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Non-indigenous freshwater fish research in Greece: Current status and future prospects

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Abstract

Many studies around the globe have documented that the introduction of non-indigenous fish species (NIFS) may pose severe impacts on native biota, ecosystem functioning and ecosystem services. In Greece, publications on NIFS have increased in recent decades, however review studies of past and current scientific research on freshwater NIFS in the country are currently lacking. The aim of the present study was to: (a) assess the current state of knowledge of freshwater NIFS in Greece based on the existing scientific literature, (b) evaluate well-studied areas of NIFS research, and (c) identify important gaps that may direct national authorities to implement appropriate research, management and conservation actions reversing the negative impacts of NIFS. A systematic literature review on NIFS research within Greek inland waters was performed by applying the PRISMA methodology (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Overall, our initial search resulted in 2,794 published articles, 87 of which met our selection criteria and were thus included for full analysis. Evidently, NIFS failed to attract the interest of ecologists in Greece until the turn of the century, at which point, publications increased at a rapid rate. However, the majority of the studies were mainly monothematic concerning NIFS distribution within freshwater ecosystems of Greece (37%), followed by studies on ecological and biological aspects of NIFS (28%). Studies based on fieldwork and laboratory experiments were by far the most common type of research, followed by studies based on bibliographic data. Despite the fact that almost half of the publications were field observational studies, most of them used only qualitative data (plain presence/absence data). Critical aspects of impact assessment were missing; 84% of the reviewed studies did not provide any concrete evidence of NIFS impacts to native biota. Overall, the focus of the studies was uneven, with important gaps in areas of theoretical and practical importance for policy-relevant detection, control and management of NIFS. Findings may assist researchers in filling scientific gaps identified by our review and guide authorities to define national priorities to prevent and control the spread of NIFS within the country.

Keywords: Alien; PRISMA; NIFS; Greece; translocated; inland waters.

Introduction

Transporting species outside their natural ranges and introducing them into novel areas dates back to prehistoric times and has been strongly linked with human trade, agriculture and aquaculture. During earlier times however, the rate of species translocation was substantially limited in comparison with recent decades. Global trade has intensified the transport and translocation of species beyond their natural distributional ranges (Hulme, 2009), including freshwater fish species, some of which have become invasive, negatively affecting native biota and local ecosystems (Leprieur *et al.*, 2009; Simberloff *et al.,* 2013). Invasive freshwater species may affect native biota by various mechanisms such as competitive exclusion, hybridization, niche displacement, predation and disease or parasites transmission; occasionally leading to species extinctions (Closs *et al.,* 2015). Despite the increased concern in biodiversity loss, invasive freshwater fish species also impose severe effects in economic terms on human health, damaged infrastructure, agriculture and fisheries (Vilà *et al.,* 2010; Van der Veer & Nentwig, 2014). Hence, supporting biological invasion science by identifying trends in alien species distributions, detecting possible vectors and pathways of introduction, classifying potential impacts and pre-screening/assessing their invasiveness into new areas could support the achievement of international policy obligations [e.g. the Convention on Biological Diversity (2010), the United Nation's Sustainable Development Goals (2015), and the 1143/2014/EU

Regulation on Invasive Alien Species].

Freshwater fishes are extremely vulnerable to human pressures, thereby being amongst the world's most threatened species (Duncan & Lockwood, 2001; Darwall *et al.,* 2008). Native fishes inhabiting Mediterranean freshwater ecosystems are forced to survive in harsh and dynamically changing environmental conditions (Vardakas *et al.,* 2017) and any additional stressor, such as the introduction of alien fish species, has the potential to increase the magnitude of impacts (Darwall *et al.,* 2014). Currently, in the updated IUCN Red List, freshwater fish species are classified as one of the most highly threatened taxa, registering alarming percentages: 23% worldwide and 38% when only the European Mediterranean freshwater fish species are considered (IUCN, 2021). In order to reverse these negative trends and decelerate biodiversity loss in the future, a deeper knowledge of the factors affecting this biodiversity change is urgently needed.

Greece, located in southeastern Europe, possesses a unique freshwater ichthyofaunal diversity within Europe and holds one of the highest degrees of fish species endemicity observed in the Mediterranean region (Reyjol *et al.,* 2007; Barbieri *et al.,* 2015). In total, 137 species are native and 47 are considered as country-specific endemics (34% of the native fish fauna that regularly resides in inland waters) (Barbieri *et al.,* 2015). According to the IUCN Red List (IUCN, 2021), 51 freshwater fish species in Greece have been classified as threatened; 20 species as Critically Endangered (CR), 15 as Endangered (EN) and 16 as Vulnerable (VU), corresponding to almost 32% of all the native ichthyofauna in the country. A large number of freshwater fishes are already under severe stress from the harsh environmental conditions and various anthropogenic pressures, as elsewhere in the Mediterranean region, which are further augmented by the introduction and spread of non-indigenous fish species (Economidis *et al.,* 2000; Caiola & de Sostoa, 2005; Hermoso *et al.,* 2011; Kalogianni *et al.,* 2019). On the other hand, non-indigenous fish species (NIFS) are not always undesirable. There is a growing interest among some scientists with a general view that the vast majority of species introductions (especially in freshwater fish taxa) are not identified as having a substantial ecological impact, and instead provide socio-economic benefits (Sagoff, 2007; Gozlan, 2008). For instance, translocations of native carps, eels, trouts and sturgeons could support aquaculture, commercial and recreational fisheries (Perdikaris *et al.,* 2010). In addition, rigorous scientifically-guided translocations (e.g. filling habitats where a species has been extirpated) could be further beneficial in a conservation context, especially when targeting re-introductions of extirpated species or scientifically-led assisted migration strategies (IUCN, 1998; Helfman, 2007; Seddon *et al.,* 2007).

Given the increasing trend of introduced fish species into neighboring countries (Innal *et al.,* 2012; Piria *et al.,* 2018) and the potential threat they pose to the local endemic fish fauna of Greece, it is of paramount importance to unravel the non-indigenous freshwater fish species (NIFS) research in the country, in order to define future research priorities. Therefore, the present study aims to compile the first comprehensive review of NIFS research in Greece. Specifically, we performed an extensive literature review of non-indigenous freshwater fish species research in Greece extending from 1950 to 2020. Our ultimate goal was to answer the following main questions: 1) what are the primary aims of the existing studies, targeting NIFS in Greece 2) is NIFS research in Greece following global trends regarding the rate of published articles over time, 3) what methodological approaches do these studies implement, 4) do these studies assess the invasiveness of NIFS or provide hard evidence of NIFS negative impacts or positive benefits and finally, 5) are these studies funded by national or European funds to target NIFS? The answers to the above questions should assist researchers to comprehend the current status of NIFS research in Greece and to direct national authorities to implement management options in order to alleviate the impacts of NIFS.

Material and Methods

A systematic literature review regarding various aspects of NIFS within Greek inland waters was performed, by applying the PRISMA approach (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher *et al.,* 2010). The search was conducted by using three main web-based bibliographic databases: Google Scholar (https://scholar.google.com), ISI Web of Science (www.webofknowledge.com) and CrossRef (www.crossref.org), on peer-reviewed literature published between 1950 and the cut-off date, 30th April 2020. The search included the following terms: "alien" OR ''non-native'' OR "non-indigenous" OR "exotic" OR "introduced" OR "invasive" AND ''freshwater fish'' OR ''inland fish'' OR ''riverine fish'' AND "freshwater" OR "aquatic" OR "river basin" OR "river" OR "lake" OR "wetland" OR "lentic" OR "lotic" AND "Greece" OR "Europe" OR "Balkans" in the title, abstract or keywords. The review was limited to English-language scientific publications and to relevant subject areas (Agricultural and Biological Sciences, Environmental Science, Earth and Planetary Sciences, Genetics and Molecular Biology). In order to draw up the full publication list, all the information was managed with the reference software Harzing (Harzing, 2007; https://harzing.com/resources/publish-or-perish).

Two independent reviewers (NK and LV) conducted the data extraction. Intercalibration between the two reviewers was obtained based on the results from all studies; in cases of data discrepancies between the reviewers' results, issues were reviewed by NK and then resolved by discussion.

The collected data (see Table S1 for the list of articles eventually included in full analysis) included information on the scope of the studies, the spatial coverage and species diversity, as well as the funding sources of their research. Specifically, the following information was retrieved from each paper (the asterisk (*) in a number of queries indicates that multiple choice answers are allowed): (1) Year of publication; (2) Non-indigenous

species-focused study: yes, no; (3) Study scope*: taxonomy, distribution, ecology/biology, fisheries/aquaculture, other; (4) Realm of the study: aquatic only, aquatic and terrestrial; (5) Aquatic taxa: freshwater only, further taxa; (6) NIFS natural environment: freshwater only, marine, brackish and freshwater; (7) Greece as the main study area: yes, no, partial; (8) Number of river basins: number, countrywide; (9) Ecosystem type*: river, lake, wetland; (10) Number of NIFS: number; (11) Survey type: bibliographical, fieldwork/laboratory, both surveys; (12) Data type: qualitative, (semi)quantitative, both types; (13) Categorization between alien and translocated species: yes, no; (14) Vectors and pathways referred: yes, no; (15) New introduction records: yes, no, not clear; (16) Georeference of introductions: yes, no; (17) Implementation of invasiveness assessment: yes, no; (18) Implementation of impacts assessment: yes, no; (19) Proofs of impacts*: competition, diseases/parasites, food web alterations, habitat degradation, hybridization, predation, socio-economic, none; (20) Benefits referred/reported*: habitat improvement, sport fishing, socio-economic, none; (21) Implementation of management actions: yes, no; (22) Climate change linkage: yes, no; (23) Management measures proposed: yes, no; (24) Funding aimed at NIFS subject: yes, no; (25) Funding sources: Greek, EU, none. To track or interpret trends in the listed references we applied descriptive statistics by using single or combined sets of the compiled data.

Results

The search initially resulted in 4,491 records, while an additional 18 relevant references that met the selection criteria, but were not flagged in the search, were also added to the review list. After discarding any duplicates, the publication list consisted of 2,794 records. Two more review rounds were performed for articles selection using studies indicating or referring NIFS introductions within lentic and/or lotic ecosystems of Greece as the primary criteria. In the first round, articles were screened based on their titles, keywords and abstract and 228 were selected. In the second round, full texts were examined for eligibility and 141 records were excluded with reasons. In total, 87 articles were included in the qualitative synthesis for full analysis (Fig. 1).

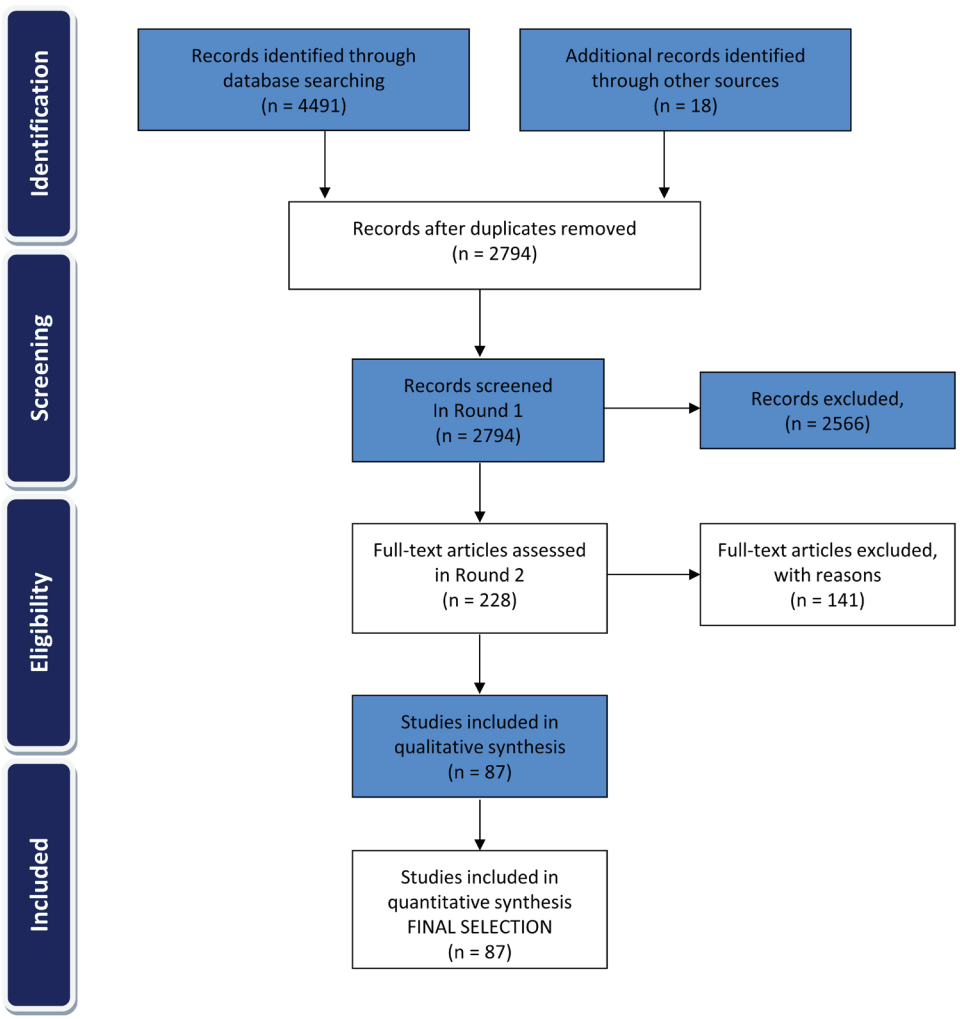

Fig. 1*:* Flow diagram of the methodology and selection process used in this systematic review by applying the PRISMA approach (Moher *et al.,* 2010).

Table 1. Alien and translocated fish species within the inland waters of Greece based on scientific publications; suspected pathways: (AN) angling/fish bait, (AQ) aquaculture, (BC) biological control, (OR) ornamental, and (UN) unintentional or unkown.

Species	Authority	Common name	Pathway	Status
Alien				
Acipenser baerii	Brandt, 1869	Siberian sturgeon	AQ	*Non established*
Acipenser gueldenstaedtii	Brandt & Ratzeburg, 1832	Danube sturgeon	AQ	*Non established*
Acipenser ruthenus	Linnaeus, 1758	Sterlet sturgeon	AQ	*Non established*
Ameiurus cf. *nebulosus*	(Lesueur, 1819)	Brown bullhead	UN	*Established*
Babka gymnotrachelus	(Kessler, 1857)	Racer goby	AN	*Established*
Carassius auratus	(Linnaeus, 1758)	Goldfish	OR	*Established*
Carassius gibelio	(Bloch, 1782)	Gibel carp	AQ/AN	*Established*
Carassius langsdorfii	(Temminck & Schlegel, 1846)	Gin-buna carp	AN	*Established*
Clarias gariepinus	(Burchell, 1822)	North African catfish	AQ	*Non established*
Coregonus albula	(Linnaeus, 1758)	Vendace	AQ/AN	*Non established*
Coregonus cf. *lavaretus*	(Linnaeus, 1758)	European whitefish	AN	*Established*
Coregonus peled	(Gmelin, 1789)	Peled	AQ/AN	*Non established*
Ctenopharyngodon idella	(Valenciennes, 1844)	Grass carp	BC	*Non established*
Gambusia holbrooki	Girard, 1851	Eastern mosquitofish	BC	*Established*
Gymnocephalus cernua	(Linnaeus, 1758)	Eurasian ruffe	UN	*Established*
Hypophthalmichthys molitrix	(Valenciennes, 1848)	Silver carp	BC	*Non established*
Hypophthalmichthys nobilis	(Richardson, 1845)	Bighead carp	AQ	*Non established*
Ictalurus punctatus	(Rafinesque, 1818)	Channel catfish	AQ	*Non established*
Ictiobus sp.	-	-	AQ	*Non established*
Lepomis gibbosus	(Linnaeus, 1756)	Pumpkinseed	OR	*Established*
Micropterus salmoides	(Lacepède, 1802)	Largemouth (black) bass	AQ	*Non established*
Misgurnus fossilis	(Linnaeus, 1756)	Weatherfish	UN	*Non established*
Mylopharyngodon piceus	(Richardson, 1845)	Black carp	BC	*Non established*
Neogobius fluviatilis	(Pallas, 1811)	Monkey goby	AN	*Non established*
Oncorhynchus kisutch	(Walbaum, 1792)	Coho salmon	AQ	*Non established*
Oncorhynchus mykiss	(Walbaum, 1792)	Rainbow trout	AQ/AN	*Non established**
Oreochromis niloticus	(Linnaeus, 1758)	Nile tilapia	AQ	*Established*
Parabramis pekinensis	(Basilewsky, 1855)	White amur bream	AN	*Non established*
Poecilia latipinna	(Lesueur, 1821)	Sailfin molly	OR	*Established*
Polyodon spathula	(Walbaum, 1792)	Mississippi paddlefish	UN	*Non established*
Pseudorasbora parva	(Temminck & Schlegel, 1846)	Topmouth gudgeon	AN/UN	*Established*
Pterygoplichthys joselimaianus	(Weber, 1991)	Gold spot pleco	OR	*Non established*
Pygocentrus nattereri	Kner, 1858	Red piranha	OR	*Non established*
Salmo letnica	(Karaman, 1924)	Ohrid trout	AQ/AN	*Non established*
Salmo salar	Linnaeus, 1758	Atlantic salmon	AQ	*Non established*
Salmo trutta	Linnaeus, 1758	Sea trout	AQ/AN	*Non established*
Salvelinus fontinalis	(Mitchill, 1815)	Brook trout	AQ/AN	*Non established*
Translocated				
Abramis brama	(Linnaeus, 1756)	Common bream	AN	*Established*
Acipenser naccarii	Bonaparte, 1836	Adriatic sturgeon	AQ/AN	*Unknown*
Alburnoides sp.	-	-	UN	*Unknown*
Barbus sperchiensis	Stephanidis, 1950	Sperchios barbel	UN	*Established*
Cobitis hellenica	Economidis & Nalbant, 1996	Louros spined loach	UN	*Established*
Cyprinus carpio	(Linnaeus, 1758)	Common carp	AQ/AN	*Established*

Continued

Table 1 continued

Species	Authority	Common name	Pathway	Status
Economidichthys pygmaeus	(Holly, 1929)	Western Greece goby	UN	*Established*
Esox lucius	Linnaeus, 1758	Northern pike	AN	*Established*
Knipowitschia caucasica	(Berg, 1916)	Caucasian dwarf goby	UN	*Established*
Luciobarbus graecus	(Steindachner, 1896)	Greek barbel	AN	*Established*
Oxynoemacheilus bureschi	(Drensky, 1928)	Struma stone loach	AN	*Established*
Pachychilon macedonicum	(Steindachner, 1896)	Macedonian moranec	UN	*Established*
Pelasgus marathonicus	(Vinciguerra, 1921)	Marathon minnow	UN	*Established*
Pelasgus stymphalicus	(Valenciennes, 1844)	Stymphalia minnow	UN	*Established*
Perca fluviatilis	Linnaeus, 1758	European perch	AN	*Established*
Rhodeus meridionalis	Karaman, 1924	Vardar bitterling	OR/UN	*Established*
Rutilus panosi	(Bogutskaya & Iliadou, 2006)	Acheloos roach	AN	*Established*
Rutilus sp.	-	-	UN	*Established*
Rutilus ylikiensis	(Economidis, 1991)	Yliki roach	AN	*Established*
Salmo cf. *farioides*	Karaman, 1924	West Balkan trout	AQ/AN	*Established*
Sander lucioperca	(Linnaeus, 1756)	Pike-perch	AN	*Established*
Scardinius acarnanicus	Economidis, 1991	Trichonis rudd	UN	*Established*
Scardinius graecus	Stephanidis, 1937	Greek rudd	UN	*Established*
Silurus aristotelis	Garman, 1890	Aristotle's catfish	AN	*Established*
Silurus glanis	Linnaeus, 1758	Wels catfish	AN	*Established*
Squalius orpheus	Kottelat & Economidis, 2006	Maritza chub	AN	*Established*
Squalius peloponnensis	(Valenciennes, 1844)	Peloponnese chub	AN	*Established*
Squalius sp.	-	-	UN	*Established*
Squalius vardarensis	Karaman, 1924	Vardar chub	UN	*Established*
Tinca tinca	(Linnaeus, 1758)	Tench	AN	*Established*

Non established:* generally *O. mykiss* has not been established in Greek freshwater ecosystems, however recently two established populations have been reported (Stoumboudi *et al.,* 2017; Koutsikos *et al.,* 2019b).

NIFS in Greece

Based on scientific publications, NIFS that have been referred within the inland waters of Greece at least once include 37 alien and 30 translocated species (Table 1). Of the 67 NIFS, at least 40 species have established viable, reproductive populations (60%), specifically 12 aliens and possibly almost all (28) translocated species. Angling/fish bait (AN) and aquaculture (AQ) are responsible for the vast majority of introductions (66%), followed by ornamental purposes (OR, 8%) and biological control (BC, 6%), however there was a substantial percentage (24%) of unintentional releases or no pathway data were available (Table 1).

Study focus and research type

All reviewed articles focused entirely on species inhabiting aquatic ecosystems. Overall, 63 studies (72%) exclusively targeted NIFS, while the remaining 24 (28%) NIFS were not within the research scope, even though they referred to the presence of alien and/or translocated fish species (Fig. 2a). The vast majority of the 87 reviewed articles focused exclusively on freshwater fish species (79 studies, 91%) while only a small number of studies (8 cases, 9%) also included other freshwater aquatic taxa (e.g. crayfish, aquatic vegetation, amphibians) (Fig. 2b). In addition, 83 studies (95%) focused solely on freshwater fish species, with a small number of studies (4 cases, 5%) including freshwater and marine fish species (Fig. 2c). Regarding the aims of the studies, most focused on the distributional range of NIFS (37%), followed by ecological and biological aspects of NIFS (28%), and to a lesser extent fisheries and aquaculture issues (8%) or taxonomy (8%) (Fig. 2d). The remaining percentage (19%) under the aim category "other" included a number of articles focusing on legislation issues or ecosystem services.

Only a small percentage of articles (18%) clearly stated new records of NIFS introductions, or reported further dispersal of previously introduced fish species to new localities (Fig. 2e). The majority of the latter studies were published after 2010 (14 of 16), with none published before 2000. Finally, most studies focused on a single NIFS (37 cases, 42%), while in 17% of the articles, more than 10 alien species were included (Fig. 3).

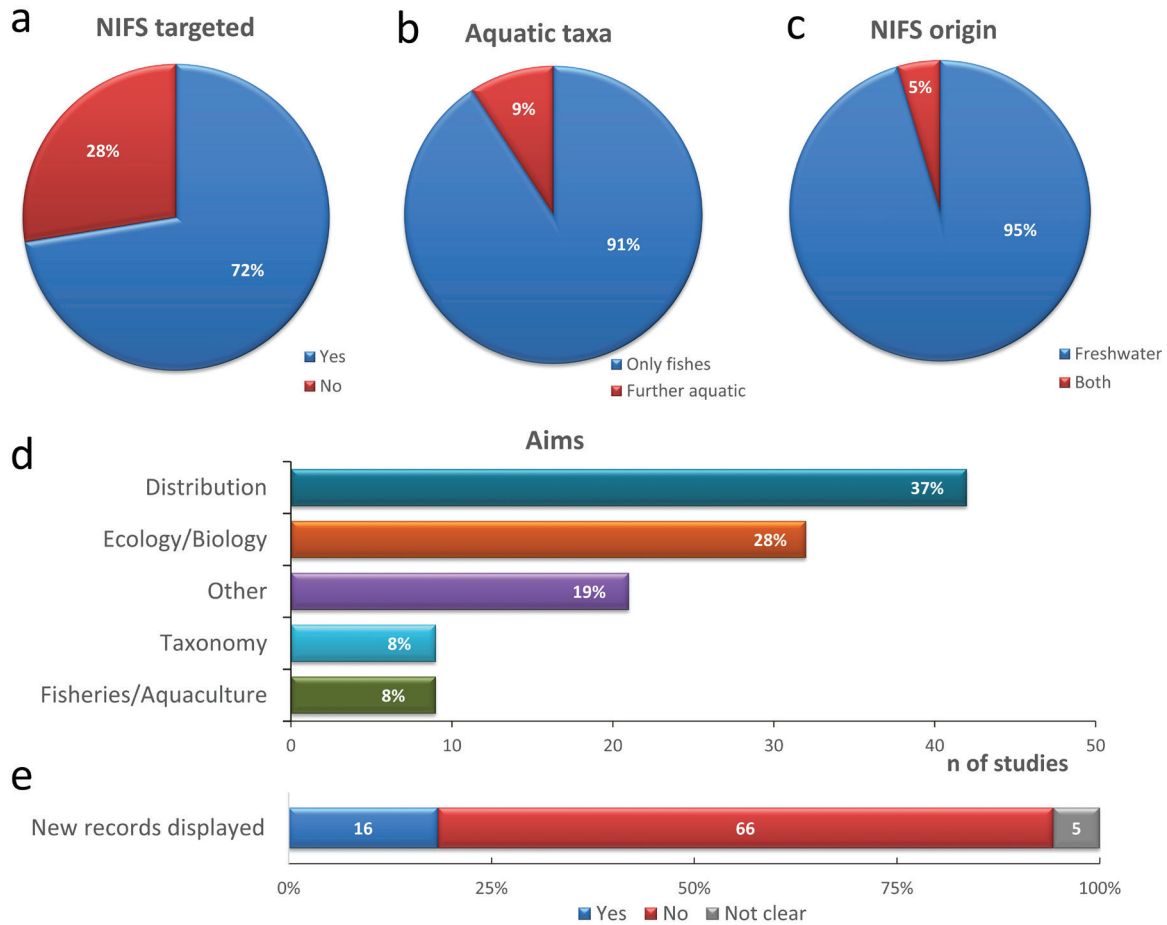

***Fig. 2**:* Focus and type of research: (a) non-indigenous species-targeted study; (b) aquatic taxa researched; (c) NIFS natural environment; (d) aim of study; and (e) new records displayed.

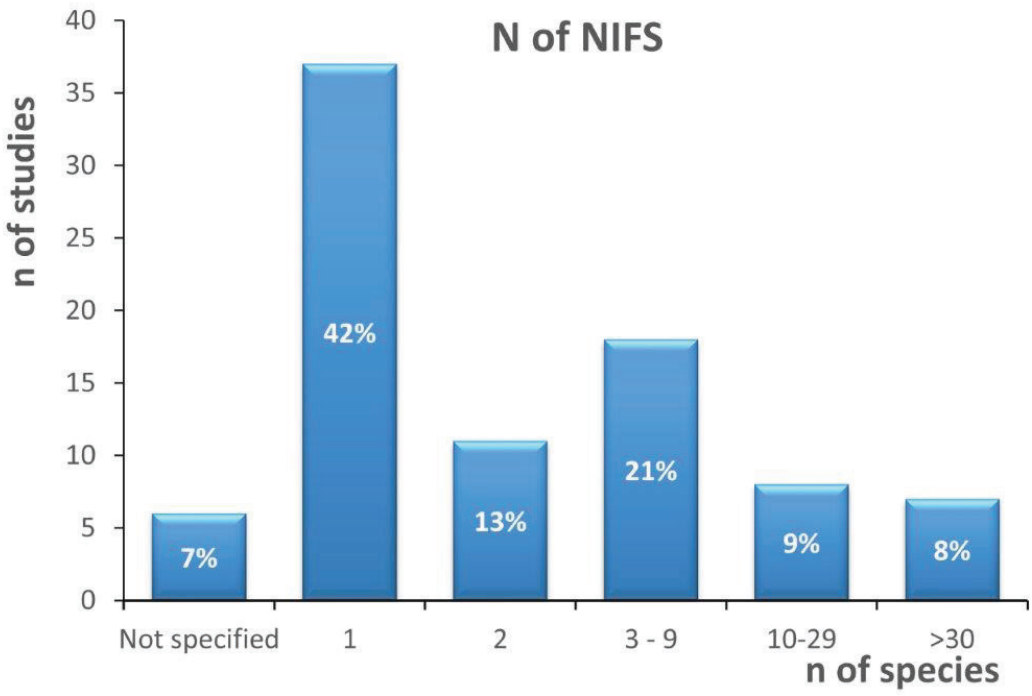

Fig. 3: Number of non-indigenous fish species reviewed per study.

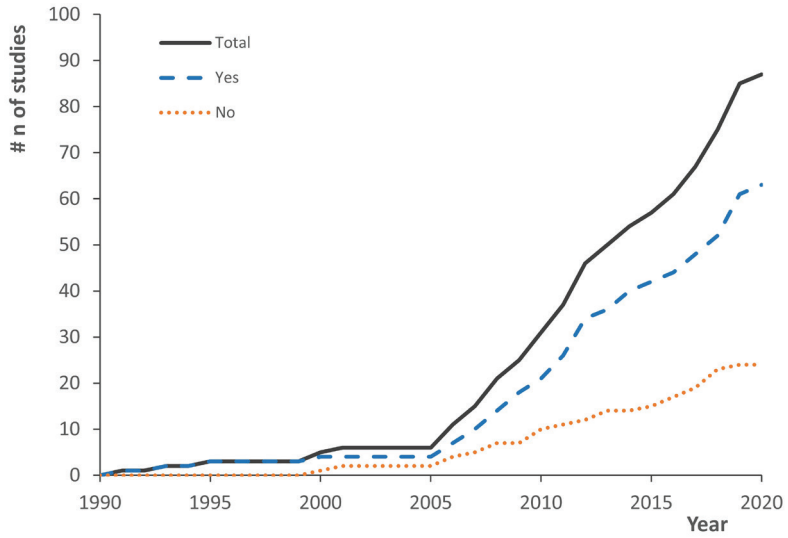

Fig. 4: Cumulative number of studies included in the systematic review, published per year from January 1950 to April 2020. Blue dashed line: studies targeting NIFS (yes); red dotted line: studies not targeting NIFS (no).

Spatiotemporal coverage of NIFS research

Based on our selection criteria that extended from 1950 to 2020, published studies referring to NIFS in Greece started to emerge after the early 1990s (Fig. 4). Within a 15-year period (1990-2005), only a few papers were published with a mean annual rate of 0.4 articles/year (total 6 articles). During the next 5 years (2006-2010), the published articles referring to NIFS rapidly increased with a mean annual rate of 5 articles/year (total 25 articles). Publications slightly increase with a mean annual rate of 5.6 articles/year (total 56 articles) within a 10-year period (2011-2020).

Spatially, more than half of the studies (53%) included Greece as the main study area, while the remaining studies either partially involved Greece as one of the study areas (26%), or included transboundary water bodies, with very few references (if any) to Greece (21%) (Fig. 5a).

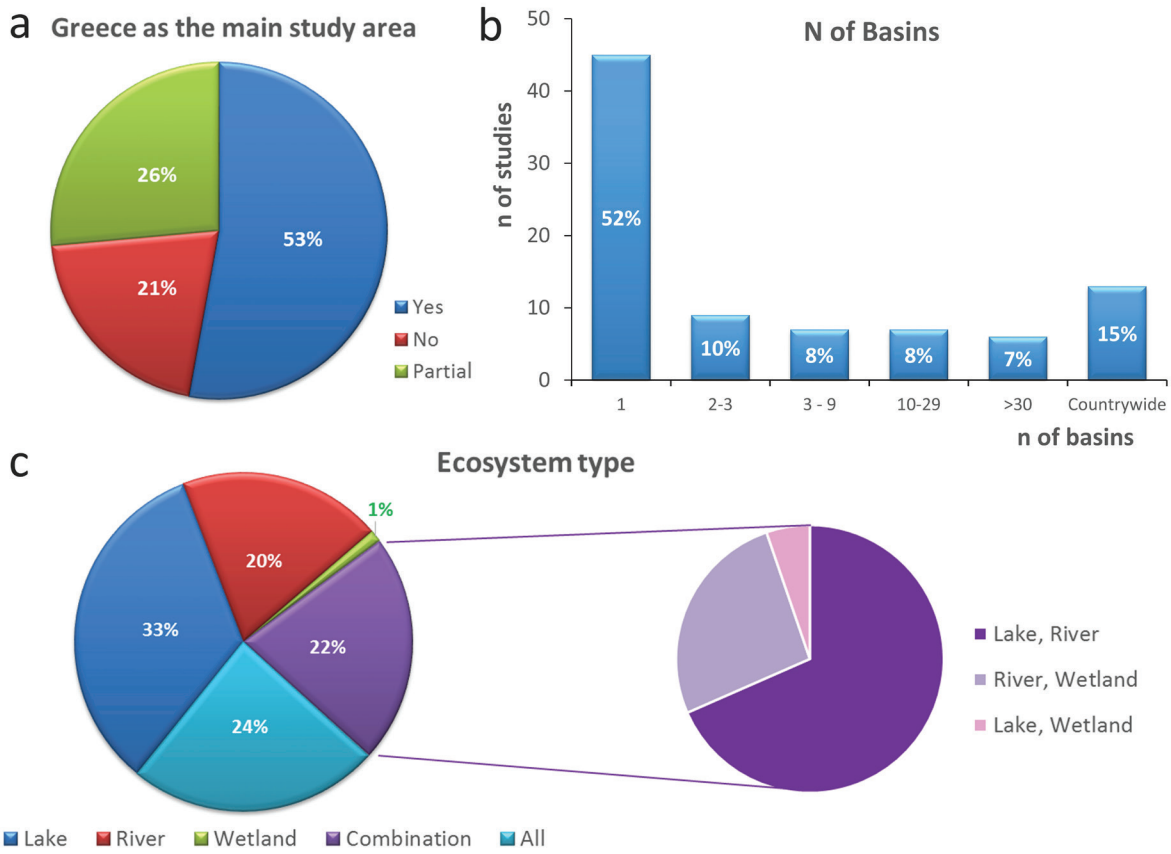

Fig. 5: Spatial coverage of the studies: (a) main study area; (b) number of basins researched; and (c) ecosystem type surveyed.

Among all studies, more than half (52%) concerned species in a single basin, while a noteworthy percentage of articles were either countrywide (15%) or included more than 10 basins (15%) (Fig. 5b). Lentic ecosystems were overwhelmingly represented (33%), in comparison to lotic ecosystems (20%), while studies on wetlands were almost absent (1%) (Fig. 5c). Although wetland habitats per se were not targetted in this study, some lentic and lotic ecosystems are encompased within wetlands and the gap is indicative. However, a substantial number of papers (24%) included case studies concerning either all aquatic environments (lakes, rivers and wetlands) or a combination of these ecosystems (e.g. river-lake (69%), river-wetland (26%), lake-wetland (5%); Fig. 5c).

Methodological aspects

Studies based on fieldwork or laboratory experiments were by far the most common type of research, representing 47% of the studies (Fig. 6a). Literature review studies comprised 35% of the published papers, while 18% of the studies reviewed combined bibliographical survey and field samplings. Overall, 69% of the studies provided qualitative description on NIFS, while 24% offered (semi)quantitative information, with relatively fewer studies (7%) including both qualitative and (semi) quantitative data (Fig. 6b).

Approximately, 85% of the studies included in our review did not distinguish between invasive and non-invasive species (Fig. 6c) or alien from translocated species (63 cases, 72%) (Fig. 6d). In addition, in almost half of the studies (45%) vectors and pathways of NIFS were not mentioned (Fig. 6e). Finally, in only 37 studies (42%) could specific locations of species introductions be obtained from geographical coordinates (Fig. 6f).

Research level and funding

Of the 87 articles, only 10 studies implemented any kind of impact assessment (Fig. 7c). Interestingly, 84% of the reviewed studies did not provided any concrete evidence of NIFS impacts (Fig. 7a). On the contrary, only 11 studies compiled evidence on the impacts of NIFS, with the most common one being competition (4%), predation (2%), food web alteration (2%), homogenization (2%) and socio-economic impacts (2%) (Fig. 7a). In a similar way, the vast majority of the studies (71%) did not demonstrate any benefits of NIFS introduction (Fig. 7b). Moreover, few studies provided evidence on the possible positive effects of NIFS socio-economically (e.g. commercial and recreational fisheries), and in habitat improvement (Fig. 7b). No studies that implemented any management actions concerning NIFS were identified (Fig. 7d) and only 12 articles presented a link between the

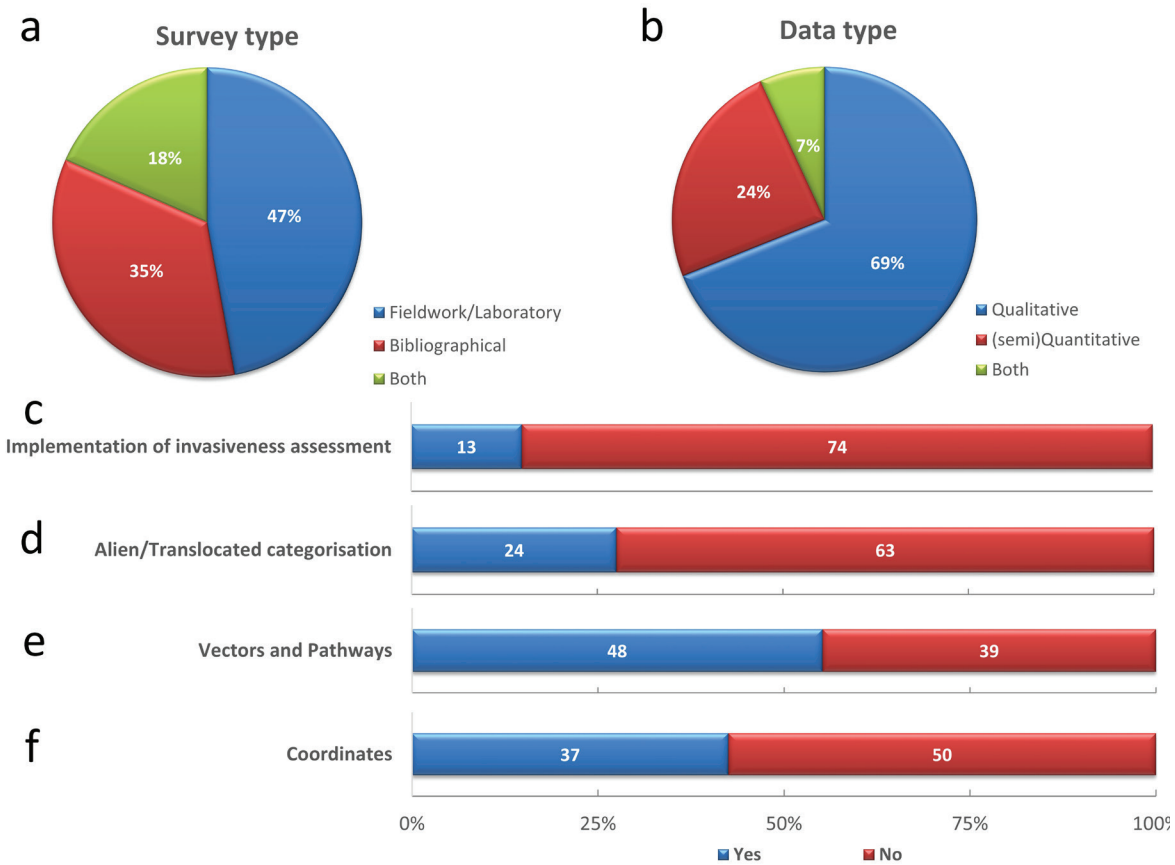

Fig. 6: Methodological aspects of the studies: (a) survey type; (b) data type; (c) implementation of invasiveness assessment; (d) categorization between alien and translocated species; (e) vectors and pathways referred; and (f) georeference of introductions referred.

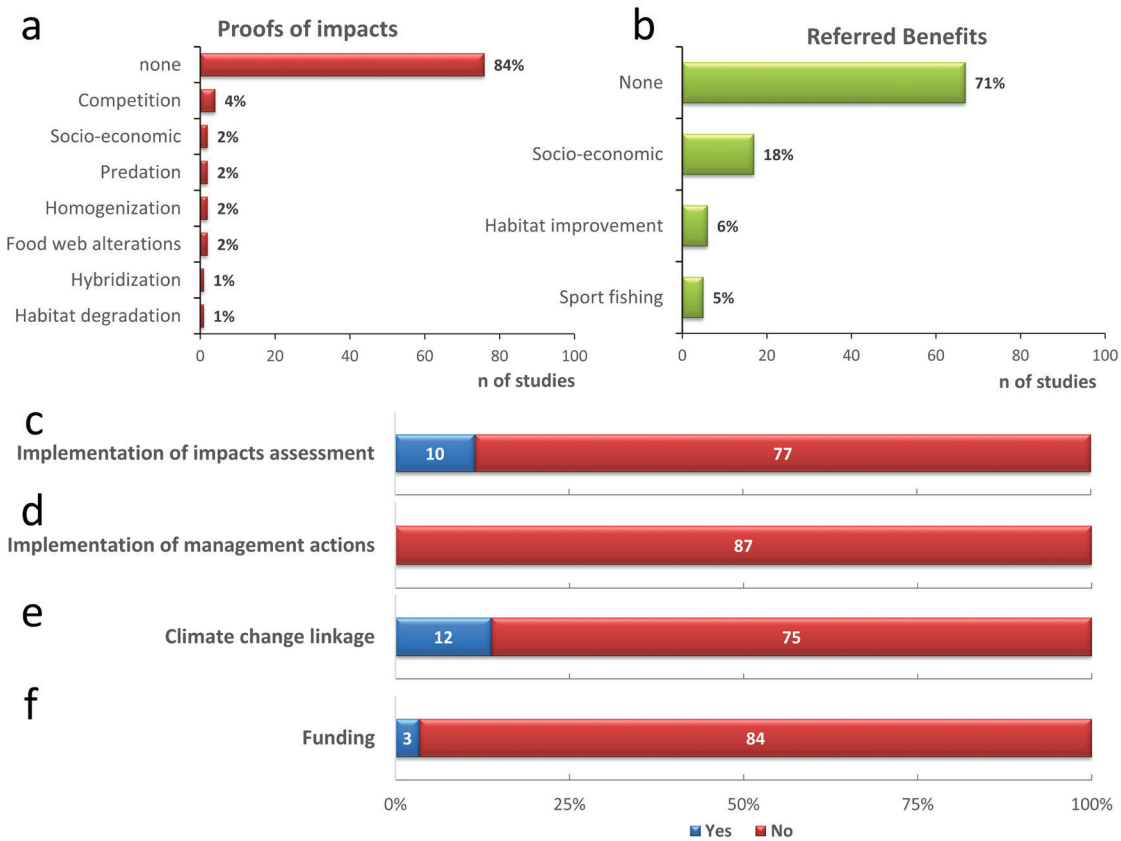

Fig. 7: Research level, risk assessments, management and funding: (a) proof of impacts provided; (b) benefits stated/reported; (c) implementation of impacts assessment; (d) implementation of management actions; (e) climate change linkage; and (f) funding aimed at NIFS subject.

spread of NIFS and climate change (Fig. 7e). Finally, 84 studies (almost 97%), had not received any form of funding targeted to NIFS research (Fig. 7f), while the remaining 3 studies were financed by Greek funding bodies.

Discussion

Main achievements

The present study is the first comprehensive review of scientific publications on the current status of NIFS research in Greece. Former reviews have mainly focused on species compilations and NIFS distributional patterns, while some have attempted to document potential impacts or specific aspects within the aquaculture sector (Economidis *et al.,* 2000; Economou *et al.,* 2007; Zenetos *et al.,* 2009; Perdikaris *et al.,* 2010; Zenetos *et al.,* 2015; Piria *et al.,* 2017; Koutsikos *et al.,* 2019a). Despite the current conservation and ecological interest in freshwater fishes (Economou *et al.,* 2016), the scarcity of historical references reflects the fact that biological invasions in Greece did not capture the interest of ecologists until the late 1990s, even though the first documented alien freshwater fish introduction in the country was reported in 1885 (Holčík, 1991), referring to the pumpkinseed (*Lepomis gibossus*) (Piria *et al.,* 2017), while the most widely known species introduction (*Gambusia holbrooki*) occurred in the mid and late 1920s (Livadas & Sfagos, 1940). Generally, research on NIFS on a global scale widely increased during the first decade of the 21st century and this trend still continues today. It is clear that Greece has lagged behind other European Mediterranean countries in reporting NIFS (Elvira & Almodóvar *et al.,* 2001; Ribeiro & Leunda, 2012; Bianco, 2014; Piria *et al.,* 2017), possible due to limited academic departments/research institutes targeting on this field. In addition, until recently relevant national or European policies to tackle NIFS issues were missing. Despite the delayed start however, Greece is following global trends regarding the number of published articles over time (Koutsikos *et al.,* 2019a; Koutsikos *et al.,* 2021a).

The majority of studies concerning NIFS in Greece have conducted basic descriptive statistics and analyses. Commonly, most studies focus exclusively on the geographical distribution of NIFS since this type of research is straightforward and widely practiced, by providing new documentation of NIFS first occurrences from field observational studies. Until recently such new documentation was relatively easy to publish. Despite the fact that almost half of the papers were field observational studies, most of them used only qualitative (plain presence/absence) data (~70%). Research merely based on presence/absence data lacks the ability to provide details on the nature of species introductions and their potential impacts. More in-depth studies utilizing quantitative data (e.g. abundances, densities, compositions) or a combination of both data types may provide support in demonstrating

broad general research issues in invasion biology, in order to draw solid conclusions or give meaningful insights to complex invasion processes (Koutsikos *et al.,* 2019a,b). In addition, more laboratory studies combined with in situ experiments are also needed in order to shed light on NIFS physiology, ethology, pathology etc. Finally, very few studies attempted to pre-screen/assess the invasiveness of NIFS in Greece (Perdikaris *et al.,* 2016; Vilizzi *et al.,* 2019) and even fewer provided hard evidence of the negative impacts (Kalogianni *et al.,* 2019) or positive benefits (Perdikaris *et al.,* 2010) of NIFS. To improve our understanding of biological invasions, it is necessary to examine in greater depth the effects of invasive species on native biota and ecosystems, including the effects in different habitat types in terms of management, restoration and broader socioeconomic costs and benefits.

What appears to be missing and why?

According to our results, research funding on NIFS in Greece is insufficient relative to the potential threats and uncertainties involved with the spreading impacts. The vast majority of the studies (~97%) include NIFS only incidentally (as a by-product of another research target) and are not directly funded by any European or national resources. There is a disconnection between the existence and/or the quality of Greek research proposals versus suitable calls and/or the allocation of funding resources specifically with concern for NIFS. This science-policy disconnection is profound and should be ameliorated through the ongoing implementation of European environmental policies and directives, including specific legislations such as the EU Regulation 1143/2014 on invasive alien species. The core of the IAS Regulation is the list of invasive alien species Union concern, yet it needs an urgent update, at least for freshwater fish species, in order to be adapted to the needs of each member state through focused assessments for invasiveness risks. Another example of this disconnection is that site selection for fish farms in freshwaters are not related to the Multiannual National Strategy Plan for the Development of Aquaculture (currently at the stage of reform until 2027, and it will soon be on public consultation; APC, 2021). In this plan, the country's current production of rainbow trout (i.e. about 4,000 tons) is planned to increase to 6,000 tons by the year 2030, while at the same time a similar national plan is also mandatory for Greece's neighbor and EU member state Bulgaria. In addition, it should be noted that the socio-economic difficulties during the last decade in Greece may have interfered with the promotion of the NIFS issue, as they have in other ecological restoration and biodiversity enforcement areas (Katsanevakis *et al.,* 2015; Zogaris *et al.,* 2017).

Although the limited research funding for "non-charismatic" inland water species is generally commonplace in Greece, our results show a substantial increase in the number of articles targeting NIFS in Greece within the last 15 years (2006-2020). Despite this recent increase in scientific interest, there are still important unmet research needs and several challenges in using the available scientific knowledge in management and conservation contexts.

Important outcomes of relevant science on NIFS in Greece indicate that only a limited number of alien fish species are widespread and abundant throughout the country's lotic ecosystems, while most aliens have a fairly restricted spatial distribution (Koutsikos *et al.,* 2019a). In the early 1990s, Greece was considered one of the least altered countries of Europe in terms of NIFS (Bianco, 1990). Nowadays however, there are worrying indications of the expansion of rather few nuisance species, forming alien fish assemblages especially in the larger river basins (Koutsikos *et al.,* 2021a). In addition, many highly invasive species have been recorded in large natural lakes (Perdikaris *et al.,* 2010; Petriki *et al.,* 2014; Catsadorakis *et al.,* 2018). Major challenges have emerged particularly in lentic environments displaying higher numbers of NIFS often overlapping with critically endangered species distributions (Koutsikos *et al.,* 2021b). Specific areas are particularly vulnerable entry-points for alien and translocated freshwater fishes and other non-indigenous aquatic biota, especially the transboundary rivers in northern Greece, such as Evros, Strymon and Axios (Ozulug *et al.,* 2018; Karaouzas *et al.,* 2020). On the other hand, other areas are obviously better studied with frequent reference to aliens, such as the major lakes of Northern Greece, particularly Greater Prespa (Shumka & Apostolou, 2018), while the majority of the country is still poorly explored; mainly the smaller river basin areas and artificial reservoirs in the southern half of the country and the islands. Ongoing tracking and data management is critically important beyond the initial scientific descriptions (Brooks *et al.,* 2004). There are many aquatic ecosystems in Greece (lakes, ponds, wetlands and small lotic habitats) that are not adequately surveyed for the presence of fish species, thus baseline species inventories and monitoring of aliens and translocated fishes are vital sources of information for researchers and policy makers.

Beyond the critical need for routine inventory and monitoring, our review provides proof that there is a remarkable lack of published studies on the following important aspects concerning NIFS and their management:

- The impacts of NIFS to ecosystem services have been studied in only a few lentic environments in Greece; impacts in wetlands and lotic environments are scarce; this is in contrast to efforts made throughout Europe in the marine environment (e.g. Katsanevakis *et al.,* 2014). The causal factors (invasive traits, propagule pressure, etc) by which species invade new areas and novel environments has also been largely neglected (Lowry *et al.,* 2013); in Greece mostly assumptions are often put forward, while evidence is lacking.
- Alien fishes have featured poorly as priority issues in Greece's protected areas; many best practice and pilot studies including adaptive applications could be better developed within protected areas (Abel *et al.,* 2007). The issue of tackling alien and translocated fishes in protected areas could supplement habitat restoration initiatives; this is sorely needed in

Greece's Natura 2000 network (Zogaris *et al.,* 2017; Koutsikos *et al.,* 2021b).

- Alien species are often omitted or combined with native fish fauna in river monitoring assessments within the context of the Water Framework Directive (WFD) (Ruaro *et al.,* 2021). Since rather few alien species are recorded in most routine surveys in Greek rivers, these are usually lumped within the whole fish assemblage as in other EU countries (Zogaris *et al.,* 2018). A revision of metrics in current bioassessment indices should be attempted to include aliens as indicators of ecosystem degradation.
- Citizen science concerning inland water fishes is quite limited in Greece and could be an additional source of early NIFS detection. This is in contrast with studies conducted in marine environments in the Mediterranean where there is widespread interest in recording, frequent publishing and tracking trends (e.g. Bianchi *et al.,* 2014).
- Transboundary rivers entering the regions of Thrace and Macedonia are critically important arrival routes for many new alien species. This problem has a socio-political aspect that requires steps to explore and identify possible solutions; these must include international cooperation and specific steps for enforcement (Dimitriou *et al.,* 2012; Ozulug *et al.,* 2018).
- The ornamental fish trade in Greece is nearly uncontrolled since species capable of establishing non-indigenous populations have been recorded while no risk assessments to scrutinize invasive species are implemented by the related authorities (Papavlasopoulou *et al.,* 2014). Appropriate legislations and trade restrictions should be urgently implemented regulating both regular and internet-based trade of aquatic organisms in order to prevent any future undesirable species introduction.
- Research studies on disease transmissions through the aquarium trade or the aquaculture sector to wild freshwater fish populations are currently missing in Greece. However, the fact that no disease has been yet reported for wild populations leads to the suggestion that they may be at least uncommon (Koutsikos *et al.,* 2019b).
- Besides the customary identification of alien species (i.e. alien on a country scale), the issue of intra-country translocated alien species is largely ignored in Greece, despite few specific references (Economidis *et al.,* 2000; Economou *et al.,* 2007). Unfortunately, for many closely related sister species, there are difficulties in detecting, identifying and assessing most translocated species (Koutsikos *et al.,* 2019a). The issue of translocated species seems to be a growing problem in many Mediterranean and Balkan countries and interest in Southeastern European countries is comparatively recent. Molecular methods are critically important to identify and confirm most such translocated species since they may be overlooked in visually-based identification during routine surveys (Grapci-Kotori *et al.,* 2020).

Future prospects and a way forward

We identified several topics that should be thoroughly addressed in the future. Baseline applied monitoring methods targeting NIFS in Greece have not yet been developed. For instance, there is a distinct lack of studies on quantitative monitoring of NIFS in Greece. Apart from the national "Monitoring Project for the Ecological Quality of surface waters" according to WDF that could provide temporal data (Economou *et al.,* 2016; Koutsikos *et al.,* 2019a), most research projects have a restricted time frame (usually less than three years), which is insufficient to cover population dynamics and demographic trends of NIFS. In addition, future studies should investigate possible interactions among highly invasive NIFS (particularly *Gambusia holbrooki*, *Carassius gibelio*, *Lepomis gibossus* and *Pseudorasbora parva*) with native fish species and especially targeting threatened native species and ecosystems with highly endemic faunas. Finally, studies on population genetics of alien and intra-country translocated alien species should be conducted in order to unravel cryptic species or unintentional translocations that may cause severe impacts on the native fish fauna. In fact, the issue of intra-country translocated species has been widely neglected, not just in Greece (Vitule *et al.,* 2019). Alien species are currently altering native fish assemblages and the biogeographic integrity of freshwaters; this will be very difficult to restore and manage. An important target in managing future impacts of NIFS rests on taking massive action to prevent new invasions (Strayer, 2010) and this should include a strong socio-political and educational component.

In conclusion, current gaps in the knowledge of NIFS research in Greece outlined in the present review, will be valuable in setting vital priorities for future research. In particular, research should focus on defining national targets for detecting and controlling invasive alien species and examining previously unexplored topics, such as interspecific interactions with native biota and the potential introduction of new NIFS or the dispersal of those already inhabiting inland waters under climate change scenarios.

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Supplementary data

The following supplementary information is available online for the article:

Table S1. The list of 87 fully reviewed articles.