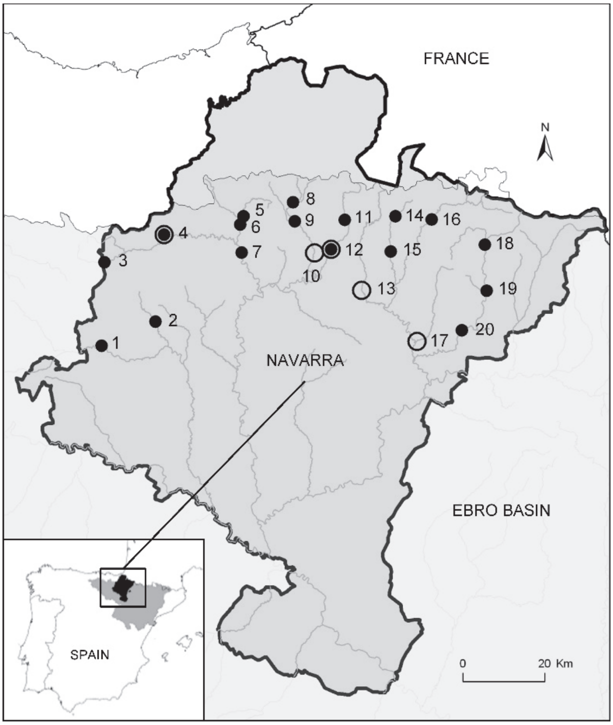

Figure 1. Distribution map of the n = 20 sampling sites for this study in Navarra region (northern Spain). Black circumferences show the n = 5 sites where behavioral interactions were recorded (see Methods section for the names of towns and rivers). Black circles show the n = 17 sites where crayfish and native fishes were sampled to evaluate their trophic overlap from both dietary and isotopic data (see Table 2 for the names of towns and rivers). Two sites (numbers 4 and 12) were used for the two approaches (i.e., behavioral interactions and trophic overlap), which are marked with both black circumferences and circles.

In the study rivers, native fish communities consist of benthic fishes, such as Pyrenean stone loach *Barbatula quignardi* (Băcescu-Meşter, 1967), Pyrenean gudgeon *Gobio lozanoi* Doadrio

& Madeira, 2004 and, less frequently, the northern Iberian spined-loach *Cobitis calderoni* Băcescu, 1962; bentho-pelagic fishes, such as Ebro nase *Parachondrostoma miegii* (Steindachner, 1866); and pelagic fishes, such as Pyrenean minnow *Phoxinus bigerri* Kottelat, 2007 and brown trout [27,28]. Specifically regarding brown trout, this salmonid species is considered as a native fish in the Iberian Peninsula, although it is also true that several stocking programs have been undertaken in the study area by using fish individuals from central European populations. This implies a certain degree of genetic introgression towards the "Iberian lineages" [29].

2.2. Behavioral Interactions

Observations were conducted during the months of June and July 2017 because during this period of the year the water clarity is generally good and the activity of fish and crayfish is high [30]. Recordings were carried out in five rivers located in Navarra region (northern Spain), from headwaters to middle reaches (towns and number of the sampling site in parenthesis): Lizarrusti (Lizarragabengoa, 4), Ulzama (Olave, 10), Arga (Idoi, 12), Erro (Lizoáin, 13), and Areta (Rípodas, 17) rivers (Figure 1). These sampling sites were selected because signal crayfish were highly abundant, which allows recording an elevated number of observations. Also, these five sites were distributed along the W–E axis in northern Navarra to accomplish a spatially representative data set (Figure 1).

As both signal crayfish and Pyrenean stone loach are more active at night, a total of 10 recording sessions (2 sessions per site on consecutive days) were carried out immediately after sunset. At each session, an area of $\approx$9 m^2 of riverbed was recorded for 3.5 h. Infrared lamps were used to illuminate and to avoid disturbing the natural behavior of signal crayfish and fishes [16,31]. The recording equipment consisted of three cameras placed above the water 1 m from the riverbed in a zenith position (GoPro4 CHDHX-401-M modified for night vision, with a 2.5 mm lens, GoPro Inc., San Mateo, Califfornia, EE.UU.) and six infrared lamps (IR100, 890 nm, 2AH, 12V, Philips Electronics Nederland BV, Eindhoven, The Netherlands) installed in a fully adjustable metallic structure. A battery (80 amperes/h) provided energy to the infrared lamps (Figure 2).

The videos from the three cameras were merged with the program Adobe Premiere Pro CS6 (Adobe Inc., San Jose, California, EE.UU.) and were visualized with VLC media player (version 2.2, VideoLAN, Paris, France). The relative body size and morphological proportions (e.g., cheliped/body ratio) were used to distinguish each crayfish individual (n = 422). To avoid pseudoreplication, only the first encounter of each crayfish individual with native fish fauna was analyzed (n = 254). The encounters were defined from the point of view of fish and categorized as (adapted from [18]) (1) "fish evasion", when only the fish individual clearly changes the direction after the encounter with the crayfish; (2) "fish dominance", when only the crayfish individual clearly changes the direction after the encounter with the fish; and (3) "no interaction", when neither the crayfish nor the fish change the direction after the encounter with the other. The life stage (i.e., juvenile/adult) and sex (i.e., male/female only for adult specimens) of crayfish individuals were also annotated, given that these factors can modulate behavior (e.g., [32,33]). To provide a high certainty on life stage and sex identifications, R. Miranda (from the author list) was the responsible person to visually classify these categories during video visualizations. This researcher is an experienced observer of signal crayfish from previous field surveys (R. Miranda, pers. observ.). More in detail, the approximate size ranges were 50 to 90 and 100 to 150 mm of total length (TL) for juvenile and adult crayfish, respectively. Previous measurements on particle sizes of substratum helped to establish these TL ranges during visualizations.

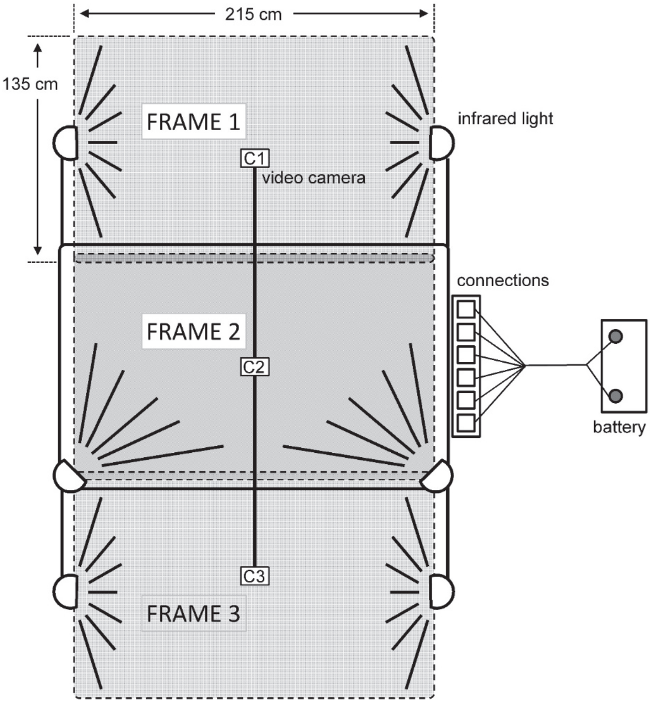

Figure 2. Recording system showing the distribution of three video cameras adapted for night vision in the zenith position and six infrared lights connected to a battery. Dimensions of the structure are also showed.

2.3. Trophic Overlap

Crayfish and fish were collected from 17 of the 20 sites in June–July 2017 to evaluate the trophic overlap between signal crayfish and native fishes (Figure 1). The remaining three sites (numbers 10, 13, and 17; see Figure 1) were not sampled by electrofishing to avoid disturbing the endangered populations of northern Iberian spined-loach. Given that mean depths were very low (15–80 cm) and that the water clarity was good enough, nocturnal hand sampling of crayfish with headlights was the most effective methodology in this type of river sections [34]. A total of $n = 85$ specimens of adult crayfish (5 per site) were collected (103–146 mm TL). Fish collection was performed by electrofishing (Hans Grassl model IG200/2D, 300–600 V, 0.2–2 A, Hans Grassl GmbH, Schönau am Königssee, Germany). According to the local abundances and conservation status, the lowest possible number of native fish specimens was collected among the sampling sites to provide a statistically representative data set from the study area. Specifically, we collected a total of $n = 85$ specimens for Pyrenean minnow (30–68 mm TL), $n = 60$ for brown trout (163–199 mm TL), $n = 25$ for Pyrenean gudgeon (30–110 mm TL), $n = 20$ for Pyrenean stone loach (50–100 mm TL), and $n = 10$ for Ebro nase (84–119 mm TL). All of the collected fish and crayfish were properly euthanized with low temperatures (cooler), as the use of anesthetic was not recommended for the subsequent stable isotope analysis (SIA) [35]. Crayfish and fish individuals were stored in ice during transport to the laboratory on the same date of collection (<2 h since capture). All field procedures complied with animal use and care regulations of Europe and Spain (specific licenses were granted for Scientific Field Research in Navarra). Crayfish and fish were collected by trained personnel (i.e., the holders of the licenses: I. Vedia and R. Miranda). Thus, no adverse effects were caused on the wildlife in the study habitats.

On arrival at the laboratory, crayfish and fish individuals were measured for TL (±1 mm) and weighed for total mass (TM, ±0.1 g). Crayfish sex (i.e., male/female) was also annotated. The pyloric and cardiac stomachs were extracted from each crayfish individual and were preserved in 70% ethanol until processing. Fish were not dissected to extract guts because data from previous surveys were used for the present study (see justification in "Data Analyses" below). For dietary analysis, stomachs were dissected and prey categories were identified to the lowest possible taxonomic level using available keys (e.g., [36]). The number of each prey category was counted using a dissecting microscope (magnification 40×). The presence of plant material and detritus was also annotated per crayfish individual.

For SIA, a sample from the abdominal muscle tissue of each crayfish specimen was extracted to determine the carbon and nitrogen stable isotopes [24]. A sample of white muscle tissue of each fish specimen was extracted from the dorsal area immediately behind the operculum [37]. Muscle samples were used because such tissue type exhibits less variation in the isotopic signatures (i.e., $\delta^{13}C$ and $\delta^{15}N$ values, see description below) than other tissues, reflecting more accurately the diet over a longer period of time [38,39]. After tissue extractions, muscle samples (≈0.2 g) were frozen (–20 °C) until processing. Muscle samples were not acidified and no debris from exoskeletons containing inorganic carbonates were included [40]. Samples were dried at 60 °C for 72 h and milled with tungsten carbide balls (Ø 3mm) in a Retsch Mixer Mill MM400 (100–240 VAC, 50/60 Hz, Retsch GmbH, Haan, Germany). The resulting homogeneous powder was then precisely weighed (1 mg) into tin cups using an electronic balance (±0.1 mg). Isotopic signatures were estimated for each crayfish and fish specimen. Firstly, total C and N proportions (% dry weight) were determined using an elemental analyzer (varioMICROCube, Elementar Analysensysteme GmbH, Langenselbold, Germany) with an analytical precision of 0.1%. Calibration was performed using sulfanilic acid. A plant reference material (algae-14th Intercomparison exercise of Organic Elemental Analysis of Reference Materials for Quality Control, MAT Control, University of Barcelona) was used to ensure that the accuracy was within 7% of the known total C and N proportions. Secondly, stable C and N isotope contents were measured using an isotope ratio mass spectrometer (Isoprime 100, Isoprime, Ltd.), operating in a continuous-flow mode. Vienna Pee Dee Belemnite (i.e., V-PDB) was the standard used as reference material for C. High-purity N_2 was the standard used as reference material for N, after calibration to the atmospheric N_2. Two sources were used to provide the N_2 standard: IAEA-N-1 (International Atomic Energy Agency) and USGS 40 (U.S. Geological Survey). Isotope data are given as $\delta^{13}C$ and $\delta^{15}N$, which are expressed per thousand (‰). These values represent the ratio between the isotopic composition of the sample and V-PDB for carbon and atmospheric N_2 for nitrogen, respectively:

$$\delta^{13}\mathrm{C}\ (‰\ \mathrm{vs.\ V\text{-}PDB}) = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$$

$$\delta^{15}\mathrm{N}\ (‰\ \mathrm{vs.\ atm\text{-}N_2}) = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000,$$

where R_{sample} is the isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for the muscle sample and $R_{standard}$ is the isotope ratio for the particular standard (V-PDB or atmospheric N_2). Accuracy below 3% was found for $\delta^{13}C$ and $\delta^{15}N$ using the same reference plant material (algae AEO, see above) as a standard. The precision of the SIA was based on the relative standard deviation (RSD) of duplicated samples. RSD was found to be within 3% for isotopic signatures.

2.4. Data Analyses

Separate contingency analyses were performed using Fisher's exact test to assess the statistical association between the categorical variables "encounter" (fish evasion/fish dominance/no interaction) with crayfish "sex" (male/female), crayfish "life stage" (juvenile/adult), and "fish species". Standardized residuals were used to reveal significant associations between particular categories in those cells where an absolute value of this parameter is >1.5 [41].

For dietary analysis, data on crayfish were pooled, as previous results (nonparametric and chi-squared tests) showed that diet composition was similar between sites, sexes, and life stages. To analyze the trophic overlap between the invasive crayfish and the native fishes, the stomach contents of signal crayfish (n = 85) were compared with the published information for specimens of Pyrenean stone loach (n = 27, 46–97 mm TL), brown trout (n = 101, 150–219 mm TL), Pyrenean gudgeon (n = 70, 30–113 mm TL), and Pyrenean minnow (n = 333, 26–69 mm TL). Given that these native fish species are subjected to conservation measures, relevant published data on greater sample sizes (see above) of fish were obtained from previous surveys in the same study area by several coauthors of this manuscript [42–47]. Dietary data from these previous fish surveys (2000–2006) were compiled at the same period as the present study (i.e., summer months). Moreover, benthic invertebrate communities and fish diet has not significantly changed in the study sites between fish and crayfish surveys, as monitored by Oscoz et al. [48] and J. Oscoz (pers. observ.). Two overall dietary indices were calculated (omitting empty stomachs) by using data sets on crayfish (present study) and fishes (previous studies). These indices were expressed as a percentage for each prey category: (1) occurrence (frequency of stomachs/guts in which a particular prey category occurred relative to the total number of crayfish/fish individuals) and (2) abundance (frequency of the number of individuals for a particular prey category relative to the total number of individuals for all prey categories). Trophic overlap between signal crayfish and native fishes was determined by using the Schoener index (S) [49] on dietary data:

$$S = 1 - 0.5 \times \left(\sum |p_{ia} - p_{ib}|\right),$$

where p_{ia} and p_{ib} are the relative proportions of each prey category i for species "a" and "b", respectively. The index ranges from 0, which indicates no dietary overlap, to a maximum overlap of 1, when all prey items are found in equal proportions. Index values >0.6 indicate significant trophic overlap [50].

At each site, pairwise comparisons were used by means of Mann–Whitney U tests to reveal significant differences between signal crayfish and native fishes for $\delta^{13}C$ and $\delta^{15}N$. This analytical approach (i.e., pairwise comparisons at each site, specifically with crayfish) was selected rather than a global analysis, as this technique did not reveal any clear pattern and results were not explanatory enough for a biological interpretation. Moreover, the isotopic baseline may differ across the study sites (e.g., [24]). The packages Stable Isotope Bayesian Ellipses and Stable Isotope Analysis in R (i.e., SIBER and SIAR, respectively) were used to estimate (1) the "size" of isotopic niche per species and (2) the trophic overlap between signal crayfish and native fishes [22,51]. SIBER and SIAR were also applied per site. The values of isotopic signatures for each individual were used to represent the isotopic niche of a particular species in a biplot, where $\delta^{13}C$ and $\delta^{15}N$ are the first and second axis, respectively. The size of isotope niche (i.e., a measure of trophic width) was quantified as the corrected Standard Ellipse Area (SEAc). These ellipses were estimated to minimize the bias caused by small sample sizes within a site, using the variance and covariance of bivariate isotopic signatures to contain approximately 40% of the data. Thus, these ellipses represent the cores of isotopic niches for the crayfish/fish population at each site. Given the coordinates of isotopic signatures for two species, the area of overlap (i.e., a measure of trophic overlap) was calculated between their respective SEAc per site (see methodology in [51]). Overall means were calculated for SEAc and Areas of Overlap to provide a better understanding on the global patterns of trophic niche width (per species) and trophic niche overlap (between crayfish and fishes) across the study area.

Statistical analyses were performed with SPSS 15.0 and R 3.1.3 (specific packages SIBER and SIAR were executed, see above) [52]. The level of significance was established at $p = 0.05$. The geographical information system software ArcGIS (version 10) was used for generating maps.

3. Results

3.1. Behavioral Interactions

A total of 28 encounters were observed with brown trout (11%), 103 with Pyrenean minnow (41%), 39 with Pyrenean stone loach (15%), and 79 with undetermined fish (31%) (Figure 3). Given that only five encounters were observed with Pyrenean gudgeon (i.e., 2% of interactions), this species was not analyzed. No association was observed between the result of the encounter and crayfish sex (F = 3.41, p = 0.197). The frequency of encounter categories significantly differed between juvenile and adult crayfish (F = 24.16, $p < 0.001$) (Figure 3). In particular, fish individuals were dominant in 25% (13% expected) of the encounters with juvenile crayfish (standardized residual = 3.4), whereas fish were dominant in only 4% (13% expected) of the encounters with adult crayfish (standardized residual = –3). For juvenile crayfish specifically, a significant association was found between the fish species and the result of an encounter (F = 21.85, p = 0.003). In 50% of the encounters with juvenile crayfish, Pyrenean stone loach was dominant (25% expected; standardized residual = 1.9). In 33% of encounters between undetermined fish and juvenile crayfish, fish individuals displayed evasion from crayfish (19% expected; standardized residual = 1.9) (Figure 3). No association was detected between the encounter category and fish species in adult crayfish (F = 9.30, p = 0.316), although observations showed a higher fish "evasion" response for Pyrenean stone loach (>30%) (Figure 3). Regarding the behavioral category "no interaction", our results showed that encounters between signal crayfish with pelagic fishes, especially brown trout, only triggered a fish response (either dominance or evasion) in <30% occasions (Figure 3).

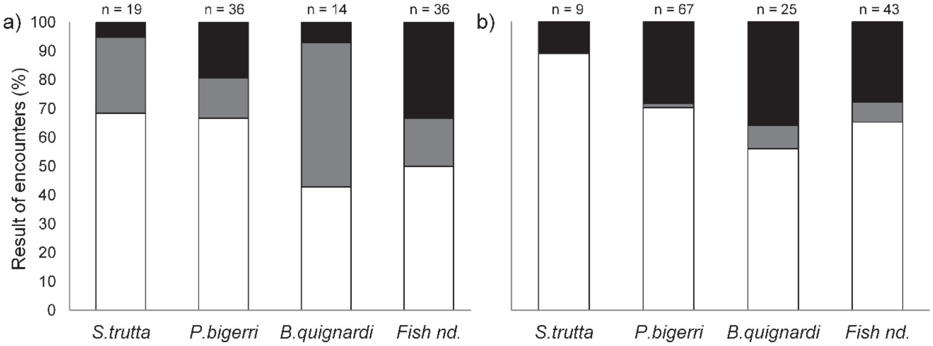

Figure 3. Percentages of encounter categories between native fishes and juvenile (**a**) or adult (**b**) signal crayfish *Pacifastacus leniusculus*. No interaction (white), fish dominance (gray), and fish evasion (black). n = number of encounters; Fish nd. = fish species not determined.

3.2. Trophic Overlap

In terms of occurrence and abundance, signal crayfish mainly preyed on Diptera, Trichoptera, and Ephemeroptera. These three prey categories were also the most important for Pyrenean stone loach, along with Crustacea, in occurrence; although Diptera larvae was clearly the most abundant prey (>75%) for this benthic fish (Table 1). Brown trout showed a diet composition highly variable, with Ephemeroptera being the most important prey in occurrence and Crustacea the most abundant. Diptera larvae were the most frequent and abundant prey for Pyrenean gudgeon and Pyrenean minnow. The Schoener index showed a significant trophic overlap between signal crayfish with Pyrenean minnow (>0.80), Pyrenean stone loach (>0.75), and Pyrenean gudgeon (≈0.70) (Table 1). Apart from prey items, a high occurrence of plant material was found in crayfish diet (>25%), such as algae and vegetation debris. Detritus was also a frequent food category (>20%).

Table 1. Diet composition of signal crayfish *Pacifastacus leniusculus* (PL), Pyrenean stone loach *Barbatula quignardi* (BQ), brown trout *Salmo trutta* (ST), Pyrenean gudgeon *Gobio lozanoi* (GL), and Pyrenean minnow *Phoxinus bigerri* (PB). Percentages of occurrences and abundances are presented. Trophic overlap values between signal crayfish and native fishes are presented by using the Schoener index.

Prey category	**% occurrence**					**% abundance**				
	PL	**BQ**	**ST**	**GL**	**PB**	**PL**	**BQ**	**ST**	**GL**	**PB**
Turbellaria	–	–	1	–	–	–	–	<1	–	–
Nematoda	–	–	39	7	2	–	–	7	1	<1
Oligochaeta	–	–	8	–	–	–	–	<1	–	–
Gastropoda	4	–	8	7	6	1	–	<1	<1	2
Hydracarina	9	–	3	5	4	3	–	<1	<1	<1
Crustacea [1]	12	32	39	35	10	6	6	46	17	2
Crayfish	–	–	1	8	–	–	–	<1	<1	–
Ephemeroptera [2]	27	64	71	15	23	13	10	26	<1	5
Plecoptera [2]	1	4	13	2	<1	<1	<1	<1	<1	<1
Heteroptera adults	–	–	1	–	–	–	–	<1	–	–
Megaloptera larvae	–	–	1	–	–	–	–	<1	–	–
Trichoptera larvae	29	56	39	48	45	22	6	15	7	28
Diptera larvae	30	84	24	98	70	53	76	2	66	51
Coleoptera larvae	2	–	4	2	2	<1	–	<1	<1	<1
Terrestrial Arthropoda [3]	8	16	22	63	35	1	1	2	7	9
Fish	–	–	2	–	–	–	–	<1	–	–
Schoener Index						–	0.77	0.39	0.69	0.84

[1] Cladocera, Copepoda, Ostracoda; [2] Nymphs and just emerged adults; [3] Adult winged insects (e.g., wasps and bees), ants, and spiders. – No data.

The isotopic values of crayfish and fish species were significantly different in several sites, mainly for $\delta^{15}N$ (Table 2; Figure 4). Particularly for this stable isotope of N, fishes always showed a higher mean value than signal crayfish (Table 2). Consequently, the isotopic niche overlap (i.e., based on the SEAc) between signal crayfish and native fish species was low (Table 3; Figure 4). According to fish results, signal crayfish had an intermediate mean SEAc value (i.e., isotopic niche). Brown trout had the widest isotopic niche (>2‰^{2}), and only this fish species showed a certain degree of isotopic overlap with crayfish (Table 3).

Table 2. Results on $\delta^{13}C$ and $\delta^{15}N$ for signal crayfish *Pacifastacus leniusculus* and native fishes. The numbers in parenthesis indicate the sampling sites represented in Figure 1. Significant differences (Mann–Whitney U test, $p < 0.05$) between signal crayfish and native fishes are marked with an asterisk (*) within each site. Results are means ± SD.

Town	**River**	**Species**	***n***	**$\delta^{13}C$**	**$\delta^{15}N$**
Zuñiga (1)	Ega	*P. leniusculus*	5	−28.9 ± 0.3	9.9 ± 0.6
		P. bigerri	5	−29.7 ± 0.3 *	10.5 ± 0.5 *
		S. trutta	5	−29.7 ± 0.7	11.5 ± 0.7 *
		G. lozanoi	5	−28.9 ± 0.7	11.1 ± 0.2 *
Artavia (2)	Urederra	*P. leniusculus*	5	−32.6 ± 0.4	6.9 ± 0.7
		P. bigerri	5	−33.2 ± 0.3 *	7.3 ± 0.2
		S. trutta	5	−33.4 ± 0.7	7.6 ± 0.7
		B. quignardi	5	−33.0 ± 0.3	7.5 ± 0.3
Ziordia (3)	Ega	*P. leniusculus*	5	−27.5 ± 0.2	9.0 ± 1.1
		P. bigerri	5	−27.7 ± 0.7	10.5 ± 0.8 *
		S. trutta	5	−27.6 ± 0.6	11.5 ± 1.4 *
		B. quignardi	5	−28.1 ± 1.0	12.2 ± 0.4 *

Table 2. *Cont.*

Town	River	Species	*n*	δ^{13}C	δ^{15}N
Lizarragabengoa (4)	Lizarrusti	*P. leniusculus*	5	−26.6 ± 0.3	3.4 ± 1.0
		P. bigerri	5	−27.4 ± 0.8	6.0 ± 1.4 *
		S. trutta	5	−29.2 ± 0.7 *	5.4 ± 0.5 *
Udabe (5)	Basaburua	*P. leniusculus*	5	−26.6 ± 0.5	7.5 ± 0.5
		P. bigerri	5	−27.9 ± 0.7 *	8.7 ± 0.5 *
		S. trutta	5	−27.9 ± 0.6 *	8.8 ± 0.5 *
Latasa (6)	Larraun	*P. leniusculus*	5	−26.0 ± 0.1	9.8 ± 0.5
		P. bigerri	5	−26.2 ± 0.6	10.8 ± 0.4 *
		G. lozanoi	5	−25.6 ± 0.5	10.4 ± 0.1
		S. trutta	5	−26.3 ± 0.5	10.6 ± 0.2 *
		P. miegii	5	−24.8 ± 0.5 *	9.4 ± 0.1
Oskia (7)	Arakil	*P. leniusculus*	5	−26.8 ± 0.3	12.0 ± 0.7
		P. bigerri	5	−28.2 ± 0.9 *	13.9 ± 0.7 *
		P. miegii	5	−26.7 ± 1.1	13.2 ± 0.3 *
Iraizoz (8)	Ulzama	*P. leniusculus*	5	−24.0 ± 0.3	8.0 ± 0.4
		P. bigerri	5	−24.0 ± 0.6	10.4 ± 0.4 *
		S. trutta	5	−23.7 ± 0.5	10.4 ± 0.7 *
Latasa (9)	Ulzama	*P. leniusculus*	5	−25.0 ± 0.1	10.6 ± 0.5
		P. bigerri	5	−25.2 ± 0.4	13.8 ± 1.6 *
		S. trutta	5	−25.7 ± 0.3 *	10.7 ± 1.0
Urtasun (11)	Arga	*P. leniusculus*	5	−27.8 ± 0.4	7.1 ± 1.0
		P. bigerri	5	−23.9 ± 0.3 *	8.1 ± 0.3
		S. trutta	5	−24.8 ± 1.7 *	7.5 ± 0.2
Idoi (12)	Arga	*P. leniusculus*	5	−25.2 ± 0.3	8.9 ± 0.6
		P. bigerri	5	−25.9 ± 0.6	9.8 ± 0.3 *
		G. lozanoi	5	−24.7 ± 0.5	9.8 ± 0.4
		B. quignardi	5	−23.5 ± 0.5 *	10.4 ± 0.1 *
Espinal (14)	Urrobi	*P. leniusculus*	5	−25.7 ± 0.3	9.7 ± 0.5
		P. bigerri	5	−25.3 ± 1.2	12.3 ± 0.6 *
		S. trutta	5	−25.5 ± 1.5	10.3 ± 1.3
Uriz (15)	Urrobi	*P. leniusculus*	5	−25.2 ± 0.6	6.9 ± 0.4
		P. bigerri	5	−23.8 ± 1.1 *	10.3 ± 0.7 *
Aribe (16)	Irati	*P. leniusculus*	5	−25.6 ± 0.3	5.6 ± 0.5
		P. bigerri	5	−25.3 ± 1.0	6.8 ± 0.3 *
		S. trutta	5	−27.4 ± 1.3 *	6.3 ± 0.5
		B. quignardi	5	−26.5 ± 0.5 *	6.5 ± 0.4 *
Ezcaroz (18)	Salazar	*P. leniusculus*	5	−25.8 ± 0.2	8.5 ± 0.4
		P. bigerri	5	−26.5 ± 0.7	10.8 ± 0.3 *
		S. trutta	5	−26.9 ± 0.7 *	10.8 ± 1.0 *
Guesa (19)	Salazar	*P. leniusculus*	5	−26.3 ± 0.7	9.0 ± 0.8
		P. bigerri	5	−27.5 ± 0.7	10.2 ± 0.5 *
		G. lozanoi	5	−25.9 ± 0.8	10.7 ± 0.4 *
Aspurz (20)	Salazar	*P. leniusculus*	5	−28.4 ± 1.0	8.2 ± 0.8
		P. bigerri	5	−28.3 ± 0.5	9.4 ± 0.4 *
		G. lozanoi	5	−27.3 ± 0.5	9.6 ± 0.4 *

Table 3. Corrected standard ellipse areas (SEAc) for each species and areas of overlap between signal crayfish *Pacifastacus leniusculus* and native fishes. Results are overall means ± SD.

Species	SEAc (‰^{2})	Area of Overlap (‰^{2})
P. leniusculus	0.80 ± 0.49	–
B. quignardi	0.68 ± 0.55	$(2.7 \pm 5.4) \times 10^{-4}$
G. lozanoi	0.62 ± 0.25	$(1.3 \pm 2.6) \times 10^{-2}$
P. bigerri	1.05 ± 0.67	$(2.8 \pm 3.2) \times 10^{-18}$
P. miegii	0.55 ± 0.65	$(4.8 \pm 6.7) \times 10^{-18}$
S. trutta	2.01 ± 2.00	0.1 ± 0.2

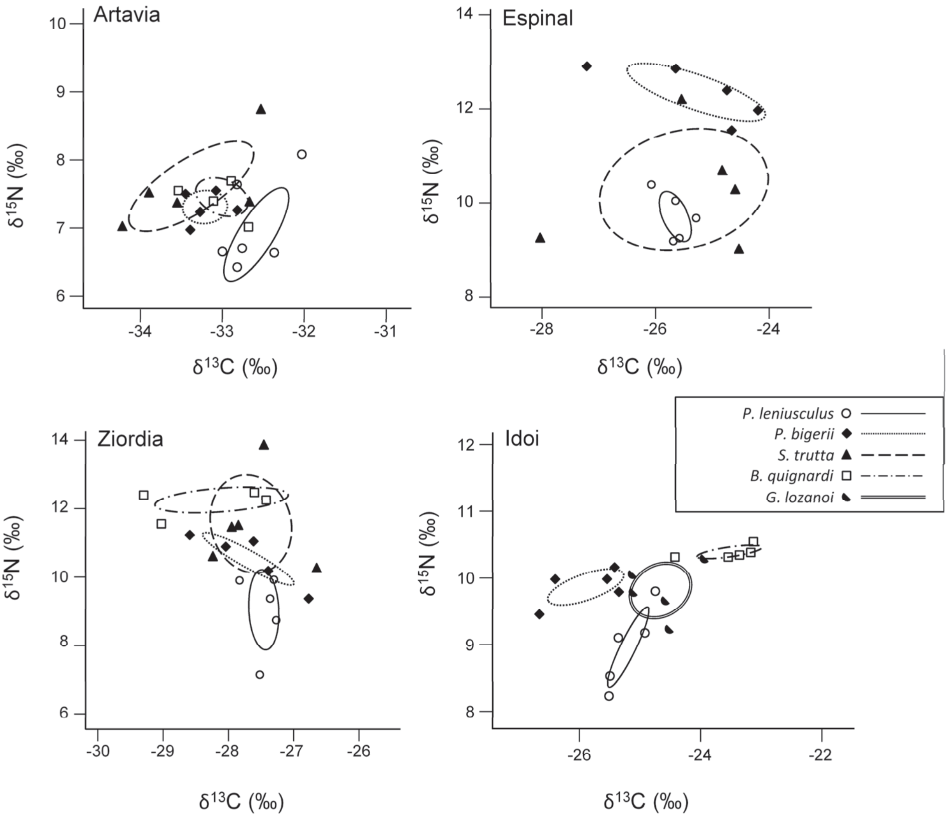

Figure 4. Biplots of isotopic signatures δ^{13}C and δ^{15}N for signal crayfish *Pacifastacus leniusculus* and native fishes at four representative sampling sites (see Figure 1 and Table 2 for details). Corrected standard ellipse areas (SEAc) are showed per species. See Table 3 for overall means of SEAc and areas of overlap.

4. Discussion

4.1. Behavioral Interactions

In agreement with our first hypothesis, observations showed that signal crayfish can physically displace native fishes, with >25% of encounters showing fish evasion under particular conditions, i.e., according to crayfish size and fish species. The result of an interaction between crayfish and fish is strongly influenced by the crayfish life stage (mainly the size). Thus, fishes were more dominant when interacting with juvenile crayfish, whereas adults displayed more dominance in their interactions with native fishes [53,54]. The present results also supported our expectations, as evasion was a behavioral response clearly observed in the benthic Pyrenean stone loach, after interacting with adult crayfish. This behavior may be related to the high spatial overlap in the habitat use between signal crayfish and a variety of benthic fishes [13]. As an example, Guan and Wiles [11] observed, in an artificial British stream, how signal crayfish displaced two benthic fishes (bullhead and stone loach) from shelters after displaying aggressive behaviors. Similar interferences have been also observed under natural conditions in an Iberian stream, the River Jarama (central Spain). Thus, field observations (via snorkeling) revealed physical displacement for habitat/refuge by signal crayfish on two endemic Iberian loaches, the northern Iberian spined-loach and the southern Iberian spined-loach *Cobitis paludica* (de Buen, 1930) (D. Almeida, pers. observ.). In North America, the benthic Paiute sculpin *Cottus beldingii* (Eigenmann & Eigenmann, 1891) reduced its use of refuges and pools, shifted into higher velocity microhabitats and spent more time fleeing in the presence of invasive signal crayfish [53]. However, we must consider that the degree of interference is strongly dependent on the overall availability of shelters. In this regard, previous studies suggest that shelter availability is limited under natural conditions because signal crayfish may be found at densities higher than or similar to the number of potential shelters within a particular site [53]. All these results, along with the present study, reinforce the idea that spatial interference occurs between benthic fish and signal crayfish where this species has been introduced in American and European fresh waters. Regarding pelagic fishes, such as brown trout in the study rivers, our previous results also indicate that signal crayfish can spatially interfere with salmonids, although these observations were recorded under laboratory conditions [14]. As a consequence of this potential spatial interference, the vulnerability of displaced fish to predation may be increased [55]. In particular, Rahel and Stein [56] observed that predation on the Johnny darter *Etheostoma nigrum* Rafinesque, 1820 was more intense by the small-mouth bass *Micropterus dolomieu* Lacépède, 1802 where the rusty crayfish *Faxonius rusticus* (Girard, 1852) was present and shelter was limiting. In our study area, signal crayfish may increase the risk of predation for benthic fishes, such as Pyrenean stone loach, by piscivorous fish (e.g., brown trout), birds (e.g., gray heron *Ardea cinerea* L., 1758), and mammals (e.g., Eurasian otter *Lutra lutra* (L., 1758)) [57–59].

4.2. Trophic Overlap

The present results partly supported the second hypothesis. Specifically, the analysis of stomach content showed that signal crayfish preyed on an important number of benthic invertebrates, mainly Diptera larvae (e.g., taxonomic families of Chironomidae and Simuliidae). Except for brown trout, benthic invertebrates were also the staple food resource for the study fish species (see bibliographic references on fish diet in Methods section), which was the reason for the high trophic overlap with crayfish by using dietary data. However, plant material and detritus were also common food categories for signal crayfish. These findings indicated a foraging strategy more "generalist" for crayfish (e.g., [40]), whereas fish species followed a more "predatory" feeding tactic on invertebrates. This would be related to an ecological role for signal crayfish as a wide omnivorous species, affecting different levels of the freshwater trophic web [60,61]. As it was stated in the Methods section, the present study used dietary data from different years (see above). Food resources can largely vary from year to year in Mediterranean freshwater habitats [62]. Thus, such a difference between survey periods could mean a confounding factor for results on diet composition. However, comprehensive monitoring surveys have been carried out on benthic invertebrates and fish diet (e.g., 48), which show that the prey use-availability have been relatively stable in the study area. This is important to state that such variation is unlikely to affect the present results regarding overlap.

Given that plant material was not used for calculation of the Schoener index (only prey items), this is the most likely cause for the discrepancies between dietary and stable isotope results on trophic overlap. The Schoener index is commonly calculated by using numbers of particular items, e.g., prey individuals to estimate overlap between obvious predatory species (e.g., [63]). Ingested mass of plant material and detritus should have been included in the gut content analysis for a more realistic comparison. Unfortunately, these data are not provided from the previous surveys, as fish species analyzed in the present study were mainly predatory on invertebrates (see bibliographic references on fish diet in Methods section), whereas signal crayfish was more omnivorous (see findings above). In these cases (i.e., discrepancies), SIA reveals feeding habits comprising a longer period, whereas dietary analysis only represents a "snapshot" of the whole foraging strategy [38,39]. More specifically, signal crayfish had lower δ^{15}N values than native fishes, which may be a consequence of the increased ingestion of algae and detritus. In this respect, Rosewarne et al. [64] analyzed the gut content of adult specimens of signal crayfish in the River Stour (UK). This study revealed a wide variety of food items, including algae, macrophytes, terrestrial detritus, benthic invertebrates, and fish. As a result, signal crayfish occupied an intermediate trophic level after performing SIA, which was similar to different omnivorous species of insect nymphs [64]. Invasive species are expected to have broader ecological, and particularly, trophic niches, as they are often generalists to exploit a wide variety of environmental resources (e.g., see [65] for a comprehensive review). Nevertheless, the niche size of signal crayfish was not as elevated as expected for this bioinvasion in the present study. This finding may be at least in part due to the size of crayfish (adult only) used in the SIA, as the isotopic niche width varies according to the ontogenetic stages [40], although other authors reported no evidence of such ontogenetic changes (e.g., [66]).

Contrary to our second hypothesis, SIA showed a lack of trophic overlap between signal crayfish and benthic fishes. A possibility to explain this result consists of potential niche alterations. More in detail, if competitively inferior individuals (i.e., fish in this case) are physically displaced and forced to feed on less suitable resources, trophic niche shifts likely occur [67]. Also, a slight isotopic overlap was found between signal crayfish and brown trout only. This can be partially explained because of the high variety of diet composition for this fish species and thus, its wide isotopic niche. The broad trophic niche of brown trout may be explained by the ontogenetic stage and seasonal variations of food resources. In particular, reliance on terrestrial and aquatic resources can be very different according to the age and period of year, irrespective of the fish density [68]. Another cause for such a variable diet may be the expansion of the trophic niches in top predators with increased population density due to high intraspecific competition, which leads to a differential diet specialization among individuals [69]. Another potential reason for this overlap is that large brown trout occasionally prey on crayfish, which are omnivores, and small brown trout commonly prey on planktonic crustaceans, such as Cladocera or Copepoda, which feed on phytoplankton. These may result in a decrease of the overall $\delta^{15}N$ level and a subsequent overlap with the isotopic crayfish niche. Other native fish species had a higher trophic level (i.e., higher $\delta^{15}N$ value) than brown trout in several sites (e.g., Pyrenean minnow and Pyrenean stone loach). These fish species frequently fed on predatory Trichoptera larvae with larger sizes, such as the taxonomic family Rhyacophilidae [43,45], which may increase the ^{15}N level in their body tissues.

Only dietary results on niche overlap (i.e., the Schoener index) suggested that trophic interference may occur between signal crayfish and native fishes, particularly for benthic invertebrates. Although we mentioned above that these results from dietary data must be limited at a specific moment (i.e., a snapshot), a certain degree of competition may be established between signal crayfish and benthic fishes, such as Pyrenean stone loach and Pyrenean gudgeon. Indeed, these three species (i.e., crayfish and two fishes) select similar shelter features in coarse substrata and feed virtually on the same benthic invertebrate communities [44,45]. Such a potential competition could be especially relevant when the abundance of this food resource is scarce (i.e., limiting) during some periods of the year. In particular, summer drought can be ecologically "severe" in northern rivers of the Iberian Peninsula under partly Mediterranean climate conditions [62]. Moreover, signal crayfish populations can reach high densities in the study rivers [70], with the pressure on benthic invertebrates being increased accordingly [61]. However, the relationship between competition and niche overlap is highly complex and difficult to evaluate in field studies. Thus, particular resources may not always limit populations and species could overlap their niches with no competition [71].

As a final remark and according to our results, a particular conservation concern arises in the study area. The benthic fish species northern Iberian spined-loach is catalogued as "Endangered" on the IUCN Red List, as their populations have undergone a steep decline across the Iberian Peninsula in recent years [72]. The present study did not analyze any direct impact of signal crayfish on this endemic loach species. However, and given that its distribution area widely overlaps with that of signal crayfish, conservation managers should apply urgent measures to control populations of this invasive species.

5. Conclusions

The present study is one of the few examples assessing crayfish behavior within its spatial natural context (e.g., [30,31]). Therefore, our findings are highly valuable for real impact assessment of invasive signal crayfish. In this regard, our field study appears to support the assumptions of previous laboratory experiments, which indicated that non-native crayfish could have detrimental effects on fish populations, especially benthic fish species (e.g., [11,13]). However, more data is necessary to accurately establish the level of ecological competition, both for food and space. In particular, these data should be generated from field surveys spending more than two years in the same study area, including different seasons (see [73] for a comprehensive review on river research). Thus, the precise environmental factors controlling habitat and prey availabilities will be better identified, as inherent fluctuations occur in crayfish/fish populations over time [55].

Author Contributions: Conceptualization, I.V., A.A.R., E.B., D.G., J.O., and R.M.; Methodology, I.V., D.E., J.M.S., and R.M.; Software, I.V. and P.M.L.; Formal Analysis, I.V.; Writing—Original Draft Preparation, I.V., P.M.L. and R.M.; Writing—Review & Editing, D.A. and R.M.; Supervision, Project Administration, and Funding Acquisition, R.M. All authors read and approved the final manuscript.

Funding: The authors are grateful to the “Asociación de Amigos-University of Navarra”, which awarded Iván Vedia a doctoral grant (2012–2016). This research was funded by the Research Program of the University of Navarra (PIUNA 2014–16).

Acknowledgments: Xabier Álvarez, Maite González, Antonio Vilches, and Gregorio Sánchez collaborated during the field surveys and laboratory studies. Gema Castillo-García helped with the text formatting. The authors thank the comments from three anonymous reviewers to improve the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest. The manuscript was approved by all authors for publication. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Effects of Nonnative Fishes on Commercial Seine Fisheries: Evidence from a Long-Term Data Set

Alexander B. Orfinger [1,2,*], Quan T. Lai [3] and Ryan M. Chabot [4]

[1] Center for Water Resources, Florida A&M University, Tallahassee, FL 32307, USA
[2] Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA
[3] Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan, District 3, Ho Chi Minh City 700000, Vietnam; laitungquan@gmail.com
[4] Inwater Research Group, Jensen Beach, FL 34597, USA; ryan.chabot@gmail.com
* Correspondence: a.orfinger@ufl.edu; Tel.: +13862902505

Received: 11 May 2019; Accepted: 2 June 2019; Published: 4 June 2019

Abstract: Dozens of introduced exotic freshwater fish species inhabit the state of Florida. These nonnative fishes interact with freshwater commercial fisheries in a variety of ways, influencing catch abundance, composition, and revenue. Using a 22-year data set collected from a commercial haul seine fishery, we aimed to explore the dynamics of yield and revenue in relation to nonnative fishes, with emphasis on the suckermouth armored catfishes (*Pterygoplichthys* spp.). Using profit index metrics and the inverse Simpson's diversity index, we found that non-native tilapia (*Oreochromis* spp.) and brown hoplo (*Hoplosternum littorale*) provided economic benefits while suckermouth armored catfishes seemed to disrupt catch consistency and lower profit index values. To reduce the negative impacts of the suckermouth armored catfishes and subsequently exert pressure on their population, we suggest marketing these edible fishes for human and/or animal consumption.

Keywords: fisheries economics; invasive species; *Pterygoplichthys*; suckermouth armored catfishes; tilapia; diversity; profit index; Simpson's diversity

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1. Introduction

Introductions of nonnative species can have impacts on the ecology, biodiversity, human and non-human health, and economies of the novel geographic range, e.g., [1] and papers therein. Such impacts may manifest as either positive, negative, or mixed outcomes [1]. Due to its island-like geography, large population, major ports of entry, and diversity of sub-tropical habitats, the state of Florida, USA has become a hotbed of both intentional and accidental species introductions [2,3]. Aquatic habitats are not immune, with at least 42 exotic fish species having been reported as established in Florida freshwater systems [4].

Among these are the South American suckermouth armored catfishes of the genus *Pterygoplichthys* (Siluriformes: Loricariidae). Members of this genus have been introduced to at least five continents and 21 countries, likely via the aquarium trade [5]. Multiple *Pterygoplichthys* species and their presumed hybrids are established throughout peninsular Florida and can form dense clusters in lakes and rivers. These fishes have been implicated in numerous negative ecological and economic impacts in their novel ranges, including, for example, competing with native fishes, inducing altered behaviors in endangered manatees (*Trichechus manatus*), causing erosion and sedimentation via nest excavation, and generating biogeochemical hotspots (e.g., [6–9]).

In Florida, members of the genus *Pterygoplichthys* are largely considered "trash" fish and unmarketable bycatch. Recent anecdotal reports from numerous commercial freshwater fishing operations have suggested a shift in catch composition and revenue in response to the presence of *Pterygoplichthys* spp. (A. Orfinger personal communications; e.g., Figure 1).

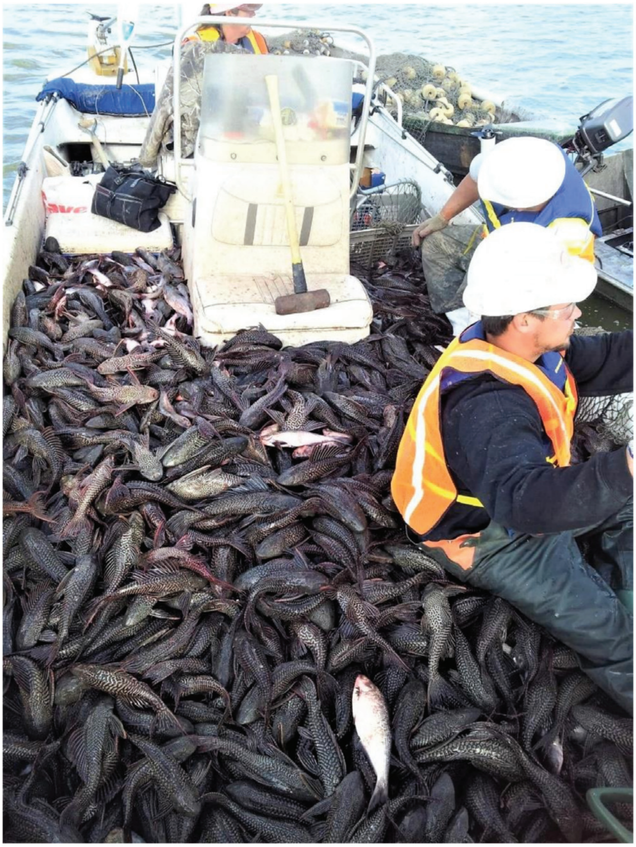


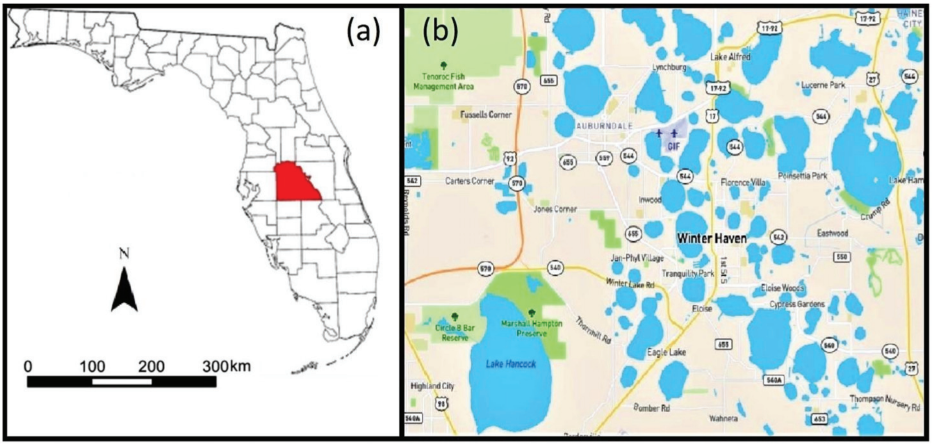
Figure 1. A representative catch illustrating the dominance of *Pterygoplichthys* spp. (dark fishes composing bulk of image) in some seining hauls. Photography by Taren Wadley.

In the present study, we aimed to explore the influence of *Pterygoplichthys* spp. and other nonnative fishes on catch composition and economic output using a long-term data set from a commercial haul seine fishery. Specifically, we anticipated that an increase in *Pterygoplichthys* spp. would correspond to a proportional decrease in marketable fish yield. Likewise, we also expected that another nonnative fish genus, tilapia, *Oreochromis* spp., would constitute the most profitable taxon. By investigating the interactions of *Pterygoplichthys* spp. and other nonnative fishes on freshwater net fisheries, we hope to provide insight into the relationships between native and nonnative fishes and the fishery operations that rely on these fish communities and make management recommendations, as appropriate.

2. Materials and Methods

2.1. Study Area

The seine fishery evaluated in this study operates out of Winter Haven, Polk County, FL, USA. Winter Haven is home to an extensive chain of natural lakes at the headwaters of the Peace River–Charlotte Harbor watershed (Figure 2). The commercial operation in question fishes in 14 of the 25 lakes. Most of the lakes (18/25) are considered impaired in terms of water quality [10]. Lake Hancock is one of the largest and the primary target of fishing activity (27°58′6.52′′ N, 81°50′20.12′′ W). Lake Hancock spans 1851 ha and reaches a maximum depth of 4.9 m, with an average depth of 1.2 m [11].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Carters Corner
Jones Corner
Inwood
Florence Villa
Poinsettia Park
Lake Ham
Winter Haven
Eastwood
Jan-Phyl Village
Tranquility Park
Winter Lake Rd
Eloise Woods
Cypress Gardens
Eloise
Circle B Bar Reserve
Marshall Hampton Preserve
Thornhill Rd
Eagle Lake
Lake Hancock
Highland City
Bomber Rd
Wahneta
Thompson Nursery Rd

Figure 2. (**a**) Polk County (red), Florida, USA. (**b**) The Winter Haven chain of lakes where data were collected. The primary lake targeted, Lake Hancock, is in the southwest corner of the map.

2.2. Sampling and Data Set

The fishery-dependent data set was generated based on collection efforts between 1995 and 2018. Fishes were collected using a haul seine with dimensions 914.4 m length, 5.2 m depth, and 7.6 cm nylon mesh. Five variables were included in the analysis: (1) year, (2) lake representation (percent overall catch), (3) taxon, (4) estimated harvest (kg), and (5) market value of harvest (USD).

Data were not collected in 1996 and 2007, resulting in a total of 22 years of data collection. Lake representation is described as percent harvest from each of the 14 lakes in relation to the overall catch each year. Lake representation also likely shifted concerning fishing efforts to the most profitable lake to optimize the harvest each year. Average total fishing effort was 57 days/year. Taxa reported (n = 6) include tilapia (nonnative *Oreochromis* spp.), catfishes (native *Ictalurus* spp.), American shad (native *Alosa sapidissima*), gar (native *Lepisosteus* spp.), suckermouth armored catfishes (nonnative *Pterygoplichthys* spp.), and the brown hoplo (nonnative *Hoplosternum littorale*). Harvest was reported for species each year. A missing harvest value of brown hoplo in 2018 was treated as zero. Market value reported was the wholesale value at dock side. Tilapia and catfishes were heavily marketable. Shad and brown hoplo were partially marketable. Gar and suckermouth armored catfishes were not marketable.

2.3. Analysis

The suckermouth armored catfishes were first recorded in the fishery operation as bycatch in 1999. We compared the impact of the suckermouth armored catfishes to the yield and economic outcomes of the fishery operation. The data set from 1995 to 1998 and from 1999 to 2018 were referred to pre-invasion and post-invasion, respectively.

The ratio of suckermouth armored catfishes is the proportion of these fishes in total yield ranging from 0 to 1. An estimate of relative catch per unit effort (CPUE) of marketable fish was calculated by dividing total yield of these fish (tilapia, native catfishes, shad, and brown hoplo) each year to the average fishing effort of 57 days/year. We used average CPUE of pre-invasion as a benchmark (hereafter referred to as CPUE-pre) in order to elucidate the impact of the suckermouth armored catfishes on the CPUE post-invasion (hereafter referred to CPUE-post).

To evaluate how the suckermouth armored catfishes' presence impacted revenue, we used the profit index to compare the revenue pre- and post-*Pterygoplichthys* invasion in Winter Haven, FL. The profit index was calculated by dividing total revenue (in USD) to total fish caught (in kg, both marketable and undesirable fish). The average profit index pre-invasion was used as a benchmark to compare the impact of the suckermouth armored catfishes on subsequent profit. Increasing the ratio of undesirable/unmarketable fish (gar, suckermouth armored catfishes) in the catch will reduce the revenue to fishers with subsequent reductions in the profit index. To further evaluate the impact of suckermouth armored catfishes in bycatch and to reduce the sensitivity of the profit index to market price, we used \$0.60, \$0.60, \$0.10, and \$2.20 kg^{-1} as the average market prices of tilapia, catfishes, shad and brown hoplo, respectively, to estimate the profit index (indicated when applied).

Next, the relationship between diversity (i.e., inverse Simpson's diversity index) and the ratio of suckermouth armored catfishes versus the profit index was assessed by using ordinary least-squares linear regression in R [12]. We calculated lake representation diversity and evenness indices to evaluate trends through time, as well as to understand the relationship of lake diversity and species diversity of the catch. Changes in catch diversity as a result of variation in the fishing effort among lakes over time could have management implications for targeted harvesting.

3. Results

3.1. Yield Impacts of *Pterygoplichthys* spp

Tilapia, a nonnative taxon, and native catfishes contributed most to the total yield. The commercial fishery relied mainly on the harvest of these two taxa for revenues. Prior to the invasion of the suckermouth armored catfishes in 1999, the yield of tilapia and native catfishes was 72,824 ± 10,169 kg/year and 8986 ± 5755 kg/year, respectively. Following the introduction of the suckermouth armored catfishes, the production of tilapia and native catfishes became highly variable at 77,454 ± 47,122 kg/year and 20,102 ± 26,020 kg/year, respectively, with large increases in standard deviation values relative to pre-invasion levels. The ratio of suckermouth armored catfishes bycatch ranged from 0 to 0.44 in the total harvest (Figure 3). The CPUE-pre was 1640 ± 542 kg/day; lower CPUE-post compared to CPUE-pre was observed in 1999, 2000, 2001, 2005, 2009, 2010, and 2011 when the ratio of the suckermouth armored catfishes in the harvest ranged from 0.15 to 0.44 (Figure 4). However, except for tilapia, the total yield of native fishes (catfishes, gar, and shad) was still higher than suckermouth armored catfish bycatch and dominated the harvest in some years (Figure 5).

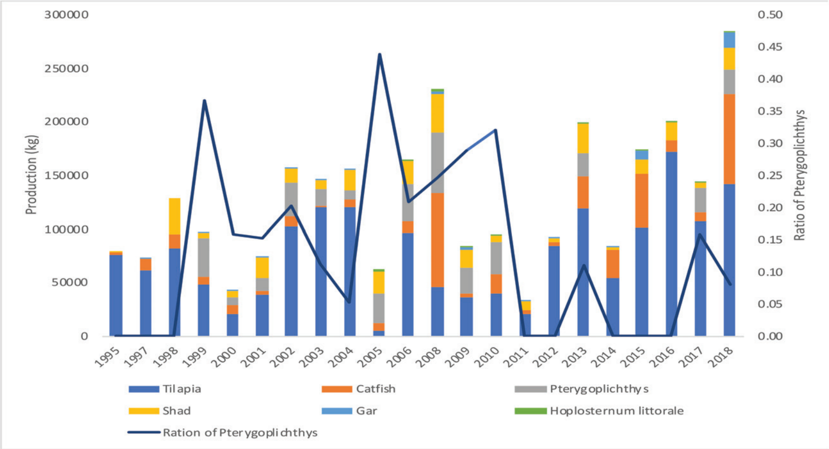

Figure 3. Total fish harvest and ratio of the suckermouth armored catfishes through the study period. The stacked bar for each year represents the production (in kg, left-hand vertical axis) represented by each taxonomic group, while the right-hand vertical axis tracks the ratio of suckermouth armored catfishes in the catch over time.

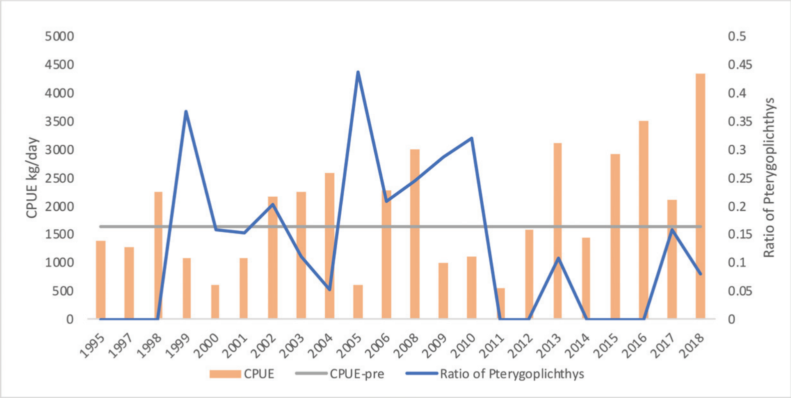

Figure 4. CPUE of marketable fish relative to CPUE-pre and ratio of suckermouth armored catfishes. Generally, years with a high ratio of *Pterygoplichthys* spp. in the catch (right-hand vertical axis) are associated with depressed CPUE values of marketable fish.

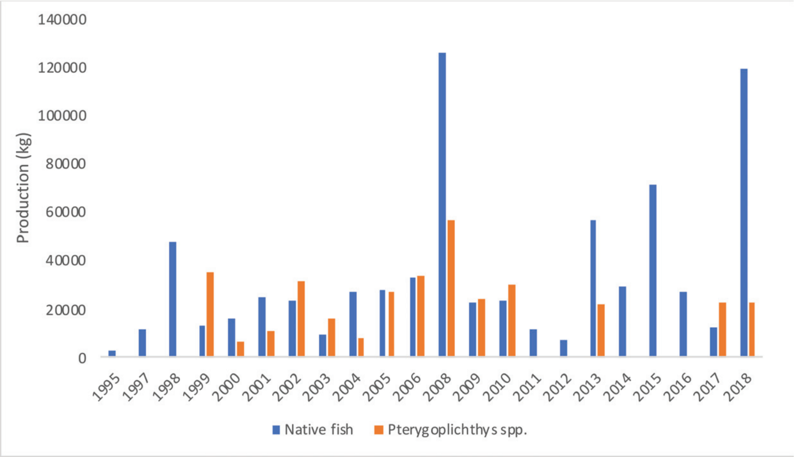

Figure 5. Yield of native fish versus suckermouth armored catfishes per year.

3.2. Economic Impact of the Pterygoplichthys Invasion

Fishing revenue stemmed mostly from tilapia and native catfishes. Shad was somewhat marketable. Nonnative brown hoplo was introduced in 2005 and is also somewhat marketable (Figure 6). The profit index benchmark was \$0.66 kg^{-1}; the lowest profit index post-invasion was \$0.19 kg^{-1} in 2005, while the highest was \$0.93 kg^{-1} in 2012 and 2015 (Figure 7). During the 1999–2018 post-invasion, there were 13 years during which the profit index was lower than the benchmark.

The profit index is driven by both fish yield and market price. However, the profit index of fishery operation in Winter Haven is driven more by market value than by yield. This is demonstrated well by data in 2018, when the highest CPUE-post was 4345 kg day^{-1} and the ratio of the suckermouth armored catfishes was 0.08 (Figure 4), but the prices of tilapia and native catfishes were just half of their pre-invasion period values (Figure 6), making the profit index in 2018 67% lower than the benchmark (Figure 7). A similar trend was observed in 2004 and 2013 with higher CPUE and a low ratio of suckermouth armored catfishes (i.e., <0.15), but also a lower unit price per marketable fish (Figures 4, 6 and 7).

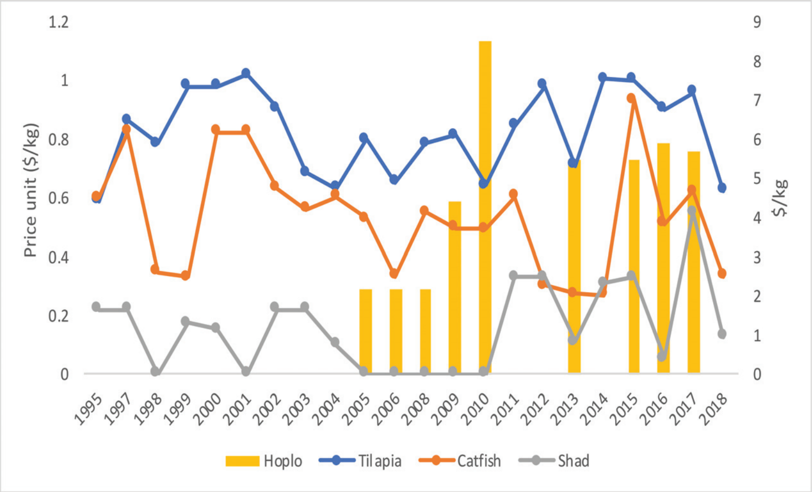

Figure 6. Price unit per marketable fish each year. Lines represent price unit ($/kg) of tilapia, catfish, and shad (left-hand vertical axis). Bars represent price unit ($/kg) of brown hoplo (right-hand vertical axis).

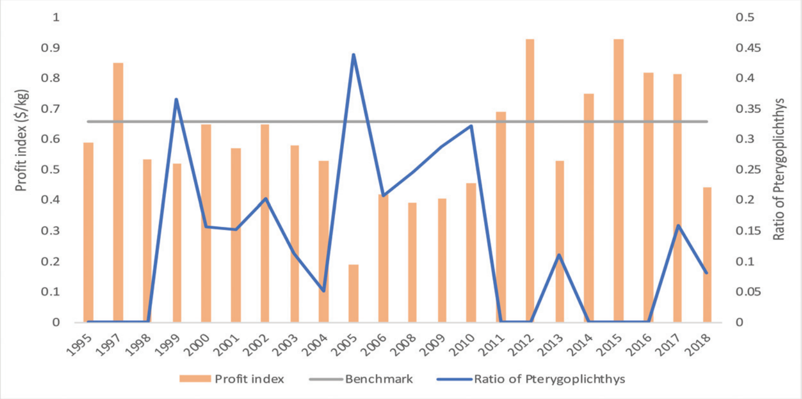

Figure 7. Profit index benchmark and post-invasion of armored catfishes.

3.3. Profit Index versus Species Diversity and Ratio of Pterygoplichthys spp

A significant negative correlation existed between the inverse Simpson's diversity index and profit index ($r^2 = 0.62$, $p = 1.497 \times 10^{-5}$; Figure 8), and also between the ratio of suckermouth armored catfishes and profit index ($r^2 = 0.60$, $p = 1.665 \times 10^{-8}$; Figure 9). As species diversity in the catch increased, there was a subsequent decrease in the profit index. Although some of this negative influence of increased species diversity is likely attributable to other unprofitable harvested species (e.g., gar), most is certainly a result of the inclusion of armored catfishes in the catch given its greater relative contribution (Figure 9). The highest profit index was associated with pre-invasion and years without suckermouth armored catfish bycatch.

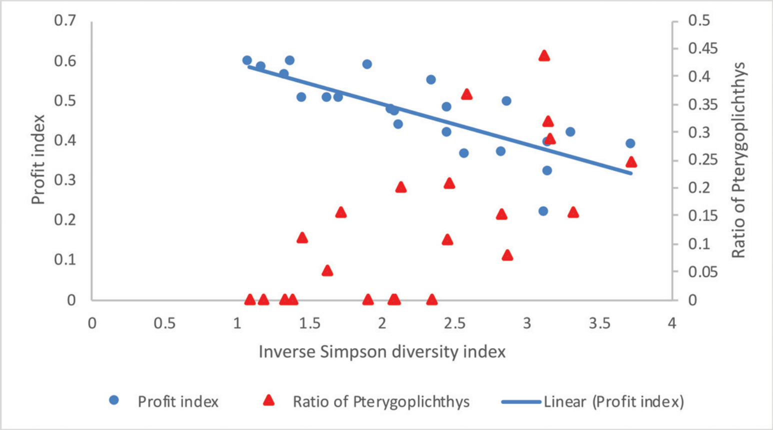

Figure 8. Negative linear correlation between diversity index and profit index (average market price applied).

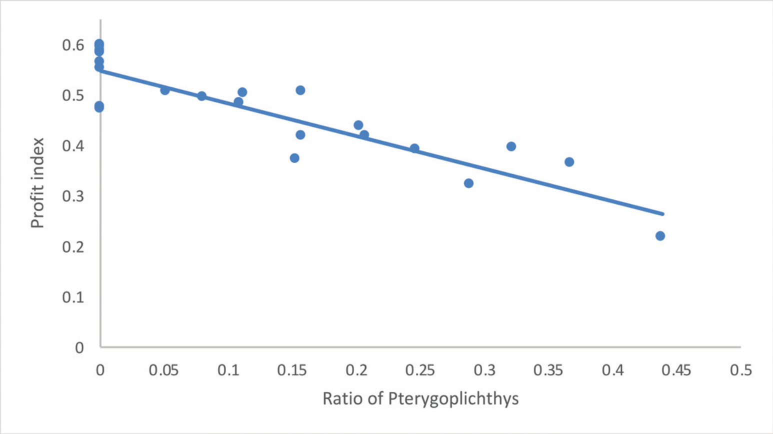

Figure 9. Negative linear correlation between the ratio of suckermouth armored catfishes in the catch and profit index (average market price applied).

3.4. Lake Representation

Except for five years (i.e., 1999, 2000, 2001, 2005, and 2008), the majority of fish were harvested from Lake Hancock with the yield ranging from 16 to 117 kg/ha. Years with a high ratio of suckermouth armored catfishes in the catch often coincided with high representation of Lake Hancock in harvests (Figure 5; Figure 10). Increases in the "spreading out" of fishing effort across multiple lakes (i.e., 1999, 2001, 2005; Figure 10) did not increase total yield or reduce the ratio of the suckermouth armored catfishes in the harvest. Primary harvest of both marketable and undesirable fish was still from Lake Hancock. No evident pattern exists between lake diversity and species diversity at low levels of lake diversity. However, high levels of species diversity were associated with high levels of lake diversity (Figure 11).

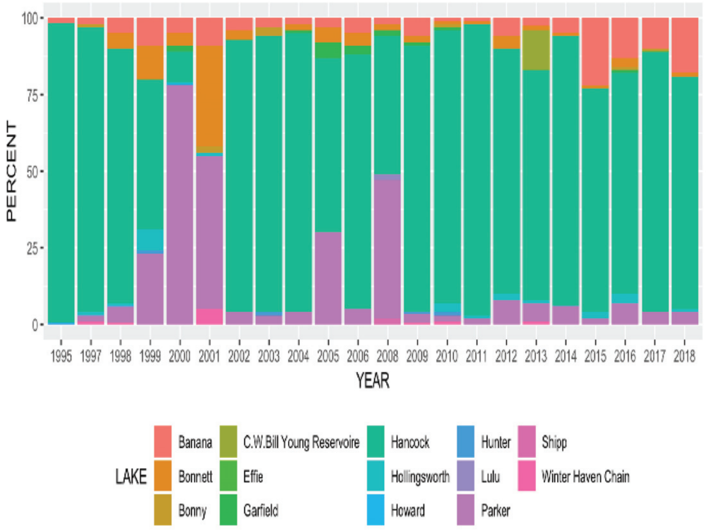

Figure 10. Lake representation of harvest per year.

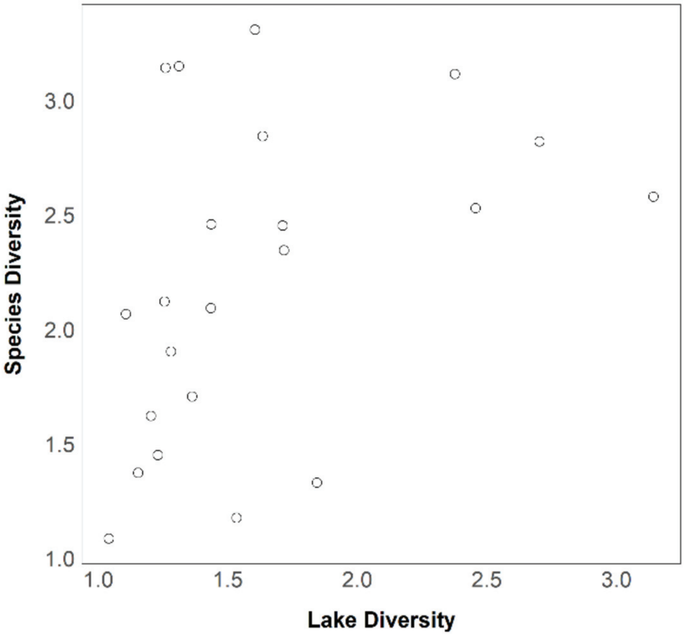

Figure 11. The relationship between lake diversity in sampling effort and species diversity of the catch. High levels of lake diversity were associated with high levels of species diversity.

4. Discussion

4.1. Observed Trends in Nonnative Taxa

Despite possible adverse ecological impacts, tilapia are of clear economic importance to Florida's freshwater haul seine industry. Brown hoplo shows slight marketability, while the data here suggest that the suckermouth armored catfishes are economically harmful to an appreciable extent. Despite constituting a large proportion of each catch annually post-invasion, the suckermouth armored catfishes did not consistently dominate the overall yield. However, the profit index through time suggests that these exotic catfishes disrupt catch consistency and lower revenue relative to pre-invasion years. This conclusion is supported to by the inverse Simpson's diversity values which demonstrate a decreasing profit index with increased species diversity (Figure 8). The increased diversity (a measure of relative abundance and richness) post-invasion can be directly attributed to the introduction of the suckermouth armored catfishes. These negative economic effects are consistent with the geographically and categorically broader findings of [4] that *Pterygoplichthys* spp. exert low to moderate negative impacts.

4.2. Recommendations

The results presented here provide implications for targeting lakes for fish harvest. Of note, broadening fishing efforts to include multiple lakes was ineffective in increasing revenue relative to fishing a primary lake (i.e., Lake Hancock). Based on the current data, then, it seems pertinent to target only the most productive lake. Further supporting this notion is the fact that high levels of lake diversity correspond to higher species diversity. When coupled with the finding that higher species diversity is negatively correlated with profit index, spreading out catch efforts among lakes could possibly contribute to a lower profit index. Therefore, targeting fewer, more productive lakes would likely result in higher revenue per unit effort.

Steps should be taken to mitigate the ongoing issues presented by the suckermouth armored catfishes. First and foremost, a concerted effort should be undertaken to market these catfishes. Eaten widely throughout the Neotropics, the suckermouth armored catfishes are readably edible and demonstrably easy to harvest. Indeed, efforts to market these fishes are already underway by some private firms. For example, in parts of Mexico, where *Pterygoplichthys* spp. are also introduced, fishermen have embraced the previous nuisance fish and now market and sell its meat locally and internationally (Acari Fish Co., Personal Communication). However, the campaign of marketing these fishes is still in its infancy, with no peer-reviewed literature to evaluate consumer acceptability beyond the fishes' native South America. Fisheries economists should seek to work with companies and consumers to better understand marketing potential of *Pterygoplichthys* spp. In addition, *Pterygoplichthys* spp. has proven a valuable commercial fish meal replacement [13], a source of antioxidants for nutrition supplements [14], and even shows promise for use in biodiesel production [15].

Government subsidization, through a bounty program or incentivization for commercial fishers, is another option for mitigation. Such programs have been employed with varying degrees of success and for various taxa globally, including in Florida [16]. For example, commercial and recreational fisheries of invasive red lionfish (*Pterois volitans*) are underway in the United States and seem to inhibit local lionfish populations [17]. Given the dense groupings formed by *Pterygoplichthys* spp., it is reasonable to suggest that at least some degree of population inhibition could be imparted by harvest-based mitigation efforts. While unlikely to eliminate the Florida populations, harvesting suckermouth armored catfishes provides an economically-sound option that bears little environmental tradeoffs compared to other options (e.g., rotenone poisoning, [18]).

5. Conclusions

Nonnative species are a double-edged sword for Florida freshwater seine fisheries. While exotic tilapia are highly marketable, other species such as the suckermouth armored catfishes are not currently marketed and seem to be negatively impacting fishery revenue. A growing body of research also suggests other negative ecological and economic impacts imparted by the genus *Pterygoplichthys* [4]. Given that large-scale eradication of these hardy and widespread fishes is unfeasible once established [4], we recommend marketing the edible suckermouth armored catfishes for consumption. In doing so, fisheries that otherwise suffer can potentially turn a profit while simultaneously exerting some degree of pressure on the introduced populations. Continued research is needed to better characterize the mechanisms of ecological and fisheries impacts of the suckermouth armored catfishes. Ongoing efforts to do so are currently underway in Florida and Vietnam.

Author Contributions: Conceptualization, A.B.O.; Methodology, A.B.O., Q.T.L., and R.M.C.; Formal Analysis, Q.T.L. and R.M.C.; Writing—Original Draft Preparation, A.B.O., Q.T.L., and R.M.C.; Writing—Review and Editing, A.B.O., Q.T.L., and R.M.C.; Supervision, Project Administration, and Funding Acquisition, A.B.O. All authors read and approved the final manuscript.

Funding: Publication of this article was funded in part by the University of Florida Open Access Publishing Fund.

Acknowledgments: The authors wish to thank Taren Wadley, owner and operator of Wadley Seine, for providing the data without which the project could not have happened, and for her insights into Florida's seining industry. We also wish to thank two anonymous reviewers for their helpful feedback that helped to improve the manuscript.

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

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water

Article

Migration of Non-Native Predator Asp (*Leuciscus aspius*) from a Reservoir Poses a Potential Threat to Native Species in Tributaries

Nikola Pfauserová [1,*], Ondřej Slavík [1], Pavel Horký [1], Jitka Kolářová [2] and Tomáš Randák [2]

[1] Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6, 165 21 Suchdol, Czech Republic; oslavik@af.czu.cz (O.S.); pavel.horky.r@gmail.com (P.H.)

[2] Research Institute of Fish Culture and Hydrobiology, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Zátiší 728/II, 389 25 Vodňany, Czech Republic; kolarova@frov.jcu.cz (J.K.); trandak@frov.jcu.cz (T.R.)

* Correspondence: pfauserova@af.czu.cz

Received: 26 May 2019; Accepted: 19 June 2019; Published: 25 June 2019

Abstract: The introduction of non-native species and human-altered habitats are currently the main threats to freshwater ecosystems. Due to predation and competition, biological invaders can cause extinctions and imperil the status of native species, and this phenomenon is enhanced by habitat alteration, for example, dam construction. In addition to river fragmentation caused by dam construction, the impact of non-native species migrating from reservoirs on native assemblages in their tributaries should be considered from a long-term perspective. The present study focused on asp (*Leuciscus aspius*), an artificially introduced piscivorous cyprinid that became established in the Lipno Reservoir (Czech Republic). Asp regularly occur in a tributary, the Vltava River, where twenty-five individuals were captured, radio-tagged, and tracked for five consecutive years. Asp occurrence in the tributary was highest during spring due to the upstream migration of spawning fish, and this was interconnected with a movement activity peak in March when the temperature reached 6 °C. The fish migrated a maximum distance of 31 km, and the probability of asp occurrence in the tributary was sex-dependent, with more females than males. Some individuals occupied the Vltava River not only for spawning but remained until the temperatures dropped below 10 °C. This study shows how non-native predators use reservoirs for wintering but feed and spawn in tributaries.

Keywords: fish; non-native species; biological invasion; radio telemetry; migration; sex-dependent; homing

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1. Introduction

The introduction of non-native species (NNS) often ensues as a result of anthropogenic disturbance and is one of the main threats to the biodiversity of freshwater ecosystems [1–3]. Biological invaders can cause extinctions and imperil native species due to predation, competition for resources, hybridization, disease transmission, and habitat degradation and alteration [4–6]. Overall, the rate of introductions and the patterns and threats associated with biotic invaders reflect the patterns of human activities, all of which have increased substantially in recent decades. Human population growth and movement, global trade, and environmental alteration have together created great opportunities for unintentional introductions [7,8]. Moreover, anthropogenic non-native fish introductions are often driven by economic benefits and often occur alongside the globalization of fish culture based on introduced species [5,9].

Trans- and intracontinental fish introductions have a long history and are driven by several motivations, including aquaculture enhancement, improvement of wild stocks, ornamental purposes, biomanipulation, and sport fishing [2]. Simply stated, an NNS is a species that is introduced intentionally or accidentally or acts as an independent invader that must disperse across barriers naturally or via indirect human action. The term "invasive species" was coined later and can be defined as an NNS with significant risk attached to its introduction [5,10].

The escape of aquaculture species and their establishment in wild local ecosystems may become a significant issue, especially in freshwater ecosystems with relatively high endemism [11–13]. Fish NNS have been introduced as aquaculture species in more than 85 countries worldwide and are known to have adverse ecological impacts [11]. However, aquaculture alone is not responsible for the spread of NNS globally; for example, *Gambusia* sp., which was introduced in more than 40 countries for the biocontrol of mosquito larvae, altered native fish assemblages [14,15], and this fish has had a negative impact on a wide range of invertebrates, amphibians, and other fish species [16]. In addition, wels catfish (*Silurus glanis*) (Linnaeus, 1758) was introduced throughout Europe as a valued game fish via intentional releases for angling purposes, aquaculture, and fisheries resources [17] and seemed to exert low pressure on well-established assemblages [18]. Regardless, there is a potential impact on native species that might be enhanced by anthropogenic disturbances and natural conditions. For particular environments (e.g., lakes), biological invaders have been recognized as one of the greatest threats to native species [17,19]. A wide adaptability range and a predisposition for successful invasion may be well illustrated, for example, by catfish using man-made fish passages as feeding sites during the spawning migrations of salmonids [20].

In addition, altered seasonal regimes (e.g., temperature and precipitation) caused by global climate change drive migrations of organisms to extended distribution as well as shifts in home ranges [18,21–23]. Moreover, species abundance is affected by many factors that are often associated with the loss of spawning grounds, lateral obstacles, and changes in temperature and flow regimes [24–26]. Human-made reservoirs are locations where these factors collectively affect fish assemblage (i.e., cause a reduction in species abundance [25,27] and an increase in the proportion of alien species [28]). In addition, man-made dams alter assemblage compositions, even in tributaries. For example, species that are originally riverine (e.g., asp (*Leuciscus aspius*) (Linnaeus, 1758) [29] and roach (*Rutilus rutilus*) (Linnaeus, 1758) [30]) influence the assemblages above reservoirs through their upstream migrations [31,32]. These migrations are well documented among cyprinid species, which generally migrate upstream and return after spawning [33–35]. This pattern has been observed in both rivers [34,36,37] and reservoirs [31,38]. The spawning migration occurs in spring, and movements in summer and autumn are driven by dispersal and/or refuge seeking [26].

In this study, we observed periodic fish migration between a reservoir and a tributary. These migrations were driven by reproduction and could be partly understood as trophic migration. Asp, a large, piscivorous, benthopelagic, rheophilic, and potamodromous predator [36,39], displays both stationary and migratory behavior during its lifetime. During spawning migrations, asp are able to move up to 60 km per day [36], and these fish are also known to migrate from reservoirs to tributaries for reproduction in spring. Their presence as a predator in tributaries may consequently affect the trophic equilibrium of native assemblages [31,38]. Asp exhibit high individual variability in diel movement within the area of their home ranges, and their activity differs seasonally [36,40]. Nonetheless, there is a lack of detailed evidence regarding how far asp migrate, how long individuals remain in rivers, and how or if the behaviors of males and females differ. Addressing these questions is important for precisely predicting their impact on native assemblages.

In this study, 25 asp individuals were captured, radio-tagged, and studied over a 5-year period. Their movement patterns within the Lipno Reservoir and its tributary, the Vltava River, were monitored by radio telemetry tracking. Here, we provide evidence that asp, an NNS artificially introduced into the Lipno Reservoir, have become successfully established and reproduce periodically in the main tributary. The findings of this study contribute to the literature regarding the consequences of riverine system fragmentation (i.e., reservoir construction) on fish migratory behavior and raises questions regarding the spread of fish to non-native ranges.

2. Materials and Methods

2.1. Study Area

This study was performed in the headwaters of the Vltava River, which flows into the Lipno Reservoir, Šumava National Park, the Czech Republic (Figure 1). The primary study area included approx. 30 km of the main free-flowing channel of the Vltava River and the first 15 km of the Lipno Reservoir. The study area was delimited upstream by the occurrence of asp in the Vltava River and downstream by the excessive depth of the reservoir. Regular attempts to track the fish outside the primary study area were conducted; however, the success was very low due to the limited range of the radio telemetry transmitters at depths greater than 5 m. The Vltava River headwaters consist of mountainous (approx. 730–756 m above sea level) and oligotrophic streams with a predominantly pristine morphology. The main channel gradient within the study area is low, where the river meets the reservoir the gradient is only 0.4 ‰. The Lipno Reservoir represents a typical heavily modified waterbody (surface area 46.5 km^2; length 42 km, maximum depth 25 m; maximum width 5 km) with multifunctional uses, including hydropower, flood protection, flow augmentation, and recreational activities, such as angling and sailing.

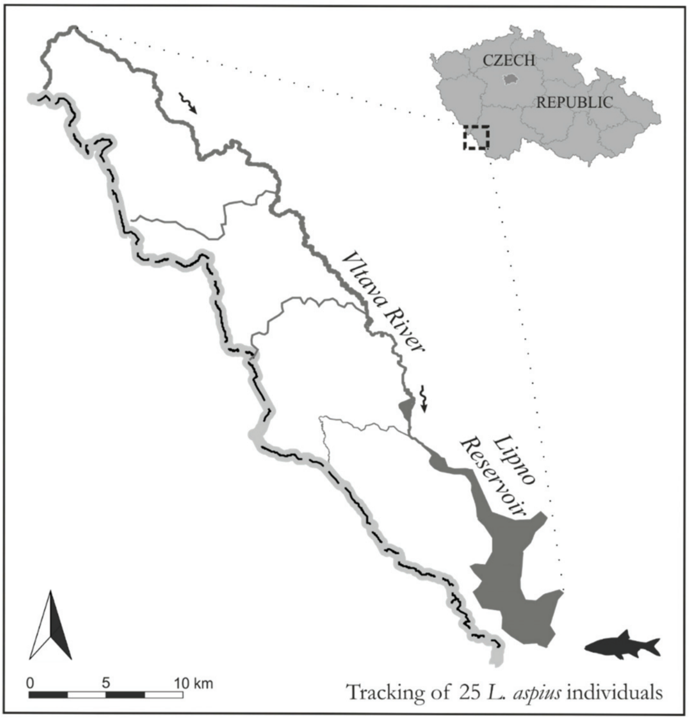

Figure 1. A map of the study area—the Lipno Reservoir and its tributary the Vltava River (Czech Republic).

2.2. Fish Origin and Tagging

In total, the 25 *L. aspius* individuals that were caught by electrofishing (650 V, 4 A, pulsed D.C.) in the Vltava River during two consecutive spring spawning seasons (2014–2015) were implanted with radio telemetry tags. The fish were anaesthetized with 2-phenoxy-ethanol (0.2 mL.L^{-1}), measured (standard length, LS; mean 517 mm, range 385–680 mm), and weighed (body size; mean 2217 g, range 1100–5620 g). Radio transmitters were implanted into the fish body cavities through a midventral incision that was closed with three separate stitches using sterile braided absorbable sutures (Coated Vicryl®, Ethicon Inc., Somerville, NJ, USA). The sex of each individual was determined during a surgical procedure. The mass of the transmitter never exceeded 2% of the body mass of the fish [41]. Two types of transmitters (Lotek Engineering, Inc., Newmarket, ON, Canada) were used in our study. Eleven *L. aspius* were tagged with MCFT2-3FM transmitters equipped with temperature sensors (operational life 1432 days), and fourteen *L. aspius* were tagged with MCFT-3L transmitters (operational life 1686 days). Prior to release in a location close to the site of capture, the fish were held in cages for approx. 30 min until they had recovered their body balance and showed spontaneous swimming activity.

2.3. Monitoring Procedures

The fish were monitored over five consecutive years from May 2014 until December 2018. The primary study area was tracked by boat once every 14 days on average. During the spring spawning migrations from February to April [36], the fish's longitudinal movements were fast (i.e., over 1 km per day upstream), and they were tracked more frequently (i.e., weekly at a minimum); during winter, the entire length of the study site was tracked at least once per month (fish were tracked by walking in the harsh conditions of ice and snow cover, which prevented fast and efficient tracking). The tracking equipment included a radio receiver (Lotek SRX_600; Lotek Engineering Inc., Newmarket, ON, Canada) and a three-element Yagi antenna. The fish's positions in the river network were recorded and stored in a GPS (GPS map 76S, Garmin LTD., Olathe, KS, USA). The temperature data from the sensor transmitters were stored automatically and subsequently downloaded from the receiver.

All of the experimental procedures complied with valid legislative regulations (Law no. 246/1992, §19, art. 1, letter c).

2.4. Habitat Measurements

The water flow (mean 4.5 m^3 s^{-1}; range 1.163–46.8 m^3 s^{-1}) was automatically recorded daily at a gauging station located within the study stretch above the reservoir. The water temperature (°C) was obtained from the sensor transmitters and was used to show the threshold when the asp entered and left the Vltava River.

2.5. Data Analyses

The position of each fish was assigned a binary variable value for 'occurrence in the Vltava River', where a value of '1' indicated occurrence in the Vltava River, and a value of '0' indicated occurrence in the Lipno Reservoir. The distance (m) between the locations of a fish for two successive tracking occasions was used as a proxy of 'movement activity'. The fish position and movement data were analyzed using Map Source version 5.3 (GPS map 76S, Garmin LTD., Olathe, KS, USA). The 'number of detections' was expressed as the number of successful tracking occasions and was counted for every individual fish. The temperature sensor transmitters (range from −6 to 34 °C; 50 stepwise temperature values; i.e., accuracy to 0.8 °C) allowed individual *L. aspius* temperature data to be saved automatically every 5 s. Every fish was assigned one mean temperature value for every tracking occasion.

2.6. Statistical Analysis

Statistical analyses were performed using the SAS software package (SAS Institute Inc., Cary, NC, USA, version 9.4, www.sas.com). When needed, the data were log$_{10}$ transformed to meet normality requirements. The asp movement activity and number of detections were analyzed using mixed models with random factors (PROC MIXED with a normal distribution for movement activity; PROC GLIMMIX with a Poisson distribution for the number of positions). Mixed models are a generalization of the standard models used (e.g., in the GLM procedure), with the generalization being that the data are permitted to exhibit correlation and nonconstant variability. This is an approach to cope with repeated measures experiments using human or animals as subjects, whereby subjects are declared random because they are selected from the larger population for which generalizations are sought [42]. Therefore, individual fish were used as random factors in the present study. More detailed information about the mixed model can be found elsewhere [43,44].

The significance of each explanatory variable (i.e., month and sex) was assessed using the F test, and least-squares means (LSM; henceforth referred to as 'adjusted means') were subsequently computed. Differences between the class variables were tested with the t test. We used Tukey–Kramer adjustment for multiple comparisons, and degrees of freedom were calculated using the Kenward–Roger method [45].

The data regarding the binary variable 'occurrence in the Vltava River' were subjected to the χ^2 test using the generalized estimating equation (GEE) approach [46] with the GENMOD procedure with binomial distributions. The GEE approach is an extension of generalized linear models and provides a semi-parametric approach to longitudinal data analysis. In this study, the GENMOD procedure was designed to estimate the probability of occurrence in the Vltava River (i.e., probability equal to 1) versus occurrence in the Lipno Reservoir (i.e., probability equal to 0) in relation to the season and sex of the tagged fish.

3. Results

Nine of the twenty-five tagged fish were never successfully detected after release. These fish presumably migrated downstream to the reservoir after tagging, as was shown for the other tagged fish, and their fate is unknown. The remaining sixteen fish that were used for the further analyses were detected during, on average, 13 tracking occasions (range 1–46 detections per individual; Table 1), and their mean movement activity was 2653 m (range 3–31,169 m) between two successive tracking intervals.

Table 1. Tagged asp individuals and their basic characteristics, including the number of detections and number of returns to the Vltava River for spawning.

Fish ID	Sex	Standard Length (mm)	Body Size (g)	Number of Detections	Number of Returns
3	male	480	1635	4	2
6	female	680	5620	8	0
7	male	560	2570	0	0
8	male	650	3990	4	1
11	male	510	1975	4	2
12	male	610	3340	8	0
13	male	660	4630	0	0
16	male	540	2240	0	0
23	female	460	1745	0	0
24	male	575	2675	6	2
32	female	455	1255	13	2
36	male	480	1930	0	0
37	male	450	1100	24	2
39	male	450	1350	18	4
57	female	580	2800	46	4
59	female	470	1565	26	3
60	male	385	1205	18	4
63	female	465	1360	24	2
140	male	530	2255	1	0
141	male	465	1540	0	0
142	male	530	2160	0	0
146	male	470	1535	0	0
150	male	465	1470	1	0
151	male	480	1540	1	0
152	male	520	1935	0	0

The occurrence of asp in the Vltava River was primarily related to spawning. This finding was supported by the fact that the probability of occurrence in the Vltava River was highest during spring ($\chi^2 = 83.93$, d.f. = 3, $p < 0.0001$; Figure 2) and that the peak of movement activity occurred in March ($F_{6,81.6} = 3.62$, $p < 0.0031$; Figure 3). In general, asp entered the Vltava River during the second half of March when the temperatures exceeded 6 °C and remained there for several weeks for spawning. During this time, large shoals of asp containing hundreds of individuals were visually observed and concentrated at the spawning sites within 4 km of the Vltava River. Homing of tagged individuals to these spawning sites during consecutive years was detected to within 1 m of the position in the previous year (mean 919 m, range 1–2600 m).

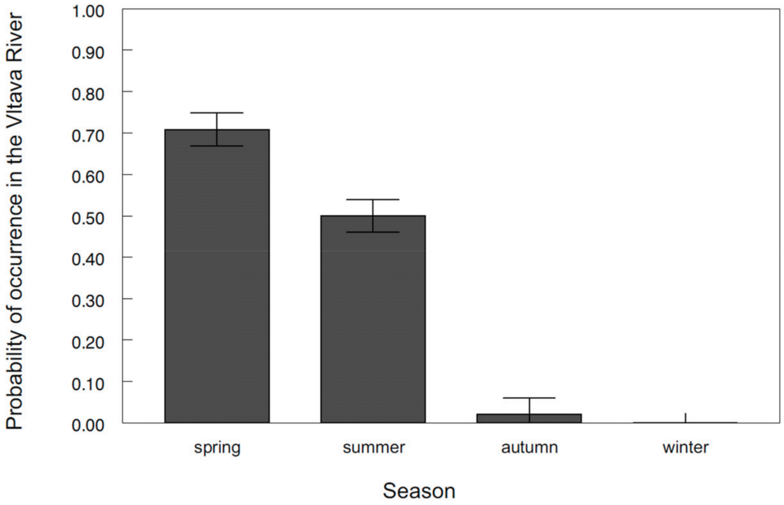

Figure 2. Probability of asp occurrence in the Vltava River across seasons. The values are the means ± the standard error (S.E.).

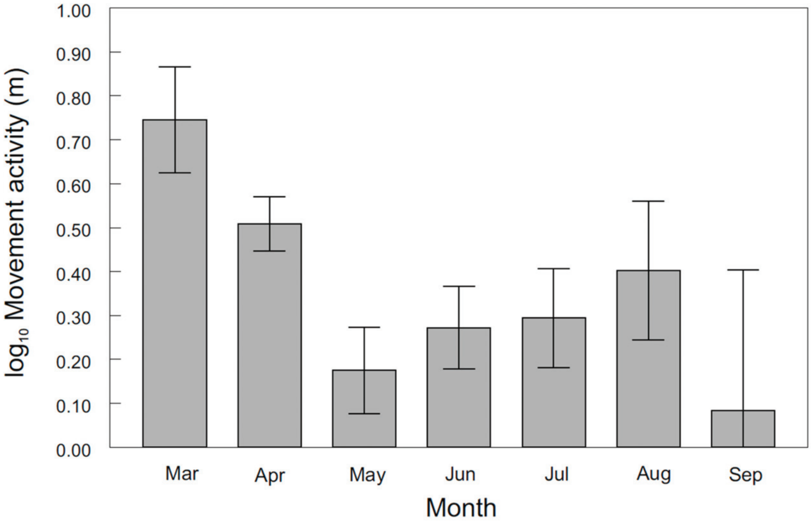

Figure 3. Asp movement activity in the Vltava River across months. The values are the means ± the standard error (S.E.).

The return of tagged individuals from the Lipno Reservoir to the Vltava River was detected, on average, during two spring spawning seasons (range from 0 to 4 returns). The probability that fish would return to the Vltava River was higher for females than for males ($\chi^2 = 6.49$, d.f. $= 1$, $p < 0.0109$; Figure 4A). Females were also detected more often than were males ($F_{1,16.24} = 5.81$, $p < 0.0281$; Figure 4B), suggesting that the females generally stayed in the shallower sections of the reservoir that were closer to the tributary where they could be easily detected. The individuals that remained in the Vltava River during summer and early autumn stayed in the downstream sections near the intake into the reservoir. All tagged fish returned to the reservoir for wintering when the temperatures in the river dropped below 10 °C.

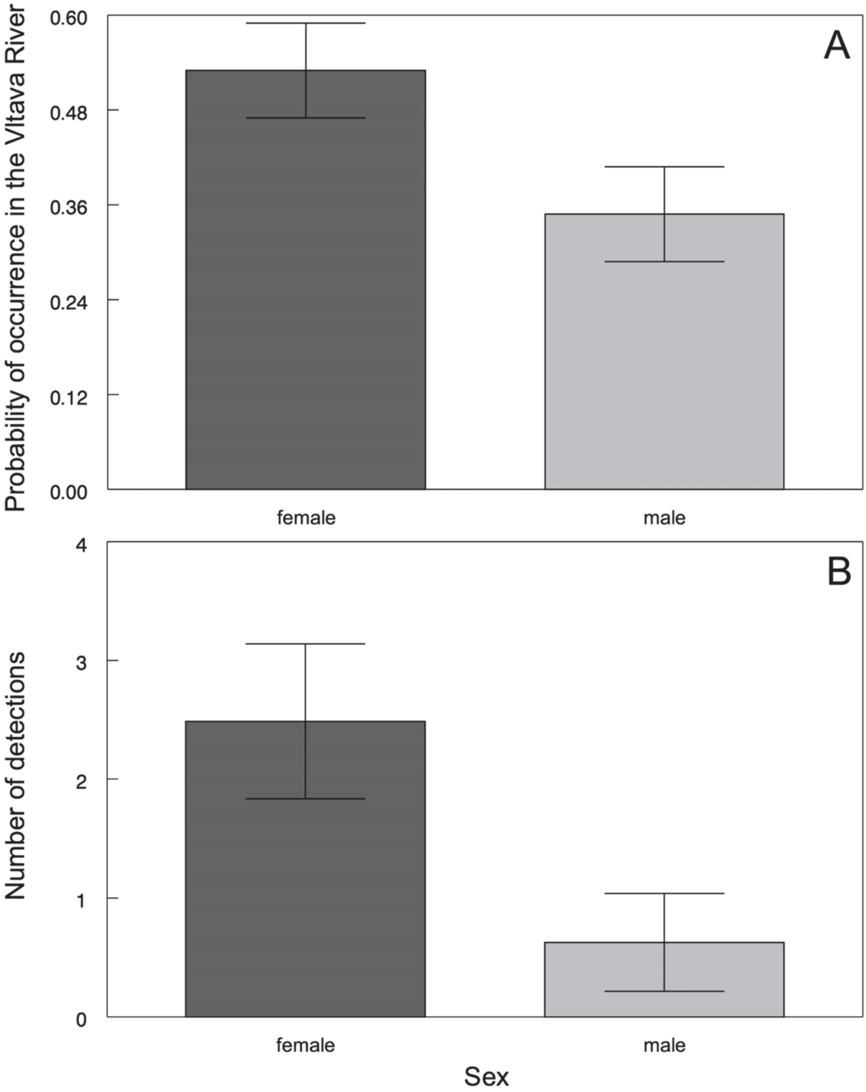

Figure 4. Sexually determined differences in the probability of asp occurrence in the Vltava River (**A**) and the number of detections (**B**). The values are the means ± the standard error (S.E.).

4. Discussion

This study shows that asp are regularly found in the Vltava River, a tributary of the Lipno Reservoir, during their upstream migrations, as driven by spawning and foraging habitat requirements. We tracked asp individuals for four consecutive spawning seasons and detected their seasonal movements. Asp, which are obligatory tributary spawners, reproduce once per year [31], and their migratory behavior peaks twice (i.e., in spring due to spawning and in autumn due to wintering migrations) [40]. In early spring, asp begin the overall spawning migration period as they migrate upstream and are followed by other cyprinid species. After spawning, asp return quickly to the reservoir, and not even juveniles remain in the river for a long time [31]. However, according to our results, some individuals do not return immediately and may compete for resources with native species in the tributary. Because the presence of predators in a river affects communities in both positive and negative ways [4], we suggest a possible effect of asp presence. The negative impact of non-native predators on fish assemblage compositions can be illustrated by decreases in species abundance [47] (e.g., the decline in haplochromines in African lakes caused predominantly by Nile perch (*Lates niloticus*) (Linnaeus, 1758) introduction) [48]). Asp is known to coexist with species such as pikeperch (*Sander lucioperca*) (Linnaeus, 1758), with reduced negative competitive interactions and utilization of different prey resources [49]. Regardless, there are no studies of the impact of asp as a trophic competitor or predator on native species when assigning food resources in an invaded river (e.g., on native salmonids).

Our data demonstrate significant differences in movement activity during the year. The Lipno Reservoir asp regularly migrate upstream to the Vltava River. Our results suggest that the peak of activity is connected to the spawning migration and that movement activity is stable during the rest of the year. After the peak in April, the activity of the fish decreased as a consequence of the stable size of the home range; after expending large amounts of energy for spawning, asp spent the oncoming months recovering, foraging, and finally migrating for wintering. Fredrich [36] distinguished two summer and winter habitats that were occupied by asp in the Elbe River; in our study, the Lipno Reservoir serves as a counterpart to the winter habitat. The activity of fish in the Elbe River is similar to the results of our study, as asp occupied their home range to forage and grow for most of the year and returned periodically to their spawning sites. Asp occurrence in the tributary appeared to be temperature limited. Although some individuals stayed in the river for a prolonged period of time, all fish returned to the reservoir for wintering (i.e., asp entered the river when the temperature exceeded 6 °C and returned to the reservoir when the temperature finally dropped below 10 °C). These results (i.e., temperature-limited migrations) are consistent with those of previous studies [31,36,40,50]; moreover, Brodersen et al. [50] suggested that temperature, partial migrations, and trophic dynamics are closely interconnected. The partial migration of asp (i.e., not all individuals returned to the reservoir after spawning [31]) might be a stimulus for the subsequent chain reaction. Most of the partial migrations of cyprinids that have been studied have focused on wintering migrations [50,51], revealing that this migration is driven by a trade-off between predation risk and growth potential (i.e., foraging efficiency) [52–55]. Apparently, migrations are tightly linked to trophic dynamics, beginning at lower trophic levels (i.e., phyto- and zooplankton and consumer abundance [50]), and culminating at the other end of the food chain (i.e., fish predators). The asp partial migration (i.e., occurrence in the river after spawning for a prolonged period of time) may encroach on native assemblages in tributaries. These migrations might directly affect prey species (e.g., roach (*Rutilus rutilus*) [56]), compete with other species (e.g., non-native salmonids largely replaced indigenous galaxiid fishes in New Zealand [57]), or could alter subsidies and affect distant food webs as a cascading reaction (e.g., the introduction of rainbow trout (*Oncorhynchus mykiss*) (Walbaum, 1792) gradually depressed riparian spider abundance in Japan [58]).

Reproductive homing is a well-studied phenomenon that is known to confer advantages with regard to reproductive success [59] and is well documented in salmonid [60] and cyprinid [30,37,61,62] species. Our results suggest that asp exhibited strong fidelity to spawning sites concentrated within 4 km of the Vltava River, and differences with respect to sex were observed. The probability of occurrence of females was higher in the tributary, and females were detected more often than were males. These results may be interpreted as a difference in preferred habitats based on sex. Detection is dependent on the section of the reservoir that an individual inhabits; in the upper, shallower part of the reservoir, fish are likely to be detected, whereas in deeper parts, they are not. Another sex-dependent phenomenon was previously observed in asp, as the amount of time spent at spawning sites differed between the sexes (i.e., males arrived at the spawning grounds earlier and departed later than did females) [31,38]. According to our results, female asp returned to the spawning grounds more often and were observed to retrace their migration more regularly than were males. Even in disturbed environments (e.g., near dams or weirs), the traditional upstream spawning migration behavior may continue; namely, fish populations may possibly utilize historical spawning sites (i.e., reproductive homing) or establish new spots to reproduce [63,64]. However, not all species are able to adapt, and for some, dam construction may be lethal (e.g., the extinction of *Coreius heterodon* (Bleeker, 1864) and *Rhinogobius typus* (Bleeker, 1871) in the upper parts of the dammed Hanjiang River in China [63]).

In conclusion, this study shows that anthropomorphically altered habitats, particularly dams, impose threats onto native communities, as illustrated by the spread of non-native species. The introduced fish predator, asp, utilized the reservoir as a wintering refuge and the tributary for a significant part of the year as a spawning habitat and foraging site, where allocated food resources may affect native species due to trophic competition or predation.

5. Conclusions

One of the threats to freshwater biodiversity is the introduction of non-native species in anthropologically altered habitats [2]. Such examples are dams that interrupt stream continuity, affect the structure of fish assemblages [27], and introduce species with a natural occurrence that does not match the location of the dam. The evidence from this study suggests that the species established in the dam undertake upstream migration to tributaries for spawning and consequently create a competitive environment for indigenous species in terms of predation or trophic competition. This phenomenon was illustrated by the predatory fish asp, which exploit tributary resources from spring to summer and utilize the dam as a wintering habitat. Because asp exhibit apparent site fidelity and regular migration between dams and tributaries, it is appropriate to consider the influence of these migrating fish as a significant manifestation of biological invasion.

Author Contributions: Conceptualization: O.S.; methodology: O.S. and P.H.; validation: N.P., O.S., and P.H.; formal analysis: P.H.; investigation: O.S. and J.K.; data curation: N.P.; writing—original draft preparation: N.P.; writing—review and editing: O.S., J.K., and T.R.

Funding: This work was supported by the European Regional Development Fund Project "Centre for the investigation of synthesis and transformation of nutritional substances in the food chain in interaction with potentially harmful substances of anthropogenic origin: comprehensive assessment of soil contamination risks for the quality of agricultural products" (No. CZ.02.1.01/0.0/0.0/16_019/0000845).

Acknowledgments: We thank Jan Turek, Vladimír Žlábek, Jan Lepič, and Roman Grabic for support with the field work.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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water

Article

Identification of the Invasive Form of *Corbicula* Clams in Ireland

Rory Sheehan [1,2,*], Emilie Etoundi [3], Dan Minchin [4], Karine Van Doninck [3] and Frances Lucy [1]

[1] Centre for Environmental Research Innovation and Sustainability (CERIS), Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland
[2] Monaghan County Council, The Glen, Monaghan Town, Co. Monaghan, H18 YT50, Ireland
[3] Department of Biology, Laboratory of Evolutionary Genetics and Ecology (LEGE), University of Namur, Namur, 5000, Belgium
[3] Marine Research Institute, Klaipėda University, University Avenue 17, Klaipėda, LT-92294, Lithuania
* Correspondence: rsheehan@monaghancoco.ie; Tel.: +353-47-30500

Received: 30 June 2019; Accepted: 5 August 2019; Published: 10 August 2019

Abstract: The basket clam genus, *Corbicula*, commonly known as the Asian clam, has become one of the most internationally high-profile and widespread aquatic invasive species. This genus is now considered to comprise a polymorphic species complex. The international invasion of *Corbicula* is characterised by four lineages, each fixed for one morphotype, genotype and haplotype combination: the American form (A) and European round form (R), the American form (C) and European saddle from (S), American form B, form round light colour (Rlc) and an intermediate between forms R and S known as Int. We investigated the genetic and morphometric makeup of each Irish population in order to establish which invasive lineages were present so as to identify the number of introductions to Ireland. A combination of morphometric, mitochondrial cytochrome oxidase subunit I (mtCOI) gene analysis and microsatellite markers were used to determine the invasive form at each Irish site. All Irish *Corbicula* samples conformed morphometrically to the invasive form A/R. All mtCOI sequences retrieved for 25 Irish individuals were identical to the international A/R form, while microsatellite markers again showed a common clustering with the international A/R forms of *Corbicula*. The combined approach of morphometries, total genomic DNA and microsatellite markers indicate only one form of *Corbicula* invaded Ireland; the international A/R form.

Keywords: Veneroida; biological invasion; Ireland; mtCOI; microsatellites; morphology

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1. Introduction

Corbicula is a freshwater bivalve genus, commonly known as basket clams. *Corbicula* clams are native to Asia, Australia, Middle East and Africa, and have become invasive in Europe and the Americas [1–4]. Interestingly, invasive *Corbicula* clams, as well as most native lineages, reproduce through a peculiar and rare asexual reproductive mode, i.e., androgenesis or strict paternal inheritance. Androgenesis in *Corbicula* occurs through the expulsion of maternal nuclear chromosomes after obligatory fertilization of an oocyte by an unreduced biflagellate androgenetic spermatozoa [5–7].

In North America, the first record of an invasive lineage of *Corbicula* was made in 1924 in British Columbia [8], with subsequent spread through many parts of the continent (reviewed in [9]). In addition, *Corbicula* has spread across much of Central and South America during the 1970s and 1980s [3,8–11]. The first records of *Corbicula* in Europe were in 1980 from the River Dordogne in France and the Tagus estuary in Portugal [1]. Subsequently, *Corbicula* has become widespread through much of Western Europe [12] and England [13].

In Ireland, *Corbicula* clams were first detected in the River Barrow in 2010 [14], where an extremely high density of 17,872 individuals/m^2 recorded at one sampling site in 2012 [15]. A subsequent investigation revealed its presence in the River Nore [16], which shares a common estuary with the River Barrow. *Corbicula* was also discovered in the Shannon system, Ireland's major navigable waterway, at Carrick-on-Shannon [17], in Lough Derg and in the Shannon River section above Portumna upstream of Lough Derg [18] and at Lanesborough, upstream of Lough Ree (R. Sheehan, Pers Obs) (Figure 1).

Invasive lineages of *Corbicula* clams have previously been described as belonging to two species, *Corbicula fluminea* and *Corbicula fluminalis* but are now assigned to forms [11] based on molecular markers because of the confused taxonomy of the genus and invasive lineages. A morphological taxonomic identification of *Corbicula* lineages has proved problematic as a wide range of shell morphology exists across invaded countries and within their native Asian range [19–24]. The use of mitochondrial analysis and the development of microsatellite markers for invasive *Corbicula* lineages in Europe, has increased our understanding of both invasive and native populations worldwide [11,24].

Internationally only four lineages of *Corbicula* have become invasive, with each being fixed for one morphotype, genotype and haplotype combination [11]: form A/R, form C/S, form B and form Rlc [11]. Form A/R is found in North and South America as well as in Europe, being the most widely distributed invasive lineage [8,11,21,25]. This form's mtCOI haplotype (FW5) has also been recorded in *C. leana* individuals, an androgenetic Japanese lineage [11]. Form R refers to the strict European populations, whereas form A strictly describes the American forms, but both have the same COI haplotype and microsatellite genotype. Form C/S (haplotype FW17) has been recorded both in South America (form C) and in Europe (form S) and appears related to *C. fluminalis* [11,21]. Form B, showing the haplotype FW1, is found in North and South America [21]. Finally, form Rlc is found only in Europe, where its distribution appears restricted to the Rhone basin in France [23,26]. Form Rlc's mitochondrial COI haplotype FW4 is closely related to that of Form B, as they differ only at one nucleotide site but are distinct at the microsatellite genotype [11].

Within invasive populations of *Corbicula*, further genetic and morphometric complexities can arise from these cryptic hybridizations between sympatric, distinct androgenetic lineages (reviewed in [27]. Indeed, when androgenetic reproduction occurs between two different lineages, the offspring are associated with the nuclear genome and thus, the morphology of the paternal lineage combined to the mitochondrial genome of a distinct maternal lineage, as mitochondria are maternally inherited. This association within one individual of the nuclear genetic information of one lineage and the mitochondrial genetic information of another is called a cytonuclear mismatch [28] and leads to cryptic hybrids. From time to time, nuclear chromosomes can be inadvertently retained within the zygote [6] leading to hybrid individuals with intermediate phenotypes between distinct forms. Such cytonuclear mismatch and hybrid individuals have been widely documented in the *Corbicula* invasive range [11,19,21,23,28,29]. A recent form D was described in North America [30] but it could be a hybrid between form A/R and form B, as the morphotype is intermediate between these two forms, the COI is from form A/R [23] and the 28S is found in form B [28], as well as in Asia [31]. Moreover, the environment can influence the phenotype, thus complicating the determination of an invasive form when it is based solely on morphotype. For example, differing *Corbicula* morphotypes in two estuaries in Portugal, possess the same haplotype FW5; their morphological differences are likely due to differing environmental factors in each estuary [22].

The phenomena of cytonuclear mismatches and cryptic hybrids in *Corbicula*, highlight the importance of combining molecular genetic methods (mitochondrial and nuclear markers) with morphological identification as a dual approach to accurately characterize invasive populations [23].

The pathways and mode by which *Corbicula* was introduced and has subsequently spread within Ireland are poorly understood [32], with recreational water activity being one possible explanation [33]. It is unclear whether only one discrete introduction occurred, with populations at other sites resulting from secondary spread or alternatively, if a number of separate introductions took place from outside Ireland to each site. Determining distinct morphotypes, genotypes and haplotypes in the various Irish *Corbicula* populations would support a hypothesis that a number of discrete introductions occurred.

The high level of phenotypic plasticity present within the invasive forms of *Corbicula* [22] and the novel reproductive strategies leading to possible cytonuclear mismatches and cryptic hybrids [23] mean any one method on its own from morphology, mitochondrial and microsatellites is likely to provide an incorrect identification of the invasive from tested. The cross-lineage genetic mixing and recent spread of androgenetic *Corbicula* lineages [27] and the presence of mismatches between mitochondrial and nuclear markers [11,23,28] further demonstrates the need for a multimethod approach.

Aims

This study aimed to demonstrate the effectiveness of combined morphological and genetic approaches (both mitochondrial and nuclear markers) in resolving invasive bivalve identification. The research also assessed the methodology as an invasion-source tool by using *Corbicula* specimens from separate invaded sites in Ireland and Belgium.

2. Materials and Methods

2.1. Specimen Collection

Five sites in Ireland with known populations at the time this study was conducted, were sampled to collect individuals for genetic and morphological study (Figure 1; Table 1). One site in Belgium, in the Meuse River at Petit Lanaye (Table 1) was sampled on the 23/10/2013 to collect individuals solely for morphological comparison with individuals from the Irish sites.

A range of standard sampling methods were used to collect *Corbicula* specimens, depending on water depth and site location, including SCUBA diving, benthic dredge, grab sampler and kick-net. Samples in Ireland were collected from five sites between 2011 and 2013; the Shannon River at Carrick-on-Shannon, Lanesborough, Portumna and Lough Derg, as well as the River Barrow, the River Nore and the Meuse River (Table 1). The Shannon River basin sites were collected from a range of river and lake settings with the St. Mullin's and Red House sites [16] being riverine.

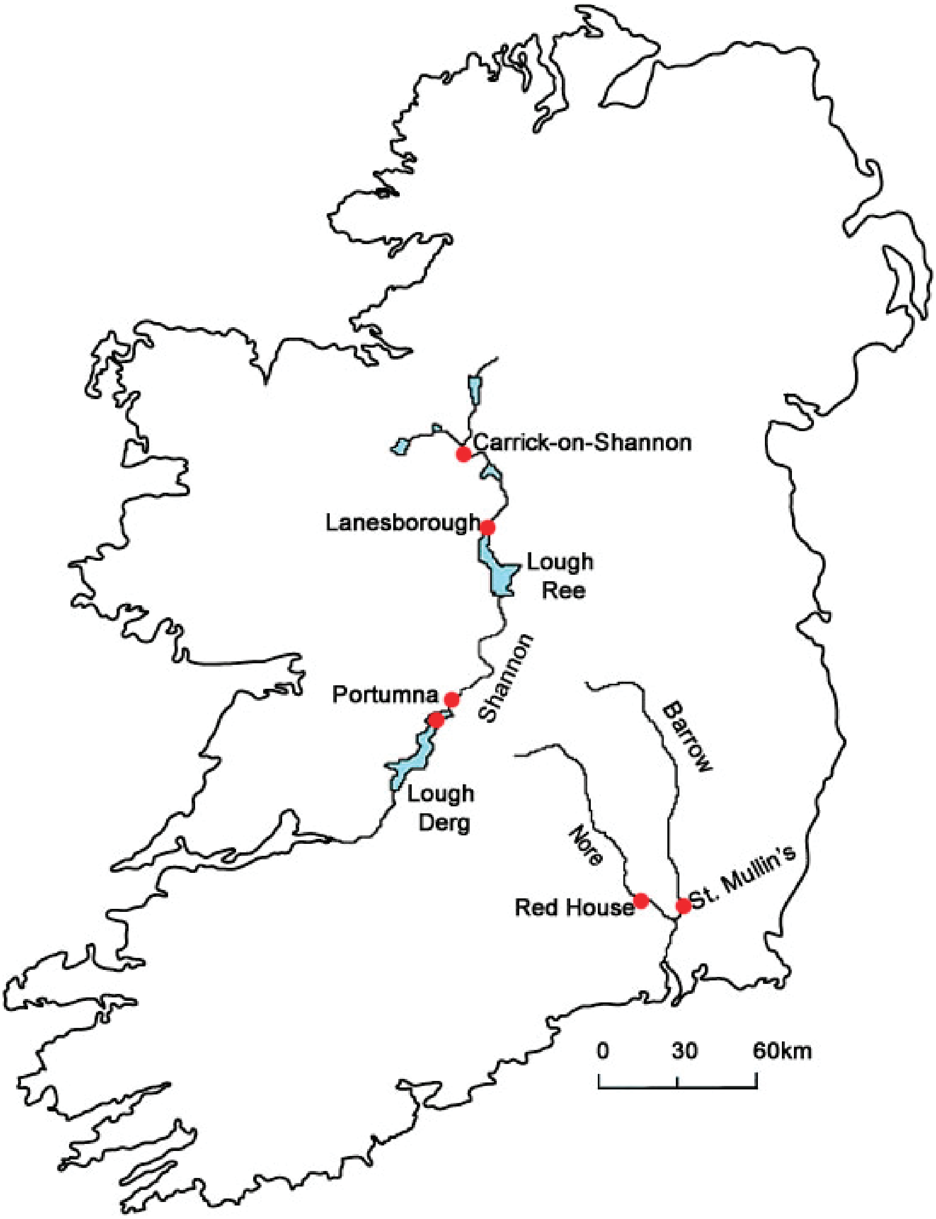
Carrick-on-Shannon
Lanesborough
Lough Ree
Shannon
Portumna
Lough Derg
Barrow
Nore
Red House
St. Mullin's
0
30
60km

Figure 1. Irish rivers containing *Corbicula* specimens, previously described as *C. fluminea*, sampled during this study, River Nore, Barrow and Shannon. Sampling sites Carrick-on-Shannon, Lanesborough, Portumna, Lough Derg, St. Mullin's and Red House.

Table 1. Sampling sites and collection methods of *Corbicula* clams for DNA and morphometric analysis.

Site	No	Site	Sampling Method	Grid Reference
Shannon (Carrick-on-Shannon)	1	1 km above town	Diving	53.951402, −8.109267
Shannon (Lanesborough)	2	Below bridge	Grab + dredge	53.669457, −7.998963
Shannon (Portumna)	3	Below bridge	Grab + dredge	53.091139, −8.195643
Lough Derg	4	Upper lake	Grab + dredge	52.928985, −8.280803
River Barrow	5	St Mullin's	Diving	52.487025, −6.926753
River Nore	6	Redhouse	Kick-net	52.469403, −7.044622
River Meuse	7	Meuse River, Petit Lanaye, Belgium	Kick-net	50.810898, −5.692482

2.2. Morphological Analysis

A morphological examination of each individual from Ireland (N = 84) and Belgium (N = 51) was carried out, separating them into the morphotypes described in Europe, R, S, Rlc and also, an intermediate morphotype (Int) [19], in order to determine the form of *Corbicula* according to the morphotype descriptions in [21] and [26]. Form R, (round form) has a shell with well pronounced concentric ridges, is round and broad and generally attains a larger size than form S. Its internal colour is white but may contain pallid purple markings [26]. Form S, (saddle form) again has concentric ridges but these are less raised then in the R form and is narrower and proportionately longer. The internal shell colour is a deep purple throughout. Form Rlc (light colour R from) is superficially similar to the R form in shape, but with a lighter surface shell colour and an off-white to yellow internal colour [26]. Form Int is similar in shape to the R form but with finer less pronounced ridges on the shell [19].

All specimens selected from Ireland and the River Meuse were measured to the nearest mm for shell height (H), length (L) and width (W) using a pair of digital calipers to determine individuals of form R, S and Intermediate (Int), Rlc was not found (Table 2). The ratio between each measurement was calculated for the Irish *Corbicula*, and compared to individuals of form R, S and Int from the River Meuse. A Principal Component Analysis (PCA) was conducted in RStudio (Version 0.98.501), on the ratios between shell length, height and width, (L/H, L/W, H/W), as described in [21].

Table 2. Numbers of individuals (forms R, S and Intermediate (Int)) used for the morphological (Ireland and Belgium), mtCOI and microsatellites (Ireland only) analyses.

Site	Described Form	Morphology	mtCOI	Microsatellites
Carrick-on-Shannon	R	11	5	9
Lanesborough	R	9	5	9
Lough Derg	R	39	5	14
River Barrow	R	10	4	9
Portumna	R	10	1	2
River Nore	R	5	5	5
Meuse River	R	10	N/A	N/A
	S	37	N/A	N/A
	Int	4	N/A	N/A

2.3. DNA Extraction

Adductor muscle tissue and foot were dissected from each of the Irish specimens for genetic analysis. The samples for the Carrick-on-Shannon, Lanesborough, River Barrow and River Nore sites were preserved in 98% ethanol and stored at ambient temperature. Samples from Lough Derg were initially preserved in methanol and transferred to ethanol after dissection.

Total genomic DNA was extracted from the adductor muscles and/or foot of 50 individual specimens (Table 2), using the DNeasy blood and tissue kit (Qiagen), according to the manufacturer's protocol. DNA extraction and microsatellite sequencing were carried out at the Laboratory of Evolutionary Genetics and Ecology, (LEGE), University of Namur, Belgium, as previously described in [11,23].

2.4. Mitochondrial COI Gene Analysis

A fragment of 710 bp of the COI gene was amplified in 25 individuals, with representatives from each Irish location. Polymerase Chain Reaction (PCR) was carried out using the universal primers LCO1490 and HCO2198 [34] following the protocol described in [23]. Amplicons were purified and sequenced with the forward universal primer HCO2198 on an automated ABI 3730XL Genetic Analyzer (Genoscreen, Lille, France). Retrieved sequences were visualized, aligned and edited using BioEdit 7.0.5.3 [35]. Corrected sequences and published sequences of the invasive lineages (FW5—Form A/R: GU721082; FW1—Form B: AF196269; FW4—Form Rlc: GU721084; FW17—Form C/S: GU721083) were added to our dataset and used to construct a haplotype median-joining network using the Network 4.6.1.2. [36].

2.5. Microsatellite Marker Analysis

Ten microsatellite markers [24] (ClA01, ClA02, ClA03, ClB03, ClB11, ClC01, ClC08, ClC12, ClE01, and ClD12) were amplified following the protocol of [24]. Microsatellite markers were read on an ABI 3130XL Genetic Analyzer with GeneScan-500 (LIZ) size standard (Applied Biosystems) and scored using GENEMAPPER (Applied Biosystems).

For each of the 50 individuals analysed, we defined a multilocus genotype (here, the unique combination of alleles for the 10 microsatellite loci). These individuals as well as 47 individuals from the European and American invasive individual's lineages previously typed (10 individuals from Form A/R, 16 from Form B, 10 from Form Rlc and 11 from Form C/S) were clustered based on their multilocus genotype using a discriminant analysis of principal components (DAPC) [37]. The DAPC analysis was performed using the package adegenet [38] implemented in R version 2.15.2 (R development core team 2008). The number of putative populations was first determined using the k-means clustering algorithm [39] for K = 1 to K = 11; 11 being the number of sampled populations added in the analysis. The appropriate number of clusters was defined using the Bayesian Information Criterion (BIC); the value at which the BIC distribution forms an elbow indicating the best clustering (Supplementary Material S1). The relationships between the BIC-defined clusters were then inferred. Six principal components and one discriminant function (98.9% of the total variance) were retained to represent the majority of the variability contained in the dataset.

3. Results

3.1. Morphology

From the morphological characterization, all Irish *Corbicula* clams were visually determined as the European form R as described in [19,23] with a round deep shell that may range from externally dark to golden, heavy ridges and a generally white interior (Figure 2).

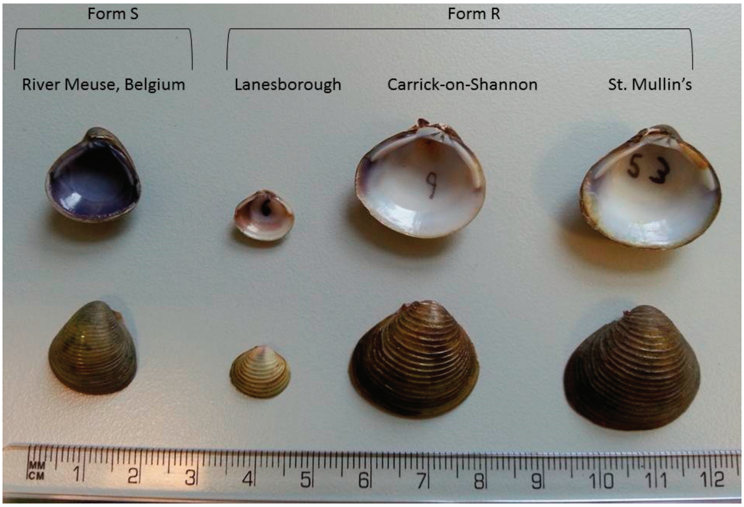

Figure 2. Examples of *Corbicula* form R from the River Shannon at Lanesborough (juvenile specimen) and Carrick-on-Shannon and the River Barrow at St. Mullin's. Also shown is an example of Form S from the River Meuse at Maastricht.

The PCA analysis (Figure 3) of shell height vs length shows the Irish *Corbicula* grouping together with the Belgium form R and the international intermediate (Int) form. The Int form displayed an intermediate morphology between R and S, with the round body shape of R and the closely spaced shell ridges and purple interior coloring of S. The Irish individuals were different from the Belgian form S. No Irish individuals had a shell height/length ratio consistent with form S.

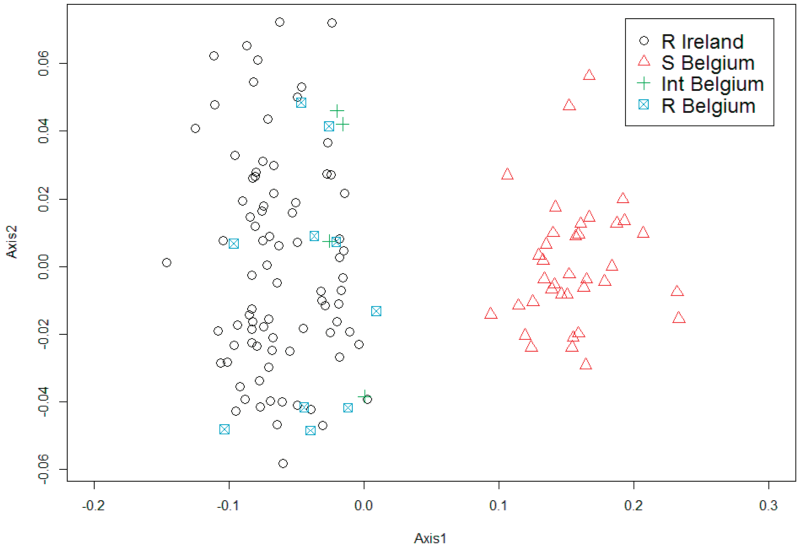

Figure 3. A principal component analysis (PCA) of the ratio between shell measurements for *Corbicula* form R from all Irish sites and forms R, S and individuals with intermediate morphotypes between form R and S (Int), from the River Meuse in Belgium.

3.2. COI Sequence

A 710 bp length fragment of the mitochondrial COI was successfully amplified for 24 of the Irish specimens (Table 2). All the COI sequences retrieved were identical to FW5, the mtCOI haplotype of form A/R distributed in Europe and Americas. All individuals sampled from the Irish populations possessed the same haplotype. No haplotypes from other invasive lineages (Figure 4) were detected from the Irish sites.

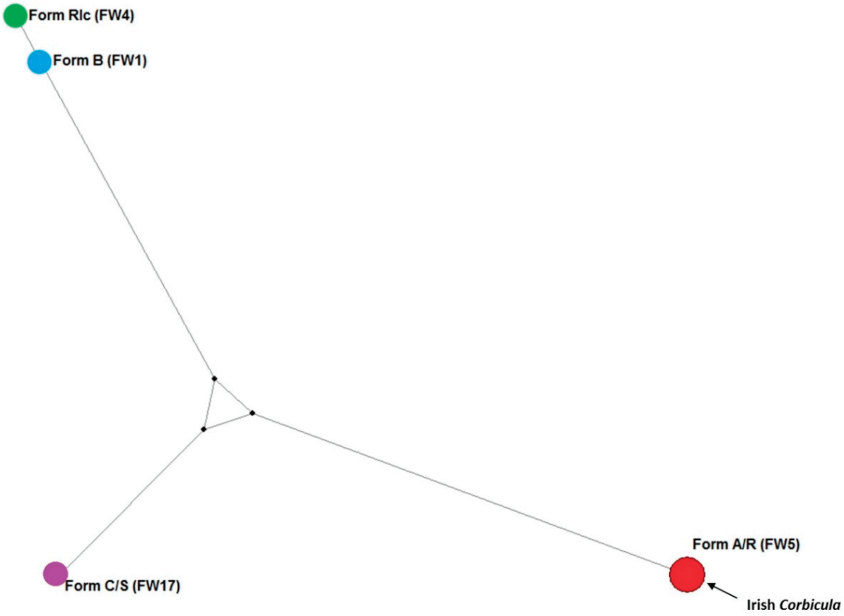

Figure 4. Haplotypic diversity and relationships in Invasive *Corbicula* clams inferred through median-joining Network. The European invasive haplotypes FW1 (Form B), FW4 (Form Rlc), FW17 (Form C/S), FW5 (Form A/R; Irish individuals) are plotted. Branch length was proportional to the number of mutations between the haplotypes and nodes proportional to the haplotype frequency.

3.3. Microsatellite Markers

Genetic diversity and genetic relationships in invasive *Corbicula* clams estimated through a Discriminant Analysis of Principal Components (Figure 5) based on multilocus genotypes from microsatellite marker amplifications show a common clustering between all Irish samples and the American and European invasive form A/R, suggesting that Irish *Corbicula* belongs to this same lineage.

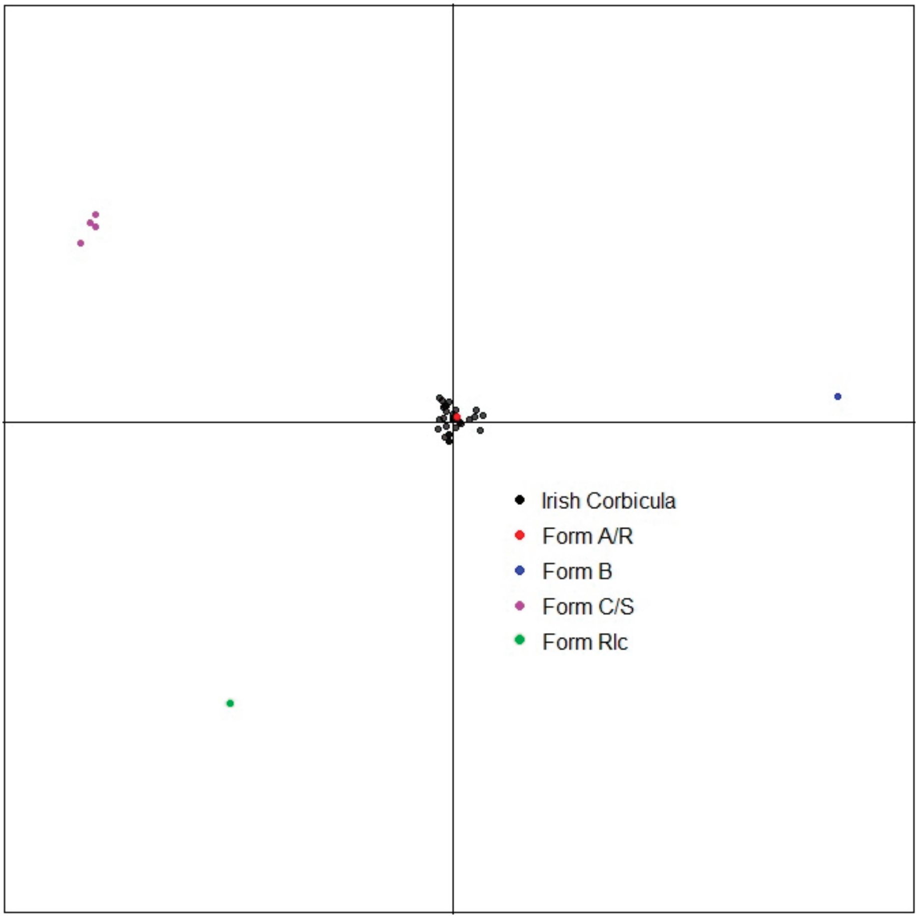

Figure 5. Genetic diversity and genetic relationships in invasive *Corbicula* clams estimated through a Discriminant Analysis of Principal Components based on multilocus genotypes. Only axes 1 and 2 are represented. One dot represents one distinct multilocus genotype; individuals showing the exact same genotype are, therefore, pictured under the same dot.

4. Discussion

Here, we used a multi-method approach, including mitochondrial COI sequencing, microsatellite genotyping and morphological analysis, to identify the invasive *Corbicula* lineages in Ireland. Such an integrative approach was necessary as the genus *Corbicula* is characterized by haplotypes capable of displaying highly divergent phenotypes in response to differing environmental conditions or following cross-lineages mixing.

Our results showed that only the invasive form A/R, the most widespread invasive lineage, was present from all samples collected in Irish waters, as determined using a combination of (a) morphological analysis, (b) mtDNA (COI) and (c) microsatellite markers.

No clams with either a narrow and fine-ridged shell or deep purple interior, as corresponding to form S [19,40] were observed. The PCA analysis carried out on the ratio between shell length and shell height (Figure 3) supports the correct classification of these individuals a belonging to form R.

Morphologically, all clams sampled from the Irish sites conform to the invasive form A/R as described in [11,21,23,28,29], with a certain variability observed (Figure 3).

All individuals sampled from the Irish sites presented the invasive FW5 mtCOI haplotype of form A/R distributed in Europe and Americas [11,21,23,28,29]. The COI sequence data supports the results from the morphological analysis: no mismatches were observed between mtCOI and morphotype, which may be potentially caused by reproduction through androgenesis between distinct lineages [28].

All Irish sampled individuals showed a common clustering for the microsatellite data with the A/R lineage. This form has proven to be clonal and, thus, shows no genetic diversity. Indeed hundreds of individuals of this lineage, sampled at different locations from across Europe, North and South America, present the exact same multilocus genotype with no genetic diversity [11]. The genetic variability detected within Irish populations is very likely due to the poor amplification of the microsatellites (Supplementary Material S2). Initial storage conditions of some samples in methanol was likely responsible for this. It is also a possible that false positives were detected from the data.

It is unlikely that other forms of *Corbicula* exist in Ireland as extensive investigations have not revealed clams displaying differing morphological characteristics [14,16,17] (Minchin Pers Ob.) or in subsequentially discovered populations [41,42]. As the level of genetic diversity among the invasive lineages of *Corbicula* clams (reviewed in [18]) was low, it was not possible to discriminate the origin of the Irish populations. The emerging field of massive parallel sequencing (MPS) to detect single nucleotide polymorphisms (SNPs) [43] could potentially complement the approaches used in our study. Similarly, it is not possible to draw any inferences on the number of discrete introductions that may have occurred [44].

The combined methodology of mitochondrial COI sequencing, microsatellite genotyping and morphological analysis has the potential to pick apart the exact identification of genetically complex species groups for which traditional ecological methods may have overlooked complex relationships such as in *Corbicula* [27] with its highly invasive clonal A/R form. The ability to discriminate between discreet introductions to a geographic area, and secondary spread is an invaluable tool in prioritising AIS biosecurity resources [45] and can inform horizon scanning [46].

5. Conclusions

The use of a combined morphological and nuclear marker approach in resolving the identity of the invasive *Corbicula*, demonstrates that only one invasive lineage of *Corbicula* has invaded Ireland, the most prevalent and widely distributed form being A/R, a form also found across Europe and America. The extremely low genetic diversity found within this invasive lineage makes the determination of differences in population origins difficult as a result, and therefore, the number of discrete introductions of *Corbicula* to Ireland remains unknown. In order to properly inform management plans and limit impacts to ecosystem services, the identity of invasive *Corbicula* populations must be resolved. The use of a combined morphological, mitochondrial and nuclear marker approach in gleaning the identity of invasive *Corbicula*, as demonstrated in this study, provides a useful tool for achieving these goals, with the possibility for extending this approach to other invasive species.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/11/8/1652/s1, supplementary S1, supplementary S2.

Author Contributions: Conceptualization, R.S., E.E. and F.L.; Methodology, R.S., E.E., F.L., K.V.D. and D.M.; Software, R.S. and E.E.; Validation, R.S. and E.E.; Formal analysis, R.S. and E.E.; Investigation, R.S, and E.E.; Resources, F.L. and K.V.D.; Data Curation, R.S., E.E., F.L. and K.V.D.; Writing—Original Draft Preparation, R.S. and E.E.; Writing—Review and Editing, R.S., E.E., F.L., D.M. and K.V.D.; Visualization, R.S. and E.E.; Supervision, F.L. and K.V.D.; Project Administration, R.S., E.E., F.L. and K.V.D.; Funding Acquisition, F.L. and K.V.D.

Funding: This research was funded by President's bursary award, Institute of Technology, Sligo. Financial support was also provided by the COST Action no. TD1209 'Alien species: Linking Information across a European Network (ALIEN Challenge)' fund. Financial support from Inland Fisheries Ireland (IFI) is also acknowledged.

Acknowledgments: Particular thanks to Alan Cullagh, Declan Cullagh, IFI Clonmel. Fergus Lynch and all of the IFI Drumsna staff. Joe Caffrey and Michael Millane of IFI Citywest. Julie Virgo of UNamur and Helen Moran of Geomara, Clarinbridge, Co. Galway, Ireland.

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

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Article

Variation in Diet Patterns of the Invasive Top Predator *Sander lucioperca* (Linnaeus, 1758) across Portuguese Basins

Diogo Ribeiro [1,*], Christos Gkenas [1], João Gago [1,2] and Filipe Ribeiro [1]

[1] MARE, Centro de Ciências do Mar e do Ambiente, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal; cgkenas@fc.ul.pt (C.G.); joao.gago@esa.ipsantarem.pt (J.G.); fmvribeiro@gmail.com (F.R.)
[2] Escola Superior Agrária—Instituto Politécnico de Santarém, Quinta do Galinheiro—S. Pedro, 2001-904 Santarém, Portugal
* Correspondence: diogorrribeiro@gmail.com

Abstract: The introduction of non-native species is recognized as a major threat to biodiversity, particularly in freshwater ecosystems. Pikeperch *Sander lucioperca*, is a recent invader to Portugal, primarily providing commercial and angling interest. The aim of this work was to study the diet of this top predator across Portuguese basins and to evaluate its potential impact on recipient ecosystems. In total, 256 pikeperch stomachs from seven basins were examined, of which 88 (n = 34%) were empty. Pikeperch diet was dominated by *R. rutilus*, *M. salmoides* and Diptera in northern populations, while *A. alburnus*, *P. clarkii* and Atyidae were important prey in more humid highlands. Variation in diet was most strongly linked to latitude and ontogeny, with both size classes showing signs of cannibalism. The population niche breadth remained low and was accompanied by higher individual diet specialization, particularly in northern populations. Pikeperch dietary patterns denoted an opportunistic ability to use locally abundant prey in each ecosystem, and was size dependent, with larger individuals becoming more piscivores, causing a higher impact in the lotic systems. This first perspective about the pikeperch diet presents a very broad view of the feeding traits of this non-native predator across Portugal, being very important to deepen our knowledge about the impact of these introduced piscivores.

Keywords: freshwaters; pikeperch; trophic ecology; diet specialization; non-native fish

Citation: Ribeiro, D.; Gkenas, C.; Gago, J.; Ribeiro, F. Variation in Diet Patterns of the Invasive Top Predator *Sander lucioperca* (Linnaeus, 1758) across Portuguese Basins. *Water* **2021**, *13*, 2053. https://doi.org/10.3390/w13152053

Academic Editors: Frédéric Santoul, Stéphanie Boulêtreau and Jan Kubečka

Received: 31 May 2021
Accepted: 24 July 2021
Published: 28 July 2021

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1. Introduction

Freshwater ecosystems are highly imperiled worldwide due to multiple pressures that result in declines of aquatic biota [1,2]. Not surprisingly, freshwater taxa such as fish or molluscs present as being under threat at high rates. When compared with terrestrial or marine ecosystems, freshwater ecosystems are generally overlooked in biodiversity studies, although they contain higher biodiversity per area and face higher declines [3,4]. Biological invasions of non-native species within freshwater ecosystems are worrisome [1,5–8], and the introduction rate of new species has been increasing [9].

Freshwater fishes are the most introduced vertebrate group, due to aquaculture, angling and commercial fisheries and for ornamental trade purposes [10–14]. In European freshwaters, recreational fisheries are one of the main drivers of fish introduction, mostly focusing on top predatory fish, particularly in southern peninsulas [15,16]. In Portuguese freshwater ecosystems, the introduction of non-native fish (NNF) has reached one new species every two years [17,18]. From a total of 62 fish species currently existent in Portuguese watersheds, 19 are non-native and some are top predators, recently established, with potentially high deleterious impacts to fish communities and aquatic food webs [19,20]. In addition, most of the native fish communities in the Iberian Peninsula evolved without predatory fish, so the introduction of predatory fish could have strong effects on the fish community [20]. The impacts of introduced predators can be devastating, leading to local native fish species extinctions [21,22] and modifying food webs and ecosystems [23,24].

Pikeperch *Sander lucioperca* (Linnaeus, 1758) is a predatory fish native to central Europe and western Asia, that has been intentionally introduced to fresh and brackish waters in Europe, Asia and Africa [25]. This species was introduced to the Iberian Peninsula in the 1970s on Catalonian reservoirs [26] and in 1998 was recorded in mainland Portugal [27]. It has now extended its distribution to most parts of the hydrographic network of Portuguese watersheds, where it has important angling and commercial interest [28–30]. Annually, pikeperch consumes several times its own bodyweight of prey, implying an impact on native aquatic communities [31]. Despite exhibiting ontogenetic shifts in its diet, it rapidly attains piscivory during its first year of life [32,33]. The predatory behavior of pikeperch has been well studied both within their native and non-native ranges (e.g., [34–38]). However, there is little information on the trophic ecology of pikeperch in relation to novel environments, which could depict distinct impacts and effects on native fish communities.

The present study uses a spatial approach to compare the diet of *S. lucioperca* across different populations in Portugal, subjected to different environmental settings. Specifically, we aim to (i) determine the diet composition of the species, (ii) quantify spatial and size-related changes in diet and (iii) identify differences in niche breadth at the population and individual level. This information will contribute to assess the potential impact of the species in an endemic rich area, such as the Iberian Peninsula, where there is an urgent need to evaluate the impact of non-native predatory fish.

2. Materials and Methods

2.1. Field Sampling and Laboratory Procedures

Pikeperch were sampled during 2017 and 2018, from April to October in selected river basins either in lotic or lentic habitats (Figure 1) and covering a total of 11 populations across mainland Portugal. The extensive coverage area of the fish population selection is important to evaluate the geographic dietary differences across the continental area of Portugal. Additionally, in these sites there was considerable commercial fishing pressure to pikeperch which provided easy access to fishes. Although this plan allowed us to use a higher geographic coverage, the sampling period was fishermen-dependent and so it could not be standardized. The majority of the specimens used in this study were provided by fishermen that used overnight gillnets of 80–150 mm mesh size as a fishing technique. Some juveniles were also captured by standardized electrofishing (300–500 V, 1–5 A).

In the laboratory, specimens were measured (Standard Length—SL, to nearest 1 mm), weighed (Eviscerated Weight—EW, to the nearest 0.01 g) and their stomachs were dissected, labelled and preserved immediately by freezing until stomach content analysis. Prey items were examined under a binocular dissecting microscope, identified to the lowest readily recognizable taxon (species and family was achieved for fishes and crayfish; order and family for insects) and counted. Identifications followed published keys and literature [39–45]. Additionally, for prey item identification, a reference collection was created with the bony parts of prey species (otoliths, scales, pharyngeal teeth). In total, we examined the stomach contents of 256 pikeperch, ranging from 9.4 to 60.3 cm SL, of which 88 individuals (n = 34%) had empty stomachs which were discarded to avoid confounding effects in the analysis of diet structure [46].

To identify size-related diet shifts, fish were grouped into two standard length classes: I $\leq$ 25 cm; II > 25 cm and differences in the contribution of prey items to the diet of individuals in each size class were based on the stomach content analysis. The definition of this limit is related with the onset of reproduction, where all the individuals belonging to Class I (ages 0 to 3) are juvenile fish, while most of Class II are adult fish (fish older than 3 years, see [29]). Given the small sample size of some fish captures (e.g., Rio Ave), to provide sufficient power in analyses, only populations with more than 5 individuals were included. For analysis, prey items were grouped into thirteen categories: *Alburnus alburnus*, *Sander lucioperca*, *Rutilus rutilus*, *Micropterus salmoides*, *Lepomis gibbosus*, Mugilidae, Diptera, Ephemeroptera, Odonata, Atyidae, *Procambarus clarkii*, “Other Fish” and “Other insects”. These categories were defined following taxonomic affinities, so that each category contributed to >1% of the total prey in at least one site. The categories “Other Fish” included native cyprinids (nase *Pseudochondrostoma* spp.), non-native Cyprinid, Iberian gudgeon (*Gobio lozanoi*) and rarely found migratory species, such as Big-scale sand smelt (*Atherina boyeri*), European seabass (*Dicentrarchus labrax*), European flounder (*Platichthys flesus*), and “Other insects” comprised rare and unidentified prey.

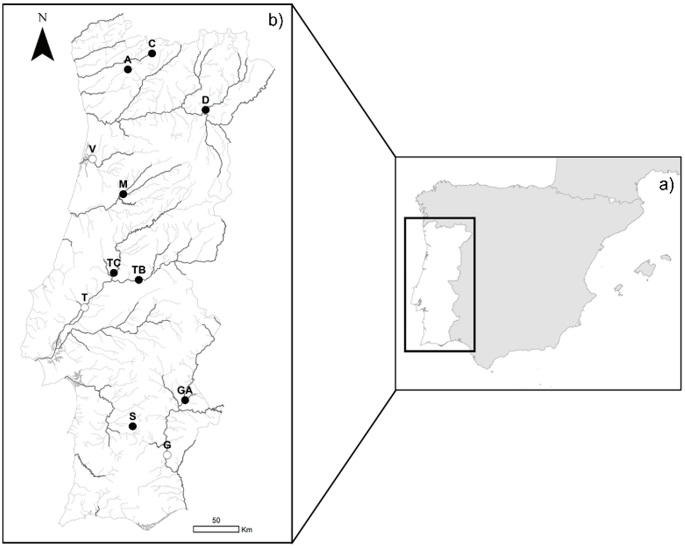

Figure 1. Map showing locations of pikeperch *Sander lucioperca* populations in (**a**) Iberian Peninsula and (**b**) mainland Portugal. White circles correspond to lotic populations and black ones to lentic populations. From north to south (Drainage—Location/Habitat): C, Cávado River—“Alto do Rabagão” reservoir; A, Ave River—“Ermal”—reservoir; D, Douro River—“Foz do Sabor” reservoir; V, Vouga River—lotic section near “Angeja”; M, Mondego River—“Aguieira” reservoir; TC, Tagus River—“Castelo de Bode” reservoir; TB, Tagus River—“Belver” reservoir; T, Tagus River—lotic section near “Santarém”; S, Sado River—“Penedrão” reservoir; GA, Guadiana River—“Alqueva” reservoir; G, Guadiana River—lotic section near “Mértola”.

2.2. Data Analyses

We described diet composition at each site using two conventional indices, the frequency of occurrence (F%) which is the proportion of non-empty stomachs containing a particular prey category, and the numerical frequency (N%), which is the proportional count of each prey category relative to the total prey count among fish [47].

We used non-metric multidimensional scaling (NMDS) analyses to visualize patterns in diet composition in the whole diet composition of pikeperch among the sites using the Bray–Curtis similarity coefficient [48]. Prior to analysis, prey proportions were determined for each individual in relation to the total prey in its stomach, and were square root transformed to reduce the influence of abundant prey in the analysis. Because sample sizes varied among sites and size classes, we averaged the mean location (i.e., centroids) from the NMDS coordinates of all the individual fish to assess geographic changes in the diet composition. Ordination results were considered to be sufficiently described in two dimensions when stress was <0.2 [49]. Finally, we performed linear regression analysis to test for the variation in diet composition with latitude, using the centroids from the NMDS scores in the first two axes as response variables in the analysis.

We then used Permutational Multivariate Analysis of Variance (PERMANOVA) with 9999 permutations based on a dissimilarity Bray-Curtis matrix [50] to test for differences between the diets of pikeperch, using the sites and two size classes (I $\leq$ 25 cm, II > 25 cm SL) as factors. Next, the Indicator Value Index (IndVal) [51] was applied to obtain the prey item indicators for sites and sites-plus-size classes. The IndVal is based on a comparison of the specificity (the relative abundance of each food resource in each group or factor) and fidelity (the relative frequency of each food resource in each group or factor), that are being tested in different groups selected a priori [52]. The greater the specificity and fidelity of an item to a particular group, the greater the value of the indicator; and this method proves robust to differences within the group, sample sizes, and differences in the abundance between the groups [53].

Niche breadth was assessed at the population and individual level, using animal prey. Population breadth was determined according to [54], as follows:

$$B = \left(\sum p_j^2\right)^{-1} \tag{1}$$

where p_j is the proportion of prey category j in the diet. The Levin's index ranges from 1 to n, where n is the number of prey categories; it is at a minimum when there is only a prey category in the diet, and at a maximum when proportions are the same in all prey categories, indicating there is no discrimination among prey categories [55].

Individual specialization (IS) was determined using the proportional similarity index (PS_i), between each individual's prey proportions and the averaged population diet distribution, using the equation by [56], as follows:

$$PS_i = 1 - 0.5 \sum_{j=1} \left| p_{ij} - q_j \right| \tag{2}$$

where p_{ij} is the proportion of prey category j in the diet of individual i, and q_j is the proportion of prey category j in the population as a whole.

This index compares each individual's diet to that of the entire population, with values ranging between 0 and 1. For individuals specializing on single or few prey types, the PS_i values tend to be low, whereas for individuals that consume resources in a similar proportion as the entire population, the PS_i values approach 1 [56]. To evaluate variation in niche indices (B and IS) between populations, we conducted 1000 bootstrap resamplings of the data for each case. Differences were significant when the 95% confidence intervals for estimates did not overlap. All analyses were conducted using the R software [57], and the significance of statistical testing was assessed at $p < 0.05$.

3. Results

In total, we analyzed 168 stomachs and 609 prey items, with sample sizes per site varying between 5 and 31 stomachs and 12–339 prey (Table 1). *Alburnus alburnus* was found in fish stomachs with the highest frequency (28%), followed by *S. lucioperca* (25.6%). Diptera larvae were the most abundant group of prey eaten by pikeperch with a contribution of 54.2%, followed by *A. alburnus* and *S. lucioperca*, but in small numbers (10.7% and 8.9%, respectively).

Diet composition varied considerably among sites and size classes (Table 1). Particularly in northern basins, large individuals consumed mostly *R. rutilus* (27.7; 50%) in the lentic sites Douro and Cávado and also preyed on high proportions of Diptera (84.6%) in the lentic Mondego and Mugilidae (42.9%) and in the lotic Vouga. Conversely, in small individuals, *M. salmoides* (100%) dominated the diet in the lentic Mondego, with Diptera making only 26.9% of the total prey in the lentic Douro, but dominated the diet in the lentic Cávado (66.7%) and the lotic Vouga (93.5%). Cannibalism occurred for both small and large individuals and increased with individual size in the lentic Douro (30.8%; 36.2%, respectively) and the lotic Vouga (0.9%; 14.3%, respectively).

Table 1. Variation in the numeric frequency (%) and in the frequency of occurrence (% in brackets) of prey categories consumed by class I (≤25 cm SL) and class II (>25 cm SL) pikeperch *Sander lucioperca* populations in Portuguese basins. Total fish contains the number of pikeperch stomachs analyzed (n) with empty stomachs presented in brackets, per population. Mean population Standard Length (SL), with size range, minimum and maximum values (min–max) presented for each analyzed population. Sites are ordered by decreasing latitude. Population acronyms according to Figure 1.

	Overall	**C**		**D**		**V**		**M**		**TC**	**TB**		**T**		**S**		**GA**	**G**
Prey Categories		**Class I**	**Class II**	**Class I**	**Class II**	**Class I**	**Class II**	**Class I**	**Class II**	**Class II**	**Class I**	**Class II**	**Class I**	**Class II**	**Class I**	**Class II**	**Class II**	**Class II**
A. alburnus	10.7 (28.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	21.3 (31.8)	0.3 (5.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	20.6 (25.0)	0.0 (0.0)	76.9 (91.7)	0.0 (0.0)	18.2 (33.3)	0.0 (0.0)	6.7 (8.3)	52.4 (72.7)	25.0 (40.0)
S. lucioperca	8.9 (25.6)	0.0 (0.0)	0.0 (0.0)	30.8 (66.7)	36.2 (54.5)	0.9 (15.8)	14.3 (28.6)	0.0 (0.0)	0.0 (0.0)	5.9 (12.5)	0.0 (0.0)	0.0 (0.0)	5.0 (9.1)	9.1 (16.7)	100.0 (100.0)	73.3 (75.0)	0.0 (0.0)	8.3 (20.0)
R. rutilus	3.4 (6.5)	33.3 (50.0)	50.0 (55.6)	0.0 (0.0)	27.7 (27.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mugilidae	1.1 (3.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	42.9 (71.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	8.3 (20.0)
M. salmoides	2.1 (5.4)	0.0 (0.0)	7.1 (11.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (100.0)	0.0 (0.0)	20.6 (18.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.8 (9.1)	0.0 (0.0)
L. gibbosus	1.3 (4.8)	0.0 (0.0)	21.4 (22.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	11.8 (25.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	8.3 (20.0)
O. Fish	2.6 (9.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0))	2.1 (4.5)	0.6 (10.5)	7.1 (14.3)	0.0 (0.0)	0.0 (0.0)	2.9 (6.3)	0.0 (0.0)	2.6 (4.2)	30.0 (54.5)	18.2 (33.3)	0.0 (0.0)	6.7 (8.3)	0.0 (0.0)	8.3 (20.0)
Diptera	54.2 (10.7)	66.7 (100.0)	21.4 (33.3)	26.9 (33.3)	0.0 (0.0)	93.5 (52.6)	0.0 (0.0)	0.0 (0.0)	84.6 (33.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	14.3 (9.1)	0.0 (0.0)
Ephemeroptera	1.8 (3.0)	0.0 (0.0)	0.0 (0.0)	19.2 (22.2)	10.6 (9.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.9 (6.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Odonata	1.3 (2.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.5 (21.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Atyidae	7.4 (12.5)	0.0 (0.0)	0.0 (0.0)	19.2 (22.2)	2.1 (4.5)	0.3 (5.3)	35.7 (14.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (100.0)	15.4 (25.0)	65.0 (54.5)	54.5 (16.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	41.7 (20.0)
P. clarkii	3.1 (5.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	15.4 (66.7)	29.4 (31.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.7 (8.3)	28.6 (9.1)	0.0 (0.0)
O. insects	2.0 (6.0)	0.0 (0.0)	0.0 (0.0)	3.8 (11.1)	0.0 (0.0)	1.8 (21.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.9 (12.5)	0.0 (0.0)	5.1 (8.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.7 (8.3)	0.0 (0.0)	0.0 (0.0)
Total prey	609	3	14	26	47	325	14	4	3	34	3	39	20	11	8	15	21	12
Total fish (n)	168 (88)	2 (3)	9 (16)	9 (7)	22 (3)	19 (9)	7 (1)	4 (1)	3 (5)	16 (7)	2 (0)	24 (8)	11 (3)	6 (4)	6 (3)	12 (8)	11 (9)	5 (1)
SL cm	29.5	23.6	34.6	19.8	32.0	12.2	38.0	21.5	42.7	40.5	19.6	29.6	15.8	29.2	22.2	39.3	40.2	41.3
min–max	9.4–60.3	22.9–24.2	25.4–53.7	12.4–24.1	28.1–39.2	9.4–19.0	31.8–60.3	20.2–22.9	31.6–48.5	25.7–52.2	15.9–23.2	25.1–38.2	9.5–24.2	27.4–30.8	19.6–23.9	25.2–56.6	32.4–43.2	31.0–51.0

In southern populations, *A. alburnus* dominated the diet of large pikeperch in the reservoirs of Alqueva (52.4%) and Belver (76.9%), with contributions of high proportions of Atyidae in the diet of the lotic sites Guadiana (41.7%) and Tagus (54.5%). Additional important prey was *P. clarkii* in the reservoirs of Castelo de Bode (29.4%) and Alqueva (28.6%). Small pikeperch preyed mostly on Atyidae in the reservoir Belver (100%) and the lotic Tagus (65%), also including "other fish" (30%) as important prey. Cannibalism was prevalent in the lentic Sado for both small (100%) and large (73.3%) pikeperch, but was found in lower numbers in the populations of the reservoir Castelo de Bode and the lotic sites Guadiana and Tagus (Table 1).

The NMDS biplot highlighted considerable diet variation in prey use among sites, with a slight separation of the lotic sites (Vouga, Tagus and Guadiana) and depicting a latitudinal gradient (Figure 2). Variation in NMDS1 scores showed no positive association with latitude ($F_{1,8}$ = 0.51, R^2 = −0.06, *p* = 0.495), but there was a positive geographic pattern (e.g., north–south trend) between the latitude and diet composition for NMDS2 ($F_{1,8}$ = 8.19, R^2 = 0.44, *p* = 0.021).

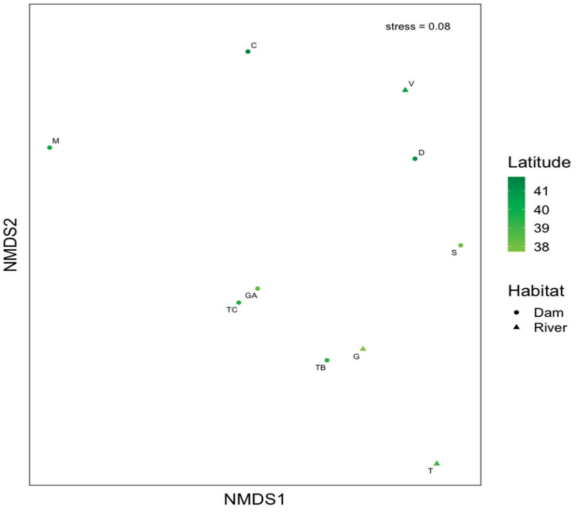

Figure 2. Nonmetric multidimensional scaling (NMDS) ordination of the diet of pikeperch *Sander lucioperca* populations in Portuguese basins using averaged mean locations (i.e., centroids). Symbols for sites are shaded by latitude from north (dark) to south (lighter).

The results of the two-way PERMANOVA (Table 2) showed that there was a significant interaction between sites x size classes (Pseudo $F_{6,151}$ = 2.38, *p* < 0.001) and also differences in the diet associated with sites (Pseudo $F_{9,151}$ = 6.56, *p* < 0.001) and size classes (Pseudo $F_{1,151}$ = 3.14, *p* = 0.004).

Table 2. Permutational Multivariate Analysis of Variance results in the diet composition of pikeperch *Sander lucioperca* populations in Portuguese basins.

Parameter	df	Pseudo *F*-Ratio	*p*-Value
Sites * Size	6	2.38	<0.0001
Sites	9	6.56	<0.0001
Size	1	3.14	0.004
Residual	151		

The most significant food items that contributed to the sites-plus-size class differentiation, were indicated by IndVal (Table 3). In northern populations, differences in prey use were consistently related to contributions of Mugilidae and *P. clarkii* to large individuals from the Vouga and Mondego, but Diptera and *M. salmoides* were also important contributors for small pikeperch in the Cávado and Mondego. In southern populations, differentiation resulted from the contribution of *A. alburnus* to large individuals in the Belver reservoir, with other contributors being important for small pikeperches including Atyidae in the Belver reservoir, and "Other fish" and *S. lucioperca* in the river Tagus and Sado reservoirs, respectively.

Table 3. Indicator Value (IndVal), *p*-values and Frequency of prey items consumed by pikeperch *Sander lucioperca* populations in Portuguese basins between sites and size classes (I ≤ 25 cm, II > 25 cm SL). Population acronyms according to Figure 1.

Prey Items	Site/Size Class	IndVal	*p*-Value	Frequency
Diptera	C (I)	0.381	0.016	18
Mugilidae	V (II)	0.545	0.002	6
M. salmoides	M (I)	0.754	0.001	9
P. clarkii	M (II)	0.432	0.009	9
Atyidae	TB (I)	0.466	0.002	21
A. alburnus	TB (II)	0.266	0.001	47
O. fish	T (I)	0.195	0.054	16
S. lucioperca	S (I)	0.309	0.001	43

The contribution of non-native prey in the diet of pikeperch populations was very high, ranging from 53% in the lotic Tagus to 100% in the lentic sites Cávado, Mondego, Sado and Guadiana (Figure 3). Contrastingly, native fish prey was found only in the lotic sites Vouga (47%), Tagus (42%) and Guadiana (29%) and accounted with lower percentages in lentic sites.

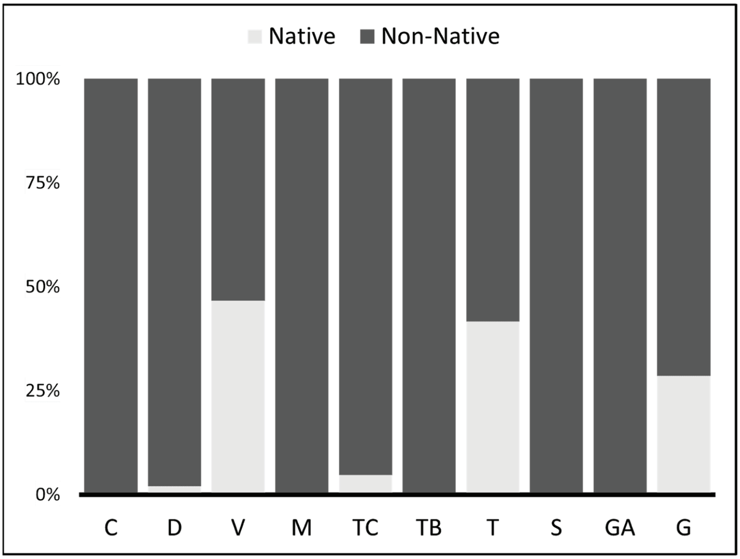

Figure 3. Consumption on Native vs. Non-Native fish preys by pikeperch *Sander lucioperca* populations in Portuguese basins. Light grey (Native), dark grey (Non-Native).

There was no substantial variation in the dietary niche breadth among populations of pikeperch (Figure 4). Levin's index showed a similar and narrow pattern, with average values ranging from one (lentic populations in the Mondego and Guadiana) to 1.32 (Castelo de Bode reservoir). The degree of individual specialization in prey use showed some considerable variation among populations. Pikeperch individuals tended to be more specialized in the northern than in southern populations (Figure 4). Nevertheless, pikeperch showed higher specialization values in the Castelo de Bode reservoir and the lotic Guadiana than in the lentic Sado and the Belver reservoir.

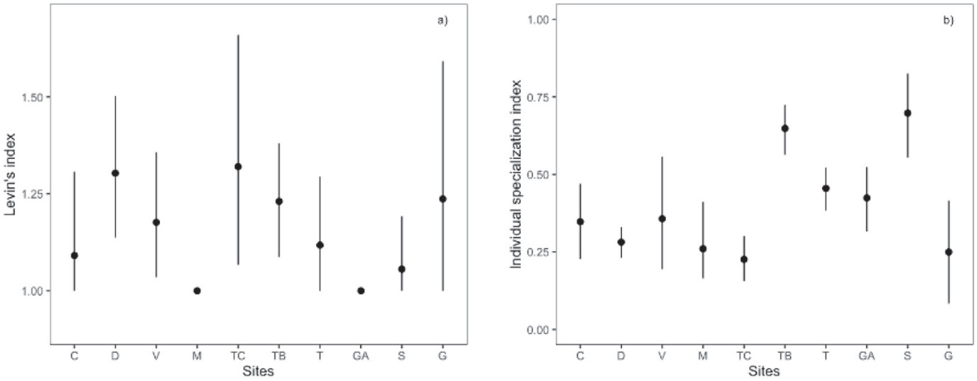

Figure 4. Population and individual niche breadth of the pikeperch *Sander lucioperca* populations in Portuguese basins: (**a**) Levin's index) and (**b**) individual specialization index. Error bars represent bootstrapped 95% confidence intervals for each index. Sites are ordered by decreasing latitude. Population acronyms according to Figure 1.

4. Discussion

Our results highlighted a variation in dietary patterns of *Sander lucioperca*, displaying a narrow niche breadth at both population and individual levels, and a highly opportunistic predatory behavior that is likely to be associated with the prey availability at a local level, which was not evaluated in this study. We observed a latitudinal gradient pattern on prey use by pikeperch, suggesting flexibility in its utilization of available prey resources. Ontogenetic fluctuations in prey use were also found, with small individuals feeding predominantly on insects, small macroinvertebrates and fish, while large specimens foraged mainly on fish and crayfish and with both size classes showing signs of cannibalism in six populations. These results are coincident with those found in lakes [58,59] and reservoirs [36,60], suggesting that fish prey constitute a staple prey for pikeperch across its non-native range. Furthermore, the majority of the pikeperch diet was composed of non-native species, although the presence of native fish preys (e.g., Mugilidae, Big-scale sand smelt, Unidentified nase, European seabass, European flounder) was found but in low numbers, but in lotic populations could reach half of the preys. Taken together, these findings suggest the importance of spatial comparisons in diet studies, supporting that non-native piscivorous fish may cause different impacts in relation to local prey richness and abundance.

The current work evaluates pikeperch dietary patterns from different populations that were collected across a wide spatial gradient, using different methodologies, encompassing distinct months and involving a limited number of individuals in some populations. However, the results presented here are generally consistent with other studies conducted in Spain and France, supporting the current observed patterns adjusted to Iberian watersheds [36,60]. The sampling period of this study might have influenced the results; however, most of the studied populations were lentic ones, related to reservoirs that are more stable environments with a limited community seasonal change [36]. Specifically, it would be important to widen the study period to encompass the entire year in riverine populations, to evaluate the predation effect on anadromous fish that spawn during the winter months [20]. Although limited sample size in some populations may have induced some shortcomings, it is unlikely to have any significant effects given the high individual specialization observed. The lack of a detailed key to identify fish bones hampered species identification in a few cases (Table 1), where identification was only possible due to the creation of a fish bone collection with eleven species associated with previous fish keys (e.g., [44]). However, we recognize that molecular techniques (e.g., DNA metabarcoding) could improve our prey resolution and unveil a wider prey breadth (e.g., [61]). Additionally, stable isotope analysis [25,62] could also allow us to understand the long-term impacts of this predatory fish, considering that traditional dietary studies can only be seen as a snapshot of feeding behavior. Latitude was shown to influence the variation in *S. lucioperca* diet and likely reflects the foraging opportunism of the species and spatial changes in local prey supply. The most frequent and numerous preys found in the northern populations were *R. rutilus, S. lucioperca, M. salmoides* and Diptera, whereas in the south they were *A. alburnus, S. lucioperca,* Atyidae, *P. clarkii* and Diptera. Those spatial changes in the consumption of prey types are often indicative of changes in the abundance or availability of those prey items [36,59]. In fact, *R. rutilus* only occurs in northern populations of Douro, Ave and Cávado [30,63], while *A. alburnus* is a recent invader (last 20 years) to Portuguese watersheds having initially invaded southern rivers and expanded northwards [30]. Similarly, there was a high prevalence of pikeperch cannibalism (six populations), reaching up to 34% and 83% of preys in the lentic sites of Douro and Sado, respectively. This was already described in several studies (see [33–36,58,60]) and is related to local prey abundance. Moreover, lotic systems exhibit higher species diversity particularly in the lower reaches of the Vouga where the occurrence of migratory fish like Mugilids is prevalent. According to previous studies, see [64], a high species richness was described in the Tagus mainstem which is consistent with the higher prey diversity found in *S. lucioperca* in this site, observed as "Other Fish" (generally native fish). Finally, a similar pattern of increasing proportions of Atyidae in the diet has been observed in another top predator, the European catfish (*Silurus glanis*), in the lotic Tagus that exhibited lower fish prey richness and higher proportions of Crustaceans, see [65].

The influence of pikeperch size on its diet has often been reported [33,36], with a rapid replacement of macroinvertebrates by fish as the increasing size of pikeperch permits the handling of bigger, more profitable prey. While, in our results the pikeperch diet contained prey fish across the range of two size classes, the consumption of macroinvertebrates were most frequently encountered in the diets of smaller individuals. The absence of suitable-sized prey fish is likely to limit the pikeperch population's ability to reach piscivory, causing a delay to growth acceleration and a high mortality due to starvation and predation in size classes [62,66]. Cannibalism seems to occur throughout the life of pikeperch. Although its importance was found modest for individuals smaller than 25 cm SL, cannibalism became more important with increasing body size [36,60]. Moreover, we found evidence suggesting that both size classes of pikeperch in the Sado were cannibalistic. This might be explained by the time of sampling, as young-of-the-year (YOY) *S. lucioperca* would not have been present in the population due to the timing of spawning [67], but also because of the high density of juveniles in the population [68]. Nevertheless, we should not exclude limitations stemming from the stomach content analyses in providing accurate dietary assessments, as it was only completed at a single time of the year.

Pikeperch showed a reduction in population prey breadth which was accompanied by an increase in individual specialization. This indicates that the specialization observed at the population level was the result of individuals specializing on a subset of resources of the prey spectrum. Similar values for population diet breadth have been found in southern Europe, namely in the Alcántara reservoir in Spain [36]. The trends towards narrower diets and the use of a small array of prey by all individuals in Mediterranean rivers may reflect conditions of reduced intraspecific competition, thus facilitating the spread and integration of this invasive species [69]. Furthermore, the use of similar prey may also be associated with changes in fish assemblages across the latitude gradient, with a low diet breadth at population and individual levels favoring prey partitioning among species.

5. Conclusions

This work highlights that pikeperch is an opportunistic predator with a specialized feeding strategy that may potentially cause severe impacts on aquatic communities. Variation in diet was most strongly linked to latitude and ontogeny. Niche breadth remained narrow to minimize the diet variation among individuals and decrease the risk of competition for available resources. These findings are important for our efforts to maintain the integrity of the highly endangered native fish existing in Iberian watersheds, where native fish communities, originally devoid of any native predator, are highly vulnerable to new predators. In fact, it is urgent to evaluate the impact of these predators in riverine fish communities, generally dominated by natives, especially during their initial colonization (but see [65]). Describing the dietary traits of top predators, understanding their behavior and knowing their potential feeding preferences, are essential pieces of information to evaluate the predator impacts on our endemic fishes and enable a better conservation strategy focusing on these unique freshwater ecosystems.

Author Contributions: Conceptualization, D.R. and F.R.; methodology, D.R. and C.G.; investigation, D.R., C.G., J.G. and F.R.; writing—original draft preparation, D.R. and C.G.; writing—review and editing, J.G. and F.R.; visualization, C.G.; supervision, F.R.; funding acquisition, F.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was conducted in the frame of the projects SONICINVADERS (FCT ref. PTDC/CTA-AMB/28782/2017), FRISK (PTDC/AAG-MAA/0350/2014) and ISO-INVA (LISBOA-01-0145-FEDER-029105 + PTDC/CTA-AMB/29105/2017) co-funded by international funds through Lisboa 2020—Programa Operacional Regional de Lisboa, in its FED ER component (Project ref. LISBOA-01-0145-FEDER-029105) and national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P (Project ref. PTDC/CTA-AMB/29105/2017). Additional support was provided by FCT through the strategic plan of the Marine and Environmental Sciences Centre (MARE) (UID/Multi/04326/2019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Acknowledgments: The authors would like to thank all the fishermen that contributed with samples that made it possible to perform this study, in particular to Carlos Serras, João Lobo and Francisco Pinto. A special thanks also, to Luís da Costa for the graphic development of Figure 1 and to the anonymous reviewers for their helpful contribution. This work was supported by project "SONICINVADERS", co-funded by Portugal 2020 and Alentejo2020 through EU ERDF funds (ALT20-03-0145-FEDER-028782), and by national funds through FCT (PTDC/CTA-AMB/28782/2017). Additional support was provided by FCT through the strategic plan of the Marine and Environmental Sciences Centre (MARE) (UID/MAR/04292/2013).

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

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