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Overlapping Areas of Non-Indigenous and Critically Endangered Freshwater Fishes: Setting Conservation Priorities in Greece †

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Abstract: The introduction of non-indigenous fish species (NIFS) to freshwater ecosystems is considered as one of the leading factors associated with freshwater biodiversity loss. Unravelling the spatial overlap between NIFS and critically endangered (CR) fish species can contribute to targeted conservation planning and actions to minimize the potential negative effects. In this study, we applied geostatistical analyses of species distribution data to investigate the potential overlapping areas of NIFS against fish species that are designated under CR status according to the International Union for the Conservation of Nature (IUCN) and the Hellenic Zoological Society (HZS) Red Lists. Distributional (presence–absence) data of all NIFS were compiled for both lentic and lotic ecosystems of Greece. In total, 800 records were utilized from 169 lakes (based on literature review) and 631 river sites from 51 river basins (based on sampling data). Our results indicate that freshwater ecosystems under high NIFS richness are located mainly in lowland areas of western, central and northern Greece usually near large cities and ecosystems with well developed commercial and recreational fisheries. On the contrary, low NIFS richness was observed in mountainous regions and small river basins. Overlapping areas of CR species with moderate to high NIFS richness (1.5–4.3 NIFS per 1 km^2) were relatively high (~50%). Many of the overlapping areas fall within NATURA 2000 network, where legal management bodies can implement management programs to minimize the negative impacts. However, some areas of conflict are in unprotected regions and may be likely to experience significant negative biodiversity impacts. The use of the CR status is useful as a proxy indicator for some of Greece's most important inland water ecosystems in order to avoid new invasions and to manage established alien and translocated species. Our findings demonstrate that many freshwater ecosystems of outstanding biodiversity conservation value in Greece are under significant invasion pressure.

Keywords: invasive species; alien; translocated; critically endangered; freshwater fishes; freshwater ecosystems; conservation; biodiversity; red list

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1. Introduction

According to the International Union for the Conservation of Nature (IUCN) more than 35,500 species (or 28% of all assessed species) are threatened with extinction worldwide, while at least 1677 species out of 15,060 assessed are threatened with extinction in Europe. Freshwater fishes are considered among the most threatened species worldwide [1]. The most recent IUCN Red List includes 2169 fish species, 20% of which are at risk of extinction as they are considered critically endangered (CR), while more than 80% of them

inhabit freshwater ecosystems. In Europe freshwater fishes display the third highest percentage of taxa being at risk; that is freshwater mollusks (59%), endemic trees (58%) and freshwater fish (40%).

Freshwater fish species inhabiting Mediterranean inland water ecosystems are considerably more vulnerable compared to the species located in the northern temperate regions mainly due to the high degree of endemism, their fragmented geographical distribution, as well as the intense anthropogenic pressures, particularly due to water scarcity [2]. Greece, located in the Eastern Mediterranean region, holds a unique ichthyofaunal diversity within Europe and displays one of the highest levels of fish species endemism in the Mediterranean region [2,3]. The complex geological processes of the wider area of the Balkans and the eastern Mediterranean has allowed multiple fish species colonisations, long-term survival of ancient taxa in refugia and enhanced speciation due to hydrographic isolation among very different biogeographic areas [3]. These factors are mainly responsible for the increased diversity and high degree of endemicity in Greece's freshwater fish fauna.

As elsewhere in the Mediterranean basin, anthropogenic alterations, such as overexploitation of water, habitat degradation and fragmentation due to morphological changes in river corridors and pollution are the main threats for freshwater fishes in Greece. These anthropogenic stresses are also augmented by the introduction of alien fishes [4]. Therefore the aim of this study was, to identify the potential overlapping areas of non-indigenous fish species (hereafter NIFS) against fish species that are designated under a CR status according to the (IUCN) and the Hellenic Zoological Society (HZS) Red Lists. This applied geographical analsis aims to support conservation planning and actions to minimize the potential negative effects that NIFS may pose to freshwater biodiversity.

2. Material and Methods

2.1. Study Area

According to the most recent checklist to date [5], 160 freshwater fish species have been recorded in Greece, where 137 are native. The country presents a substantial proportion of country-specific endemics, 47 in total (or 34% of the native fish fauna). Moreover, 10% of the freshwater fish species are endemic or near endemic (i.e., occurring also in shared transboundary freshwater lake basins); by excluding alien and species of a marine origin, the percentage raises up to 56%. In Greece, 51 fish species are considered threatened at a global scale (i.e., CR, Critically Endangered (20); EN, Endangered (15); VU, Vulnerable (16)) corresponding to 31.8% of all native inland water fish species in Greece based on the IUCN Red List and Barbieri et al. [5].

2.2. Data Acquisition

We obtained geographic range data for fish species designated as critically endangered (CR) based on the International Union for the Conservation of Nature (IUCN) Red List integrated with the critically endangered fish species listed by the Hellenic Zoological Society (HZS) Red List. In addition, we included three additional fish species not yet evaluated displaying restricted distributional range in Greece. In total 25 freshwater fish species were included in our dataset, 19 by IUCN, three species under the HZS Red List (*Barbus pergamonensis, Knipowitschia goerneri, Oxynoemacheilus theophilii*) and three additional species which are not yet formally evaluated (*Caspiomyzon graecus, Eudontomyzon* sp. Almopaios, *Valencia robertae*) displaying however a very restricted distributional range (Table 1). The European eel, *Anguilla anguilla*, a eurihaline fish of marine origin, was excluded from our dataset due to its wide distributional range in the country.

The geographical distribution and the expanse of NIFS in Greece, was compiled based on distributional data obtained from two different sources: (a) a bibliographical survey for the lentic ecosystems and (b) survey data by electrofishing in lotic waters; derived from standardized field sampling in rivers, streams, canals and springs, in the

framework of various regional surveys. The derived matrix summarized NIFS records from 169 lakes (107 artificial and 62 natural) within the Greek territory (Figure 1). Data of fish species in lentic waters—lakes, ponds and reservoirs—was obtained from an ongoing project that is reviewing both field serveys and the literature (see Acknowledgements). Fish sampling data in lotic waters were obtained from various research surveys conducted between the years 2001–2017 by the Hellenic Centre for Marine Research (HCMR); these cover the entire mainland as well as the major islands of Greece. Most of the sampled data are publicly available and have been recently published in a number of studies [4,6]. Field sampling was conducted primarily through a standardized electrofishing procedure following the FAME research project guidelines with some modifications; for a detailed description of the sampling procedure see Economou et al. [7]; in some cases seine nets and other methods were also used to ascertain fish presence. In total, 265 sites from 51 different river basins with at least one NIFS in Greece were compiled (Figure 1). Barbieri et al. [5] was used for species taxonomy and nomenclature. Species that are introduced by humans beyond their native freshwater ecoregion, but are native to a part of the country, are designated as translocated species [4]. NIFS include both alien and translocated species in this analysis.

Table 1. Freshwater fish species of Greece listed as Critically Endangered (CR) fish species under IUCN and/or the HZS Red Lists. Categories: CR, Critically Endangered; EN, Endangered; VU, Vulnerable; LC, Least Concern and DD, Data Deficient. Transl. in brackets denotes species that are introduced as translocated populations not the original wild stock.

Number	Species	Authority	IUCN Red List	HZS Red List
1	*Acipenser naccarii*	Bonaparte, 1836	CR	[Transl.]
2	*Acipenser stellatus*	Pallas, 1771	CR	DD
3	*Acipenser sturio*	Linnaeus, 1758 N	CR	DD
4	*Alburnus macedonicus*	Karaman, 1928	CR	CR
5	*Alburnus vistonicus*	Freyhof & Kottelat, 2007	CR	CR
6	*Alosa vistonica*	Economidis & Sinis, 1986	CR	CR
7	*Aphanius almiriensis*	Kottelat, Barbieri & Stoumboudi, 2007	CR	CR
8	*Barbus euboicus*	Stephanidis, 1950	CR	CR
9	*Barbus pergamonensis*	Karaman, 1971	LC	CR
10	*Caspiomyzon graecus*	Renaud & Economidis, 2010	-	-
11	*Caspiomyzon hellenicus*	Vladykov, Renaud, Kott & Economidis, 1982	CR	CR
12	*Cobitis stephanidisi*	Economidis, 1992	CR	CR
13	*Eudontomyzon* sp. Almopaios	-	-	-
14	*Huso huso*	(Linnaeus, 1758)	CR	[Transl.]
15	*Knipowitschia goerneri*	Ahnelt, 1991	DD	CR
16	*Knipowitschia milleri*	(Ahnelt & Bianco, 1990)	CR	VU
17	*Oxynoemacheilus theophilii*	Stoumboudi, Kottelat & Barbieri, 2006	LC	CR
18	*Pelasgus epiroticus*	(Steindachner, 1896)	CR	CR
19	*Pelasgus laconicus*	(Kottelat & Barbieri, 2004)	CR	CR
20	*Pungitius hellenicus*	Stephanidis, 1971	CR	CR
21	*Salaria economidisi*	Kottelat, 2004	CR	LC
22	*Scardinius graecus*	Stephanidis, 1937	CR	VU
23	*Squalius* sp. Evia	-	CR	-
24	*Valencia letourneuxi*	(Sauvage, 1880)	CR	CR
25	*Valencia robertae*	Freyhof, Kärst & Geiger, 2014	-	-

2.3. Spatial Analysis

Spatial analyses of species distributional data were preformed in order to investigate the potential overlapping areas of NIFS against fish species that are designated under a

CR status. The geographical range of each CR fish species was projected within Grid cells of 1 × 1 km in a geographical information system (ESRI—ArcGIS v. 10.4), and the final matrix generated by the total number of CR species in each cell. The predictive distribution of NIFS richness was generated by using presence/absence NIFS data through the Kriging interpolation analysis in ArcGIS. The overlapping "conflict areas" were spatial delimited by the produced NIFS distribution which extents within the geographical range of CR fish species. Finally, we compared the distribution of CR fish species richness of the overlapping areas for protected areas (i.e., Greece's Natura 2000 sites) versus areas outside protected regions.

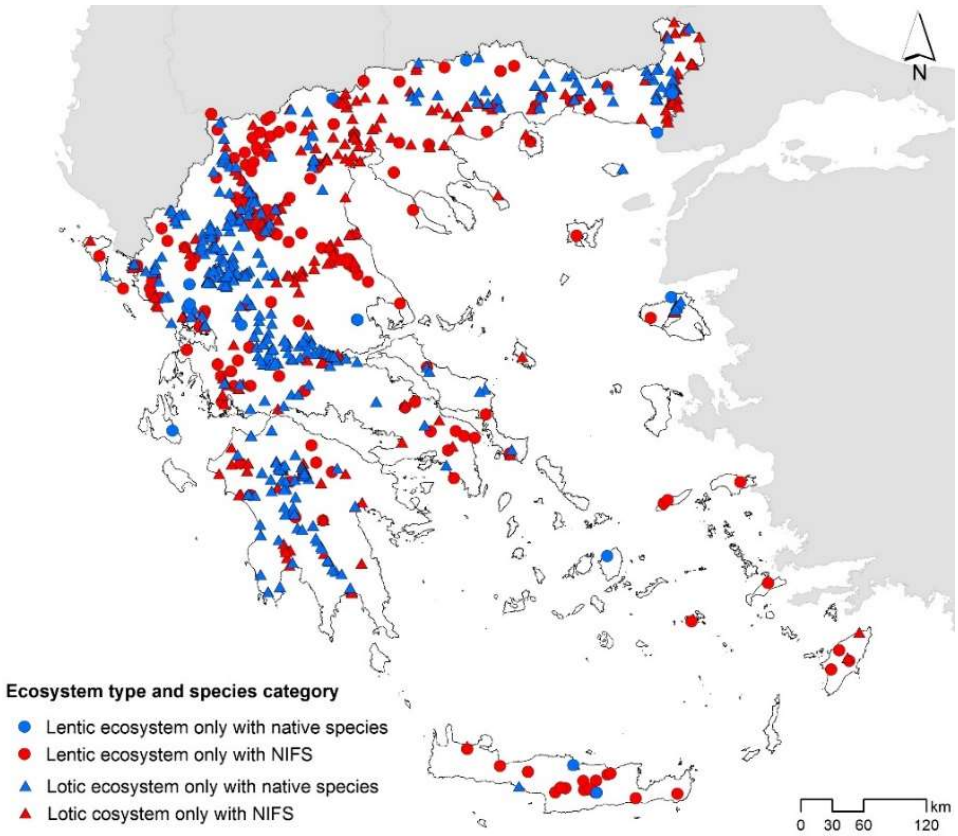

Figure 1. Map of Greece indicating the location of lentic (points) and lotic (triangles) ecosystems used in this study. Blue symbols indicate sites only with native species and red symbols sites occupied with NIFS.

3. Results—Discussion

Overall, we recorded 52 NIFS in 154 lentic and 51 lotic ecosystems (265 sites) of which 17 were categorized as alien, and 35 as translocated. The five most widespread NIFS within Greece included four alien species, namely *Gambusia holbrooki* occurring in 223 locations (53.1%), *Carassius gibelio* occurring in 187 locations (44.52%), *Lepomis gibbosus* occurring in 113 locations (26.9%), *Pseudorasbora parva* occurring in 93 locations (22.14%) and one translocated species *Cyprinus carpio* occurring in 109 locations (25.95%). Evidently, most recorded NIFS display limnophylic (lacustrine) life history strategies. NIFS richness ranged from one (176 records) up to 12 fish species (1 site—Lake Pamvotis). Our results indicate that freshwater ecosystems under high NIFS richness are located mainly in lowland areas of western, central and northern Greece (Figure 2a) usually near large cities and lentic ecosystems with well developed commercial and recreational fisheries. On the contrary, areas with low NIFS richness were observed in mountainous regions, and at small river basin areas in southern Greece and the Aegean islands (Figure 2a).

According to the latest red list survey at the European level, more than 37% of the freshwater fish species are considered as threatened; 15% as Vulnerable, 10% as Endangered and 12% as CR status [8]. According to the IUCN, Greece hosts the most species under a threatened status and the most critically endangered freshwater fish species in Europe. The distribution of the CR freshwater fish species in Greece was scattered throughout the aquatic ecosystems of the country, covering approximately 28% (36,708 km^2) of the entire area of Greece. In most areas, only one CR fish species occurs (69.9%) however in some ecosystems two or more species co-occur (Figure 2b). The highest species richness of CR fish species was observed in the north and northeastern, western and central parts of Greece, while no CR fish species are recorded in the mountainous areas of northern and central Greece, in the majority of the Aegean or Ionian islands, or the island of Crete (Figure 2b).

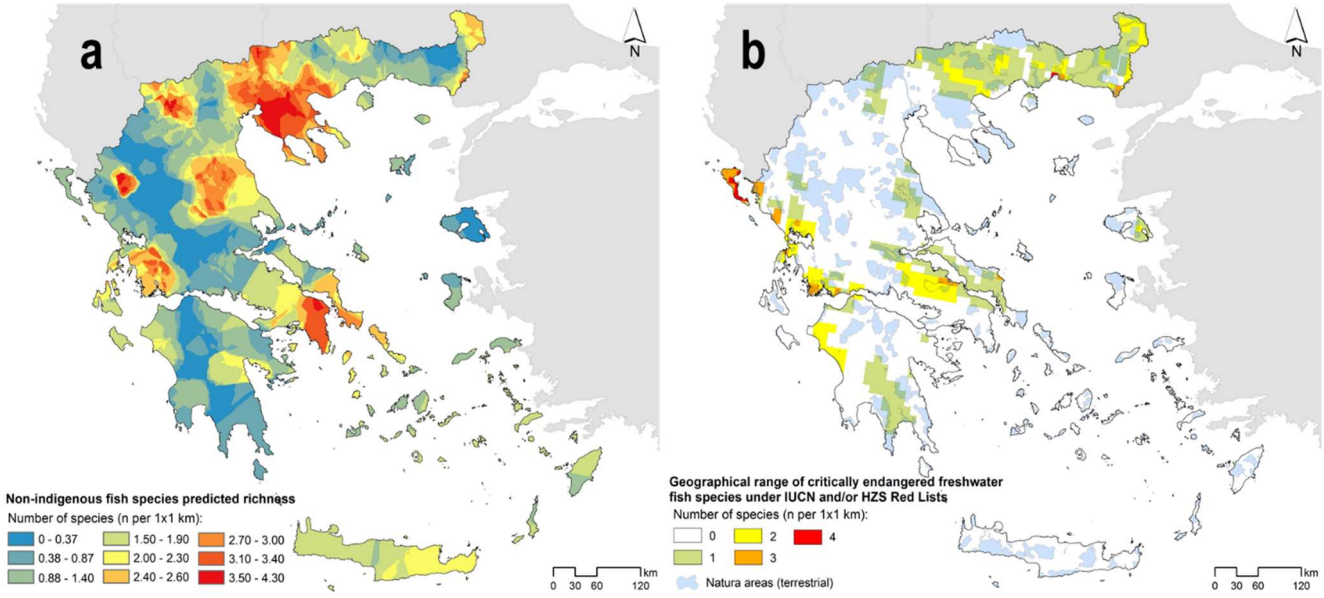

Figure 2. (**a**) Predicted richness of non-indigenous fish species (indicating high invasion pressure) and (**b**) geographical distribution range of critically endangered freshwater fish species and protected areas (Natura 2000 network).

Overlapping areas of CR species with moderate to high NIFS predicted richness (1.5–4.3 NIFS per 1 km^2) were relatively high (~50%) (Figure 3, Table 2). These areas were located in the western, central-east and northern part of Greece (Figure 3). Shared absences indicating both low CR species and NIFS and thus low overlapping areas were observed in the mountainous regions of central Greece, the Aegean Islands and the Island of Crete. The CR fish species co-occurring in areas with moderate to high NIFS richness were: *Pelasgus epiroticus* (Lake Pamvotis), *Valencia robartae*, *Salaria economidisi* (Acheloos basin), *Scardinius graecus* (Lakes Yliki and Paralimni), *Cobitis stephanidisi* (Lake Karla basin), *Alburnus macedonicus* (Lake Doirani). From the aforementioned species *P. epiroticus* and *C. stephanidisi* are considered on the brinkof extinction; while *Alosa vistonica* has recently been assessed by a local study as extinct [9]. Some other formerly widespread species, with marginal distributions in the country, may now be extirpated from Greece; these include wild populations of all the sturgeons (*Huso huso*, *Acipenser sturio*, *Acipener naccarri*, *Acipenser stellatus*). Once more, the most common NIFS within the overlapping areas were Greece's four most widespread and abundant aliens *G. holbrooki, C. gibelio, L. gibbosus and P. parva* as well as the translocated *C. carpio*, *Salmo farioides* and *Economidichthys pygmaeus* [4].

Table 2. The proposed class boundaries (low, moderate, high) of the overlapping areas of non-indigenous and critically endangered freshwater fish species, the actual areas and the total percentage for each category.

Category		Area (km²)	Precent (%)	Total Area (km²)	Total Precent (%)
Low	0–0.37	4.270	11.6		
	0.38–0.87	5.906	16.1	18.408	50.1
	0.88–1.40	8.232	22.4		
Modarate	1.50–1.90	7.154	19.5		
	2.00–2.30	3.926	10.7	14.997	40.9
	2.40–2.60	3.917	10.7		
High	2.70–3.00	1.637	4.5		
	3.10–3.40	1.371	3.7	3.303	9.0
	3.50–4.30	295	0.8		
Total		**36.708**	**100**		

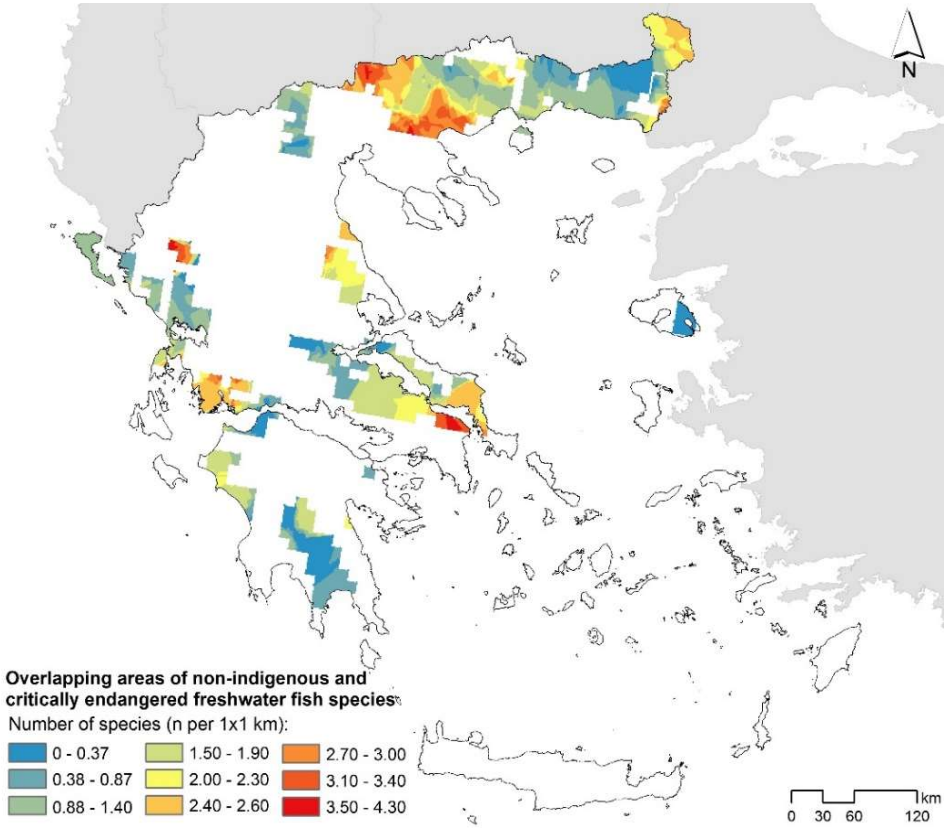

Figure 3. Overlapping areas between the distributions of NIFS and CR freshwater fish species in Greece.

Even though there has been a rapid growth in developing protected areas [10], such efforts are not usually optimally designed for freshwater biodiversity. Many conservationists believe that freshwater conservation must relate to a separate ecological realm, beyond the terrestrial or marine, whose specific recognition has important consequences for both biodiversity conservation and the wider water management issues [11]. In Greece, a quarter of the overlapping areas fall well within the NATURA 2000 network (Figure 4a), where management bodies could implement management, control or mitigation programs to minimize the negative impacts.

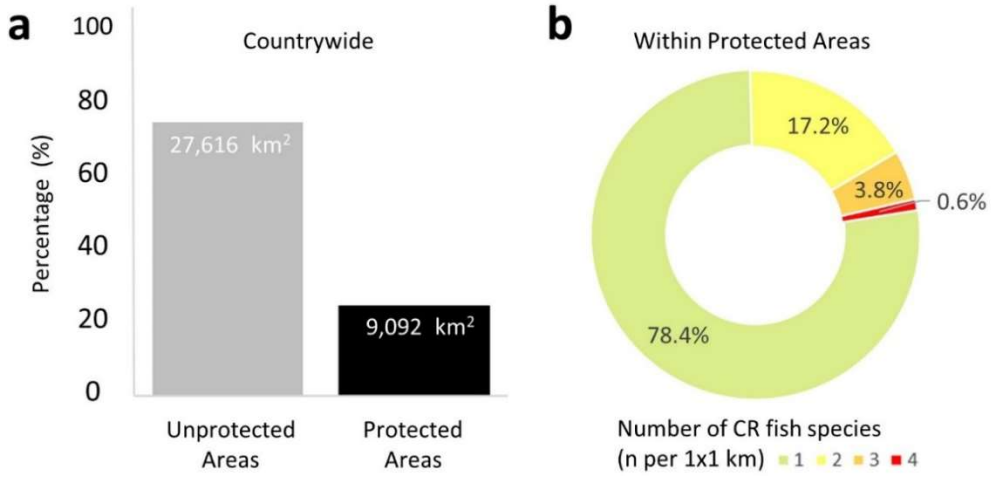

Figure 4. (**a**) The frequency distribution of CR freshwater fish species countrywide in unprotected areas (grey) and protected areas (black); and (**b**) the area coverage (%) of CR freshwater fish species richness within the protected areas.

However, the majority of the distribution areas of CR fish species in Greece are located outside of protected areas (75.2%; Figure 4a) and any additional anthropogenic stressor have the potential to increase pressures and threats to the populations of these CR species. Moreover, the vast majority of the overlapped areas within the protected zones encompassed only one or two CR fish species per 1 km^2, 78.4% and 17.2%, respectively (Figure 4b), while three or four CR fish species per 1 km^2 covered considerably smaller areas (4.4% in total; Figure 4b).

Finally, IUCN and national red listing assessment schemes will change some listed species categories as new reviews of vulnerability status are revised (i.e., some species that are Critical may be downgraded to Endangered). The Hellenic Zoological Society's Greek Red List procedure was last reviewed in 2009, lagging far behind many other EU states and must be revised as soon as possible. Recently, a new review of Mediterranean freshwater fish species has begun by IUCN experts, to be completed in 2021. In this work, we solely utilized the highest extinction threat level category (CR) as a proxy indicator for identifying areas of outstanding conservation interest in a rapid screening process that may be repeated and is easily transferable to other states and for future monitoring. Although the new red listing revisions may alter the status of some species, in our opinion, most of the species utilized here should remain as important guiding species for conservation planning.

4. Conclusions

Recent studies have indicated that freshwater biodiversity is declining at a rapid rate, suggesting that the sixth mass extinction of species is under way [12]. Whole assemblages of fishes are rapidly changing as the impacts of non-indigenous invasives spread into Greece [13]. Until fairly recently, Greece was a country that had few alien species compared to other European states [14]. Our findings demonstrate that many freshwater ecosystems in Greece are under significant invasion pressure and also exhibit high biodiversity conservation value. The use of the CR status is useful as a proxy indicator for identifying some of Greece's most important and most vulnerable inland water ecosystems in order to avoid new invasions and to manage established alien and translocated species. Finally, the effectiveness of protected areas has been reviewed [15], thus, the need for optimal well-designed protected zones for freshwater biodiversity is a necessity for CR fish species in Greece's freshwaters.

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

Abbreviations

The following abbreviations are used in this manuscript:

CR	Critically Endangered
DD	Data Deficient
EN	Endangered
HZS	Hellenic Zoological Society
IUCN	International Union for the Conservation of Nature
LC	Least Concern
NIFS	Non-Indigenous Fish Species
VU	Vulnerable

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