Alien and Translocated Fish Species
in lotic ecosystems of Greece

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Alien and translocated fish species in lotic ecosystems of Greece

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Ξενικά και αλλότοπα είδη ιχθύων στα οικοσυστήματα των ρεόντων υδάτων της Ελλάδας

Νικόλαος Κούτσικος

Η παρούσα Διατριβή
κατατέθηκε στο Τμήμα Περιβάλλοντος,
Σχόλη Περιβάλλοντος,
Πανεπιστήμιο Αιγαίου
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για την απονομή του τίτλου
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ΥΠΕΥΘΥΝΗ ΔΗΛΩΣΗ

Είμαι ο αποκλειστικός συγγραφέας της υποβληθείσας Διδακτορικής Διατριβής με τίτλο «Ξενικά και αλλότοπα είδη ιχθύων στα οικοσυστήματα των ρεόντων υδάτων της Ελλάδας». Η συγκεκριμένη Διδακτορική Διατριβή είναι πρωτότυπη και εκπονήθηκε αποκλειστικά για την απόκτηση του Διδακτορικού διπλώματος του Τμήματος Περιβάλλοντος κάθε βοήθεια, την οποία είχα για την προετοιμασία της, αναγνωρίζεται πλήρως και αναφέρεται επακριβώς στην εργασία. Επίσης, επακριβώς αναφέρω στην εργασία τις πηγές, τις οποίες χρησιμοποίησα, και μνημονεύω επώνυμα τα δεδομένα ή τις ιδέες που αποτελούν προϊόν πνευματικής ιδιοκτησίας άλλων, ακόμη κι εάν η συμπερίληψή τους στην παρούσα εργασία υπήρξε έμμεση ή παραφρασμένη. Γενικότερα, βεβαιώνω ότι κατά την εκπόνηση της Διδακτορικής Διατριβής έχω τηρήσει απαρέγκλιτα όσα ο νόμος ορίζει περί διανοητικής ιδιοκτησίας και έχω συμμορφωθεί πλήρως με τα προβλεπόμενα στο νόμο περί προστασίας προσωπικών δεδομένων και τις αρχές Ακαδημαϊκής Δεοντολογίας.

PRELIMINARY NOTE

The present dissertation includes published papers on peer-reviewed journals or manuscripts in a final draft version. These articles are the output of collaborative effort reflected in the list of co-authors.

As doctoral candidate, I was responsible for the scientific design followed during this study, the development of datasets, the statistical analyses and the writing. The advisors were intensely involved on the dissertation's conceptual framework and on all stages of work development, and also as co-authors of the articles. Moreover, additional colleagues supported this dissertation by providing assistance in field work, laboratory work, critical opinions, informal discussions, etc. Whereas the dissertation is a personal synthesis, I declare that I was highly benefited directly by the participation of all co-authors.

The ichthyological data for this study were obtained within the framework of several National as well as European research projects: i. Ichthyological Index for upland rivers and streams; ii. RIPIDURABLE – Gestion durable de ripisylves, [INTERREG III C SUD]; iii. Study of the ichthyofauna and recommendations for its conservation at the hydroelectric dam of Ilariona; iv. Monitoring river ecological quality in Eight Regions of Greece; v. Biodiversity of Attika Wetlands; vi. National monitoring and recording of the Water Status in Greece (2012-15, 2018-23; 2000/60 WFD); and vii. Development of an integrated management system for river basin, coastal and marine zones (KRIPIS). Finally, a number of field surveys were carried out with the individed help of several colleagues voluntary under no specific research programme.

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“There are two things in life
for which we are never truly prepared:
twins.”
— Josh Billings

For
Triantafyllos-P. and Vaggelis-Ch.

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OUTLINE

The dissertation is divided into one introductory section and six (6) chapters.

GENERAL INTRODUCTION provides background information on fish species introductions and several aspects of invasion biology. Legislation, implications and future perspectives concerning non-indigenous fish species are also discussed. The study aims of the dissertation are presented.

CHAPTER 1 presents a bibliographical review of alien freshwater fish species inhabiting Balkan’s inland waters and focuses on Greece, providing a historical overview of introductions, their impacts, as well as the current dispersal of alien fishes in the region.

CHAPTER 2 tests a readily transferable screening procedure on invasive patterns of alien and translocated fish species in lotic ecosystems of Greece at different spatial scales, contributing to the application of the EU Regulation on IAS; suggesting gaps and uncertainties, and proposing conservation and management actions.

CHAPTER 3 develops a novel classification framework based on network analysis to identify and prioritize non-indigenous fish assemblage types in lotic ecosystems, rather than focusing on particular species. Results contribute to the design of effective post-invasion management actions dealing with specific NIFS assemblages and provide valuable information for the protection of high-priority water bodies.

CHAPTER 4 evaluates the establishment and the spreading potential of the sailfin molly worldwide, with emphasis in Europe and the Mediterranean, as target regions, through the use of climate matching.

CHAPTER 5 deals with one of the world's worst alien invasive species, the rainbow trout, and assesses its establishment , in Greek lotic ecosystems and explores the factors affecting the success or failure of establishment.

CHAPTER 6 explores the potential use of introduced species into scientific research, by investigating the presence and abundance of microplastics within Kifissos (Attica) river basin, via translocated fish species as bio-indicators.

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EXTENDED SUMMARY

Native freshwater fishes inhabiting Mediterranean lotic ecosystems survive in harsh and dynamically changing environmental conditions and many populations live near the edge of their physiological limits. Major threats related to hydrological fluctuations and hydromorphological alterations of habitats, make Mediterranean rivers and streams even more vulnerable, since their conditions are changing further and threatened in various ways. In fact, a large number of species are now under severe stress due the latter anthropogenic activities. Any additional anthropogenic stresses, such as the introduction of non-indigenous fish species (hereafter as NIFS), has the potential to increase the magnitude of threats, and also to incur changes in the biogeographic characteristics over various spatial scales. Hence, while the Mediterranean region is a hotspot of freshwater fish diversity, it has become one of the top global invasion hotspots.

Riverine ecosystems within the Balkan Peninsula are characterized by high biological diversity and endemism, and NIFS represent a major threat for their biodiversity. To date, 60 fish species have been introduced, to the Balkan Peninsula, of which 36 have become naturalised in inland waters. Since the Balkans are one of the world's 35 biodiversity hotspots, this large presence of alien fish species poses a serious threat for the integrity of freshwater ecosystems, the survival of the native ichthyofauna, and biodiversity in general. The reasons behind the introductions, and the historical timeline, vary within individual Balkan countries. Despite recent attempts to implement and align legislations aimed at preventing the introduction of potentially invasive species, and the implementation of rigorous controls of introductions and increased protection of open waters, today the majority of introductions remains intentional, primarily via aquaculture. The first chapter focuses on Greece, and provides a historical overview of introductions, the reasons behind them, their impacts and the current dispersal of alien freshwater fishes in the Balkans. The current issues, implications and future perspectives concerning alien non-indigenous fish species are also discussed.

Mediterranean running waters are poorly monitored for NIFS and since these systems are stressed by multiple anthropogenic pressures, it is important to build robust procedures to track NIFS distribution and spread. Therefore, in the second chapter, multi-faceted assessment of NIFS in the lotic ecosystems of Greece at different spatial scales is applied by providing: a) a historical review of temporal patterns and entry pathways of fish introductions in river basins of Greece (140 basins) across 100 years; b) an analysis of occurrence and abundance data of NIFS assemblages at the lotic site scale (644 electrofished sites); c) the mapping of NIFS distributional patterns at river basin (75 basins) and regional scales (7 freshwater ecoregions); and, d) a vector analysis of fish translocations using an ecoregional framework. In total, 55 NIFS were recorded (25 alien and 30 translocated); however, there is a low incidence of NIFS in lotic waters at the site scale (30 NIFS recorded in the field samples; 10 alien and 20 translocated). NIFS introductions in Greece appear to be influenced by specific socio-historical periods, indicating a gradual increase since late 1970s. Despite this increase, our study provides evidence that only four alien species are currently widespread and common in the rivers and streams of Greece: *Gambusia holbrooki, Carassius gibelio, Pseudorasbora parva, and Lepomis gibbosus* (in order of recorded abundance). NIFS tend to be absent or distributed in very low numbers in upland streams and in smaller river basins. However, the issue of translocated fish species is shown to be a sorely neglected problem that is difficult to track. This chapter a) tests a readily transferable screening procedure, b) contributes to the application of the European Union Regulation on Invasive Alien Species, c) suggests gaps and uncertainties, and finally d) proposes conservation and management actions.

The design and implementation of appropriate management actions to tackle the spread and negative impacts of non-indigenous fish species (NIFS) in freshwater ecosystems still remains a complex task. So far, aquatic managers either apply risk assessments of the invasiveness potential of a single species, during the pre-invasion phase, or implement more drastic post-invasion measures, with, often however, limited success and/or, more importantly, unintentional negative impacts on the native fauna. In an effort to address the limitations of current managerial approaches, our study develops and applies a classification framework to define non-indigenous fish assemblage types (FATs) in Mediterranean riverine ecosystems and identifies the linkage with various regional, local, biotic and abiotic environmental factors; this framework contributes to a pre-invasion stage screening and to the design of effective post-invasion "tailor made" management actions dealing with specific NIFS assemblages, rather than focusing on single species. A network analysis approach was applied in order to extract NIFS community level information from 393 samples obtained from 51 river basins of Greece, while a multivariate ordination analysis was applied to detect the factors best explaining the structure and distribution of FATs. Lastly, the association patterns of the study's inputs and outcomes are illustrated through an alluvial diagram, providing insights across different spatial scales. In total, five FATs were generated where the major NIFS contributors of average similarity within each modularity class revealed the key indicator species (*Gambusia*–FAT; *Carassius/Lepomis*–FAT; *Pseudorasbora*–FAT; Salmonids–FAT and Carp–FAT). Overall, the identified FATs varied spatially, indicating different community structure, mainly based on the diverse habitat preferences and life-history traits of indicator species. Alien FATs were mainly distributed within large and transboundary rivers, while Translocated and Salmonids FATs mostly occupied ecoregions with relatively depauperate faunas and often in biodiversity hotspots. The results of this study can identify conservation priorities within FATs, inform specific-type post-invasion management actions tackling NIFS, while in addition may provide valuable information for protecting high-priority water bodies before invasion.

The invasion process contains a series of stages, from introduction, survival and reproduction to dispersal, with species having to overcome specific barriers to reach the next step, with differing invasion success. Thus, predicting the establishment as well as the spread of alien species may help to establish management actions and to prevent future invasions. Chapters four and five provide insights into current distribution, establishment status and potential spread of two alien fish species, through the use of spatial distributions, demographic criteria, propagule pressure, climate matching and other environmental factors. The two case studies were based on a) the limnophilic *Poecilia latipinna*, a popular ornamental fish, that has been introduced throughout the world and b) the rheophilic *Oncorhynchus mykiss*, probably the most widely introduced fish species globaly via aquaculture and fisheries.

Particularly, the aim of the fourth chapter was to evaluate the spreading potential of the sailfin molly (*P. latipinna*) worldwide, with emphasis in Europe and the Mediterranean region. Literature review conducted for this chapter, indicated that the sailfin molly presents a non-indigenous occurrence in 29 countries worldwide with a total of 100 occurrences, predominantly within tropical and subtropical climatic zones, usually clustered near ornamental trade centers and in areas affected by malaria. Its invasion potential assessed by Climatch tool indicated a low environmental match (5% of the assessed sites) within Europe where in the peri-mediterranean area this percentage rose to 60%, despite the fact that the species is recorded at a single location. The overall distribution of *P. latipinna* reveals a warm-stenothermic tolerance. The findings suggest that intolerance to prolonged temperature drops is the leading factor constraining establishment success in Europe, but it is not the sole determinant of invasive spread. Constrains on the spreading capacity of sailfin molly are imposed by a combination of propagule supply, other ecological constraints, as well as of reduced reproductive or physical fitness due to breeding manipulations of commercially available strains.

Continuing with NIFS, the fifth chapter aimed to assess rainbow trout establishment in Greece and explored the factors affecting the success or failure of establishment. Rainbow trout (*O. mykiss*) is ranked as one of the world's worst alien invasive species; in Europe, however, the extent of established populations remains localized and poorly reported. Fish samples and site-specific environmental attributes were analysed. All available literature on the distribution patterns of rainbow trout were reviewed in parallel to those of the Greek native *Salmo* trout species and demographic criteria were applied to infer potential establishment. Data indicating poor persistence of populations and population structure support the argument that recruitment of rainbow trout is extremely limited in Greece. Lack of suitable environmental conditions is not the main factor leading to the failure of rainbow trout to become established. Genetic factors affecting reproduction, possibly through a combination of outbreeding depression resulting from the admixture of unrelated intraspecific lineages, and maladaptive behaviour resulting from domestication in captivity, remain probable causes of poor establishment for the Greek populations of rainbow trout. Overall, the threat of *O. mykiss* as a highly invasive species in Greece is lower than suggested by recent risk assessments.

For decades now, the use of plastics has provided a plethora of applications and socio-economic benefits. However, an unsustainable use of substantial amounts combined with the inappropriate waste management of plastics have created an emerging, harmful contaminant for natural ecosystems and their biota called microplastics. Microplastics are synthetic polymers smaller than 5 mm and created by fragments of larger plastics due to processes such as photo-degradation, and physical, chemical or biological interactions. So far, most research efforts on microplastics have been focused on the marine environment and their impacts on marine organisms. Despite of the fact that the vast majority of plastic waste (~80%) derive from terrestrial sources and pass initially through freshwater lotic ecosystems, which may act as natural filters for the marine environment, studies of plastic contamination in freshwater ecosystems and their biota remain quite scarse. Non-indigenous fish species with widespread distribution, high level of biomass and dominance, ease of capture and ability to adapt laboratory settings could be indicative of reflecting the abiotic and biotic state of a freshwater environment. Thus, biomonitoring investigations based on translocated fish species of a given area may provide valuable information regarding pollution and its impacts on native fish species and/or their sister species populations within the country. The aim of the last chapter was to explore the potential use of introduced species into scientific research, by utilizing translocated fish species (*Squalius vardarensis*) as bio-indicators in order to detect the occurrence and the abundance of microplastics in Kiffisos River (Attica) in Greece. The study area was selected due to the fact that Kifissos Att. River is a heavily modified urban river and vastly impacted due to the insertion of high loads of point and non-point source wastes. The river flows through the largest part of the Metropolitan area of Athens and is expected to uncover pollution from microplastics.

Overall, 321 microplastic items were detected within the water sample, while 16 mesoplastic (5 mm to 2 cm) were also identified and excluded from further analyses.

The abundance of microplastics in the freshwater column calculated at 8.1 items/m^3. The major polymer types of microplastics identified by FT-IR analysis were: polyethylene (PE), polyvinyl alcohol (PVA) and polypropylene (PP). Results indicated moderate prevalence and abundance of microplastics in *S. vardarensis* specimens, with almost the one third of fish specimens (35%) contained microplastics. In total, 19 microplastics were found in 11 specimens of *S. vardarensis*. Thus, outcomes suggested that the gastrointestinal tract of non-indigenous fish species could be used as a tool for the qualitative assessment of microplastics pollution, as they provide a suitable bio-indicator for freshwaters, especially for urban ecosystems.. Although more thorough research is needed to assess the microplastic contamination of river sediments and whether the microplastic ingestion could compromise the health of fish species or whether these effects are dependent on species traits, feeding habits and/or plastic type.

Concluding, the introduction of new fish species into lotic systems of Greece represents a relatively recent but constantly rising threat, impacting indigenous species through ecological processes, interspecific hybridization and new diseases or parasites. The lack of organized monitoring of NIFS and scarcity of quantitative data at the local scale presents an important obstacle to the flow of accurate information needed to support NIFS prevention and management measures in Greece. However, based on the results of the current study, despite the increasing entry of introduced fish species in Mediterranean-climate freshwater ecosystems, only a small group of alien species is widespread and has invaded the lotic waters of Greece. The most neglected and insidious NIFS problem within Greek riverine ecosystems concerns translocated species, which could generate impacts on native ichthyofauna that may exceed the impacts of alien species.

On the other side of the coin, alien and translocated fish species are not all bad or undesirable. While rainbow trout has been listed among the worst invasive alien species in the continent, it plays a vital role in the economy as the backbone of European inland aquaculture and fisheries. Moreover, in many cases there are beneficial and suitable reasons for scientifically-guided introduction, for example "conservation translocations" for a very few threatened species, including range-restricted poor-dispersing endemics in Greece. In addition, the use of introduced species to scientific research, instead of using individuals of indigenous species, could be of a great value to the native ichthyofauna.

It is almost certain that new introductions will continue in Greece due to increasing river reservoir/water development works, a continuous angling interest, low public biodiveristy awareness, and very poor law enforcement. However, preventing further harmful NIFS introductions remains the most important and immediate measure needed in country’s inland waters. Greece has the opportunity to prevent the spread of NIFS in its river, stream and spring waters, since many areas are still free from NIFS.

ΕΚΤΕΝΗΣ ΣΥΝΟΨΗ

Τα ενδημικά είδη ψαριών των εσωτερικών υδάτων και συγκεκριμένα αυτά των ρεόντων οικοσυστημάτων της Μεσογείου, συχνά επιβιώνουν σε ακρέες και δυναμικά μεταβαλλόμενες περιβαλλοντικές συνθήκες, ενώ πολλοί από τους πληθυσμούς τους ζουν κοντά στα φυσιολογικά τους όρια. Οι μεγάλες απειλές που σχετίζονται με τις υδρολογικές διακυμάνσεις και τις υδρομορφολογικές μεταβολές των ενδιαιτημάτων καθιστούν ακόμη πιο ευάλωτα τα εν λόγω οικοσυστήματα, καθώς οι συνθήκες τους μεταβάλλονται περαιτέρω απειλώντας τα επιπλέον με διαφόρους τρόπους. Στην πραγματικότητα, ένας μεγάλος αριθμός ειδών υφίσταται ήδη έντονες πιέσεις λόγω αυτών των ανθρωπογενών δραστηριοτήτων. Οποιαδήποτε λοιπόν επιπρόσθετη ανθρωπογενής δραστηριότητα σε αυτά τα πλέον ευάλωτα οικοσυστήματα, όπως η εισαγωγή αλλόχθονων (ξενικών και αλλότοπων) ειδών, έχει τη δυνατότητα να αυξήσει το μέγεθος των απειλών και να επιφέρει αλλαγές στα βιογεωγραφικά χαρακτηριστικά τους, σε διαφορετικές χωρικές κλίμακες. Ως εκ τούτου, ενώ η Μεσογείος θεωρείται ως ένα από τα σημαντικότερα και μεγαλύτερα «hotspot» βιοποικιλότητας των ιχθύων των εσωτερικών υδάτων, είναι ταυτόχρονα μία από τις περιοχές με τις περισσότερες εισαγωγές ειδών σε παγκόσμιο επίπεδο.

Αντιστοίχως, τα εσωτερικά ύδατα της Βαλκανικής χερσονήσου θεωρούνται από τα πλουσιότερα σε αριθμό «αυστηρά ενδημικών» και ενδημικών ειδών ιχθυοπανίδας, τόσο της Ευρώπης όσο και της ευρύτερης περιοχής της Μεσογείου. Η εισαγωγή αλλόχθονων ειδών αποτελεί μια από τις σημαντικότερες απειλές για τη βιοποικιλότητα της περιοχής. Μέχρι σήμερα στη Βαλκανική Χερσόνησο έχουν εισαχθεί 60 είδη ψαριών, εκ των οποίων τα 36 έχουν δημιουργήσει βιώσιμους πληθυσμούς στα οικοσυστήματα των εσωτερικών υδάτων. Δεδομένου λοιπόν ότι τα Βαλκάνια είναι ένα από τα 35 σημαντικότερα σημεία της βιοποικιλότητας στον κόσμο, αυτή η μεγάλη παρουσία αλλόχθονων ψαριών αποτελεί σοβαρή απειλή για τη σταθερότητα των οικοσυστημάτων, την επιβίωση της ενδημικής ιχθυοπανίδας και της βιοποικιλότητας γενικότερα. Οι αιτίες, καθώς και το χρονικό αυτών των εισαγωγών ποικίλλουν ανάλογα από το εκκάστοτε βαλκανικό κράτος. Παρ’ όλες τις πρόσφατες προσπάθειες εφαρμογής και εναρμόνισης της νομοθεσίας που αποσκοπεί στην πρόληψη της εισαγωγής των δυνητικά χωροκατακτητικών ειδών, την εφαρμογή αυστηρών ελέγχων των εισαγωγών και της αυξημένης προστασίας των υδάτων, η πλειονότητα των εισαγωγών παραμένει ακόμα και σήμερα εκούσια, κυρίως μέσω των υδατοκαλλιεργειών και των εμπλουτισμών. Το πρώτο κεφάλαιο της διαδκτορικής διατριβής επικεντρώνεται στην Ελλάδα, παρέχοντας μια ιστορική επισκόπηση των εισαγωγών, τους λόγους πίσω από αυτές, καθώς και τη σημερινή εξάπλωση των ξενικών ψαριών βάσει των στοιχείων της διαθέσιμης βιβλιογραφίας. Επιπροσθέτως, αναλύονται οι επιπτώσεις καθώς και μελλοντικά ζητήματα σχετικά με τις εισαγωγές των ξενικών ψαριών.

Οι ποταμοί, τα ρέματα και οι πηγές της Μεσογείου, παρακολουθούνται ανεπαρκώς όσον αφορά την παρουσία αλλόχθονων ειδών. Δεδομένου ότι τα συστήματα αυτά απειλούνται από πολλαπλές ανθρωπογενείς πιέσεις, όπως έχει ήδη αναφερθεί ανωτέρω, είναι σημαντικό να δημιουργηθούν συστηματικές διαδικασίες με στόχο την παρακολούθηση της κατανομής και της εξάπλωσης των εισαχθέντων ψαριών. Στο δεύτερο λοιπόν κεφάλαιο εφαρμόζεται μια πολύπλευρη εκτίμηση των αλλόχθονων ειδών στα ρέοντα οικοσυστήματα της χώρας σε διαφορετικές χωρικές κλίμακες, παρέχοντας: α) μια ιστορική ανασκόπηση των χρονικών προτύπων και των βασικών οδών εισαγωγής των αλλόχθονων ιχθύων στις λεκάνες απορροής της Ελλάδας (140 λεκάνες) κατά τον τελευταίο αιώνα, β) μια ανάλυση της παρουσίας και της αφθονίας των συναθροίσεων τους σε χωρική κλίμακα σημείου (654 σταθμοί δειγματοληψίας), γ) τη χαρτογράφηση των προτύπων κατανομής των αλλόχθονων σε επίπεδο λεκάνης απορροής ποταμού (75 λεκάνες) καθώς και σε βιογεωγραφικό επίπεδο (7 περιοχες) και τέλος δ) μια διανυσματική ανάλυση μεταφοράς των αλλότοπων ιχθύων στις λεκάνες απορροής εντός του βιογεωγραφικού πλαισίου της χώρας. Συνολικά, καταγράφηκε η παρουσία 55 αλλόχθονων ειδών (25 ξενικά και 30 αλλοτοπικά είδη). Ωστόσο, η εικόνα των λεκανών απορροής παρουσιάζει μια χαμηλή συχνότητα εμφάνισης αλλόχθονων ιχθύων σε υφάλμυρα ύδατα σε χωρική κλίμακα σημείου (30 αλλόχθονα είδη από τα δεδομένα πεδίου: 10 ξενικά και 20 αλλοτοπικά). Οι εισαγωγές των ειδών στην Ελλάδα φαίνεται να επηρεάζονται από συγκεκριμένες κοινωνικο-ιστορικές περιόδους, γεγονός που υποδηλώνει σταδιακή αύξηση από τα τέλη της δεκαετίας του '70. Παρά την αύξηση αυτή, η μελέτη μας αποδεικνύει ότι μόνο τα τέσσερα ξένα είδη είναι σήμερα ευρέως διαδεδομένα και κοινά στα ποτάμια και τα ρέματα της Ελλάδας, συγκεκριμένα τα: *Gambusia holbrooki, Carassius gibelio, Pseudorasbora parva* και *Lepomis gibbosus* (κατά σειρά καταγεγραμμένης αφθονίας). Τα αλλόχθονα είδη τείνουν να απουσιάζουν ή να κατανέμονται σε πολύ μικρούς αριθμούς στα ορεινά συστήμα μεγάλου υψομέτρου (π.χ. της οροσειράς της Πίνδου), καθώς και στις μικρότερες σε μέγεθος λεκάνες απορροής (π.χ. της Δυτικής Ελλάδας). Ωστόσο, το θέμα των αλλότοπων ιχθύων αποδεικνύεται ότι είναι ένα σοβαρά παραμελημένο πρόβλημα, όπου παρουσιάζει ταυτόχρονα μεγάλη δυσκολία εντοπισμού. Το κεφάλαιο αυτό εξετάζει μια εύκολα μεταβιβάσιμη διαδικασία ελέγχου, συμβάλλει στην εφαρμογή του κανονισμού της Ευρωπαϊκής Ένωσης για τα χωροκατακτητικά ξενικά είδη, εντοπίζοντας τα όποια κενά προτείνοντας δράσεις διατήρησης και διαχείρισης.

Ο σχεδιασμός και η εφαρμογή των κατάλληλων διαχείριστικών δράσεων με σκοπό την αντιμετώπιση της εξάπλωσης αλλα και των αρνητικών επιπτώσεων των αλλόχθονων ψαριών στα οικοσυστήματα των εσωτερικών υδάτων, παραμένει ακόμη ενα δύσκολο και πολύπλοκο έργο. Στο στάδιο πριν από την εισαγωγή ενός είδους σε ένα οικοσύστημα εφαρμόζονται συνήθως στοχευμένες εκτιμήσεις κινδύνου της πιθανότητας εισβολής του συγκεκριμένου είδους. Αντιθέτως, σε περιπτώσεις μετά την εισβολή του είδους στο οικοσύστημα εφαρμόζονται πιο δραστικά μέτρα, ωστόσο όμως, με περιορισμένη επιτυχία ή/και συχνά με ακούσιες αλλα σοβαρές επιπτώσεις στα αυτόχθονα είδη. Σε μια προσπάθεια αντιμετώπισης των όποιων περιορισμών των ήδη υπαρχόντων διαχειριστικών προσεγγίσεων, το συγκεκριμένο κεφάλαιο αναπτύσσει και εφαρμόζει ένα πλαίσιο ταξινόμησης για τον ορισμό συναθροίσεων των αλλόχθονων ψαριών (FAT) στα ποτάμια οικοσυστήματα και ταυτόχρονα προσδιορίζει τη σύνδεση με διάφορους περιφερειακούς, τοπικούς, βιοτικούς και αβιοτικούς περιβαλλοντικούς παράγοντες. Το πλαίσιο συμβάλλει στο σχεδιασμό αποτελεσματικών και προσαρμοσμένων δράσεων διαχείρισης πρίν αλλά και μετά την εκάστοτε εισβολή, εστιάζοντας στις συναθροίσεις των ειδών και όχι απλά σε μεμονωμένα είδη. Για τους παραπάνω λόγους εφαρμόστηκε η τεχνική της ανάλυσης δικτύων, ως μια νέα προσέγγιση, με στόχο την εξαγωγή πληροφοριών σε επίπεδο κοινότητας των αλλόχθονων ψαριών από συνολικά 393 δείγματα σε όλη την Ελλάδα.

Επιπρόσθετα εφαρμόστηκε μια ανάλυση πολυπαραγοντικής ταξιθέτησης για την εύρεση των παραγόντων που εξηγούν καλύτερα τη δομή αυτων των κοινοτήτων καθώς και τη χωρική κατανομή τους. Τέλος, οι συσχέτισεις των δεδομένων και των αποτελεσμάτων των αναλύσεων, απεικονίστηκαν μέσω ενός διαγράμματος ροης (alluvial/sankey diagram), παρέχοντας συγκεντρωτικές και συνδιαστικές πληροφορίες για διαφορετικές χωρικές κλίμακες. Συνολικά, δημιουργήθηκαν πέντε διαφορετικές συναθροίσεις και ανιχνεύτηκαν τα κυρίαρχα είδη όπου περιγράφουν καλύτερα την κάθε συνάθροιση (*Gambusia*–FAT, *Carassius/Lepomis*–FAT, *Pseudorasbora*–FAT, Salmonids–FAT και Carp-FAT). Οι συναθροίσεις ποικίλλαν χωρικά, υποδηλώνοντας διαφορετική δομή κοινότητας, βασισμένη κυρίως στις διαφορετικές προτιμήσεις των ενδιαιτημάτων καθώς και ιδιαίτερα φυλλογενετικά χαρακτηριστικά των ειδών. Οι συναθροίσεις των ξενικών ειδών κατανεμηθηκαν κυρίως σε ποταμούς της Κεντρικής και Βορείου Ελλάδας, μεγάλου μεγέθους, συνήθως με διασυνοριακά τμήματα. Αντιθέτως, οι συναθροίσεις των αλλότοπων ειδών καθώς και της ομάδας Salmonids–FAT (ρεόφιλα-ψυχρόφιλα είδη) κατέλαβαν ως επί το πλείστον περιοχές με σχετικά «φτωχή» πανίδα (ως προς τον αριθμό των ειδών) αλλά συχνά σε σημαντικά σημεία για βιοποικιλότητα της χώρας. Τα αποτελέσματα του κεφαλαίου μπορούν να προτεραιοποιήσουν τις συναθροίσεις και τους κινδύνους τους, να συνδράμουν σε συγκεκριμένες δράσεις διαχείρισης που έπονται το στάδιο της εισαγωγής των ειδών, ενώ έχουν περαιτέρω την δυνατότητα να παρέχουν πολύτιμες πληροφορίες για ανεπηρέαστα ρέοντα οικοσυστημάτα από εισαγωγές αλλόχθονων ψαριών, όπου αποτελλούν υψηλή προτεραιότητα για την βιοποικιλότητα της χώρας.

Η διαδικασία της εισβολής ενός είδους σε ένα νέο οικοσύστημα περιλαμβάνει μια σειρά από στάδια, από την εισαγωγή, την επιβίωση και την αναπαραγωγή του, μέχρι και την εκτενή διασπορά του σε όλο το σύστημα, ή και κατ’ επέκταση σε καινούρια. Αντίστοιχα, η επιτυχία ή μη της όλης διαδικασίας, εξαρτάται από το κατά πόσο το εκάστοτε είδος έχει την ικανότητα να ξεπερνά συγκεκριμένα εμπόδια, ώστε να φτάσει έως το τελικό στάδιο της διασποράς. Η πρόβλεψη λοιπόν της πιθανής εγκατάστασης βιώσιμων πληθυσμών ξενικών ειδών ή/και της περαιτέρω εξάπλωσης τους, μπορεί να συμβάλει στη δημιουργία διαχειριστικών δράσεων, καθώς και στην πρόληψη μελλοντικών εισαγωγών. Για αυτό τον σκοπό, στα κεφάλαια τέσσερα και πέντε που ακολουθούν, διερευνήθηκε η παρούσα κατανομή, η εγκατάσταση των πληθυσμών και η δυνητική εξάπλωση δύο ξενικών ιχθύων, που έχουν ήδη εισαχθεί στα εσωτερικά ύδατα της χώρας. Οι δύο περιπτωσιολογικές μελέτες βασίστηκαν στην χρήση των χωρικών κατανομών, διαφόρων δημογραφικών κριτηρίων, στην ένταση και συχνότητα εισαγωγών, στην αντιστοίχιση κλιματικών μεταβλητών και άλλων περιβαλλοντικών παραμέτρων. Τα είδη που χρησιμοποιήθηκαν ήταν α) το *Poecilia latipinna*, ένα λιμνόφιλο δημοφιλές ψάρι ενυδρείου, όπου έχει εισαχθεί σε αρκετές περιοχές του κόσμου και β) το ρεόφιλο *Oncorhynchus mykiss*, ένα από τα πιο ευρέως εισαχθέντα ξενικά είδη ιχθύων παγκοσμίως, κυρίως μέσω των υδατοκαλλιεργειών και της αλιείας.

Πιο συγκεκριμένα, στόχος του τέταρτου κεφαλαίου ήταν η αξιολόγηση της δυνητικής εξάπλωσης του είδους *P. latipinna* παγκοσμίως, με έμφαση στην Ευρώπη καθώς και την ευρύτερη περιοχή της Μεσογείου. Βάση της βιβλιογραφικής ανασκόπησης το *P. latipinna* έχει εισαχθεί σε 29 χώρες παγκοσμίως, με συνολικά 100 καταγραφές, κυρίως εντός τροπικών και υποτροπικών κλιματικών ζωνών, συνήθως σε εγγύτητα με κέντρα εμπορίας διακοσμητικών ειδών και σε περιοχές προσβεβλημενες με ελονοσία. Η δυνητική εξάπλωση του είδους προσομοιώθηκε με το κλιματικό μοντέλο Climatch, και παρουσίασε χαμηλή περιβαλλοντική αντιστοιχία (5% των περιοχών) στην Ευρώπη, ενώ περιμετρικά της Μεσογείου το ποσοστό αυτό αυξήθηκε στο 60%, παρά το γεγονός ότι το είδος εντοπίζεται σε μία μόνο περιοχή (Λίμνη Βουλιαγμένης, Αττική). Συμπερασματικά, η συνολική κατανομή του είδους παρουσιάζει μια στενοθερμική ανοχή. Πιθανολογείται ότι η περιορισμένη ανθεκτικότητα στην παρατεταμένη μείωση της θερμοκρασίας είναι ο κύριος παράγοντας που περιστέλλει την επιτυχία της εγκατάστασης του είδους στην Ευρώπη, εντούτοις δεν είναι ο μοναδικός καθοριστικός παράγοντας της περιορισμένης του εξάπλωσης. Τα εμπόδια στην ικανότητα διασποράς του *P. latipinna* προέρχονται από ένα συνδυασμό περιοριστικών παραγόντων, όπως: η συχνότητα απελευθέρωσης ατόμων του είδους στο φυσικό περιβάλλον, η μειωμένη αναπαραγωγική ικανότητα λόγω γεννετικά τροποποιημένων ποικιλιών, καθώς και άλλων διαφόρων οικολογικών παραγόντων.

Η αμερικάνικη ή ιριδίζουσα πέστροφα (*O. mykiss*) κατατάσσεται ως ένα από τα 100 πιο χωροκατακτητικά ξενικά είδη στον κόσμο. Ωστόσο, στην Ευρώπη η έκταση των εγκατεστημένων πληθυσμών παραμένει περιορισμένη και ανεπαρκώς αναφερθείσα στην βιβλιογραφία. Το πέμπτο κεφάλαιο αποσκοπεί στην αξιολόγηση της εγκατάστασης αναπαρωγικών πληθυσμών της ιριδίζουσας πέστροφας στην Ελλάδα και διερευνά τους παράγοντες που επηρεάζουν την επιτυχία ή την αποτυχία της εγκατάστασης. Τα ιχθυολογικά δεδομένα καθώς και οι περιβαλλοντικές παράμετροι που χρησιμοποιήθηκαν συλλέχθηκαν από το σύνολο της επικράτειας. Η διαθέσιμη βιβλιογραφία των προτυπων της γεωγραφικής κατανομής του είδους αναλύθηκε παράλληλα με την αντίστοιχη των ελληνικών ειδών πέστροφας (*Salmo* spp.), ενώ επίσης εφαρμόστηκαν αναλύσεις βάσει δημογραφικών κριτηρίων για την εξεύρεση πιθανών εγκατεστημένων πληθυσμών. Τα δεδομένα της πληθυσμιακής δομής και των ηλικιακών κλάσεων του είδους υποδεικνύουν λίγους αναπαραγωγικά βιώσιμους πληθυσμούς, με εξαιρετικά περιορισμένη παρουσία νεαρών ατόμων στα ιχθυοαποθέματα της ιριδίζουσας πέστοφας στην Ελλάδα. Η έλλειψη λοιπόν των κατάλληλων περιβαλλοντικών συνθηκών δεν είναι ο κύριος παράγοντας της αποτυχημένης εγκατάστασης του είδους. Γενετικοί παράγοντες που επηρεάζουν την αναπαραγωγή, είτε μέσω ετερογαμικής καταστολής (διασταύρωση γενετικά ανόμοιων πληθυσμών), είτε μέσω δυσπροσαρμοστικής συμπεριφοράς κατά την εκτροφή σε συνθήκες αιχμαλωσίας, αποτελούν πιθανές αιτίες για τη χαμηλή επιτυχία εγκατάστασης της ιριδίζουσας πέστροφας στην Ελλάδα. Συμπερασματικά, η απειλή του *O. mykiss* ως ιδιαίτερα χωροκατακτηρικού είδους στην Ελλάδα είναι μικρότερη από ότι έχει εκτιμηθεί σε διάφορες πρόσφατες αξιολογήσεις εκτίμησης κινδύνου.

Η χρήση των πλαστικών εδώ και πολλές δεκαετίες παρέχει στον άνθρωπο πληθώρα εφαρμογών και κοινωνικο-οικονομικών οφελών, ωστόσο η μη βιώσιμη χρήση μεγάλων ποσοτήτων τους και σε συνδυασμό με την ακατάλληλη διαχείριση μετά την χρήση τους δημιουργεί τα μικροπλαστικά, μία ανερχόμενη και αρκετά επιβλαβή μορφή ρύπανσης για το περιβάλλον και την βιοποικιλότητα. Τα μικροπλαστικά είναι συνθετικά πολυμερή μικρότερα από 5 mm και δημιουργούνται από θραύσματα μεγαλύτερων πλαστικών λόγω των εξής διαδικασιών: φωτοαποικοδόμηση ή/και άλλες φυσικές, χημικές και βιολογικές αλληλεπιδράσεις. Μέχρι στιγμής, το μεγαλύτερο βάρος της έρευνας για τα μικροπλαστικά έχει επικεντρωθεί στο θαλάσσιο περιβάλλον, καθώς και στις επιπτώσεις τους στους θαλάσσιους οργανισμούς. Αντιθέτως, έρευνες για την ρύπανση των μικροπλαστικών στα οικοσυστήματα των εσωτερικών υδάτων παραμένουν μέχρι στιγμής αρκετά λίγες, παρά το γεγονός ότι η συντριπτική πλειονότητα των πλαστικών απορριμμάτων (~80%) προέρχονται από χερσαίες πηγές ενώ διέρχονται αρχικά μέσω των οικοσυστημάτων του γλυκού νερού, τα οποία λειτουργούν ως φυσικά φίλτρα για την θάλασσα. Τα κυρίαρχα αλλόχθονα είδη ψαριών θα μπορούσαν να είναι ενδείκτες της αβιοτικής και βιοτικής κατάστασης ενός οικοσυστήματος που έχουν εισαχθεί λόγω της εκτεταμένης κατανομής και της υψηλής βιομάζας τους, καθώς επίσης και της εύκολης σύλληψης τους. Έρευνες λοιπόν που έχουν ως σκοπό την βιοπαρακολούθηση μιας συγκεκριμένης περιοχής μπορούν να πραγματοποιηθούν με βάση τα αλλότοπα είδη ψαριών παρέχοντας πολύτιμες πληροφορίες σχετικά με τη ρύπανση και τις επιπτώσεις της είτε στα αυτόχθονα είδη ψαριών είτε στους πληθυσμούς των αντίστοιχων συγγενών ειδών τους της χώρας. Στόχος του τελευταίου κεφαλαίου λοιπόν, ήταν να διερευνηθεί η πιθανή χρήση των αλλόχθονων ειδών στην έρευνα, χρησιμοποιώντας τα αλλότοπα είδη ψαριών (συγκεκριμένα άτομα του είδους *Squalius vardarensis*) ως βιοδείκτες με σκοπό την ανίχνευση και την αφθονία των μικροπλαστικών στον ποταμό Κηφισό της Αττικής. Η περιοχή μελέτης επιλέχθηκε λόγω του γεγονότος ότι ο Κηφισός Αττικής θεωρείται ως ένας πολύ τροποποιημένος αστικός ποταμός που επηρεάζεται σε μεγάλο βαθμό από υψηλά φορτία σημειακών και μη σημειακών αποβλήτων καθώς ο ποταμός ρέει μέσω του μεγαλύτερου μέρους της ευρύτερης περιοχής της Αθήνας.

Συνολικά στο δείγμα του νερού ανιχνεύθηκαν 321 μικροπλαστικά, ενώ βρέθηκαν και 16 μεσοπλαστικά (από 5 mm έως 2 cm) τα οποία εξαιρέθηκαν από περαιτέρω αναλύσεις. Η αφθονία στη στήλη γλυκού νερού υπολογίσθηκε σε 8,1 μικροπλαστικά/m^3. Οι κύριοι τύποι των πολυμερών προσδιορίστηκαν με Φασματοσκοπία Υπερύθρου Μετασχηματισμού Fourier (FT-IR) και ήταν: το πολυαιθυλένιο (PE), η πολυβινυλική αλκοόλη (PVA) και το πολυπροπυλένιο (PP). Τα δείγματα του είδους *S. vardarensis*, παρουσίασαν μια συγκριτικά μέτρια αφθονία μικροπλαστικών, ενώ πάνω από το ένα τρίτο των ψαριών (35%) περιέχε μικροπλαστικά. Συνολικά, βρέθηκαν 19 μικροπλαστικά σε 11 δείγματα *S. vardarensis*. Επομένως, ο γαστρεντερικός σωλήνας των αλλότοπων ψαριών μπορεί να χρησιμοποιηθεί ως εργαλείο για την ποιοτική αξιολόγηση της ρύπανσης από μικροπλαστικά, ειδικά για εκείνα τα οικοσυστήματα που διαρρέουν μεγάλες αστικές περιοχές. Παρ΄όλα αυτά απαιτείται περαιτέρω έρευνα για την εκτίμηση της ρύπανσης μικροπλαστικών στα ιζημάτα καθώς και σε μεγαλύτερο αριθμό ατόμων ή/και σε περισσότερα είδη. Επιπρόσθετα, θα πρέπει να διερευνηθούν οι πιθανές παρενέργειες που πιθανόν να προκαλεί η κατάποση των μικροπλαστικών από την ιχθυοπανίδα και κατ΄ επέκταση οι παράγοντες που επηρεάζουν τις παρενέργειες, όπως τα χαρακτηριστικά των ειδών, οι διατροφικές τους συνήθειες, ο τύπος και η προέλευση των μικροπλαστικών κ.ο.κ.

Συμπερασματικά, η εισαγωγή αλλόχθονων ιχθύων στα ρέοντα συστήματα της Ελλάδας αντιπροσωπεύει μια σχετικά πρόσφατη αλλά συνεχώς αυξανόμενη απειλή, η οποία επηρεάζει τα ενδημικά και τα αυτόχθονα είδη μέσω οικολογικών διαδικασιών, διαειδικού υβριδισμού, νέων ασθενειών ή/και μεταφορά παράσιτων. Η απουσία ενός προγράμματος οργανωμένης παρακολούθησης τους σε συνδιασμό με τη έλλειψη ποιο-ποσοτικών δεδομένων, αποτελούν τα σημαντικότερα εμπόδια προς την σωστή ενημέρωση με έγκυρες και ολοκληρωμένες πληροφορίες που απαιτούνται για την λήψη μέτρων πρόληψης και διαχείρισης του προβλήματος στην Ελλάδα. Ωστόσο, βάσει των αποτελεσμάτων της παρούσας μελέτης, παρά την αυξανόμενη είσοδο των εισαγόμενων ειδών στα Μεσογειακά οικοσυστήματα, μόνο μια μικρή ομάδα ξενικών χωροκατακτητικών ιχθύων έχει εισαχθεί στα ρέοντα οικοσυστήματα της χώρας. Ο υπόλοιπος μικρός αριθμός των ξενικών ειδών εντοπίζεται σε λίγες και μικρές λεκάνες απορροής, σχετικά απομονωμένες από το δίκτυο των μεγάλων λεκανών απορροής, διατηρώντας μικρούς πληθυσμούς, που συχνά δεν είναι ικανοί να αναπαραχθούν. Αντιθέτως, επι του παρόντος, η παρουσία των αλλότοπων ιχθύων στα ελληνικά ποτάμια οικοσυστήματα δεν αντιμετοπίζεται ως σοβαρό θέμα. Υπάρχει η τάση να υποβαθμίζεται ως γεγονός, ενώ θα έπρεπε ήδη να θεωρείται ως ένα επιπρόσθετο και μάλιστα ύπουλο πρόβλημα που απειλεί την δομή και τις λειτουργίες των οικοσυστημάτων. Τα είδη αυτά, μη έχοντας βιοκλιματικά εμπόδια και μεγάλες αποστάσεις να προσπεράσουν, μπορούν άμεσα να εισαχθούν και να εγκαταστήσουν βιώσιμους χωροκατακτικούς πληθυσμούς, προκαλώντας σοβαρές επιπτώσεις στην αυτόχθονη ιχθυοπανίδα της χώρας οι οποίες πιθανότατα και να υπερβαίνουν αυτές των ξενικών ειδών.

Το ερώτημα που εύλογα προκύπτει είναι εάν η παρουσία όλων των αλλόχθονων ειδών, ξενικών και αλλότοπων, πρέπει να θεωρείται αποκλειστικά και μόνο επιβλαβή και ανεπιθύμητη. Ένα χαρακτηριστικό παράδειγμα είναι η ιριδίζουσα πέστροφα, όπου ενώ κατατάσσεται μεταξύ των χειρότερων χωροκατακτητικών ειδών στην ηπειρωτική Ευρώπη, διαδραματίζει ζωτικό ρόλο στην οικονομία, ως σπονδυλική στήλη της Ευρωπαϊκής υδατοκαλλιέργειας, καθώς και της αλιείας των εσωτερικών υδάτων. Αντιστοίχως, υπάρχουν πολλές περιπτώσεις όπου κατάλληλοι λόγοι ευνοούν την επιστημονικά τεκμηριωμένη εισαγωγή ειδών, για παράδειγμα "εισαγωγές διατήρησης" για πολύ λίγα απειλούμενα είδη, συμπεριλαμβανομένων ενδημικών που διαβιούν σε ελάχιστες περιοχές και περιορισμένες έκτασεις στην Ελλάδα. Επιπλέον, η εκκάστοτε χρήση των ήδη εισαγμένων ειδών, αντί της χρησιμοποίησης των αυτόχθονων, στην επιστημονική έρευνα, θα μπορούσε να αποτελέσει ένα τρόπο αξιοποίησή τους και ταυτόχρονα ένα ουσιαστικό μέτρο διαχείρισής των πληθυσμών τους.

Είναι σχεδόν βέβαιο ότι οι νέες εισαγωγές θα συνεχιστούν στην Ελλάδα λόγω της αύξησης των έργων υδατοκαλλιέργειας του συνεχιζόμενου ενδιαφέροντος για την αλιεία, της χαμηλής ευαισθητοποίησης του κοινού σε θέματα βιοποικιλότητας και της ανομίας ή/και ελλιπέστατης επιβολής των νόμων. Ωστόσο, η πρόληψη περαιτέρω επιβλαβών εισαγωγών αλλόχθονων ειδών παραμένει το πιο σημαντικό και άμεσο μέτρο που απαιτείται στα εσωτερικά ύδατα της χώρας. Η Ελλάδα παρουσιάζει σημαντικές ευκαιρίες για να αποτρέψει την εξάπλωση των αλλόχθονων στα ρέοντα οικοσυστήματα, καθώς πολλές περιοχές δεν επηρεάζονται ακόμα από τα αλλόχθονα ψάρια, τα οποία είναι αρκετά έως πολύ διαδεδομένα σε άλλες ευρωπαϊκές χώρες.

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‘A supposedly daring insight came up, disguised as a question: “Dr. Cole, aren't humans the most invasive species of all?” She'd fielded that one many times before, during public lectures and even in her days as a teaching assistant [...], "I'm not unsympathetic to that line of thinking,” she answered, “but even if it's true, we're also the only species in any position to do anything about it.” ‘

— **Joe Pitkin,** *Analog Science Fiction and Fact,* June 2012

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General Introduction

INTRODUCTION

Freshwater ecosystems under immense pressures

Water is the most vital natural resource necessary for the survival of humans and other organisms. The important functions performed by aquatic ecosystems provide a plethora of goods and services essential for human societies that they use to their advantage. However, since the beginning of the 21st century, the planet has entered into a water crisis era, caused by excessive human development, which has led to a global deterioration in the quality and a vast decrease in the quantity of freshwater ecosystems.

Freshwater ecosystems can be considered as systems of high habitat diversity and biodiversity, as they include 2.3% of all known fish species, although they occupy 1% of the land surface and constitute 0.01% of all water on the planet (Nelson, 2006). They include habitats with a wide variety of physicochemical and hydrological characteristics, consisted of surface waters (e.g. rivers, lakes, wetlands, estuaries) and subsurface waters (hyporheic and phreatic) as well as riparian systems (Ward and Tockner, 2001). Although freshwater ecosystems are characterized by the presence of freshwater, they also include brackish ecosystems, such as lagoons and enclosed lakes. This diversity in habitats, combined with the geographic isolation of freshwater ecosystems through geological times, have led to the speciation of many organisms, creating a large number of range restricted species occurring in a single lake, spring or river.

In spite of their significant ecological importance, freshwater ecosystems are under immense pressure, confronting multiple and often interacting anthropogenic stressors (see Dudgeon et al., 2006 and references therein; Ormerod et al., 2010). The most intense pressures derive from industrial and agricultural activities, urban wastes, overexploitation of water for irrigation needs, hydro-morphological alterations (e.g. dams, fragmentations, etc.) and last but not least the introduction of non-indigenous species (Baron et al., 2002; Nilsson et al., 2005; Xenopoulos and Lodge 2006). Due to these stressors, freshwater organisms have been classified as the most threatened biota globally (Balian et al., 2008; Strayer and Dudgeon, 2010).

Freshwater fishes are extremely vulnerable to human pressures, such as habitat degradation, water pollution, over-exploitation of water and habitat fragmentation (Bruton, 1995; Maitland, 1995; Cowx, 2002), thereby are amongst the world's most threatened species (Duncan and Lockwood, 2001; Darwall et al., 2008). Several species occurringin the Mediterranean basin are predominantly threatened, where the majority of them are endemics with restricted distributional ranges, surviving near the edge of their physiological limits (Abell et al., 2008; Hermoso and Clavero 2011; Darwall et al., 2014). Native species inhabiting Mediterranean freshwater ecosystems are forced to survive in harsh and dynamically changing environmental conditions. Hence, a large number of endemic species inhabiting Mediterranean rivers and streams are already under severe stress due the latter anthropogenic pressures. Any additional stressor, such as the introduction of non-indigenous fish species, has the potential to increase the magnitude of threats, and to incur changes in the biogeographic characteristics over various spatial scales. Currently, in the updated worldwide IUCN Red List, freshwater fish species are classified as one the most highly threatened taxa, registering alarming percentages, 37% worldwide (Vié et al., 2009), and 56% when only the Mediterranean endemic species are considered (Smith and Darwall, 2006). Hence, to reverse these negative trends and decelerate biodiversity loss, a deeper knowledge of biodiversity patterns and the factors affecting this biodiversity change is urgently needed, before the opportunity is totally squandered (Vardakas, 2017).

Definitions/Terminology: Alien, exotic, introduced, non-native and non-indigenous species are the same thing

Invasive alien species (IAS) research is considered as an emerging science where its terminology is continuously evolving. Currently, the science of IAS has not established a consistent terminology that could provide a comprehensive set of definitions based on fully understood processes. Various terms have been used to define an organism that has been deliberately or accidentally transferred to an area that was not occurring naturally. Terms that are used by most scientists include the words: alien, non-native, non-indigenous, exotics and others (no less than 27 in total are listed by Colautti and MacIsaac, 2004) and all these terms are referring, more or less, to the same thing. However, not all alien species are invasive or could become invasive. Misusing terms in any scientific field could create confusing conditions between scientists and policy makers, hindering understanding and processes.

Generally, alien species can be defined as those species that are introduced either intentionally or unintentionally in a location, area or region by humans, outside of their natural distributional range. According to the Convention on Biological Diversity (CBD) “Alien species” *refers to a ‘species, subspecies or lower taxon, introduced outside its natural past or present distribution; including any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce’*. The introduction and dispersal of these non-indigenous species is mainly attributed, in a general context, on the increasing extent of global trade (Hulme, 2009). Despite the fact that some of these species are considered as valuable for generating income into various economic sectors (e.g. agriculture, aquaculture, fisheries, aquarium trade etc.), the vast majority of these species can have severe impacts when released into their new environments. A number of alien species can become invasive under some circumstances, leading to severe ecological impacts and eventually drive native biota to extinction (Gido and Brown, 1999; Olden et al., 2004; Light and Marchetti, 2007).

Invasions of alien species are now widely considered to be one of the main threats to biodiversity and the second leading cause of animal extinctions, after habitat destruction (see MEA, 2005; Clavero and García-Berthou, 2005). Currently in Europe there are over 12,000 alien species of which 15% are invasive, thus are considered as one of the most severe danger for the European threatened native biota. Approximately 680 species extinctions listed by the IUCN were caused by invasive alien species (Clavero and García-Berthou, 2005). In terms of economical impacts, it is estimated that their effects on agriculture, forestry and fisheries can rise up to 12 billion Euros per year in Europe alone (Scalera et al., 2012).

Even though several definitions have been proposed for the term “invasive” (reviewed by Heger et al., 2013; Pereyra, 2016), two groups of definitions prevail in the scientific literature: the “ecological definitions” and the “policy definitions” (Heink et al., 2018). Ecological definitions emphasize the ecological aspects of species invasions and do not include any reference to broader impacts (Blackburn et al., 2011; Colautti and Richardson, 2009). Policy definitions largely follow the definition of an invasive alien species provided by the World Conservation Union (IUCN): ‘*a species which becomes established in natural or semi-natural ecosystems or habitats, is an agent of change, and threatens native biological diversity*’ (IUCN, 2000). This definition and conceptually similar definitions adopted by the Convention on Biological Diversity (CBD, 2002) and the EU Regulation 1143/2014 on Invasive Alien Species, implicitly draw a connection between ‘invasiveness’ and ‘impacts’ and require an evaluation of harmfulness. Only those alien species that have a demonstrable ecological or economic impact should be considered as invasive, based on a comprehensive risk assessment. Which definition for invasive species is adopted is largely a matter of research focus and motivation (Heger et al., 2013).

Translocations of native fish species

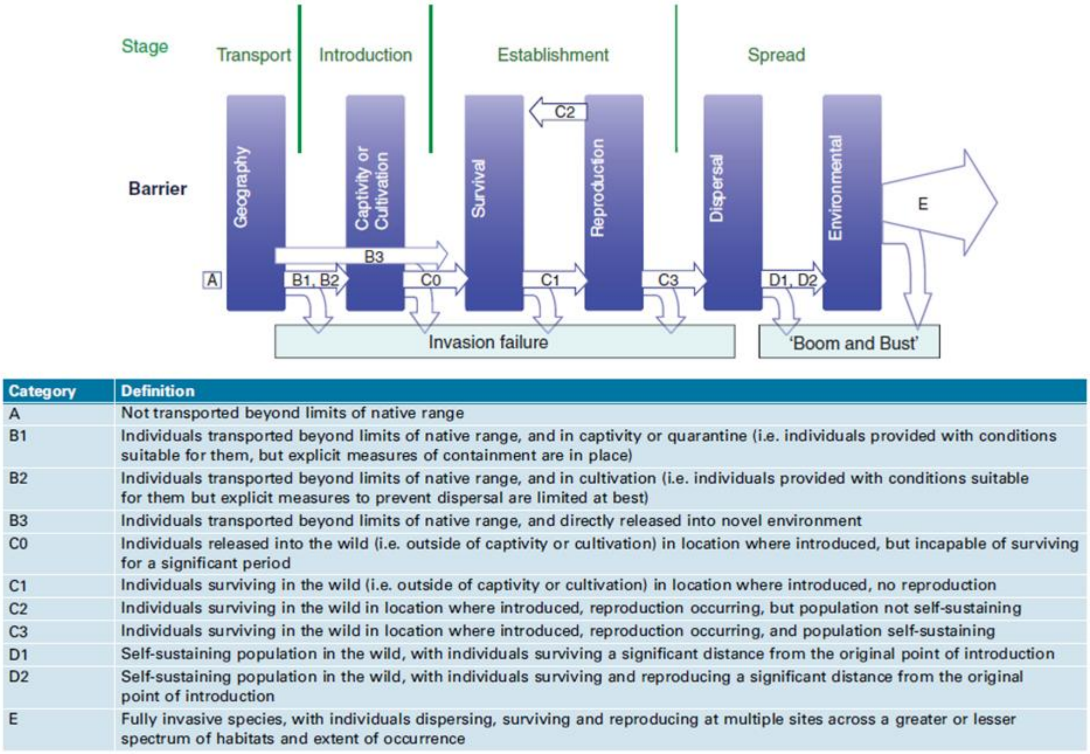
Besides the introductions of exotic fishes from abroad there are also transplants of native fish species from one part of a political entity (e.g. country) in which it is native to another part of the same country outside of its native distributional range (Copp et al., 2005). The invasion potential of translocated species is enhanced by the geographic proximity between the source and receiving areas, which increases the transport possibilities and release frequencies. Most translocated species can easily establish reproductive populations due to the greater likelihood of adapting to the country’s general natural flow, habitat and temperature regimes in contrast with alien species (Ribeiro et al., 2008). However, introduction pathway data are often unavailable and this is a serious impediment to stemming their spread (Hulme et al., 2017). Additionally, many translocated species are difficult to identify and may go unnoticed since they physically resemble local "sister species" (related similar-looking species from neighbouring ecoregions). During fish surveys translocated species could easily be overlooked by research teams as "on site" identification is "subjective and observer-dependent" (Sousa-Santos et al., 2018). However, some translocations may have a considerable impact on the genetic composition and future survival of many endemic fish assemblages. Interspecific hybridization due to translocations seems to be an ongoing pressure and an increasing threat, but without appropriate monitoring and genetic screening, this kind of impact is difficult to track and quantify. Fishes have a great potential for successful interspecific introgressive hybridization (Scribner et al., 2000) and there are numerous examples of gene pool erosion of native species following the introduction of aliens and translocated species (Largiadèr, 2007; Apostolidis et al., 2008).

The expansion of translocated species is poorly reported and not yet considered as a significant threat even in protected area assessments; this may be similar to the situation of underreported alien threats in marine protected areas (Mazaris and Katsanevakis, 2018). As emphasized by Buoro et al. (2016) the global effects of translocated introductions may exceed those induced by aliens species. The impacts of translocated native invaders are often appreciated at the individual level on sister species and wild conspecifics, however ripple-effects at the community and ecosystem levels could also be serious (Helfman, 2007; Moyle, 2013).

Freshwater fish introductions and invasive species

Despite the relatively recent problems and the dangers that aquatic ecosystems are facing, Europe's flora and fauna have been evolving for millions of years. Rivers, lakes as well as wetlands, have distinct species assemblages and a large number of different habitats allowing a huge range of biodiversity to thrive. However, the expansion of international trade as well as the simplification of travel have eliminated the borders on a global scale, bringing various species into direct contact with one another.

The introduction of alien fishes to freshwater ecosystems is considered as one of the leading factors for freshwater biodiversity loss (Saunders et al., 2002; Dudgeon et al., 2006; Liu et al., 2017) and combined with habitat loss, hydrological alteration and pollution can lead to the extinction of native freshwater fish species (Arthington et al., 1983; Kennard et al., 2005; Dudgeon et al., 2006; Olden et al., 2010). Although native species are resistant to local "known" pests and/or diseases, they often have little or no natural defenses against introduced organisms. These risks can range from simple competition at the level of individuals or populations to literally species extinction. Indigenous species in normal conditions, maintain balanced populations under the control of predators, unlike the introduced species, which can reproduce rapidly and even spread to new localities due to the absence of such control. Once an non-indigenous fish species has established a viable population into a new locality it could produce severe ecological damage by affecting native species at various levels of biological organization through predation, competition, habitat degradation, food web alteration, hybridization and disease transmission (Copp et al., 2005; Savini et al., 2010; Ribeiro and Leunda, 2012; Havel et al., 2015).



Category	Definition
A	Not transported beyond limits of native range
B1	Individuals transported beyond limits of native range, and in captivity or quarantine (i.e. individuals provided with conditions suitable for them, but explicit measures of containment are in place)
B2	Individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them but explicit measures to prevent dispersal are limited at best)
B3	Individuals transported beyond limits of native range, and directly released into novel environment
C0	Individuals released into the wild (i.e. outside of captivity or cultivation) in location where introduced, but incapable of surviving for a significant period
C1	Individuals surviving in the wild (i.e. outside of captivity or cultivation) in location where introduced, no reproduction
C2	Individuals surviving in the wild in location where introduced, reproduction occurring, but population not self-sustaining
C3	Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining
D1	Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction
D2	Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction
E	Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence

FIGURE I.1. The unified framework for biological invasions proposed by Blackburn et al (2011).

However, the invasion process contains a series of stages, from introduction, survival and reproduction to dispersal, with species having to overcome several "barriers" to reach the next step, with differing invasion success (see Fig. I.1.; a proposed framework by Blackburn et al., 2011). At different stages of the invasion process, non-indigenous species have to overcome several barriers (biotic and abiotic) in order to invade successfully a new area (Moyle and Marchetti, 2006). The first two steps of this process are “the arrival stage” of the species to the invading ecosystem through anthropogenic transport, and its” survival” throughout it (Sakai et al., 2001; Moyle and Marchetti, 2006). Thus, when suitable ecological conditions and habitats for a species do exist at a large scale, non-native range expansion may be constrained by the limited possibility of anthropogenic transport and thus entry into new aquatic systems. Yet, the quantification of human influences on freshwater fish invasions remains a challenge, due to the lack of effective indicators to express the degree of human activity (García-Berthou, 2007).

In order for a non-indigenous species to become resident into a novel environment, propagule pressure is critical in determining which introductions are going to lead to establishment (Marchetti et al., 2004). High propagule pressure usually, but not always, leads to high success rates of colonization (Moyle and Marchetti, 2006) and the establishment of a viable, self-sustaining population (Sakai et al., 2001). Finally, spread and integration are local processes, conditioned by the interplay between abiotic and biotic factors (Moyle and Marchetti, 2006). If the species falls through any one of the barriers at any stage of the invasion process, fails to become an invader (Fig. I.1; Blackburn et al., 2011). In this sequence of events, propagule pressure, habitat suitability and appropriate ecological conditions are the critical determinants for the establishment success of an introduced species to an ecosystem and its further spread.

Invasive species are often superior competitors in relation to the evolutionary isolated native species populations (Mills et al., 2004; Townsend, 1996) while they acquire specific ecological traits that make them tolerant to a wide environmental spectrum, being thus able to thrive also in degraded habitats (Courtenay and Meffe, 1989; Kennard et al., 2005). Several attempts have been made to classify alien species according to the magnitude of their environmental impacts, based on the mechanism of impact through which they exert their effect, that is predation, competition, hybridization, disease transmission (see Blackburn et al., 2014). The effects of invasive species can range from minimal to massive, and the various stages of the invasion process require different management interventions, with special emphasis on the halting of their secondary spread, following the establishment of isolated populations (Blackburn et al., 2011; Vander Zanden and Olden, 2008). However, under current climate change scenarios, invasive alien species are expected to accentuate climatic stress effects by reducing the number of native species and/or their functional types within the ecosystem and by increasing ecosystem susceptibility to climatic perturbation (Masters and Norgrove, 2010).

Vectors and pathways of alien freshwater fish introductions

The terms vector and pathway are often misconceived by many authors. In brief, a vector is the actual physical mean or mechanism which assists the transfer of an organism from one location to another while a pathway is broadly defined as the route, purpose or activity which an alien species can be transported to a new location, deliberately or unintentionally. Vectors operate along a combination with pathways, since they are the mean to move species along the pathway. So for instance, a hobbyist carrying a fishbowl with a few goldfish ready to unfill it into a lake is a vector, whereas the aquarium trade and international flights are the pathways. Nevertheless, determining the mode (vector) and source (pathway) of non-indigenous species introduction is essential in order to apply management and control actions (Fig. I.2, Ruiz and Carlton, 2003).

Freshwater fish are the most widely introduced aquatic animal group (Gozlan, 2008) and most introductions have been conducted deliberately (Stohlgren et al., 2006). The vectors for the introduction of alien freshwater fishes include the aquaculture industry (e.g. escapes), the government agencies (for stocking purposes, fisheries enhancement and bio-control programmes), the ornamental fish trade, recreational anglers and inter-basin transfer schemes (Savini et al., 2010; Strayer, 2010). The main pathways of introduction of freshwater alien fish species are highly related with many human activities such as aquaculture, fisheries, bio-control projects, ornamental trade and inter-basin transfers (Maitland, 1995; Ruesink, 2005; Jeschke and Strayer, 2006; Stohlgren et al., 2006).

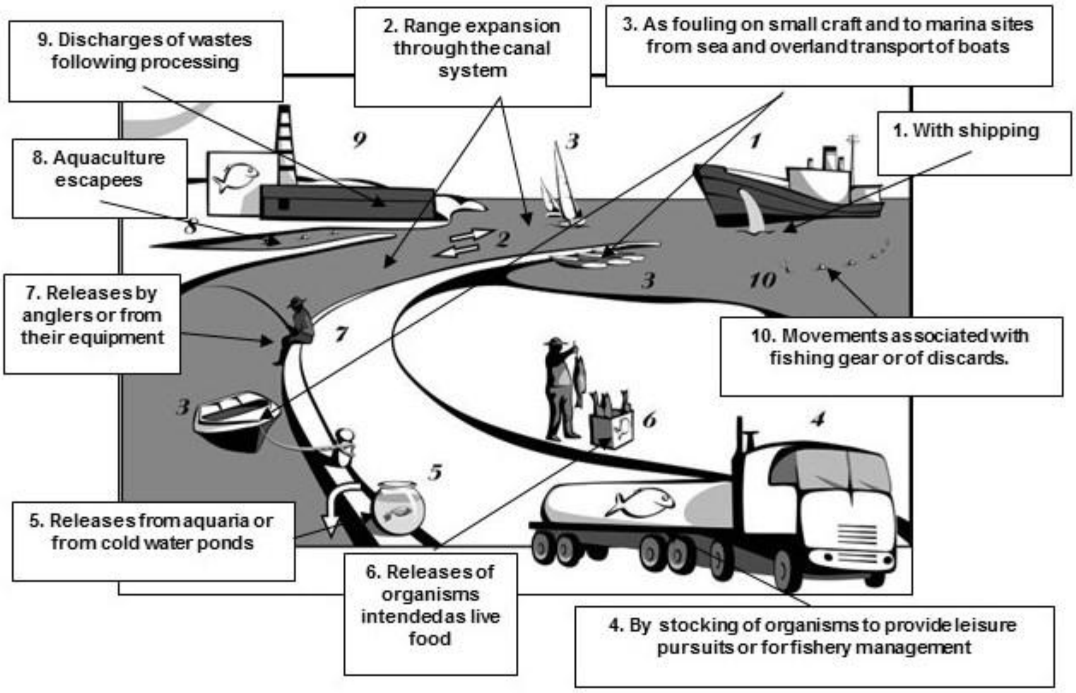


FIGURE I.2. Vectors and pathways of non-indigenous species introductions into a new environment. *Retrieved from http://archive.iwlearn.net/globallast.imo.org/the-invasive-aquatic-species-2/index.html*

Aquaculture is one of the fastest growing primary industry in the world (Tacon et al., 2010). The general trend in Europe is to cultivate mostly non-indigenous species (Turchini and De Silva, 2008), highly responsible for the introduction of many harmful species, often leading to irreversible ecological impacts (Naylor et al., 2001; Gozlan, 2008; Keller et al., 2011).

Several non-indigenous fish species have been stocked in the wild, in order to promote commercial fisheries and/or recreational angling (Gherardi et al., 2009; Tricarico, 2012). Beside deliberately stocking, escapes of live baits or bait fish discards from anglers, which is a common practice in order to provide bait for future trips (Lintermans, 2004), makes fisheries and angling two of the principal causes of biodiversity loss in both lentic and lotic ecosystems worldwide (Cambray, 2003).

Additionally, many freshwater fish species have been released as biological agents, for the biological control of mosquitoes, aquatic weeds, nematodes etc. (Pípalová, 2006; Pyke, 2008; Froese and Pauly, 2010). Nevertheless, most introduced fish species do not meet the basic requirements for biological control due to the fact that fish feeding habits are usually broader than a single-target organism (Nunes et al., 2015).

Ornamental fish introduced in the wild through the dumping of aquarium species and escape from garden ponds or breeding farms (Copp et al., 2005; Duggan et al., 2006). The ornamental trade in the European continent involves a vast number of different species and has been identified as an important source of fish introductions (Copp et al., 2010; Maceda-Veiga et al., 2013).

Finally, non-indigenous fish species may also disperse into neighboring river basins via shipping and shared waterways. The European network of inland waterways, connects catchments of southern European seas to northern European seas through >28000 km of navigable rivers and constructed canals (Nunes et al., 2015). These artificial river corridors have enabled introductions and further spread of many aquatic species within European catchments that were previously well isolated (Bij de Vaate et al., 2002; Galil et al., 2007; Panov et al., 2009).

The EU Regulation on Invasive Alien Species

In recent decades, the European Union has enacted a wide range of environmental legislations, establishing some of the strictest environmental standards in the world. The Water Framework Directive 2000/60/EU (WFD) is currently the cornerstone of water management in Europe, by adopting an innovative and comprehensive approach for the management and protection of water resources, aiming to achieve "good ecological quality" for all its surface waters (rivers, lakes, coastal waters). However, the WFD as well as other legislations only partially address the issue associated with alien species introductions (Shine et al., 2010).

The EU Regulation on Invasive Alien Species (IAS) (1143/2014/EU) was entered into force on 2015 to fulfill this legal gap by providing the necessary tools for the Member States of the European Union in order to comprehensively address the issue of the invasive alien species. This new Regulation aims to terminate any fragmentary measures of each country by establishing rules and practices for the prevention and management of the introduction and dispersal of invasive alien species within its

Member States, with the ultimate goal of limiting and at the same time controlling the growing threats posed by the introduction of these species (EU, 2014). Specifically, this regulation targets to prevent, minimize and mitigate the adverse effects of alien species on both the biodiversity and ecosystems of the European Union and on human health and the economy. In addition, it makes a distinction between “alien species” and “invasive alien species”, where the latter are defined as those found “*to threaten or adversely impact upon biodiversity and related ecosystem services*”, and specifies that management is taken up for those invaders which are “widely spread”.

The Regulation promotes the creation of a list of IAS of Union concern (the Union List), to be updated at regular intervals, for which member states are required to apply restrictive, preventive or eradication measures in order to minimise the risks of their introduction, establishment and spread (EU, 2014). The creation of lists of IAS of regional and of Member State concern is also envisaged. Inclusion of a species in the Union List and the general listing and prioritisation process should be based on a comprehensive risk assessment, which must include information relevant to the evaluation of threats (e.g. species ranges, reproduction, spread patterns and documented impacts), with a due consideration of various socio-economic aspects (EU, 2014). It is explicitly stated that only IAS for which the available scientific evidence indicates capacity for establishment and spread shall be considered for inclusion. Member states are required to establish surveillance systems to monitor the occurrence and spread of invasive alien species, and also to assess the effectiveness of intervention measures, making use of all available relevant information, e.g. data from monitoring systems established by Union law (e.g. WFD monitoring). It is obvious that, for an effective implementation of this regulation, appropriate data on alien species establishment rates or capabilities, persistence through time and spreading potentials must become available.

Once the countries of the European Union compile the list of alien species of "EU interest", they have three years to draw up action plans to determine the priority pathways and prevent the unintentional introductions and spread of alien species in their territory. European countries must take measures to assist in the restoration of degraded or damaged ecosystems, unless demonstrated with reasonable certainty, through cost-benefit analysis based on available data, that the cost of such measures will be high and disproportionate to the benefits of restoration. Then, within 18 months from the date of issue of the list, the Member States must implement monitoring systems as well as actions of rapid elimination/eradication of alien species.

The core of EU Regulation 1143/2014 is the Union list, which for the first time entered into force on 3 August 2016. The species included on the Union list are subject to restrictions and measures *"on keeping, importing, selling, breeding and growing"* (1143/2014/EU). The first update of the Union list entered into force on 2 August 2017 and the second one on 15 August 2019. Up until now three freshwater fish species are included as Invasive Alien Species (IAS) in the consolidated Union list, namely, the Pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758); the Amur sleeper *Percottus glenii* Dybowski, 1877; and the Stone moroko, *Pseudorasbora parva* (Temminck & Schlegel, 1846) (see Table A.I.1 in Appendices).

Greece as a hotspot of freshwater fish biodiversity. Do non-indigenous fish species pose a threat?

Greece is located in the Balkan region (southeastern Europe), with a territory approx. 132.000 km^2, and is characterised by a highly fragmented hydrographic network with a large number of medium and small-sized river basins dominated by mountainous landscapes but fringed by a long convoluted coastline (Economou et al., 2007; Skoulikidis et al., 2009). The complex geological processes of the wider area of the Balkans has allowed multiple colonisations of many species throughout long periods, where combined with hydrographic isolation has led to speciation (Economidis and Bănărescu, 1991). These factors are mainly responsible of the increased diversity and high degree of endemicity observed now on the freshwater fish fauna of Greece. From a biogeographical perspective, Greece is divided into eight freshwater ecoregions (Fig. I.3). Each ecoregion hosts distinctive assemblages of freshwater fish species and other aquatic/semi aquatic life forms, many being endemic to each region (Zogaris and Economou, 2017). The spatial scale of the ecoregion is widely used as standard geographical and non-political framework for conservation/ecological evaluations (Abell et al., 2008) and biological assessments

(Zogaris et al., 2018). Greece is an example of a state facing lotic ecosystem conservation challenges, especially with respect to freshwater fish.

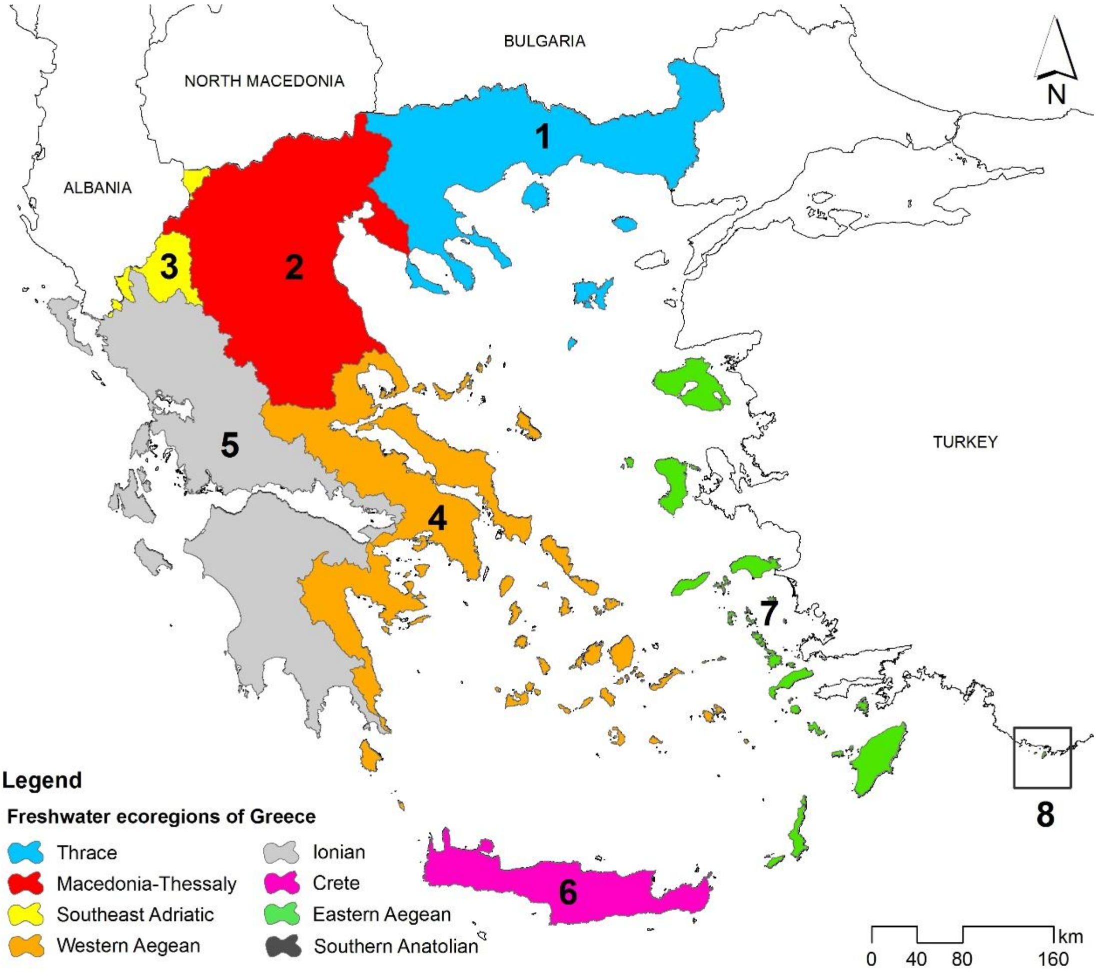


FIGURE I.3. The eight freshwater ecoregions of Greece. Numbers in the first map denote the freshwater ecoregions: 1. Thrace; 2. Macedonia -Thessaly, 3.Southeast Adriatic; 4. Western Aegean; 5. Ionian; 6. Crete; 7. Eastern Aegean; 8. Southern Anatolian.

Greece holds a unique ichthyofaunal diversity within Europe (Economou et al., 2007) and displays the highest level of fish species endemism in the Mediterranean region (Crivelli, 1996). According with the most recent nationwide checklist released (Barbieri et al., 2015), to date, 160 freshwater fish species have been recorded in Greece, where 137 are considered as native. The country presents a substantial proportion of country-specific endemics, 47 in total (or 34% of the native fish fauna, Fig. I.4). Moreover, 10% of fish species are endemic or almost endemic (occurring also in transboundary freshwater water bodies) and if alien and species with a marine origin are excluded, the percentage raises up to 56%.

According to the IUCN, many of the freshwater fish species occurring in Greece are under a threatened status. Specifically, 53 species are considered threatened at a global scale (i.e., CR, EN, VU) corresponding to 39% of all native inland water fish species in Greece. As elsewhere, anthropogenic alterations, such as overexploitation of water for irrigation, habitat degradation and fragmentation due to morphological changes in river corridors and pollution are the main threats that freshwater fish species of Greece are dealing with. These anthropogenic stresses are also augmented by localized overfishing and especially by introduced alien fishes (Economidis et al., 2000; Caiola and de Sostoa, 2005; Kalogianni et al., 2019). In fact, whole assemblages of fishes are rapidly changing as the impacts of non-indigenous invasives spread into Greece; a country that once had few alien species compared to other European states (Bianco, 1990).

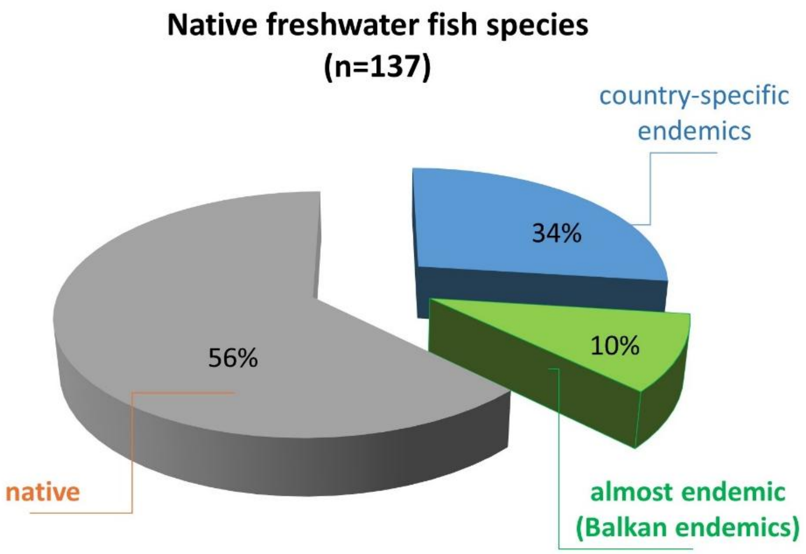


FIGURE I.4. The Native freshwater ichthyofauna of Greece.

Non-indigenous invasives include both alien fishes from abroad as well as translocated fishes present in other ecoregions within Greece. Even though many studies have reported adverse impacts of non-indigenous freshwater fish species into native biota and their environment many aspects of introduced species in Greece are lacking in order to tackle this increasing threat. Specifically, the distribution of non-indigenous freshwater fish species in Greece is poorly documented and even qualitative survey compilations are very recent (Economou et al., 2007; Corsini-Foka and Economidis, 2007; Zenetos et al., 2009; Koutsikos et al., 2012; Leonardos, 2016). Most studies referring to alien freshwater fish species are mainly from lentic environments (e.g. Tsekos et al., 1992; Rosecchi et al., 1993; Crivelli et al., 1997; Economidis et al., 2000; Perdikaris et al., 2005; Leonardos et al., 2007).whereas translocated species are usually neglected and there is no surveillance of them for the entire country. Quantitative data of introduced fish populations in lotic ecosystems are especially scarce (Economou et al., 2016). However, even under these data-scarce conditions, some risk assessment studies have been recently implemented at broad spatial scales and thematic contexts (Papavlasopoulou et al., 2014; Perdikaris et al., 2016; Piria et al., 2018). With regard to the freshwater fish fauna of Greece, reviews and checklists produced by Stephanidis (1939), Economidis (1973, 1991) and Economou et al. (2007) indicate an increasing rate of occurrence of non-indigenous fish. An overview of the introduced fish species to the inland waters of Greece published by Economidis et al. (2000) contains comprehensive information for each species listed. Recently, more data on the introduced species in individual drainages have been provided by numerous authors, i.e., Economou et al. (2004), Economou et al., (2007), Leonardos et al. (2007) and Barbieri et al., (2015). Despite periodical reviews, site-based inventory and monitoring of species and assemblage distributions are poorly developed and coordinated in Greece.

Two sides to every story

The past decades have seen enormous attention and effort focus upon the research and the management of invasive species. Yet, as every major scientific issue, the negative role of introduced species *per se* has been entered into a deep dispute. Specifically, consensus is lacking regarding the potential role of every introduced species on harming other natives or in influencing the integrity of ecosystems (Copp et al., 2005). The common view within the scientific community is that the introduction of non-indigenous species is harmful and fundamentally negative for biodiversity (Courtenay and Moyle, 1992; Moyle and Leidy, 1992; Cooke and Cowx, 2004; Vitule et al., 2006, 2009; Simberloff, 2007, 2013; Leprieur et al., 2009). On the other hand, there is a growing interest among a portion of scientists with a general view that the vast majority of species introductions (especially in freshwater fish taxa) are not identified as having an ecological impact, while having great socio-economic benefits (Sagoff, 2005, 2007; Brown and Sax, 2007; Gozlan, 2008, 2009). The scientific debate caused quite a stir and reached to a point of allegations for pseudoscience made by skeptics (Theodoropoulos, 2003; Thompson, 2014), which resulted counteraccusations of science denialism by invasion biologists (Russell and Blackburn, 2017; Ricciardi and Ryan, 2018). According to Frank (2019) the charges of "invasive species denialism" degenerated into name-and-shame style publications triggering further responses to the latter accusations (Davis and Chew, 2017; Munro et al., 2019; Sagoff, 2020).

Scope and aims of the current thesis

Given the increasing trend of introduced fish species into neighboring countries and the potential threat they pose to the local endemic fish fauna of Greece, it is of paramount importance to track the current distribution of non-indigenous fish species, as well as their vectors and pathways, and their ecological effects on native biota, in order to design and implement appropriate control and mitigation measures.

The present study is the first attempt to track alien and translocated freshwater fish species inhabiting lotic ecosystems of Greece. Overall, the main objectives of the current research were to:

- provide a literature review of alien freshwater fish species inhabiting Balkan's inland waters with a focus on Greece, by providing a historical overview of introductions, their impacts, as well as the current dispersal of alien fishes in the region.
- test a readily transferable screening procedure on invasive patterns of alien and translocated fish species in lotic ecosystems of Greece at different spatial scales, contributing to the application of the EU Regulation on IAS; suggesting gaps and uncertainties, and proposing conservation and management actions.

- develop a novel classification framework based on network analysis to identify and prioritize non-indigenous fish assemblage types in lotic ecosystems, rather than focusing on particular species. The results from this study can contribute to the design of effective post-invasion management actions dealing with specific NIFS assemblages and provide valuable information for the protection of high-priority water bodies.
- evaluate the establishment and the spread potential of a highly traded aquarium fish species (*Poecilia latipinna*) worldwide, with emphasis in Europe and the Mediterranean, as target regions, through the use of climate matching.
- assess the establishment of rainbow trout, one of the world's worst alien invasive species, in Greek lotic ecosystems and explore the factors affecting the success or failure of establishment.
- explore the potential use of introduced species into scientific research, by investigating the presence and abundance of microplastics via translocated fish species as bio-indicators.

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Chapter 1

ALIEN FRESHWATER

FISH SPECIES

IN BALKAN PENINSULA: ONE OF THE WORLD'S BIODIVERSITY HOTSPOTS

BASED ON*:

Piria M., Simonović P., Kalogianni E., Vardakas L., Koutsikos N., […] et al., **2018**.
Alien freshwater fish species in the Balkans – Vectors
and pathways of introduction.

FISH AND FISHERIES 19(1): 138-169

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INTRODUCTION

The introduction of non-indigenous fish species (hereafter NIFS) into natural, semi-natural and novel aquatic ecosystems has been consistently reported as one of the principal causes of biodiversity loss (Dudgeon et al., 2006; Butchart et al., 2010; Liu et al., 2017). Freshwater fish introductions have increased exponentially during the last decades; although, not all introduced fish species develop established populations, and from those that do many have minor effects on their host ecosystems. The ecological damage as well as the negative socio-economic damages of human-induced fishes introductions have been well established by a constantly growing literature (Hulme, 2009; Vitule et al*.,* 2009; Gozlan et ajll*.,* 2010; Cucherousset and Olden, 2011). Moreover, the introduction of non-indigenous species together with further anthropogenic stresses such as habitat loss and degradation, hydromorphological alteration and pollution often interact towards the reduction or even the extinction of native fauna (Arthington et al., 1983; Kennard et al., 2005; Dudgeon et al., 2006; Olden et al., 2010). NIFS introductions may produce severe ecological damage by affecting native species at various levels of biological organization through predation, competition, habitat degradation, food web alteration, hybridization and disease transmission (Copp et al., 2005; Savini et al., 2010; Ribeiro and Leunda, 2012; Havel et al., 2015).

Inventories of non-indigenous species have been compiled for several countries (Elvira and Almodovar 2001; Gherardi et al., 2008; Gollasch and Nehring 2006), and recently a European list of introduced freshwater alien species has been compiled by the European Alien Species Information Network (European Alien Species Information Network, EASIN; Katsanevakis et al., 2012). In a recent study of the first Europe-wide assessment of the major pathways of first introductions for freshwater alien species has been compiled yet, the countries of the Balkan Peninsula were excluded (Nunes et al., 2015). Detailed analyses, of all European regions may provide useful insights into patterns and drivers of biological invasions, contributing to developing strategies for the management of alien species in different spatial scales and could provide information on key recipient areas of introduction for the purposes of surveillance and prevention, in line with the requirements of the new European Regulation on Invasive Alien Species (EU 2014).

The Balkan Peninsula (Balkans) is one of the world's 35 biodiversity hotspots (Mittermeier et al. 2011) and together with the Iberian and Apennine Peninsulas, harbours vast genetic and species diversity (Hewitt 2011). More specific Balkans hosts a highly unique native freshwater ichthyofauna with several endemic genera and many endemic species (Simonović et al. 2013), including Greece, the country with the highest proportion of endemic fishes in Europe (Bobori and Economidis 2006; Economou et al. 2007; Barbieri et al. 2015). Lentic and lotic ecosystems within the Balkan Peninsula are characterized by high biological diversity and endemism; however, the introduction of non-indigenous species represent a major threat for their biodiversity. Many aquatic animals have been long ago naturalized in the Balkans, though there are several recently introduced species, even in isolated lakes (Banarescu 2004). Thus, the high rate of alien fish species introductions in the Balkans presents threats to the local endemic fauna and possible socio-economic damages, thus identification of introduction pathways, their current dispersal and the ecological implications of their introduction, becomes of great importance for designing and implementing appropriate post-invasive management actions. However, there are no complete lists of the alien freshwater fishes in the Balkan area that follow invasions from their very beginning, and consequently, the initial pathway and subsequent dispersion of many introduced species remains unknown.

The current study reviews alien fish species of the Balkan Peninsula, and focuses on Greece that possess the highest proportion of range restricted endemic fish species in Europe (Kottelat and Freyhoff 2007; Barbieri et al., 2015). Furthermore, attempts to fill this gap in our knowledge on alien species in the Balkans by providing the first comprehensive review of alien species introductions, aiming to advance the knowledge of the history of introductions, vectors and pathways of fish species introductions, based on a thorough review of the scientific and grey literature. Future prospects concerning alien fish dispersal in the Balkans, especially under current climate change scenarios are also discussed.

STUDY AREA

The Balkan Peninsula lies between continental Europe and Western Asia, and is surrounded by the Adriatic and Ionian Seas in the west, the Mediterranean Sea in the south and the Aegean, Marmara and Black Seas in the east. Its high fish biodiversity is a result of the region's geological and palaeoclimatic history, and the geophysical variety of its inland water bodies (Griffiths et al., 2004; Skoulikidis et al., 2009). The major biogeographical barrier in the Balkans is the Dinarides–Hellenides mountain chain that separates the western and eastern faunas, with the early isolation of the Western Balkans in the Miocene leading to a rich endemic fauna (Bianco 1986; Gasc 1997, Skoulikidis et al.,, 2009; Ćaleta et al., 2015). In contrast, the Eastern Balkans, have a lower degree of endemicity, but a higher richness of aquatic biota, and are influenced by adjacent biogeographical regions, such as the Black Sea and Western Anatolian regions.

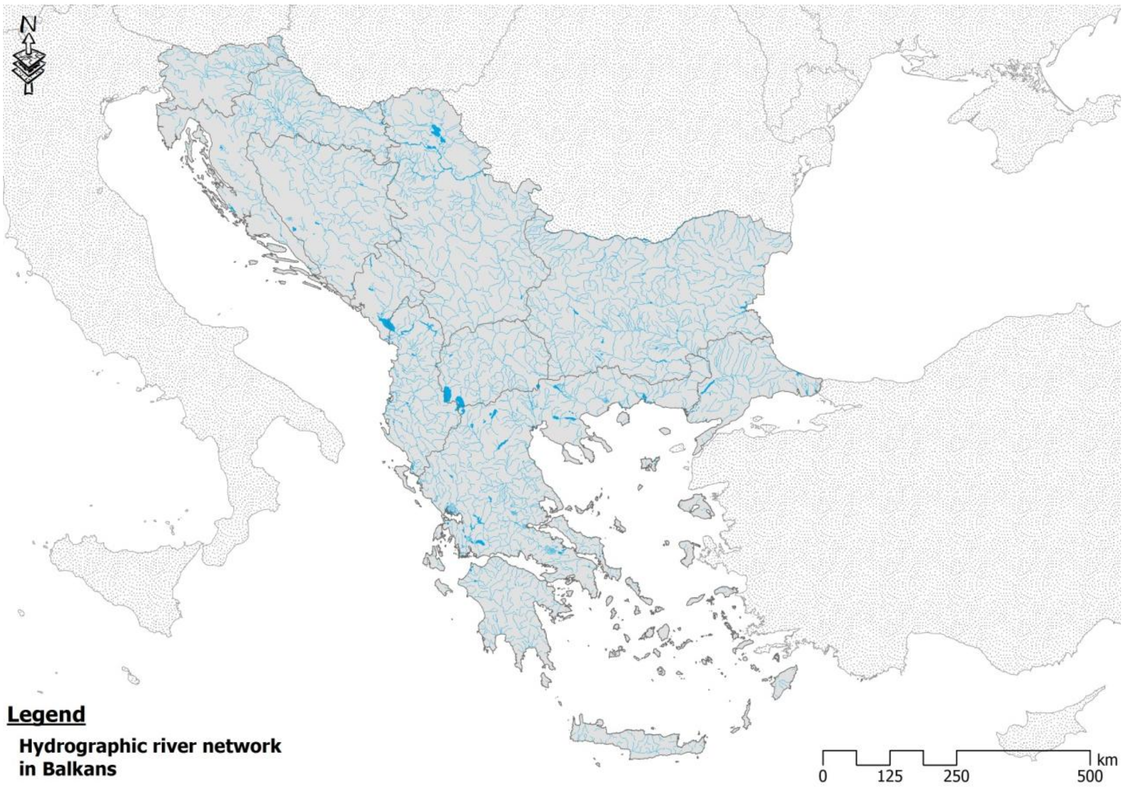
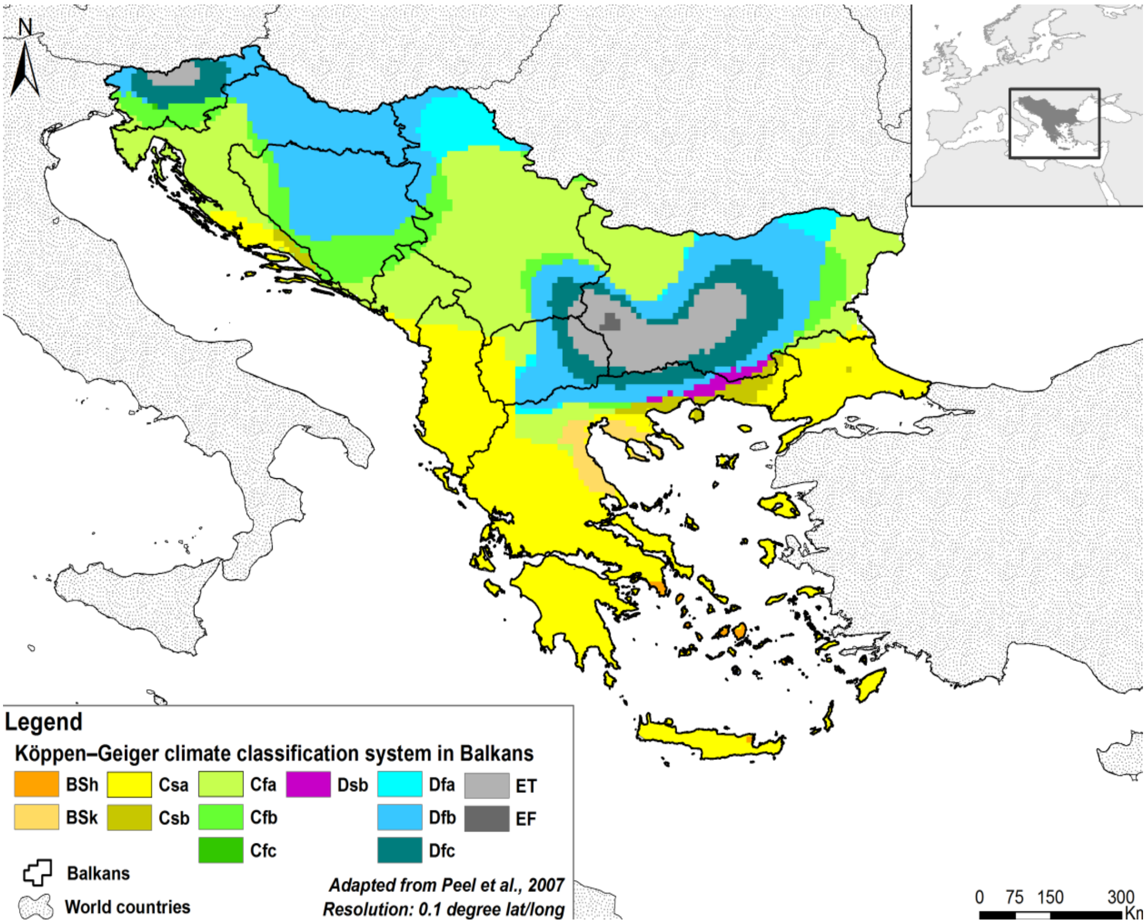


FIGURE 1.1 Hydrographic network of the Balkan Peninsula.

The inland waters of the Balkans are characterized by extreme hydrographic fragmentation, with hundreds of autonomous river basins, numerous natural lakes and artificial large and small dams/reservoirs (Fig. 1.1). The largest river networks are those of Serbia and Bulgaria. Serbia's extended river network of 65,980 km is dominated by streams and small to medium length rivers less than 100 km in length (Gavrilović and Dukić 2014), while Bulgaria has a dense network of some 540 rivers and streams (Bulgarian Ministry of Environment and Water resources 2016) with a total length of 19,761 km. In the northwestern Balkans, Slovenia's dense river network (river density of 1.33 km/ km2) is also extended (26,989 km) and consists of 59 rivers, which like the rivers of Croatia and Bosnia-Herzegovina, are divided hydrologically into the Black Sea and the Adriatic drainages.



Adapted from Peel et al., 2007
Resolution: 0.1 degree lat/long

FIGURE 1.2 Koppen Geiger climate classification system in Balkans.

The hydrological network of Montenegro is also divided into two hydrographical catchment areas, i.e. the Black Sea and Adriatic Sea drainage basins, which are almost equally represented (52% and 47.8% respectively). In the southern part of the Balkan Peninsula, the river network of Greece is both extended and highly fragmented, with 22 major rivers, 12 draining into the Aegean Sea, nine into the Ionian Sea and one, the Aoos/Vjosë River flowing via Albania into the Adriatic Sea. Most of the larger rivers are located in Northern Greece and are transboundary rivers, i.e. Axios, Strymon, Nestos and Evros. Evros (Meriç/Maritsa) is also the largest river of Eastern Thrace (Turkey), followed by Rezovo River (Rezve Deresi Mutludere) flowing through Turkey and via Bulgaria into the Black Sea. Other large rivers in Greece are the Aliakmon (northern Greece) and Pinios (central Greece) that flow into the Aegean Sea, while Kalamas, Acheron, Louros, Arachthos and Acheloos are large rivers situated in Western Greece that flow into the Ionian Sea.

The climatic differences between the various parts of the Balkans further contribute to these biogeographic differences. Climate is generally characterized by a distinct bimodal seasonality and a strong N–S gradient, with increasing temperature and decreasing precipitation towards the S–SE (Skoulikidis et al., 2009). Furthermore, the eastern Balkans are characterised by much lower precipitation than the Western Balkans. According to Peel et al., (2007), with subsequent corrections by Koutsoyiannis et al., (2008), the modified Köppen-Geiger climate type map of the Balkans shows there are four main climate types in the region (Fig. 1.2). The dominant climate type by land area is temperate (C, 61.3%), followed by cold (D, 31.1%), polar (E, 5.6%) and arid (B, 2.1%). In contrast, Europe as a whole is dominated by the cold climate type (D, 44.4%), followed by arid (B, 36.3%, Peel *et al.* 2007). Overall, there are 13 subtypes within the Balkan Peninsula, the majority of which belong to the temperate/mesothermal climates (group C, Fig. 1.1) with the two dominant temperate types, Csa and Cfa, encompassing more that 50% of the Balkans.

RESULTS

Alien species in freshwater ecosystems of Balkans

Owing to the high level of endemism and the great conservational value of the Balkan freshwater fish species, introductions can have large negative and irreversible impacts on the ichthyodiversity of the Peninsula (Karapetkova and Zhivkov 1995; Sušnik et al., 2007; Snoj et al., 2007; Mrdak et al., 2012; Barbieri et al., 2015; Ćaleta et al., 2015; Povž et al., 2015). Although, recent surveys from several Balkan countries, however, have revealed that 15–23% of their fish faunas are non-indigenous (Simonović et al., 2013; Piria et al., 2016a,b; Barbieri et al., 2015), with certain catchments, such as the Danube River and Pamvotis Lake (Greece), having an ichthyofauna comprised of more than 50% and 80% of introduced fishes respectively (Simonović et al., 2013; Leonardos et al., 2008).

In total, 60 fish species have been introduced in the Balkan Peninsula (Table 1.1), with the motivation for the introductions, and the historical timeline, vary in individual Balkan states. The first introductions were documented in the 19th century in Bulgaria (two species), Croatia (one species) and Slovenia (four species). Known introductions were performed, from 1901 to 1920, in Bosnia-Herzegovina and Croatia, from 1921 to 1940 in Croatia, Serbia, Bosnia-Herzegovina and Bulgaria and after 1940 in Albania, Greece, Montenegro and Serbia. In most countries, the highest number of introductions took place between 1960 and 1980. Since 2000, Turkey has not documented any new alien fish species introductions, as opposed to Croatia, which has recorded the highest number of new alien species since then (Ćaleta 2011a,b; Jelkić and Opačak 2013; Safner et al., 2013; Šanda et al., 2013), though this may be the result of intensive ichthyological research in the period after 2000 rather than new introductions.

The first attempts of non-indigenous fish introductions into Europe are not known, as they predate the modern era of introductions (Holčik, 1991). Introductions and/or translocations of fish in Europe may be traced back to the beginning of first century A.D., when some species of fish, mostly marine, were held in piscinae, fish ponds, by the Romans (Balón, 1969). The common carp, *Cyprinus carpio* Linnaeus, 1758, appears to have been the first freshwater species transferred from its native range, first to Rome and then elsewhere in Europe (Balón, 1995). With the spread of Christianity, the construction of ponds became a necessity, and most monasteries or parishes kept fish transferred from neighbouring streams. Later, the nobility adopted this custom and during the Renaissance, fish farming and ornamental ponds became a widespread trend throughout Europe. The idea of transferring fish from other continents likely arose during Renaissance times (Holčik, 1991).

However, documentation of fish introductions into the Balkan area began in the 19th century with introductions of common carp and goldfish *Carassius auratus* (Linnaeus, 1758) (Copp et al., 2005; Povž and Šumer, 2005; Vilizzi, 2012). These introductions were followed by North American salmonid species (Holčik, 1991). Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) was extensively introduced from the USA to various European countries (Crawford and Muir, 2008) including the Balkan region, where records date back to 1878 in Bulgaria (Uzunova and Zlatanova, 2007). The latter introductions were followed with those of brown bullhead *Ameiurus nebulosus* (Lesueur, 1819) and, possibly, black bullhead *Ameiurus melas* (Rafinesque, 1820) in Croatian ponds in 1905 (Fijan et al., 1989). The negative effects of brown bullhead introductions into Croatia and the unintentional introduction of the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) were observed as early as 1946 (Plančić, 1946). In the mid 1960s, a molly population originated from an ornamental domestically-bred black form of *Poecilia latipinna,* (Lessuer, 1821) releashed in a geothermically-heated lake in Greece (Chintiroglou et al., 1996; Koutsikos et al., 2012, 2017). To our knowledge, this remains the only confirmed established population of sailfin molly in Europe (Koutsikos et al., 2018). In the latter half of the 20th century, the introduction of alien fishes intensified (Copp et al., 2005), including the unintentional introduction of topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel 1846) (Záhorská et al., 2009), and the disease agents and parasites accompanying them (Pinder et al., 2005; Taraschewski, 2006), which threatened European fish diversity (Gozlan et al., 2005).

In the early 1970s, discussions began on the consequences of introductions into open waters, for species such as the brown bullhead, pumpkinseed (Sabioncello, 1971), grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) (Hristić, 1977) and gibel carp *Carassius gibelio* (Bloch 1782) (Vuković and Knežević, 1978) in the Balkan region. The first comprehensive review of fish introductions and their impacts on native fish species was given by Vuković and Kosorić (1978). Their concern was primarily focused on the uncontrolled introductions of fish species into inland waters, particularly into Lake Skadar in Montenegro and translocations occurring in Bosnia and Herzegovina. However, intentional introductions continued following this, as several non-indigenous fish species were introduced and translocated. For example, the European whitefish *Coregonus lavaretus* (Linnaeus, 1758) was successfully introduced into Croatia (Habeković, 1978), Slovenia (Povž and Šumer, 2005), and the inland waters of Bulgaria (Karapetkova and Zhivkov 1995), where their introduction failed.

Finally, other introductions included the Lake Ohrid letnica trout *Salmo letnica* (Karaman, 1924), Atlantic salmon *Salmo salar* Linnaeus, 1758, brook trout *Salvelinus fontinalis* (Mitchill, 1814), and coho salmon *Oncorhynchus kisutch* (Walbaum, 1792), which were introduced in Greece (Tsekos et al., 1992; Crivelli et al., 1997; Economidis et al., 2000a) and Serbia (Janković and Raspopović, 1960a,b). Since the late 1980s, many new fish species were recorded in the inland waters of the Balkans (Kostov et al., 1998; Simonović et al., 1998; Sekulić et al., 1999; Economidis et al., 2000b; Uzunova and Zlatanova, 2007; Simonović et al., 2010; Ćaleta et al., 2011a,b; Safner et al., 2013), including recent records of ruffe *Gymnocephalus cernua* (Linnaeus, 1758) (Petriki et al., 2014).

TABLE 1.1 Alien freshwater fish species introduced in the Balkan Peninsula. AL, Albania; BG, Bulgaria; BiH, Bosnia–Herzegovina; CRO, Croatia; GR, Greece; KS, Kosovo; ME, Montenegro; MK, North Macedonia; RS, Serbia; SLO, Slovenia; TR, Turkey-Thrace.

Species name	Authority	Common name	AL	BG	BiH	CRO	GR	ME	MK	RS	SLO	TR
Abramis brama	(Linnaeus, 1758)	common bream							●			
Acipenser baerii	Brandt, 1869	Siberian sturgeon					●					
Acipenser gueldenstaedtii	Brandt & Ratzeburg, 1832	Danube sturgeon					●		●			
Acipenser ruthenus	Linnaeus, 1758	sterlet							●			
Ameiurus melas	(Rafinesque, 1820)	blacklbullhead			●	●				●	●	
Ameiurus nebulosus	(LeSueur, 1819)	brown bullhead		●	●	●	●	●	●	●	●	
Babka gymnotrachelus	(Kessler, 1857)	racer goby			●	●				●		
Carassius auratus	(Linnaeus, 1758)	goldfish	●			●	●				●	●
Carassius carassius	(Linnaeus, 1758)	crucian carp	●									●
Carassius gibelio	(Bloch, 1782)	gibel carp	●		●	●	●	●	●	●	●	●
Carassius langsdorfii	(Temminck & Schlegel, 1846)	Gin-buna carp					●					
Clarias gariepinus	(Burchell, 1822)	North African catfish		●		●					●	
Coregonus albula	(Linnaeus, 1758)	Vendace		●								
Coregonus lavaretus	(Linnaeus, 1758)	European whitefish		●		●	●				●	
Coregonus peled	(Gmelin, 1789)	peled		●	●	●				●		
Ctenopharyngodon idella	(Valenciennes, 1844)	grass carp	●	●	●	●	●	●	●	●	●	

Species name	Authority	Common name	AL	BG	BiH	CRO	GR	ME	MK	RS	SLO	TR
Cyprinus carpio	(Linnaeus, 1758)	common carp			●						●	
Gambusia holbrooki	Girard, 1851	Eastern mosquitofish	●	●	●	●	●	●	●		●	●
Gasterosteus aculeatus	Linnaeus, 1758	three-spined stickleback								●		
Gymnocephalus cernua	(Linnaeus, 1758)	Eurasian ruffe					●		●			
Hypophthalamichthys molitrix	(Valenciennes, 1848)	silver carp	●	●	●	●	●	●	●	●	●	
Hypophthalmichthys nobilis	(Richardson, 1845)	bighead carp	●	●	●	●	●	●	●	●	●	
Ictalurus punctatus	(Rafinesque, 1818)	channel catfish		●					●			
Ictiobus bubalus	(Rafinesque, 1818)	smallmouth buffalo		●								
Ictiobus cyprinellus	(Valenciennes, 1844)	bigmouth buffalo		●								
Ictiobus niger	(Rafinesque, 1819)	black buffalo		●								
Lepomis gibbosus	(Linnaeus, 1756)	pumpkinseed	●	●	●	●	●	●	●	●	●	●
Megalobrama amblycephala	Yih, 1955	Wuchang bream	●									
Megalobrama terminalis	(Richardson, 1845)	black Amur bream	●					●				
Micropterus salmoides	(Lacepède, 1802)	largemouth (black) bass			●	●	●			●	●	
Mylopharyngodon piceus	(Richardson, 1845)	black carp	●	●				●			●	
Neogobius fluviatilis	(Pallas, 1811)	monkey goby			●	●	●			●		
Neogobius melanostomus	Pallas, 1811	round goby				●				●		
Oncorhynchus kisutch	(Walbaum, 1792)	Coho salmon					●					

Species name	Authority	Common name	AL	BG	BiH	CRO	GR	ME	MK	RS	SLO	TR
Oncorhynchus mykiss	(Walbaum, 1792)	rainbow trout	●	●	●	●	●	●	●	●	●	●
Oreochromis mossambicus	(Peters, 1852)	Mozambique tilapia		●								
Oreochromis niloticus	(Linnaeus, 1758)	Nile tilapia				●	●				●	
Parabramis pekinensis	(Basilewsky, 1855)	white amur bream	●				●		●			
Perccottus glenii	(Dybowsky, 1877)	Chinese sleeper		●		●				●		
Piaractus brachypomus	(Cuvier, 1818)	pirapatinga				●						
Poecilia latipinna	(Lesueur, 1821)	sailfin molly					●					
Poecilia reticulata	Peters, 1859	guppy		●								
Polyodon spathula	(Walbaum, 1792)	Mississippi paddlefish		●		●				●	●	
Ponticola kessleri	(Günther, 1861)	bighead goby			●	●				●		
Proterorhinus semilunaris	Heckel, 1837	tubenose goby			●					●		
Pseudorasbora parva	(Temminck & Schlegel, 1846)	topmouth gudgeon	●	●	●	●	●	●	●	●	●	●
Pterygoplichthys pardalis	(Castelnau, 1855)	spotted sailfin catfish								●		
Pygocentrus nattereri	Kner, 1858	red piranha					●					
Salmo letnica	(Karaman, 1924)	Ohrid trout					●			●		
Salmo salar	Linnaeus, 1758	Atlantic salmon					●					
Salmo trutta	Linnaeus, 1758	sea trout	●		●	●	●	●		●		
Salvelinus alpinus	(Linnaeus, 1758)	Arctic char			●	●				●	●	

Species name	Authority	Common name	AL	BG	BiH	CRO	GR	ME	MK	RS	SLO	TR
Salvelinus fontinalis	(Mitchill, 1815)	brook trout		●	●	●	●	●	●	●	●	
Salvelinus umbla	(Linnaeus, 1758)	lake charr						●				
Sander lucioperca	(Linnaeus, 1758)	pikeperch							●			
Scardinius erythrophthalmus	(Linnaeus, 1758)	rudd	●									
Silurus aristotelis	Garman, 1890	Aristotle's catfish		●								
Squalius cephalus	(Linnaeus, 1758)	chub	●									
Thymallus thymallus	(Linnaeus, 1758)	grayling	●	●				●				
Tinca tinca	(Linnaeus, 1758)	tench	●									

Pathways of introductions

Data revealed that the two main modes of alien species introduction in the Balkan countries were: 1) intentional, i.e. for recreational and sport fishing, aquaculture, ornamental trade, and biological control), and 2) unintentional, i.e. the accidental spread of a species as a consequence of introduction for aquaculture purposes, or the natural spread of a species outside its natural habitat (Fig. 1.3).

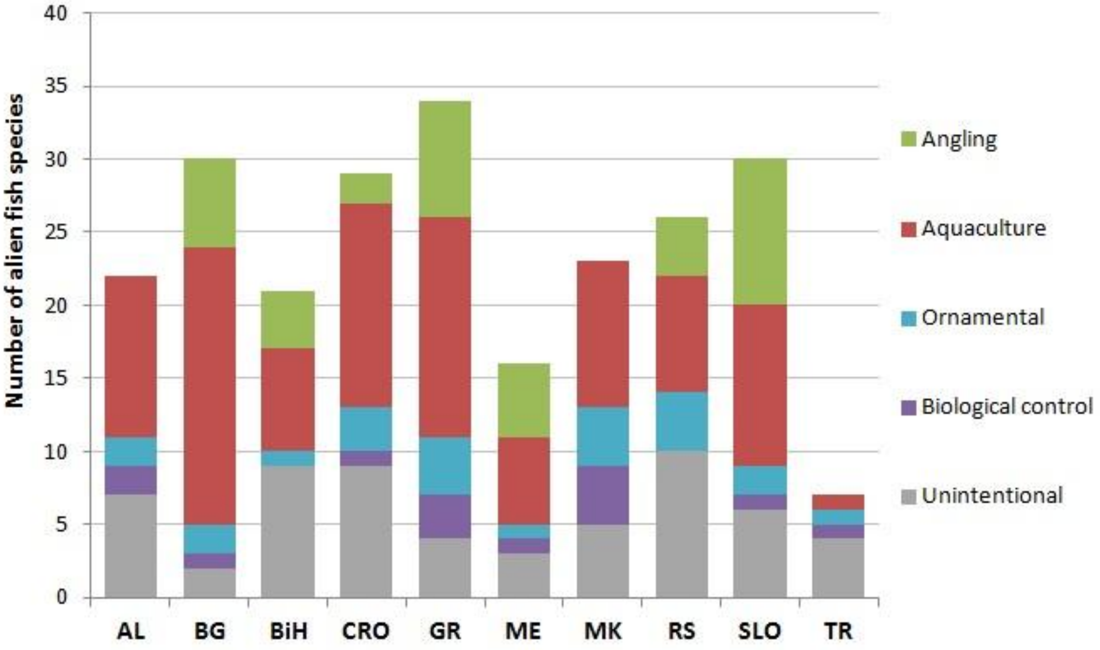


Figure 1.3. Pathways of fish introductions of alien fish species into the Balkan Peninsula. AL = Albania; BG = Bulgaria; BH = Bosnia–Herzegovina; CRO = Croatia; GR = Greece; KS = Kosovo; ME = Montenegro; MK = North Macedonia; RS = Serbia; SLO = Slovenia; TR = Turkey-Thrace), since the beginning of the 20th century.

Data since the late 19th century indicate that introductions continued at an immense rate right up to the 1980s. By country, the highest number of unintentionally introduced species over the last 15 years has been recorded in Serbia and Croatia. The main pathway for introductions, with the highest number of fish species in most Balkan countries, was aquaculture, except in Serbia and Bosnia-Herzegovina, where most species were introduced unintentionally. Recreational and sport fishing (angling) is another major route of alien species introduction and dispersal. In Slovenia, this has been a common practice for more than 100 years, while in other Balkan countries, recreational fisheries and stocking activities began after World War II. Another important introduction route was aimed at biological control. This practice began in Albania, and spread throughout the Balkans after the 1960s. It is interesting that no introductions of this type have ever been reported for Serbia or Bosnia-Herzegovina. Finally, though it appears that the ornamental trade is not a highly important route of introduction, some have warned that this will become an increasing problem in Europe and the Balkans (Chucholl, 2013).

While freshwater aquaculture represents a major pathway for the inter-watershed dispersion of many alien species in Balkans, the freshwater aquaculture sector in Greece was very limited until the 1980s (Perdikaris et al., 2010), focusing mainly on cold-water (salmonid or trout-like) species with rainbow trout (*O. mykiss*) as the predominant species. Sport and recreational fishing (angling) is also a major pathway of the spread of alien freshwater fishes in the Balkans, through their translocation between watersheds, an activity that is very difficult to monitor. In Greece, in contrast, the scale of angling and commercial fisheries in large lakes and reservoirs is scarce, and thus the spread of alien species through these practices is rather limited, in comparison to the other Balkan countries. The ornamental trade has also been recognized as an important pathway for the introduction of invasive species, linked to over 150 species' invasions in natural ecosystems around the world (Fuller 2003; Siguan 2003; Padilla and Williams 2004). The vast majority of the studies on the ornamental trade pathway have been conducted in North America, with few studies conducted in Europe (Padilla and Williams 2004; Duggan et al., 2006; Copp and Fox 2007; Maceda-Veiga et al., 2013). For the Balkan region, data collection and monitoring of the ornamental fish trade is virtually non-existent, as no official datasets are available. Recently, Papavlasopoulou et al., (2014) concluded that the aquarium fish sector is virtually not under any control in Greece, given the existence of threatened species, species potentially harmful to humans and species capable of establishing non-indigenous populations, if released into the wild. Finally, only three species have been introduced in the Balkans for biological control: the grass carp (*Ctenopharyngodon idella*), the silver carp (*Hypophthalmichthys molitrix*) and the highly invasive Eastern mosquitofish (*G. holbrooki*). The grass carp was introduced to control the growth of aquatic vegetation (Economidis 1991) and the silver carp to control planktonic assemblages (Vuković and Kosorić 1978), though these introductions had negative implications for inland water communities (Domaizon and Dévaux 1999; Leonardos et al., 2008). The Eastern mosquitofish was introduced in the Balkans and elsewhere in the Mediterranean in the 1920s for mosquito control, with questionable results (Stephanides 1964). Today, the eastern mosquitofish is the second most widespread non-indigenous fish species in the Mediterranean region and the most widespread in Greece, occurring in 49.5% of its river basins (Economou et al., 2007).

Origin of introductions

The majority of introduced species in the Balkans are of North American origin (25.0%), followed by Asian (23.3%) and Eastern European species (23.3%), which coincides with the chronology of the first introductions. Until the early 1950s, introductions were primarily of North American and Asian species (Nežić 1938; Livadas and Sphangos 1941; Fijan et al., 1989; Holčik 1991; Copp et al., 2005; Povž and Šumer 2005), while the interest for species from Northern and Western Europe arose later. Other introduced species originated from Northern Europe (8.3%), South America (5.0%), Asia/Europe (6.7%), Africa (5.0%) and Western Europe (3.3%) (Fig. 1.4). Ichthyofaunal research in Kosovo is sparse, and the ichthyofauna is poorly known in most of the country (Gashi et al., 2016).

The majority (38.46%) of species in the Balkan Peninsula are distributed in restricted territories, including alpine lakes (e.g. arctic charr in Slovenia), reservoirs (e.g. pikeperch in North Macedonia), artificial lakes and reservoirs (e.g. Aristotle's catfish in Bulgaria) and isolated and small river basins (e.g. grayling in Montenegro). In the current study, species distributed in all major basins and waterbodies of a country (rivers, lakes) are considered widespread species, and 33.17% of all introduced fishes are widespread.

The most widespread species are gibel carp, pumpkinseed, brown bullhead, Eastern mosquitofish and topmouth gudgeon, due to their highly invasive potential

(Simonović et al., 2013; Piria et al., 2016b; Perdikaris et al., 2016). Species with a moderate distribution (15.38% of the total) are those found in a single basin of a country, and includes species such as monkey goby and bighead goby in Croatia, goldfish in Albania and Greece, silver and bighead carp in most countries. For 12.98% species, their Balkan distribution is unknown, including species found/caught only once (e.g. short snouted pipefish in the Danube River), sporadically (e.g. Mississippi paddlefish in Serbia, Croatia and Bulgaria) or introduced, but their status remains unknown (e.g. brook trout in Skadar Lake), (Fig. 1.5A). The prevalence of the alien species in the Balkan countries (Fig. 1.5B) suggests that most have become widespread in Serbia, Slovenia, Croatia, Turkey and Albania.

FIGURE 1.4 Origin* of introduced alien fish species in the Balkan Peninsula. Numbers in arrows denote the number of alien fish species of varying origins introduced to the Balkans. The standard Jenks Natural breaks classification method (ArcGIS, version 10.1) was used to define the class ranges of species per 1,000 km^2 groups. *AFR = Africa; AS = Asia; EE = Eastern Europe; NE = Northern Europe; NA = North America; SA = South America; WE = Western Europe.

The majority of these species have a restricted distribution in Greece, Bulgaria, North Macedonia and Montenegro. Most introduced fish species in the Balkans have naturalized (Fig 1.5C), with the exception of Turkey, where most introduced fish have acclimatized (Fig 1.5D).

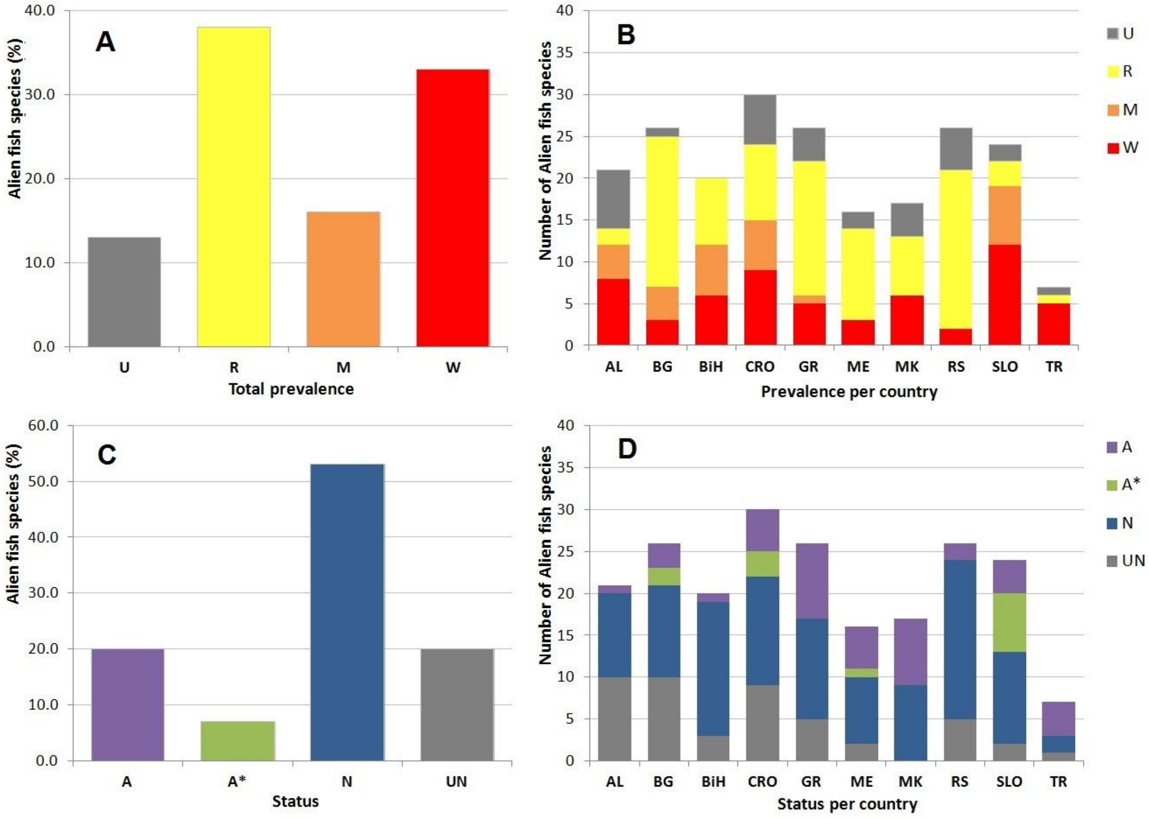


FIGURE 1.5 Alien species (A) total prevalence; (B) prevalence per country; (C) status of acclimatization; (D) status of acclimatization of introduced species per country. W = widespread species; M = moderate distribution; R = restricted; U = unknown distribution; A = acclimatized; A* = acclimatized in restricted areas; N = naturalized; UN = unknown status.

Ecological implications

The introduction of new species can have unexpected negative consequences (Gozlan et al., 2005), it may not have any reported ecological impact on the native ecosystem (Gozlan 2008), or it may disappear without causing any ecological impacts (Lehtonen, 2002). In the Balkans, there are few reports on the ecological impacts of introduced fishes. Most publications are studies outlining the general risks associated with the introduction of non-indigenous fish species (Vilizzi 2012). Many introduced alien species cause adverse effects (Economidis et al., 2000a,b; Copp et al., 2005; Gozlan et al., 2005; Gozlan et al., 2010; Britton et al., 2011), though there is an ongoing scientific debate about whether, instead of causing significant disturbances in the ecosystems, the introduction of non-indigenous species can result merely in an increase of biodiversity (Gozlan, 2008), or can even have positive impacts on native species (Rodriguez, 2006; Schlaepfer et al., 2011). In some natural lakes (and reservoirs) with an impoverished fish fauna or no fish fauna, the introduction of alien species through concerted state-sponsored stocking programmes and private initiatives could be deemed beneficial, by increasing fisheries production or by contributing to the ecological balance of eutrophic lentic ecosystems (for Greece, see Economidis et al., 2000a). In many other lentic systems, however, introductions of alien species have dramatically altered their ecological communities. Extensive stocking, mainly for commercial purposes and sport fishing, coupled with unintentional introductions, have radically affected the fish fauna of those aquatic systems, leading even to the extinction of native species.

In addition to other negative impacts of alien species on freshwater ecosystems, the introduction of non-native infectious agents represents one of the greatest risks associated with this global movement and introduction of species (Britton et al., 2011). From the perspective of the entry of pathogens into Balkan freshwaters, few studies have given reports (Nikolić and Simonović 2002; Nikolić et al., 2007), but none have focused on the consequences and/or impact mechanisms on native populations. Little is still known about the consequences of the introduction of freshwater fishes and of various pathogens, indicating the need for future monitoring and research efforts concerning this issue (Britton et al., 2011). On the other hand, due to climatic reasons, several alien species have rare to no natural reproduction in the Balkans and as a result, dispersion is often limited.

Climate change

Under current climate change scenarios, impacts on Balkan freshwater biodiversity are expected to increase as a general rule, since the number of alien species involved is growing and the vulnerability of ecosystems to invasions is increasing. Rising water temperatures will particularly affect freshwater fishes (Cochrane et al., 2009) and the problem is expected to be severe, for example, in Greece, where 39% of all native fish species are already classified as threatened (Barbieri et al., 2015). Moreover, changes in the sea level with the consequent flooding, erosion and salinization of coastal lowlands and estuaries (Handisyde et al., 2007) can cause further alterations in the balance of aquatic systems and create opportunities for tropical and subtropical alien species to invade fragile Balkan coastal wetlands, lagoons and spring-fed pools, which host strictly endemic species with highly localized distributions. One such example is Greece, with the endemic species almiri toothcarp (*Aphanius almiriensis*, Cyprinodontidae), Corfu toothcarp (*Valencia letourneuxi*, Valenciidae) and others. On the other hand, the high degree of intermittency in the freshwater resources of the southern Balkans may possibly be a restrictive factor for the spread of invasive species. For instance, the Adriatic basin is characterised by small isolated river basins, many of which are subject to extreme hydrographic fragmentation, which could reduce the range expansion of newly introduced freshwater fish species. Nevertheless, climate change induced alterations of ecosystem conditions, i.e. in the large number of large-scale transboundary river basins in the Balkans, may enable the spread of invasive species and favour the creation of habitats and conditions suitable for newly introduced invasive species (EPA 2008). Indicative of that, is the finding that the majority of freshwater alien species in the inland waters of Greece have been recorded in Northern Greece, where most lentic and lotic systems are transboundary. Thus, climate change may either create additional opportunities for invasion or, alternatively, conditions unsuitable for certain invasive species, thus the relative importance of climate change impacts on invasive freshwater fish species in the Balkans depends primarily on the ichthyoregion considered and the traits of each fish species..

DISCUSSION

As a consequence, primarily of aquaculture activities but also other motives for introduction, such as angling, biological control and to a lesser degree the ornamental trade, is the recent increase in the number of the total fish species in each country of the Balkan Peninsula suggesting an ongoing process of biotic homogenization. Studies of alien fish introductions in Mediterranean-climate regions have emphasised the loss of faunal uniqueness, albeit with a concurrent increase in the total number of fish species. A 7% average increase in taxonomic and functional similarity of freshwater fish fauna has been documented as a result of human activities (Marr et al., 2013). All Mediterranean regions around the world are displaying taxonomic and functional homogenisation in more than 50% of their catchments, with the exception of the southwestern Cape, Central Anatolia and the Aegean Sea drainages (Marr et al., 2013). Overall, catchments exhibiting taxonomic homogenisation are also homogenised in terms of their functional trait composition, which may have important consequences for the functioning of these ecosystems (Olden et al., 2016). These studies on biotic homogenization, however, also included translocated species, which may possibly play a stronger role than exotic species (Villéger et al., 2014), but were not considered in the current study. Thus, the temporal changes in genetic, taxonomic and functional dissimilarities for freshwater fishes in Bakans could be even greater and further research on this topic is certainly required.

For the Csa climate type, which is the predominant type in the Mediterranean regions of most of the Balkan countries, as well as Spain and Italy (Peel et al., 2007), concerns have been expressed over impacts on freshwater biota (Vilizzi 2012). Furthermore, the Balkan Peninsula, which is dominated by the Csa climate type, is the locality for the largest number of endemic species (Oikonomou et al., 2014) that are directly threatened by the introduction of non-indigenous species (Snoj et al., 2007; Ribiero and Leunida 2012; Ćaleta et al., 2015; Piria et al., 2016b). The Cfa climate type is also found in Asia and North America, where the Dfb climate type is also significantly represented (Peel et al., 2007). It is from the Cfa and Dfb climate areas of North America that the pumpkinseed, brown bullhead, black bullhead and Eastern mosquitofish originate (Froese and Pauly 2016), and therefore, the climatic conditions in the Balkans are suitable for their acclimation and naturalisation (Fox et al., 2007; Vidal et al., 2010; Movchan et al., 2014). Most of Northern and Western Europe are characterised by the Dfa and Cfb climate types (Peel et al., 2007), which also corresponds to part of the Balkan Peninsula, and therefore the introduced species from these areas are also capable of naturalisation, as seen in the case of European whitefish and peled (Uzunova and Zlatanova 2007; Hamzić et al., 2011). In contrast, the African and South American climate types do not correspond to the climatic conditions in the Balkans. Therefore, the survival of species of that origin is limited to thermal waters, such as the Nile tilapia in Slovenia (Povž 2009), though there is a realistic threat of naturalisation in Csb areas, for example, the Nila tilapia in Greece (Perdikaris et al., 2010; Barbieri et al., 2015).

In addition to the diverse impacts of alien species on freshwater ecosystems, the introduction of non-native infectious agents represents one of the greatest risks associated with this global movement and introduction of species (Britton et al. 2011). As such, topmouth gudgeon in Europe is a carrier of several pathogens (Gozlan et al. 2005), which poses a risk not only for the freshwater aquatic species, but this threat has also expanded to the production of marine fishes, e.g. as with the transfer of the S. destruens pathogen to European seabass *Dicentrarchus labrax* (Linnaeus, 1758) production (Ercan et al. 2015). From the perspective of the entry of pathogens into Balkan freshwaters, few studies have given reports (Nikolić and Simonović 2002; Nikolić et al. 2007), but none have focused on the consequences and/or impact mechanisms on native populations. Little is still known on the consequences of the introduction of freshwater fishes and of various pathogens, indicating the need for future monitoring and research efforts concerning this issue (Britton et al. 2011).

There have been several attempts to clasify alien speceis according to the magnitude of their environmental impacts, based on the mechanism of impact throuh which they exert their effect i.e. predation, competition, hybridization, disease transmission (see Blackburn et al., 2014). The obvious aim remains a standardized method of impact assessment that would be applicable at a wide range of spatial scales, from global to regional and national. The effects of alien species can range from minimal to massive and the various stages of the invasion process require different management itervention, with special emphasis on the halting of their secondary spread, after having established isolated populations (see Vander Zanden and Olden 2008; Blackburn et al., 2011). However, under current climate change scenarios, invasive alien species are expected to accentuate climatic stress effects by reducing the number of native species and/or their functional types within the ecosystem and by increasing ecosystem susceptibility to climatic perturbation (Masters and Norgrove 2010).

Although national legislations are in place that prohibit introductions in the countries of the Balkan Peninsula, as in for many other European countries (Copp et al., 2005) these legislations are rarely enforced by national environmental agencies and are practically ineffective. Furthermore, in most Balkans countries, measures to effectively control the introductions or translocations of non-indigenous fish species are still lacking. In addition, within the framework of the recent European IAS regulation, several issues related to invasive species control which are by definition cross-border cannot be addressed without a collaboration between the various countries, especially since EU Member States along the EU's borders are potentially at greater risk of new bioinvasions from their non-EU neighbors (EC, 2008), since non-EU bordering countries are not obliged to enforce EU laws concerning IAS. Thus, a common framework guiding the implementation of IAS legislation between EU and neighboring non-EU countries are therefore urgently required (Piria et al., 2017) as well as a congruence of the legal framework betwee Balkan states. The large number of transboundary rivers and lakes in the Balkans acting as corridors of dispersal beyond state boundaries makes this common framework an even more urgent priority. Transboundary agreements such as the recent one betwen Greece and Bulgaria for River Nestos or for North Macedonia, Greece and Albania for Prespa Lakes on coordinated management and planning are positive initiatives towards that goal.

Pursuant to this, it would be desirable to prioritise the Balkan Peninsula for the purpose of actions to remediate and/or control introduced as well as translocated fishes, which has been lacking, following the example of countries where progress has been made in the management of invasives, e.g. of common carp in Australia or topmouth gudgeon in England and Wales. Nonetheless, these attempts demonstrate that eradication may not be possible, though management efforts can aim to control their distribution and dispersal in the environment and reduce their impacts on natve species and ecosystems (Britton et al., 2011).

The current study has shown that many alien species in the Blakans are widespread. A major corridor for their spread are the transboundary rivers and lakes, such as, for example, the Ohrid-Drin-Skadar river system draining a large number of countries in the Balkans, the Danube River draining a large portion of Eastern Europe and a number of several basins (Aoos/Vjose; Axios/Vardar; Strymon/Struma; Nestos/Mesta; Evros/Meric; Prespa Lakes) draining Greece with four other southern countries of the region. In the Balkans, most major rivers and lakes are transboundary creating conflicts of interest since water resources are unevenly distributed between the different countries. Thus, there is a strong need for coordination in river management issues, such as pollution, hydroelectric energy production and the control of alien fish species. In addition, there are also many gaps in our knowledge on the current state of dispersal and the impacts of many alien species in the Balkans. These gaps should be targeted through coordinated research that could inform proper preventive and mitigating conservation efforts. In the Balkan Peninsula, there remains an immeasurable wealth of freshwater fish diversity, despite the fact that there are very few freshwater ecosystems left intact by introduction, translocation or stocking with alien genetic material. Above all, there is a great obligation and responsibility to preserve these unique ecosystems for future generations, which can be saved only through rational and joint long-term conservation actions.

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Chapter 2

TRACKING

NON-INDIGENOUS

FISH SPECIES IN LOTIC ECOSYSTEMS: INVASIVE PATTERNS AT DIFFERENT SPATIAL SCALES IN GREECE

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INTRODUCTION

Freshwater ecosystems and their biota are under immediate threat, confronting multiple and often interacting anthropogenic stresses (Ormerod et al., 2010). One of the leading drivers for freshwater biodiversity loss and degradation of ecosystems is the human-induced introduction of non-indigenous fish species (hereafter NIFS) (Saunders et al., 2002; Dudgeon et al., 2006; Liu et al., 2017). The introduction of NIFS may produce severe ecological damage by affecting native species at various levels of biological organization through predation, competition, habitat degradation, food web alteration, hybridization and disease transmission (Copp et al., 2005; Savini et al., 2010; Ribeiro and Leunda, 2012; Havel et al., 2015). Apart from the ecological perspective, NIFS may also create serious socio-economic impacts (Pimentel et al., 2000; Helfman, 2007).

However, although NIFS are frequently blamed for adverse environmental impacts, the evidence for this is often weak and circumstantial (Ribeiro and Leunda, 2012). We leave aside the questions of how to identify and quantify impacts of non-indigenous species (for related discussions and debates, see: Gozlan, 2008; Leprieur et al., 2009; Ricciardi et al., 2013; Russell and Blackburn, 2017; Crowley et al., 2017; Briggs, 2018; Ricciardi and Ryan, 2018; Sagoff, 2018). We further emphasize that if some kind of (negative) ecological impact is generated by an invader, the magnitude of this impact depends greatly on the invader's abundance and spatial distribution (Ricciardi et al., 2013; Havel et al., 2015; Laverty et al., 2017; Sofaer et al., 2018). An important implication of this reasoning is that the capacity for establishment, proliferation and spread constitutes a precondition for designating a species or population as a harmful invader. Lockwood et al. (2013) put the argument thus: "Typically it is only when a population is widespread and abundant that it will cause some sort of ecological or economic harm, and thus earn the name invasive”. Exceptions nevertheless exist, as is the case of impacts induced by pathogens, which can emerge immediately after a species arrives in a new environment (Jeschke et al., 2014).

These considerations highlight the importance of establishing appropriate data collection methods for evaluating the invasion risk of non-indigenous species.

However, detailed information on species occurrence, distributions and population dynamics is rarely available in sufficient detail to guide management priorities and options (e.g. prevention, control or eradication). A world-wide review of studies providing information on risk assessments of alien species (including freshwater fish) indicated that only 5% of the studies included information on abundance and spread (McGeoch et al., 2012). In Europe, comparisons and evaluations of some of the risk assessment methods revealed substantial uncertainties and inconsistencies in the assessment outcomes that were largely attributed to the lack of essential baseline data, such as on establishment and spread rates, thus necessitating an excessive use of expert judgment (Rabitsch et al., 2012; Verbrugge et al., 2012; Matthews et al., 2017; Vanderhoeven et al., 2017; Roy et al., 2018).

Another important problem in invasion studies is a lack of common understanding of terms, concepts and conservation goals across stakeholders and policy makers (Heger et al., 2013; Humair et al., 2014; Davies, 2016; Heink, 2018). For instance, there are still conceptual problems concerning non-indigenous status (Essl et al., 2018), and the terminology varies depending on management contexts (Colautti and Richardson, 2009; EU, 2014). Species translocations (i.e. movements of fish across zoogeographical boundaries within the same political entity (country) (Copp et al., 2005) are often difficult to track and thus often omitted from nation-wide risk assessments and other surveys (e.g. see Zenetos et al., 2009 for Greece). However, translocated fish species may generate impacts that may exceed those of alien fishes due to higher possibility of introgressive hybridisation between populations of closely related species (e.g. Buoro et al., 2016). The issue of translocation may be an overlooked invasive species problem despite the fact that translocated species are currently widely considered an important part of the NIFS spectrum (Helfman, 2007).

Greece is an example of a state facing lotic ecosystem conservation challenges, especially with respect to freshwater fish. The country hosts a diverse freshwater fish fauna with a substantial proportion of country-specific endemics, 47 in total (or 35% of the native fish fauna). Many endemic and globally threatened fishes are burdened by various anthropogenic pressures mainly attributable to water abstraction, habitat alteration, resource extraction, climatic variability/climate change, pollution and the presence of non-indigenous species. The latter represents a rather recent but increasing form of biological pollution that is especially difficult to track (Economidis et al., 2000; Zenetos et al., 2009). The distribution of NIFS in Greece is poorly documented and even qualitative survey compilations are very recent (Economou et al., 2007; Corsini-Foka and Economidis, 2007; Zenetos et al., 2009; Koutsikos et al., 2012; Leonardos 2016). Most studies referring to NIFS are mainly from lentic environments (e.g. Tsekos et al., 1992; Rosecchi et al., 1993; Crivelli et al., 1997; Economidis et al., 2000; Perdikaris et al., 2005; Leonardos et al., 2007) and there is no surveillance of NIFS for the entire country. Quantitative data concerning non-indigenous fish populations in lotic ecosystems are especially scarce (Economou et al., 2016). However, even under these data-scarce conditions, some risk assessment studies have been recently implemented at broad spatial scales and thematic contexts (Papavlasopoulou et al., 2014; Perdikaris et al., 2016; Piria et al., 2017).

How the risks posed by non-indigenous species are assessed varies widely in approach, objective, implementation and taxa covered; and improvements are needed (Copp et al., 2009; Verbrugge et al., 2012; Ferincz et al., 2016; Vanderhoeven et al., 2017; Roy et al., 2018). Most screening methods still use qualitative criteria and the need to develop quantitative approaches is increasingly recognised and recently promoted (Roy et al., 2018). Apart from sparse work and reviews beyond Europe (e.g. Gido et al., 2004; Mitchell and Knouft 2009; Fitzgerald et al. 2016) most studies in Europe and the Mediterranean countries concentrate on areas of intensive invasion problems such as lentic environments, rarely focusing on site-scale lotic waters or tracking invasions at multiple spatial scales. Assessing species distributions and prevalence in all lotic waters at various scales is important for underpinning many components of NIFS policy and detecting invasiveness, for example in the application of European Union Regulation on Invasive Alien Species (IAS) No 1143/2014 (EU, 2014) and decision-making frameworks at various policy levels (Roy et al., 2018).

In order to address the latter issues, this study applies a multi-faceted assessment procedure for lotic ecosystems at the state-wide level and provides: a) a review of temporal patterns and arrival pathways of fish invasions/translocations in Greece’s inland waters across 100 years; b) an analysis of occurrence and abundance of NIFS assemblages within lotic ecosystems at the site scale; c) the mapping of NIFS distributional patterns at river basin and ecoregional scales; and, d) a vector analysis of fish translocations using an ecoregional framework.

MATERIAL AND METHODS

Study area

Greece is characterised by a highly fragmented hydrographic network with a large number of medium and small-sized river basins dominated by mountainous landscapes but fringed by a long convoluted coastline (Economou et al., 2007; Skoulikidis et al., 2009). From a biogeographical perspective, Greece, is divided into eight freshwater ecoregions (see Fig. 2.7). Each ecoregion hosts distinctive assemblages of freshwater fish species and other aquatic/semi aquatic life forms, many being endemic to each region (Zogaris and Economou, 2017). The spatial scale of the ecoregion is widely used as standard geographical and non-political framework for conservation/ecological evaluations (Abell et al., 2008) and biological assessments (Zogaris et al., 2018).

Data acquisition and analysis

In order to compare the historical with distributional fish fauna records of each river basin and freshwater ecoregion, data where obtained from two different sources: a) a bibliographical survey; from all available literature at each river basin system (including lakes) and b) lotic waters electrofishing survey data; derived from standardised field sampling in rivers, streams, canals and springs, in the frame of various regional surveys including the EU Water Framework Directive (WFD) monitoring. An overview of the procedure to assess the spread of NIFS based on available knowledge (Fig. 2.1).

Literature review data were based on all available scientific and grey literature, using as main references Economidis et al. (2000), Economou et al. (2007), Koutsikos et al. (2012), Barbieri et al. (2015) and Piria et al. (2017). Data from complementary sources, including reports from environmental agencies, were assessed and used when appropriate. Through identifying species richness within river basins and analyzing the distribution as well as the fish assemblages across basins, presence-absence values were used. The derived matrix summarised the ichthyofaunas of 140 drainage basins within Greek territory; including all species inhabiting the entire watershed, whether the system consisted of only lotic and/or lentic catchments. On the other hand, the electrofishing samples were entirely within lotic systems.

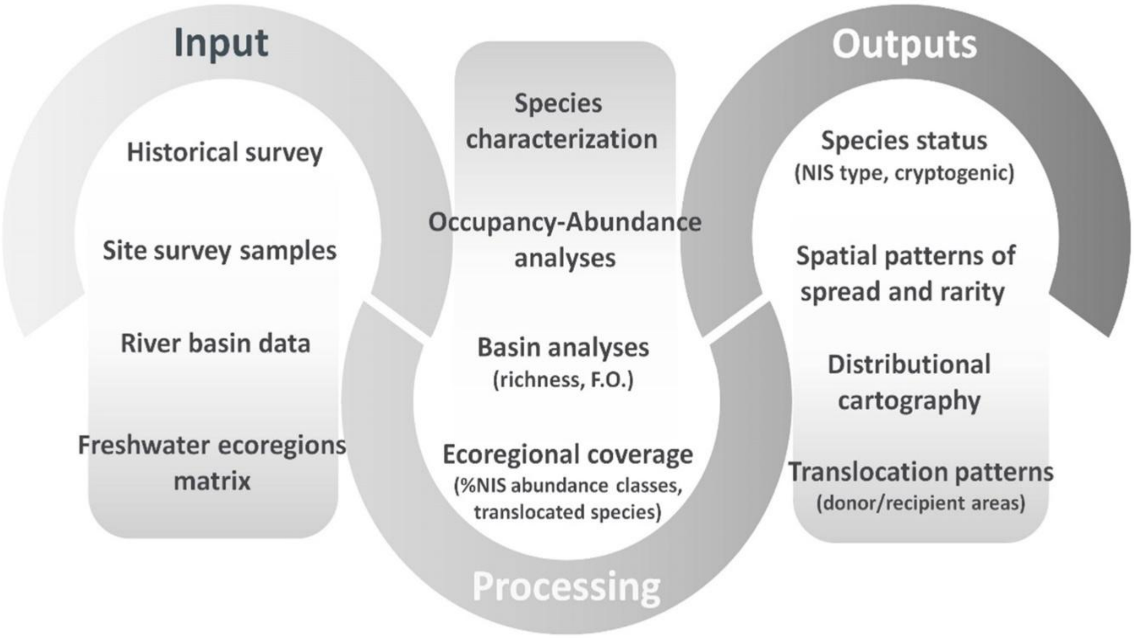

FIGURE 2.1 Assessment scheme applied in this study: highlighting inputs, processing methods, and outputs of the country-wide screening procedure.

Electrofishing surveys were carried out primarily from May to October in the years 2001–2015, during the rivers' reduce summer flows. In total, 644 sites were sampled comprising 954 different samples from 75 different drainage basins in Greece. Fish sampling was conducted through using appropriate electrofishing equipment (backpack, shore-based and boat-based electrofishing) depending on river size (e.g. depth) and flow conditions (from small fast flowing upland streams to main-stem rivers close to river mouths). The procedure is standardised basically following CEN (2003) guidelines for electrofishing and sampling targeted river bioassessment during most samples (Zogaris et al., 2018); for method description see Economou et al. (2016).

Taxonomy and nomenclature follow Barbieri et al. (2015). A distinction between alien and translocated species is made here; this categorization is used widely in recent years (e.g. Copp et al., 2005; Koutsikos et al., 2012; Tarkan et al., 2015; Xiong et al., 2015; Buoro et al., 2016; Pofuk et al., 2017; Trochine et al., 2018). Alien species are considered those whose native distributional range did not include any river basin within inland waters of Greece. Translocated species are native fish species that have been introduced into a given catchment, within Greek territory, but outside their historical native range. Euryhaline native species of marine origin were excluded from the analyses. When alien and translocated species are referred together they are cited as non-indigenous fish species (NIFS).

The arrival pathway of each species was categorised as AQ, aquaculture; OR, ornamental; AN, angling or bait fish; BC, biological control and UN, unintentional. Invasion phases were classified according to Blackburn et al. (2011). This classification scheme views invasions as a series of stages (transport, introduction, establishment and spread), in each of which there are "barriers" that need to be overcome for a species or population to pass on to the next stage. Species (or populations) are catagorised according to their position in the invasion process, and only those which have reached the final stage are regarded as invasive. The abundance classification followed Macdonald et al. (2003). Both of the latter classifications referred on the status of each NIFS at the country level. Year of introductions represent the earliest date of introduction or detection of the NIFS and when the latter information was not available we provided an approximate estimation (e.g. mid 1950s entered in database as 1955, etc.).

In addition, the native ichthyofauna was used to guide assemblage characteristics by virtue of their abundance and distributional range. We employed the quadrant graph technique originally used by Ono (1961; after Soto 1986) to illustrate the position of native species in occupancy and abundance axes, with lines drawn to show the means and 95% confidence limits of site occupancies and local abundances. Species site occupancy (number of sites in which a species was recorded) and mean local abundance (mean number of individuals per site in the sites of its occurrence) were estimated for all native fish species. The lines divide the graph area into four quadrants characterizing the species along occurrence frequency (from restricted to widespread) and abundance (from locally rare, hereafter referred to as rare, to abundant). Subsequently, based on the latter occupancy-abundance classification produced by native species patterns, we projected the NIFS into the graph to comparatively depict spatial patterns of spread and rarity of alien and translocated fish species.

To further investigate invasiveness at an ecoregional scale, we calculated the NIFS prevalence per 1000 km^2, for both bibliographic and field survey data, the percent of NIFS abundance, as well as the percentage of species per status (native/alien/translocated), on each of the Greek freshwater ecoregions separately (for names see Fig. 2.7). To define the class ranges of each group we used logical cut-off values for the measured values of % NIFS abundance and the Jenks Natural breaks (Smith et al., 2015) in NIFS per 1000 km^2, as one of the standard classification methods provided in ArcGIS (version 10.4).

The visualization of human-induced movements (e.g. the species transplants into different ecoregions) allows for the identification, quantification and direction flows of their spatial patterns (Abel and Sanders, 2014). Thus, to identify the major donor and recipient areas of translocated fish species in the country, we charted the flows of translocations among Greek freshwater ecoregions for both bibliographical and sampling survey data by assigning each translocated species to its native ecoregion(s). The analysis is depicted using circular flow plots created using a R syntax (package version 3.4.3; R Core Team, 2017) adapted from Abel and Sander (2014) and Van Kleunen et al. (2015). The origins and destinations of translocations within the seven ecoregions were each assigned a color and represented by the circle’s segments (one ecoregion, Southern Anatolia was excluded since the island and islets within Greek territory do not have lotic waters with fishes). The direction of the translocation flows was encoded by an arrow head between the flow and the destination segment, whereas the width of the flow (at the beginning and at the end points) indicated the volume of translocations. The tick marks on the circle segments represented the number of translocations (species inflows and outflows), while number in brackets indicated the native species richness of each ecoregion in either bibliographical or survey data.

RESULTS

Arrival pathways and temporal patterns of introductions

The two major pathways for alien species were aquaculture with 28% (7 species) and angling/fish bait with 20% (5 species); and/or both pathways together (category AQ/AN, Table 2.1) with an extra 16% (4 species). In contrast, the dominating pathway for translocated species was the category of angling/fish bait by scoring 43.3% (13 species). Although there is anecdotal evidence to support contamination of stocking as an important specific pathway of spread, this is not confirmed in many cases and here this means of entry is subsumed in both aquaculture and angling/fish bait categories. However, for another 13 translocated species, the 43.3% of already established translocations within inland waters, no pathway data were available (category UN, Table 2.1).

Power relationships were applied for the cumulative number of both alien (R^2=0.953) and translocated fish species (R^2=0.916) through the years of introductions (Fig. 2.2). Patterns were similar for both categories of NIFS, however differences were observed at the number of species per time periods. Until the early 1920s (period I) no record of NIFS was documented into the inland waters of Greece. The first documented translocations in the country took place in the 1920s (period A). After this period there was a paucity of introductions for the next two decades (from the 1930s to late 1940s, period II). From 1950 and until the late 1960s (period B) nine more fish species were introduced, raising the total number of the NIFS to 13. Finally, the third and the largest wave of fish introductions in the country, started in the late 1970s (period C) with an introduction of a further 25 species, and during the last two decades (period D) another 17 NIFS were introduced. The average rate of NIFS introductions to Greece since the 1920s is 5.5 species per decade, but separately for the periods C and D (Fig. 2.2) increases to 8.4 species per decade.

Bibliographical versus field survey data

The bibliographical research compiled a total list of 151 freshwater fish species present within the 140 studied Greek river basins. Overall, 126 species were native (83%), 25 species alien (17%), while 28 native species (22% of Greek native) have been translocated into new catchments. The five most commonly recorded NIFS within Greek drainage basins were: the Eastern mosquitofish, *Gambusia holbrooki* Girard, 1859 with 73 occurrences (52.1% F.O.), followed by the Prussian carp *Carassius gibelio* (Bloch, 1782), the common carp, *Cyprinus carpio* Linnaeus, 1758 and rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), all with 29 occurrences (20.7% F.O.), and the grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) with 14 occurrences (10% F.O.) (Table 2.1).

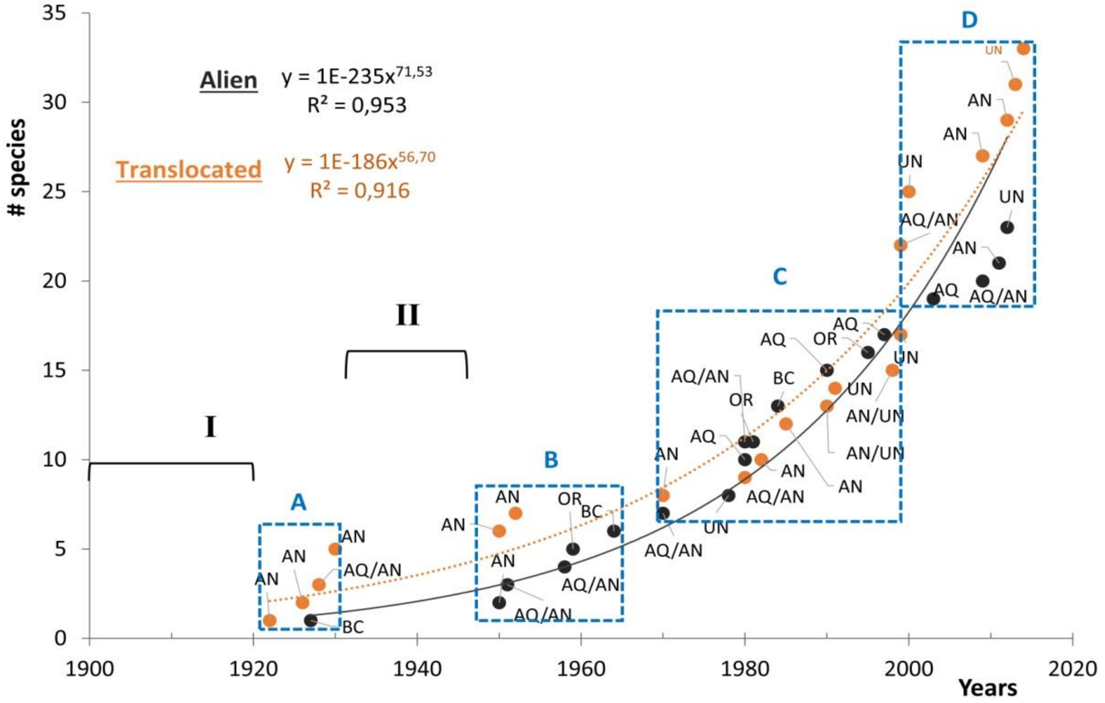

FIGURE 2.2 Temporal patterns of NIFS introduction into Greek river basins. Cumulative number of alien and translocated fish species. Time periods: I, until 1922; A, 1922-1931; II, 1932-1949; B, 1950-1969; C, 1970-1999 and D, 2000-2018. Pathways: AN, angling or bait fish; AQ, aquaculture; BC, biological control; OR, ornamental; UN, unkown.

Throughout the 15 years of standardised river sampling surveys, approximately 240 small, mid-sized and large tributaries and/or main river channels, of 75 river basins were sampled. The current ichthyological survey covered more than half (54%) of the aforementioned drainage basins (n=140) that are known to sustain native freshwater ichthyofauna in Greece (Table 2.1). In total, 953 samples out of 644 sites were surveyed and over 325.000 individual fish were recorded. Specifically, 115 freshwater fish species were collected, of which 105 were native (91%) and 10 alien (9%). Furthermore, 20 translocated fish species were collected, representing approximately an extra 17% of Greek native species sampled.

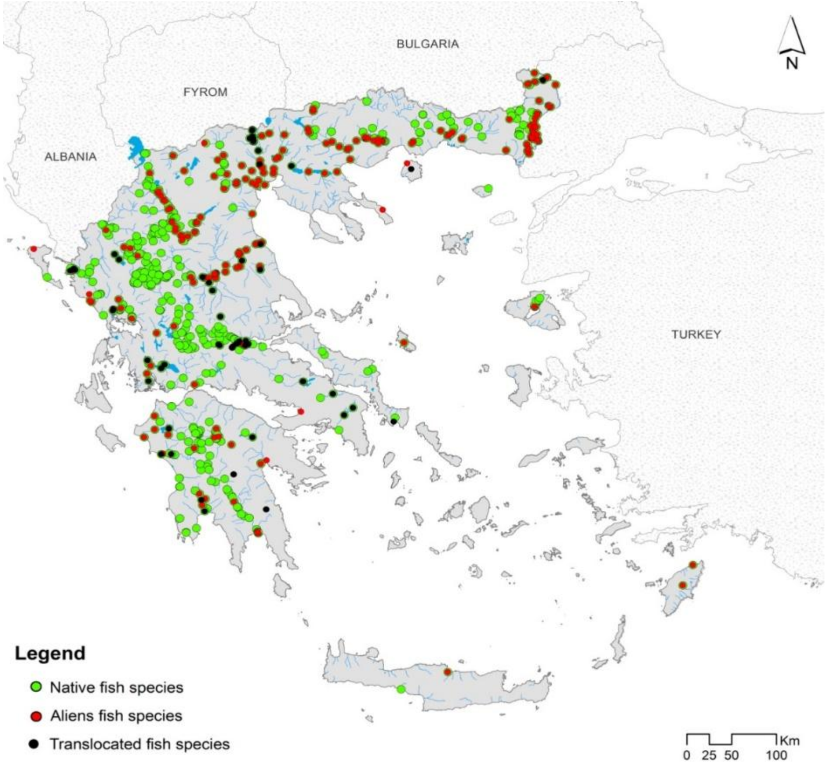

FIGURE 2.3 Lotic sampling sites, showing the distinction among sites where wholly native exist and sites inhabited also by non-indigenous fish species (alien and translocated).

NIFS are distributed throughout the Greek peninsula as well as the Aegean and Ionian Islands. On the contrary, the absence of established NIFS introductions along the Pindos mountain range and in parts of the southern half of Greece (in relatively small river basin areas) is conspicuous (Fig. 2.3). Alien fish species were mainly located in the country's central, northern and northwestern river basins. In contrast, the majority of translocated fish species were located within basins of the Ionian and Western Aegean ecoregions, with some exceptions in Thrace (Axios/Vardar, Evros, and Thassos) and in the Thessalian Pinios basin of the Macedonia-Thessaly ecoregion.

The five most frequent NIFS sampled within the 75 Greek river basins were: *G. holbrooki* with 42 occurrences (56% F.O.), followed by *C. gibelio* with 24 occurrences (32% F.O.), *O. mykiss* and the pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758) with 11 occurrences (14.7% F.O.) respectively, and the topmouth gudgeon, *Pseudorasbora* *parva* (Temminck and Schlegel, 1846) with 10 occurrences (13.3% F.O.) (Table 2.1). In total, 55 NIFS were recorded (25 alien and 30 translocated); however, there is a low incidence of NIFS in lotic waters at the site scale (30 NIFS recorded in the field samples; 10 alien and 20 translocated). Out of 55 NIFS listed in Table 2.1, 28 (10 alien and 18 translocated) were common in the both inventories of bibliographical and sampled data. Three species, *Alburnoides sp.*, Western Greece goby, *Economidichthys pygmaeus* (Holly, 1929) and an unidentified chub *Squalius* sp., are referred here as translocated, for the first time, in the Evros, Lake Taka and the Charadros river basins respectively (Table 2.1). The invasion categories of the annotated list, five NIFS (9.1%) have been categorized as "E" (fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence), 10 species (18.1%) in category "D2" (self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction) and 11 (20%) in "D1" (self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction).

Under the "C" categories of the invasion phases, 12 NIFS (21.82%) have been categorised as "C3" (individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining), seven (12.7%) as "C1" (individuals surviving in the wild in location where introduced, no reproduction), while in the remaining two categories, "C2" (individuals surviving in the wild in location where introduced, reproduction occurring, but population not self-sustaining) and "C0" (individuals released into the wild in location where introduced, but incapable of surviving for a significant period), record only one species (1.8%), respectively. Finally, the remaining seven NIFS (12.7%) belonged to the category "B3" of the classification scheme (individuals transported beyond the limits of their native range, and directly released into novel environment) of Table 2.1. The abundance classification according to Macdonald et al. (2003), showed that only four NIFS (7.3%), the aliens *G. holbrooki* and *P. parva*, and the translocated Struma stone loach, *Oxynomacheilus bureschi* (Drensky, 1928) and Vardar chub, *Squalius vardarensis* Karaman, 1928 were classified as "widespread" within the catchments (category "3").

Table 2.1 Non-indigenous fish species (NIFS type: Alien and Translocated) in Greece, from bibliographical and survey data. Invasion phase and abundance classification followed Blackburn et al. (2011) and Macdonald et al. (2003), respectively, both at the country level.

Species	NIFS Type	River basin Occurrence (Bibliographical data)	Bibliographical data F.O. (%) (n=140)	River basin Occurrence (Survey data)	Survey data F.O. (%) (n=75)	Invasion phase	Abundance code
*Abramis brama**	T	1	0.7	-	-	C2	1
Acipenser baerii	A	1	0.7	-	-	B3	1
Acipenser gueldenstaedtii	A	3	2.1	-	-	C1	2
Acipenser naccarii	T	1	0.7	-	-	C0	1
Alburnoides sp.[1]	T	-	-	1	1.3	B3	1
Ameiurus cf. nebulosus	A	1	0.7	-	-	C3	2
Barbus sperchiensis	T	1	0.7	1	1.3	D2	3u
Carassius auratus	A	7	5.0	1	1.3	C3	3l
Carassius gibelio	A	29	20.7	24	32.0	E	3l
Carassius langsdorfii	A	1	0.7	-	-	B3	3l
Cobitis hellenica	T	1	0.7	-	-	C3	2
Coregonus cf. lavaretus	A	2	1.4	-	-	C3	3l
Ctenopharyngodon idella	A	14	10.0	1	1.3	C1	3l
Cyprinus carpio	T	29	20.7	9	12.0	E	3l
Economidichthys pygmaeus[1]	T	4	2,9	5	6.7	D2	3l
*Esox lucius**	T	2	1.4	-	-	D1	2
Gambusia holbrooki	A	73	52.1	42	56.0	E	3
Gymnocephalus cernua	A	1	0.7	-	-	C3	2

Species	NIFS Type	River basin Occurrence (Bibliographical data)	Bibliographical data F.O. (%) (n=140)	River basin Occurrence (Survey data)	Survey data F.O. (%) (n=75)	Invasion phase	Abundance code
Hypophthalmichthys molitrix	A	11	7.9	-	-	C1	3l
Hypophthalmichthys nobilis	A	2	1.4	-	-	C1	3l
Knipowitschia caucasica[1]	T	1	0.7	1	1.3	D1	2
Lepomis gibbosus	A	12	8.6	11	14.7	E	3l
Luciobarbus graecus	T	3	1.4	-	-	C3	2
Micropterus salmoides	A	1	0.7	-	-	B3	1
Neogobius fluviatilis	A	1	0.7	1	1.3	B3	1
Oncorhynchus kisutch	A	5	3.6	1	1.3	C1	2
Oncorhynchus mykiss	A	29	20.7	11	14.7	C1 (C3)	3u
Oreochromis niloticus	A	6	4.3	-	-	C3	2
Oxynoemacheilus bureschi	T	1	0.7	1	1.3	D2	3
Pachychilon macedonicum	T	1	0.7	1	1.3	D2	3l
Parabramis pekinensis	A	1	0.7	-	-	C3	2
Pelasgus marathonicus	T	1	0.7	1	1.3	D1	3l
Pelasgus stymphalicus	T	3	1.4	1	1.3	D1	2
Perca fluviatilis	T	2	1.4	-	-	E	3l
Poecilia latipinna	A	1	0.7	-	-	C3	2
Pseudorasbora parva	A	11	7.9	10	13.3	E	3
Rhodeus meridionalis	T	1	0.7	1	1.3	D2	3l
Rutilus panosi	T	2	1.4	1	1.3	D2	2

Species	NIFS Type	River basin Occurrence (Bibliographical data)	Bibliographical data F.O. (%) (n=140)	River basin Occurrence (Survey data)	Survey data F.O. (%) (n=75)	Invasion phase	Abundance code
Rutilus sp.	T	1	0.7	1	1.3	C3	1
Rutilus ylikiensis	T	1	0.7	-	-	D1	2
Salmo farioides	T	4	2,9	1	1.3	D2	3u
Salmo letnica	A	1	0.7	-	-	C1	2
Salmo salar	A	2	1.4	-	-	C1	1
Salmo trutta	A	2	1.4	1	1.3	B3	1
Salvelinus fontinalis	A	3	2.1	-	-	B3	2
Sander lucioperca	T	1	0.7	-	-	E	3l
Scardinius acarnanicus	T	1	0.7	-	-	D1	2
Scardinius graecus	T	2	1.4	-	-	D1	2
Silurus aristotelis	T	3	2.1	1	1.3	D2	3l
Silurus glanis	T	5	3.6	2	2.7	D2	3l
Squalius orpheus	T	1	0.7	1	1.3	D1	2
Squalius peloponensis	T	2	1,4	1	1.3	D1	2
Squalius sp.[1]	T	-	-	1	1.3	D1	2
Squalius vardarensis	T	1	0.7	1	1.3	D2	3
Tinca tinca	T	9	6.4	2	2.7	D1	2

**: population of the species probably extirpated*

1: first occurrence record of species population (this survey)

The majority of introduced species recorded in category “2” (isolated population in small restricted region/s) with 22 NIFS (40%), followed by category “3l” (widespread in lower catchment) with 17 NIFS (30.9%) and category “1” (very rare; probably a single record) with 9 NIFS (16.4%).

Occurrence, co-occurrence and dominance

The mean number of NIFS individuals varied among species, sites and samples. The most abundant species was *G. holbrooki* with a total of 8,677 individuals in 154 sites (mean abundance 88.1 ±21.01 per sample) and the two rarest species were coho salmon, *Oncorhynchus kisutch* (Walbaum, 1792) and *Alburnoides* sp., represented by a single specimen (Table 2.2). Of the total 30 NIFS recorded through the field surveys, only eight species exceeded 10 sample occurrences. The most widespread species in the samples were *G. holbrooki, C. gibelio, L. gibbosus, P. parva,* and *O. mykiss* (Table 2). On the contrary, 18 NIFS (five alien and 13 translocated species) were caught in no more than a single site.

In terms of dominance, while the translocated Sperchios barbel, *Barbus sperchiensis* Stephanidis, 1950*,* Maritza chub, *Squalius orpheus* Kottelat & Economidis, 2006*, Squalius sp.* and Stymphalia minnow, *Pelasgus stymphalicus* (Valenciennes, 1844), as well as the alien common goldfish, *Carassius auratus* (Linnaeus, 1758) and brown trout, *Salmo trutta* Linnaeus, 1758 were sampled only in one site, they dominated 100% the sites sampled. Moreover, 63.3% of the presence of West Balkan trout, *Salmo farioides* Karaman, 1938 within the samples were numerically dominant, and followed by Peloponnese chub, *Squalius peloponensis* (Valenciennes, 1844)*, O. mykiss* and *G. holbrooki* with a 50%, 30% and 21.7% dominant presence per samples, respectively. Overall, 50% of the NIFS (15 species) co-occurred in all cases with other introduced species among the samples (Table 2.2). In addition, almost half of the NIFS (14 species, 46.7%) typically co-occurred with other translocated species, while over 23 NIFS (76.6%) co-occurred with other alien species.

Table 2.2 Non-indigenous fish species sampled in Greek lotic ecosystems, occurrence, abundances, dominance, richness and co-occurrences with other species within samples.

Species	NIFS code	Occurrence in sites (n=664)	Occurrence in samples (n=953)	Total abundance (min-max)	Mean abundance per sample (SE)	Dominance per sample (%)	Richness (min-max)	Native species co-occurrence richness (min-max)	Only NIFS within samples (%)	Co-occurrence with aliens (%)	Co-occurrence with translocated (%)
Alburnoides sp.	*Alb*.sp.	1	1	1	1	0	13	9	0	100	0
Barbus sperchiensis	*B.sper*	1	1	150	150	100	3	1	0	0	100
Carassius auratus	*C.aur*	1	1	5	5	100	1	0	100	0	0
Carassius gibelio	*C.gib*	128	187	5392 (1-814)	28.8 (5.05)	4.3	2-20	0-16	16.0	81.3	12.8
Ctenopharyngodon idella	*C.ide*	1	1	2	4	0	11	7	0	100	0
Cyprinus carpio	*C.carp*	16	19	32 (1-3)	1.7 (0.19)	5.3	4-15	0-8	10.5	89.5	47.4
Economidichthys pygmaeus	*E.pyg*	6	9	451 (3-129)	50.1 (16.92)	11.1	2-9	0-4	0	100	33.3
Gambusia holbrooki	*G.hol*	154	212	18677 (1-4015)	88.1 (21.01)	21.7	1-20	0-16	33.5	58.5	15.1
Knipowitschia caucasica	*K.cau*	1	1	50	50,0	0	3	1	0	100	0
Lepomis gibbosus	*L.gib*	81	115	2445 (1-198)	21.3 (3.36)	6.1	2-20	0-16	13	86.1	11.3

Species	NIFS code	Occurrence in sites (n=664)	Occurrence in samples (n=953)	Total abundance (min-max)	Mean abundance per sample (SE)	Dominance per sample (%)	Richness (min-max)	Native species co-occurrence richness (min-max)	Only NIFS within samples (%)	Co-occurrence with aliens (%)	Co-occurrence with translocated (%)
Neogobius fluviatilis	*N.flu*	1	1	8	8,0	0	18	12	0	100	0
Oncorhynchus kisutch	*O.kisu*	1	1	1	1	0	3	1	0	100	0
Oncorhynchus mykiss	*O.myk*	23	30	455 (1-77)	15.2 (4.22)	30	1-10	0-8	70	3.3	26.7
Oxynoemacheilus bureschi	*O.bur*	8	10	259 (1-64)	25.9 (8.01)	0	5-17	4-13	20	80	0
Pachychilon macedonicum	*P.mac*	1	4	39 (1-19)	9.8 (3.68)	0	3-7	0-2	0	100	0
Pelasgus marathonicus	*P.mar*	3	4	43 (2-31)	10.8 (6.80)	0	4-16	2-12	0	100	0
Pelasgus stymphalicus	*P.sty*	1	1	14	14	100	3	1	0	100	0
Pseudorasbora parva	*P.parv*	76	117	2529 (1-271)	21.6 (3.47)	0.9	3-20	0-16	19.7	80.3	8.5
Rhodeus meridionalis	*R.mer*	4	7	129 (1-44)	18.4 (5.96)	0	7-15	3-10	0	100	57.1
Rutilus panosi	*R.pan*	1	1	4	4,0	0	5	0	0	100	100
Rutilus sp.	*Rut.*sp.	1	1	3	3	0	9	8	100	0	0

Species	NIFS code	Occurrence in sites (n=664)	Occurrence in samples (n=953)	Total abundance (min-max)	Mean abundance per sample (SE)	Dominance per sample (%)	Richness (min-max)	Native species co-occurrence richness (min-max)	Only NIFS within samples (%)	Co-occurrence with aliens (%)	Co-occurrence with translocated (%)
Salmo fariodes	*S.far*	10	11	243 (1-101)	22.1 (8.62)	63.3	1-7	1-7	81.8	18.2	0
Salmo trutta	*S.tru*	1	1	2	2,0	100	1	0	100	0	0
Silurus aristotelis	*S.ari*	1	1	3	3,0	0	9	4	0	100	100
Silurus glanis	*S.gla*	5	5	17 (1-11)	3.4 (1.91)	0	5-10	1-6	20	80	60
Squalius orpheus	*S.orph*	1	1	162	162	100	1	0	100	0	0
Squalius peloponensis	*S.pel*	1	4	278 (38-108)	69.5 (14.52)	50	2	0	0	25	0
Squalius sp.	*Sq.sp*	1	1	66	66	100	2	1	100	0	0
Squalius vardarensis	*S.vard*	1	1	59	59	0	3	1	0	0	100
Tinca tinca	*T.tin*	3	5	25 (1-15)	5 (2.61)	0	4-8	1-5	40	60	40

Site occupancy and local abundance relationships

The position of native and non-indigenous species according to the quadrant graph technique is displayed in Fig. 2.4. The species enclosed in the constituent quadrants can be characterised as “I: restricted and rare”, “II: restricted and abundant”, “III: widespread and abundant” and “IV: widespread and rare”, respectively. The majority of the native ichthyofauna (49.5%, 52 species) plotted in quadrant I, suggesting numerical scarcity and localized distribution.

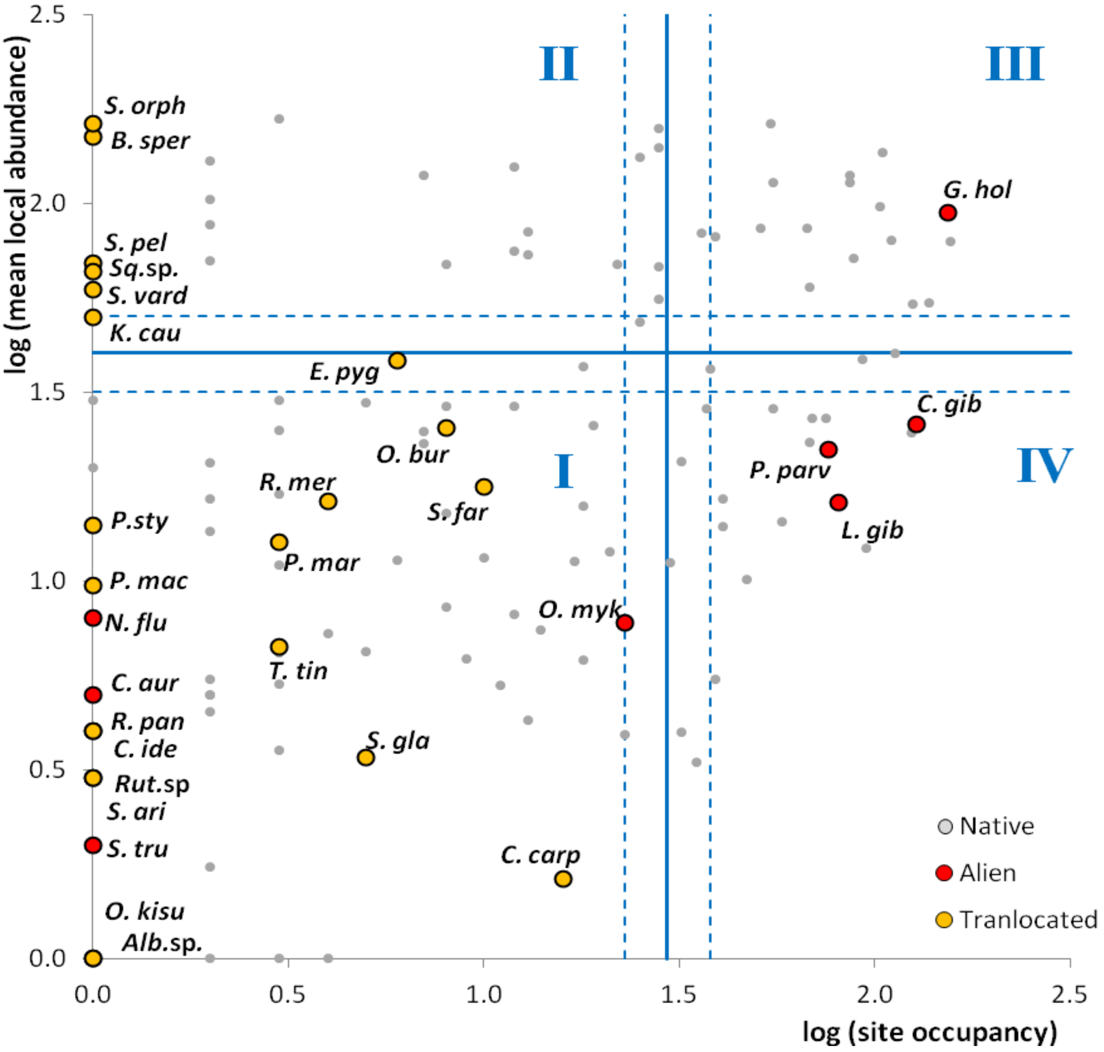

FIGURE 2.4 Species site occupancies plotted against species abundances in Quadrant graph. Blue lines show the means and dashed lines indicate the upper and lower confidence limits of site occupancies and local abundances. Quadrants: I, restricted and rare; II, restricted and abundant; III, widespread and abundant; and IV, widespread and rare. For NIFS code see Table 2.

The remaining native species are distributed almost equally in the three quadrants II, II and IV with 18, 16 and 19 species, respectively. Species in quadrant III are both frequent and abundant indicating that these native species are found throughout most of the sites sampled. Thus, in proportion to the native ichthyofauna's occupancy-abundance patterns (Fig. 2.4), of all NIFS only one alien (*G. holbrooki*) is categorised as widespread and abundant (quadrant III), whereas none of the translocated species is distributed in the two areas of widespread classification (quadrants II and III).

Translocated species distributed primarily in quadrant I are restricted and locally rare (14 species), while the 6 remaining species plotted in quadrant II are restricted and abundant. Most of the alien species (60%) similarly to the majority of translocated species in quadrant I, are restricted and locally rare. On the contrary, the alien species *C. gibelio, P. parva* and *L. gibbosus* have been placed in quadrant IV, suggesting a widespread distribution and numerical scarcity.

Patterns of NIFS dominance at basin scale

Although the major larger river basins hold most NIFS species (Fig. 2.5A), NIFS are more prevalent within the fish assemblage in the smaller species-poor southern basins (Fig. 2.5B). In terms of proportional abundance, the representation of NIFS is low, with the exception of certain rather smaller basins, most of which are dominated by lakes, some being incidentally adjacent to urban centers (Athens, Thessaloniki, Ioannina; Fig. 2.5C).

As is immediately apparent when assemblage species density among alien, translocated and natives is graphed, the prevalence of NIFS is sparse when all sampled basins are considered (Fig. 2.6). In particular, very few samples and basins are dominated by NIFS (see inflection points of dominance – III, in both A anb B graphs of Fig. 2.6).

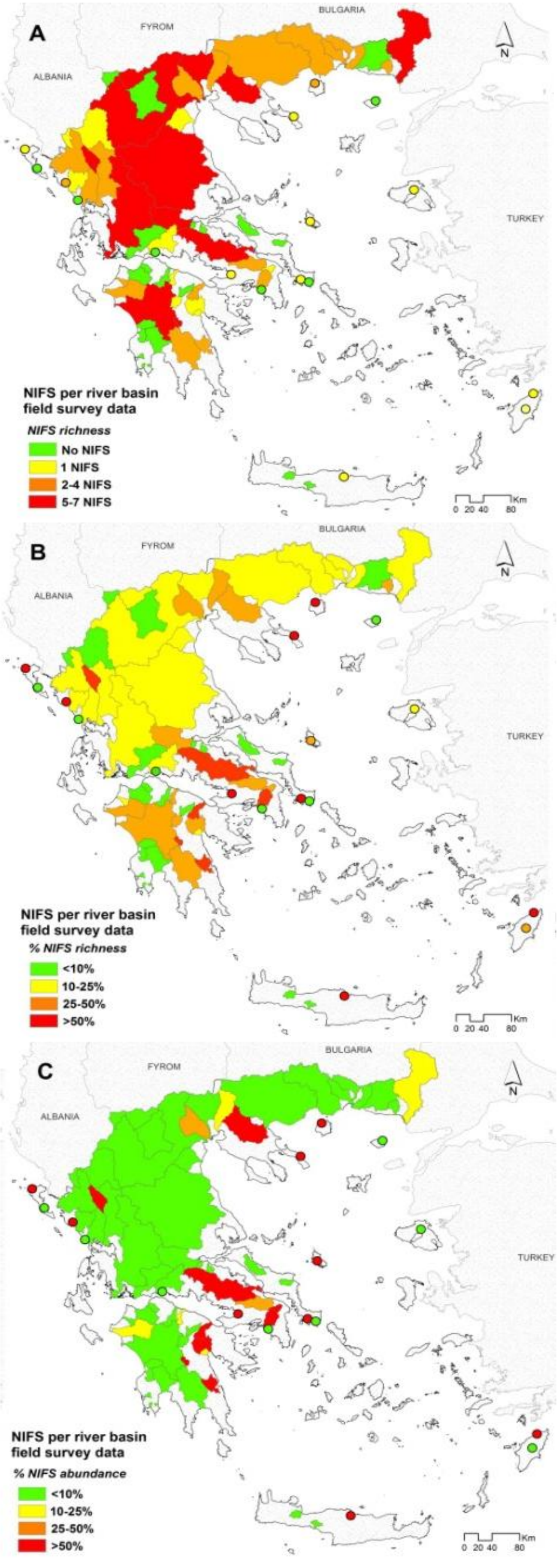

FIGURE 2.5 Basin scale analyses of NIFS in Greek lotic ecosystems using field survey data: A, NIFS richness; B, %NIFS richness and C, %NIFS abundance in four arbitrary categories respectively. Small-sized river basins were depicted as circles (with each category colour) for visualisation purposes.

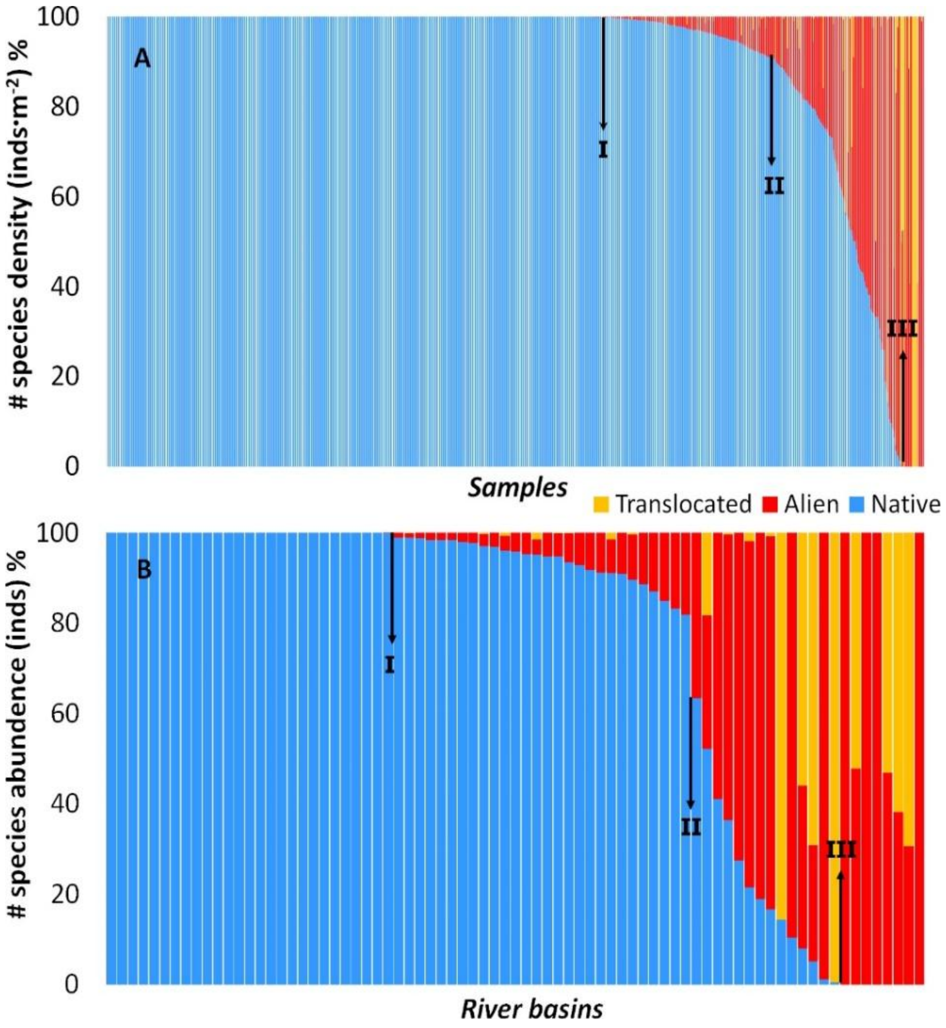

FIGURE 2.6 Species density (A) and species abundance (B) at reach scale and basin scale samples ranked by native species in each sample. Inflection points (I, II, III) show the beginning, the step-change and point of dominance of NIFS in the samples, respectively.

Ecoregional scale coverage: prevalence and donor/recipient regions

Jenks natural breaks defined four classes of the distributional prevalence of NIFS per 1000 km^2 for each group (in bibliographical and survey data, respectively) within the freshwater ecoregions in Greece (Fig. 2.7A). Bibliographical data ranked NIFS prevalence in ecoregions, from highest to lowest, as follows: 1. Southeastern Adriatic; 2. Thrace, Ionian, E. and W. Aegean; 3. Crete and 4. Macedonia-Thessaly. On the other hand, prevalence based on survey data classified ecoregions in the following descending order: 1. Thrace; Southeastern Adriatic and W. Aegean; 2. Ionian; 3. Macedonia-Thessaly and 4. E. Aegean and Crete.

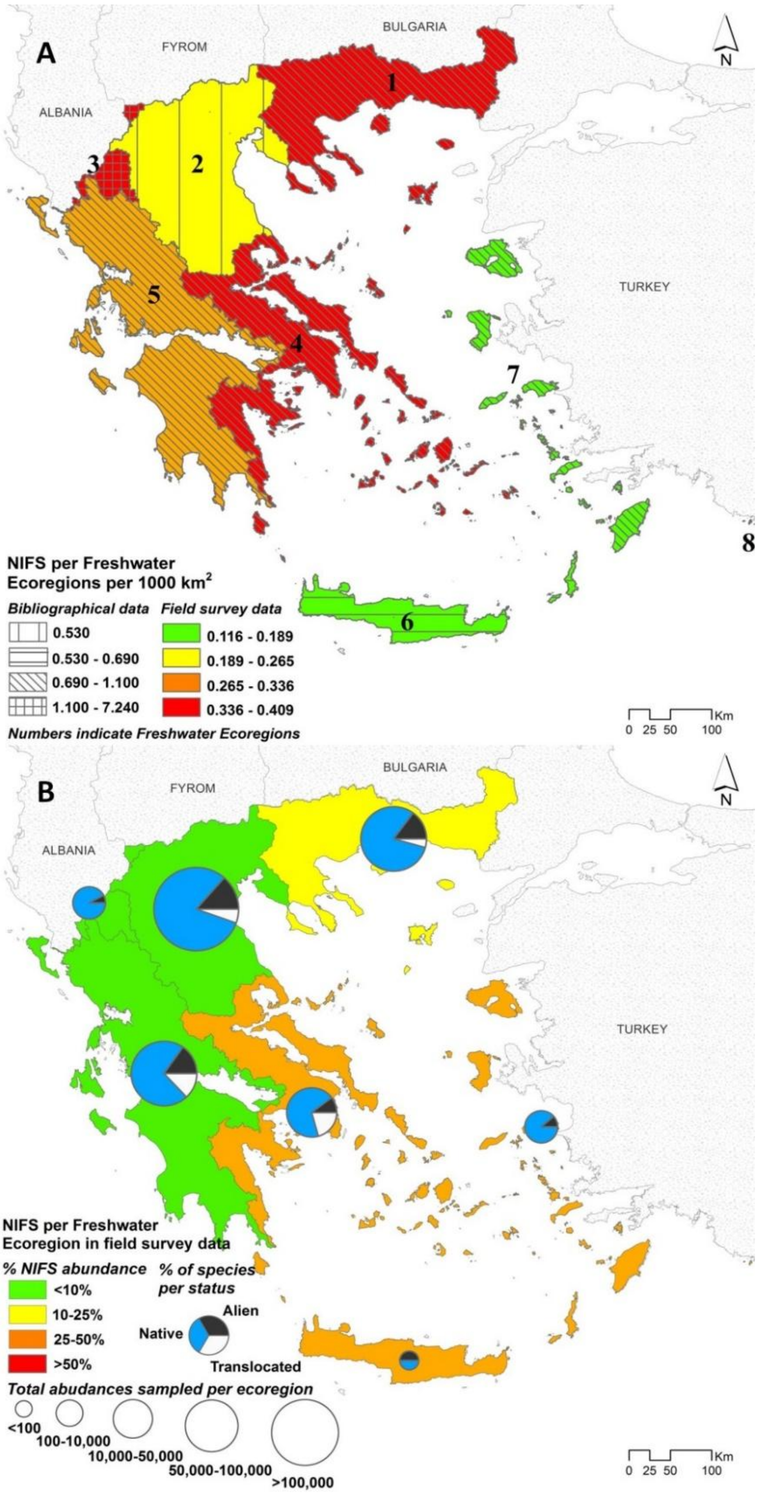

FIGURE 2.7 NIFS presence at the ecoregional scale within Greek lotic waters: A. density of NIFS per areal coverage (both for bibliographic at river basin scale and field survey site data); B. NIFS abundance per freshwater ecoregion in the field survey site data. Numbers in the first map denote the freshwater ecoregions:
1. Thrace; 2. Macedonia - Thessaly, 3.Southeast Adriatic; 4. Western Aegean; 5. Ionian; 6. Crete; 7. Eastern Aegean; 8. Southern Anatolian.

NIFS prevalence was also defined within four categories (from minimum to high), through the percent of NIFS individuals sampled towards the total abundance per freshwater ecoregion (Fig. 2.7B).

The first category of minimum prevalence (% NIFS abundance <10%) included Macedonia-Thessaly with 9,339 NIFS individuals out of 138,898 (6.72%), Ionian with 4,597 NIFS individuals out of 70,763 (6.50%) and S.E. Adriatic with 3 NIFS individuals out of 5,273 (0.06%). In the second category of low prevalence (% NIFS abundance 10-25%) followed the ecoregion of Thrace with 10,208 NIFS individuals out of 82,160 (12.42%). Crete, Western and Eastern Aegean were in the category of medium invasive prevalence (% NIFS abundance 25-50%), with 43 NIFS individuals out of 93 (46.24%), 549 NIFS individuals out of 1,664 (32.99%) and 7,144 NIFS individuals out of 27,600 (25.88%), respectively. Finally, as would be expected, none of the ecoregions recorded the highest category of invasive prevalence (% NIFS abundance >50%).

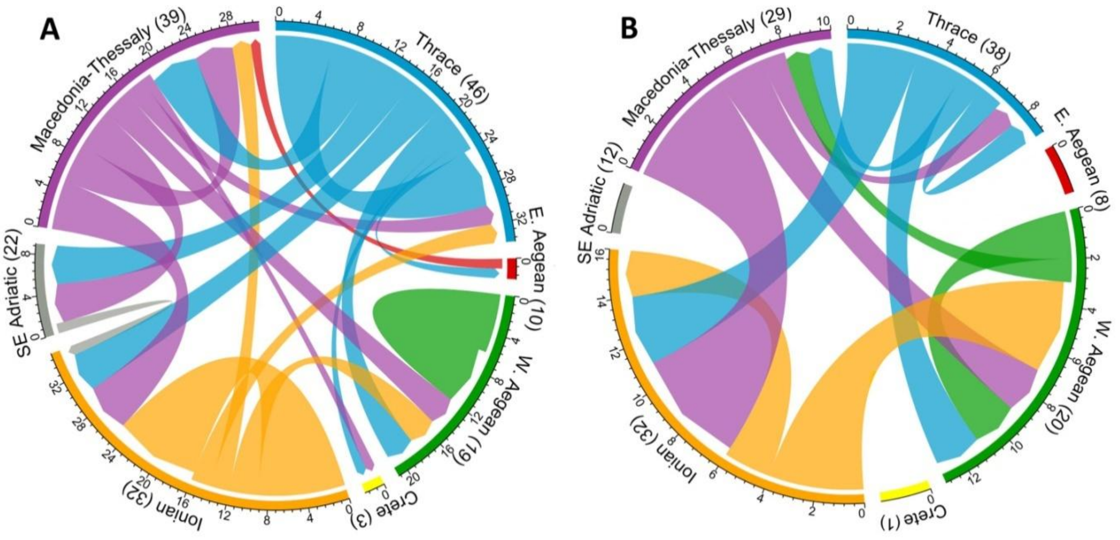

FIGURE 2.8 Flows of translocated fish species among the freshwater ecoregions of Greece, on A bibliographical and B. survey data. Tick marks on the circle segments show the number of translocations, while number in brackets denotes the native species richness of each ecoregion per bibliographical and per survey field data.

An important portion of NIFS spread concerns translocated non-indigenous species; thus it is important to track donor and recipient regions. The first circular plot in Fig. 2.8A visualises the translocation flows according to bibliographical data. The northern ecoregions, namely Thrace and Macedonia-Thessaly, were the major donor areas within Greek river basins, while Ionian, W. Aegean, S.E. Adriatic, E. Aegean and Crete are identified as the main recipient regions. Despite the fact that the Ionian outranked

16 species in absolute numbers, more than half (nine species) were donated in basins within the ecoregion itself, raising the total amount of intake translocation to 19. Finally, on the basis of survey data (Fig. 2.8B), the majority of translocated fish species sampled within lotic ecosystems followed similar trends already described from the bibliographical data. Thrace and Macedonia-Thessaly were the main donors of translocations, while Ionian as well as W. Aegean are defined as the major recipient ecoregions. The main differences between of the two circular flow plots were that Crete, W. Aegean and E. Aegean in survey data neither supplied nor received any translocated species.

DISCUSSION

The importance of history

NIFS introductions into freshwaters in Greece appear to be influenced by specific socio-historical circumstances. The first documented introductions (including *G. holbrooki*) occurred in the mid and late 1920s (Livadas and Sfagos, 1940), a late start for invasive fish entry by European standards (Piria et al., 2017 and references therein). From the early-1930s, when the Great Depression hit Greece (Chouliarakis and Lazaretou, 2014) until the end of the late 1940s the country was frequently war-torn, including periods of civil strife and mass migration, leading to widespread land abandonment (Pantelouris, 1980), thus no NIFS are known to have been introduced then. A second wave of introductions took place after World War II and the Greek civil war, when aquaculture and stocking were proposed as a food shortage solution; six additional species were introduced, including the highly invasive *C. gibelio*. In 1956 the first hatchery for rainbow trout, *O. mykiss*, was created (Louros river), which facilitated widespread salmonid stocking operations (MacCrimmon, 1971; Economidis et al., 2000). Greece's per-capita GDP between 1950 and 1973 increased rapidly outperforming all the major European economies, with an annual growth averaged of 7.7%, a rate second only to Japan's during the same period (Singh, 2010).

The greatest wave of fish introductions begun in the late-1970s after the end of a seven-year military dictatorship (1974) (Period C, Fig. 2.2). During this period state policy was strongly directed to fisheries and aquaculture development. Financial incentives also increased and new species where tested for aquaculture and inland fisheries, especially after Greece’s entry into the European Community (1981). Large quantities of hatchery-reared or wild-caught fry were released into lakes and rivers (primarily of carp and rainbow trout, but also other salmonids, perch, Asian "grass-carps", catfish, tench, coregonids, eels, grey-mullets, etc). By 1999, 23 more species had been added to the list of introduced species of which at least 14 have been established in their new areas or have hybridised with local species (Economidis et al., 2000). Practically no care was taken for genetic stock management or recording/monitoring species translocations, something especially problematic for native trout (Apostolidis et al., 1997, 2008). Furthermore, during this period, there was a rapid spread of particularly nuisance species such as *P. parva* and *L. gibbosus,* perhaps primarily as contaminants in ad hoc stocking activities. Finally, from 2000 until the present-day (period D in Fig. 2.2) another nine NIFS were discovered in the country’s inland waters; recording of these new species was assisted by increasingly standardised sampling efforts (i.e. EU WFD monitoring). The increasing invasion of lotic waters by NIFS shows an expanding trend through site-based monitoring during this period (Economou et al., 1999, Koutsikos et al., 2012; Zogaris et al., 2018).

Low richness and limited spread of alien fish species in lotic waters

Despite the recently increasing entry of NIFS in lotic waters, our review provides both qualitative and quantitative evidence that alien fish species are not widespread in the rivers and streams of Greece (Figures 2.4–2.7). Only four alien fishes are currently commonly encountered: *G. holbrooki, C. gibelio, P. parva, L. gibbosus* (in order of sampling catch abundance). Of these species, *P. parva* is broadly regarded as a highly invasive species with a pan-European distribution and is already included in the Union List of the EU IAS Regulation (Tsiamis et al., 2017).

The four species mentioned above represent 16% of all alien species reported from the freshwaters of Greece (25 species). They can be characterised as "widely spread" in the sense of the IAS Regulation, i.e. their population have gone beyond the naturalisation stage and have spread to colonise a large part of the potential range.

Similar numbers of widespread established alien species, with proportions to the total number of species introduced ranging between 10 and 20 per cent, have been reported from some central and north European countries, including Germany (five species; Wolter and Rohr, 2010), England and Wales (six species; Britton et al., 2010), Czech Republic (four species; Lusk et al. 2010) and Lithuania (three species; Rakauskas et al. 2016). By contrast, generally high invasion rates, with numbers of alien established species ranging from 15 to 55, and establishment success rates ranging from 50 to 80 per cent, have been reported from other Mediterranean countries (Garcia-Berthou et al., 2007; Ribeiro et al., 2009; Maceda-Veiga, 2013; Bianco, 2014). However, when fine-scale or quantitative analysis was conducted, only a fraction of the species introduced into Mediterranean areas appeared to be widespread and/or abundant (Nocita et al., 2017; Teletchea and Beisel, 2018). Beisel et al. (2017) remarked that of the 25 species considered to be established in France, most still have limited distributions around the country and their abundances have remained (and are likely to remain) low. These authors raised the need for additional research on the spatial distribution of species in order to develop criteria for prioritising intervention measures. In a more general European context, Rabitsch et al. (2013) asserted that only a minority of introduced alien species become invasive, and considered that reported establishment rates between 36 and 64% at the continental and/or global scale constitute significant overestimations.

The rather low incidence of alien fish species in Greece relative to other European countries was first noted by Bianco (1990) and to the best of our knowledge it seems that at least in lotic waters this state has until recently persisted in Greece (Economou et al., 2016, Marr et al., 2013; Zogaris et al., 2018). We hypothesize the following non-mutually exclusive reasons for this state of affairs: i) Low invasion rates could be due to the relatively low density of dams in Greece as compared to other Mediterranean-climate countries which have many major river transfers and much higher numbers of aliens in their inland waters; such as Spain (Clavero and Hermoso, 2011). Proximity to dams is not a guarantee of NIFS spread at the assemblage/site level (see Gido et al., 2004) but it is frequently and widely shown to be a direct contributor to alien species spread over broader scales (Clavero et al., 2004; Johnson et al., 2008; Moyle, 2013;

Crook et al., 2014). ii) Greece's small seasonally desiccated stream basins may have some capacity to resist species invasions due to their highly variable and fragmented conditions. This includes their swings in hydrological regime, temperature, and desiccation events (Cid et al., 2017) that may not allow NIFS to persist or disperse. These conditions are not conducive for sustaining most temperate lotic and lacustrine species that usually spread in larger more stable European temperate/northern lotic waters (Irz et al., 2004; Marr et al., 2010). iii) Alien cold-water hatchery-raised salmonids have not prospered in Greece. Although hatchery-raised rainbow trout have been widely stocked in Greece since the 1950s, for reasons concerning the domesticated and hybridized stock of this species reproduction in the wild is extremely rare and localized (Koutsikos et al., 2012; Stoumboudi et al., 2017). This echoes the general situation for rainbow trout in Europe, where this species is recently re-evaluated as a non-invasive element (Stanković et al., 2015; Koutsikos et al. 2019). iv) A relatively low number of recreational anglers use Greece's inland waters, and rather few using live fish bait or target predatory fishes (Lachanas et al., 2016). Since there was a rather low recreational fishing interest, stocking for angling occurred historically far later than in other Mediterranean countries. It has been shown that in the Mediterranean climate-regions occurrence and spread of NIFS was not significantly related to the invasive species life-history traits, but more to historical introduction date (Villa-Gispert et al., 2005) often relating to local fishing/angling cultural practices (Cerri et al., 2018; Mar et al., 2010, 2013).

Translocations as a priority issue

Our analysis is the first to explore the issue of translocated native invaders using quantitative field survey data in Greece; and to use freshwater ecoregions as a benchmark for species provenance. In contrast to alien species, we provide evidence for overlooked introductions of many translocated species in Greece’s lotic waters. As derived by the occupancy-abundance analyses, 1/3 of the translocated species are locally restricted and abundant producing thriving populations, and these may impact community and ecosystem dynamics (quadrat II in Fig. 2.2). The remaining 2/3 of translocated species may currently be locally restricted and rare but range within areas occupied by many range restricted endemics and threatened species (quadrat I in Fig. 2.2); this includes species co-existing in endemicity-rich lowland lotic waters.

The northern ecoregions of Greece (Thrace and Macedonia-Thessaly), that include large transboundary river basins and support rich fish faunas (Economou et al., 2016), are defined as the major donor areas of translocated species (Fig. 2.8). Ecoregions in the western and southern parts of the country (e.g. Ionian, W. Aegean, S.E. Adriatic), which can be characterized as depauperate in terms of regional species richness (Economou et al., 2016), were identified as the main recipient areas. The two latter trends support the argument that NIFS mainly originate from river basins with richer native ichthyofaunas than basins of the invaded fish community (Fitzgerald et al., 2016). Insular ecoregions (E. Aegean, Crete) neither supplied nor received any translocated species within the survey data; this may be due to the fact that until very recently the xerothermic areas of Southern Greece and the Greek islands had very few artificial reservoirs (Georgiadis et al., 2010) and also since inland waters angling in the Greek islands is nearly non-existent (Catsadorakis and Paragamian, 2007; Zogaris pers. obs.). In total, we document for the first time 20 translocated NIFS; while approximately 1/3 of these species are locally abundant at the sites they inhabit (Fig. 2.4). This number is much higher than a recent review for neighbouring Turkey (Tarkan et al., 2015).

The invasion potential of translocated species is enhanced by the geographic proximity between the source and receiving areas, which increases the transport possibilities and release frequencies. Moreover, most translocated species establish reproductive populations easily because of the greater likelihood of adapting to the county’s general natural flow, habitat and temperature regimes than can alien species (Ribeiro et al., 2008). However introduction pathway data are often unavailable and this is a serious impediment to stemming their spread (Hulme et al., 2017). In Greece, local fishers have mentioned that translocated species may frequently be an unintentional by-product of ad-hock carp re-stocking since this species is often moved in an unregulated manner from certain lakes with a rich native fauna. Additionally, many translocated species are difficult to identify and may go unnoticed since they physically resemble local "sister species" (related similar-looking species from neighbouring ecoregions). During electrofishing surveys translocated cyprinid species could easily be overlooked by research teams as "on site" identification is "subjective and observer-dependent" (Sousa-Santos et al., 2018). Although some translocations may not entail visible negative ecological or socio-economic consequences, they may have a considerable impact on the genetic composition and future survival of many endemic fish assemblages.

Interspecific hybridization due to translocations seems to be an ongoing pressure and an increasing threat in Greece, but without appropriate monitoring and genetic screening, this kind of impact is difficult to track and quantify. Fishes have a great potential for successful interspecific introgressive hybridization (Scribner et al., 2000) and there are numerous examples of gene pool erosion of native species following the introduction of aliens and translocated species (Largiadèr, 2007; Apostolidis et al., 2008). One of the greatest translocation problems concerns threatened local trout populations; translocation of *S. farioides* from the Acheloos drainage (western Greece) to the Aliakmon and Nestos drainages (eastern and northern Greece), where distinct *Salmo* species exist (Pelagonian trout, *Salmo pelagonicus* Karaman, 1938 and Macedonian trout, *Salmo macedonicus* Karaman, 1924), has resulted in serious hybridization problems (Apostolidis et al., 1997, 2008). Recently, genetic screening identified hybrid minnows of the endemic genus *Pelasgus* in the Peloponnese (Viñuela Rodríguez, 2016). The expansion of translocated species is poorly reported and not yet considered as a significant threat even in protected area assessment; this may be similar to the situation of underreported alien threats in marine protected areas (Mazaris and Katsanevakis, 2018). As emphasized by Buoro et al. (2016) the global effects of translocated introductions may exceed those induced by aliens species. The impacts of translocated native invaders are often appreciated at the individual level on sister species and wild conspecifics, however ripple-effects at the community and ecosystem levels could also be serious (Helfman, 2007; Moyle, 2013).

The multi-scaled distributional assessment, uncertainties and gaps

Lotic waters are rarely investigated in the Mediterranean basin specifically for NIFS. These ecosystems sustain specialized and often vulnerable biota and it has been widely shown that deteriorating lotic conditions may be associated with NIFS invasion (Aparicio et al., 2011; Milardi et al., 2018). Site-based river and stream sampling using a common method (standardized electrofishing) is the main workhorse in our documentation and assessment; although this has been used to screen aliens in rivers outside of the Mediterranean (Mitchell and Knouft, 2009) such applications are scarce for lotic waters in the Mediterranean countries. Most European country-wide studies use either presence/absence lists, historical and incidental records and expert judgment desk studies (e.g. Elvira and Almodovar 2001; Leunda, 2010; Hesthagen and Sandlund, 2007; Musil et al., 2010; Almeida et al., 2013; Anastacio et al., 2017).

Simple yet robust analyses at different spatial scales can provide insights on co-occurrence and spatial interaction with native fishes, signaling out the most prevalent invaders. Our multi-scale investigation supports conceptual approaches showing that native to non-native distributional relationships in rivers vary across spatial scales (Guo and Olden, 2014). Distinct evolutionary histories in different biogeographical regions strongly influence invasion of intact communities (Fitzgerald et al., 2016) and may explain the conflicting or varied relationships cartographically depicted for native and non-native species richness parameters found at different spatial scales. Comparative studies that examine patterns across multiple regions and spatial scales provide an understanding of fish assemblages at the community level (Angermeier and Winston, 1998) and only recently have these approaches been used in invasive aquatic species studies (Gido et al., 2004; Mack et al., 2007; Guo and Olden, 2014).

Our distributional assessment displays some important gaps and uncertainties. Complete representation of the majority of rivers and streams across Greece is not available, thus the results from this study should be viewed in light of areal coverage and sampling constraints. Areas such as Crete for example were at the time of the data compilation poorly explored, although there is evidence that a few alien species are already widespread in this island's lentic and heavily modified lotic waters (Tigilis, 2000; Barbieri et al., 2013). Also, although the EU CEN standardized electrofishing sampling has many positive aspects in terms of standard protocols and repeatability it is well known that certain benthic fishes often escape electrofishing catches in larger water bodies (e.g. catfishes; see Ruetz et al., 2007). Nevertheless, for nearly all river types in Greece, electrofishing has shown a rather high level of catch consistency and broad selectivity (Zogaris et al., 2018). Unfortunately electrofishing and other complementary sampling methods are not yet fully standardized in lakes or other lentic waters, so we deem it is not yet appropriate to comparatively explore lentic waters within this country-wide review (but see Petriki et al., 2017).

Furthermore, in our account, incidental or single observation data of NIFS are not used (i.e. casual observations, citizen science compilations) since data from amateur anglers and naturalists are poorly documented in Greece. Finally, there are taxonomic complications concerning NIFS which may hide species (i.e. cryptogenic species *sensu* Essl et al., 2018) and some translocated species many not be easily identified in field survey conditions. Taxonomic problems and uncertain identifications may include hatchery-reared salmonids and their hybrids, Asian *Carassius* spp. (Koutsikos et al., 2012); hybrid *C. carpio* varieties (Balon 1995) and recently-translocated cyprinids and/or hybrid cyprinids (Barbieri et al., 2015; Viñuela Rodríguez, 2016). Regular genetic screening for NIFS is a serious unmet need in Greece. Finally, new methods such as eDNA have not yet been applied to track NIFS in Greece, despite the fact that these are being developed recently in several Mediterranean countries (e.g. in Turkey, see Keskin et al., 2014).

Policy relevance

The issues and problems associated with alien species introductions are addressed, arguably incoherently, by a large number of EU policies, e.g. related to biodiversity conservation, ecological status evaluations, fisheries and aquaculture (Shine et al., 2010). The EU Regulation on IAS (No 1143/2014) introduces a framework for developing standardised procedures concerning non-indigenous species at the European level (EU 2014). The Regulation makes a distinction between "alien species" and "invasive alien species", where the latter are defined as those found "to threaten or adversely impact upon biodiversity and related ecosystem services", and stipulates that management is taken up for those invaders which are "widely spread". The Regulation promotes the creation of a list of IAS of Union concern (the Union List), to be updated at regular intervals, for which member states are required to apply restrictive, preventive or eradication measures in order to minimise the risks of their introduction, establishment and spread (EU 2014). The creation of lists of IAS of regional and of Member State concern is also envisaged. Inclusion of a species in the Union List and the general listing and prioritisation process should be based on a comprehensive risk assessment, which must include information relevant to the evaluation of threats (e.g. species ranges, reproduction, spread patterns and documented impacts), with a due consideration of various socio-economic aspects (EU 2014, 2017). It is explicitly stated that only IAS for which the available scientific evidence indicates capacity for establishment and spread shall be considered for inclusion. Member states are required to establish surveillance systems to monitor the occurrence and spread of invasive alien species, and also to assess the effectiveness of intervention measures, making use of all available relevant information, e.g. data from monitoring systems established by Union law (e.g. WFD monitoring).

It is obvious that, for an effective implementation of this regulation, appropriate data on alien species establishment rates or capabilities, persistence through time and spreading potentials must become available. The present work can contribute in this direction by providing data and evaluations that may assist in future risk assessments and the prioritisation procedure of alien fish species.

Conservation implications

NIFS have already negatively impacted the distinctiveness and biogeography of freshwater fish assemblages in many rivers in all Mediterranean climate regions (Moyle et al., 2003; Marr et al., 2010, 2013) and in Europe in general (Sommerwerk et al., 2017). It is almost certain that new introductions will continue in Greece due to increasing river reservoir/water development works, a continued angling interest, low public biodiveristy awareness, and very poor law enforcement (Aperghis and Ghaetlich, 2006; Zenetos et al., 2009; Lachanas et al., 2016). The poorly organized monitoring of NIFS and scarcity of quantitative data at the local scale presents an important obstacle to the flow of accurate information needed to support NIFS prevention and management measures in Greece. In such situations where distributional information is highly uncertain the influence of misinterpretations, gross generalizations and subjective values may create confusion and conflict (Humair et al., 2014), thus hindering effective conservation actions.

Preventing further harmful NIFS introductions remains the most important and immediate measure needed in Greece's inland waters. Prevention strategies require the "management of humans and human behaviour" (Wolter and Rohr, 2010) primarily with strategic investments and multi-party engagement in relevant research, monitoring, education, awareness, and policy enforcement (Galil et al., 2016). Defining targets and prioritizing objectives that address NIFS invasion in inland waters may lead to much better planning and effective management. The following issues are shown to be important in this review:

- Focus must not be restricted to aliens *per se* since translocated species and intraspecific genetically-modified strains and hybrids are also pivotal to tracking non-indigenous invasions (Maric et al., 2006).
- Site-based assemblage inventories such as electrofishing should be expanded to include specifically for NIFS in all lotic waters since this provides standardized quantitative data at the fish community level. Efforts for standardizing lentic water surveys should continue.
- Molecular identification methods are critically important for tracking NIFS especially at the stage of “early detection monitoring” (Trebitz et al., 2017). Advancing technologies for molecular identification (in-country capacity building) and rapid assessments using eDNA must also be promoted.
- Tackling NIFS demands collaboration, harmonization and sharing of data. Citizens can provide substantial contributions by reporting NIFS sightings. Citizen support should supplement state non-indigenous species surveillance systems (Tsiamis et al., 2017). Openness, accessibility of databases and appropriate public interest/awareness must be enhanced (Olenin et al., 2014).
- It is important to prioritize based on identifying highly invasive species (e.g. Gozlan et al., 2009); in Greek rivers aliens such as *G. holbrooki, C. gibelio L. gibbosus,* and *P. parva,* are spreading and should be regarded as the most harmful invasive fishes in country's lotic waters.

- Efforts must aim at preventing entry of NIFS in currently NIFS-free waters (Hermoso and Clavero, 2011). Invasive species control programs should focus on the areas of highest value for biodiversity and those most at risk from non-indigenous invaders (Saunders et al., 2002).

Our assessment shows that Greece presents important opportunities to prevent the spread of NIFS in its river, stream and spring waters since many areas are still not impacted by NIFS that are otherwise widespread in other European countries (Clavero and Hermoso, 2011). As in other European and Mediterranean countries, most NIFS occupy lentic habitats (Irz et al., 2004) and lower river courses (Crivelli, 1995; Elvira, 1995). The lower courses of the main rivers and associated diverse wetland habitats in the lowlands of Greece represent the areas with a highest degree of the endemism, rare fish habitat types and concentrations of threatened species (Economou et al. 1999; Catsadorakis and Paragmian, 2007; Barbieri et al., 2015). These lowland areas are often most vulnerable to NIFS expansion and good-quality lowland habitats with good ecological integrity are much scarcer than upland lotic habitats in Greece (Zogaris et al., 2018). Finally, on the "other side of the coin", alien and translocated fish species are not all bad or undesirable in a conservation context. In many cases there are beneficial and suitable reasons for scientifically-guided introduction (Helfman, 2007; Hoegh-Guldberg et al., 2008). Conservation-relevant species introduction to fill habitats where a species has been extirpated has had some success (Minckley, 1995; IUCN, 2013). "Conservation translocations" could be promoted more frequently in the Mediterranean, for example in climate-change adaptation programmes (Wolter and Rohr, 2010) and for managing novel lotic and lentic ecosystems (Moyle, 2013). Ichthyologists have recently began isolated translocation studies and applications for a very few threatened species, including range-restricted poor-dispersing endemics in Greece (Zogaris et al., 2017).

The need for methodological consistency and standardized approaches in collecting and analysing data on alien species is increasingly recognised (Matthews et al., 2017; Vanderhoeven et al., 2017; Roy et al., 2018). In this study we introduce a readily transferable screening procedure utilizing standardized site-level quantitative data, qualitative (presence/absence) data, and historical survey at different spatial scales for the identification of potentially high risk non-indigenous species. Using occupancy-abundance sampling data brings an integrated perspective that allows the simultaneous consideration of a wide array alien, translocated, and native species across the state. Although we found that only a small group of alien species has invaded lotic ecosystems in Greece, we assert that one of the most neglected and insidious NIFS problems concerns translocated species. Our review identifies aquaculture and fisheries-related activities as the main vectors of introduction; this includes frequent contamination of stockings. Consequently, action on the priorities identified here ultimately should benefit not only conservation and sustainable river management at the country level, but also broader efforts to better assess the risk and interpretation problems concerning NIFS at a broader European level.

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Chapter 3

DEFINING NON-INDIGENOUS FISH ASSEMBLAGES TYPES IN MEDITERRANEAN RIVERS: NETWORK ANALYSIS AND MANAGEMENT IMPLICATIONS

AN EARLIER VERSION OF:

Koutsikos N., Vardakas L., Vavalidis T., Kalogianni E., Kalatzi O-I, Dimitriou E. and Zogaris S. **(submitted)**.
Defining non-indigenous fish assemblages types in Mediterranean rivers:
Network analysis and management implications.

INTRODUCTION

The increasing volume and extent of global trade has resulted in the dispersal of various species outside their native distributional range, causing severe socio-economic and ecological impacts (Hulme, 2009). Agriculture, aquaculture and recreational activities have also promoted both the intentional and unintentional introduction and spread of non-indigenous species, resulting in an unprecedented rise of biological invasions (Kolar and Lodge, 2001; Mack, 2003). Currently, management actions to tackle species invasions focus largely on the pre-invasion phase, by applying risk assessments of the potential risk of a species to be invasive (Pheloung et al., 1999; Copp et al., 2005; 2016). Following a species' introduction and establishment, aquatic managers have limited management options, with the most common approach for controlling and mitigating the negative impacts of an invasive species to be trial eradication. However, these active removal measures are highly costly and often have limited success (e.g. Davies et al., 2020) and in those cases where the use of a piscicide is involved, though more successful than physical removal (Rytwinski et al., 2019), may negatively affect native biota (Vinson et al., 2010).

The introduction of non-indigenous fish species (hereafter NIFS) into natural, semi-natural and novel aquatic ecosystems has been consistently reported as one of the principal causes of biodiversity loss (Dudgeon et al., 2006; Butchart et al., 2010; Liu et al., 2017). The spread of NIFS in aquatic environments that already confront multiple anthropogenic pressures, such as hydrological perturbation and pollution, notably in Mediterranean rivers and streams (Skoulikidis et al., 2017), increases the magnitude of stress, leading to species extinctions and biotic homogenization (Scott and Helfman, 2001). Regardless of the above well-established threats, spatial patterns and environmental factors that are correlated with NIFS are poorly investigated in riverine ecosystems in the Mediterranean basin, since organized inventories and targeted field monitoring are quite limited (Clavero et al., 2004; García-Berthou et al., 2005; Piria et al., 2018; Radinger et al., 2018) and usually not adequate for multiple policy-relevant targets (Economou et al., 2016).

The use of fish assemblages to describe river ecosystem properties is applied worldwide with important policy-relevant applications (Angermeier and Winston, 1999; Aarts and Nienhuis, 2003). Despite the value of defining and characterizing rivers using native fish-based assemblage structures (Schlosser, 1985; Lyons, 1996; Jackson et al., 2001), or analyzing native fish assemblage structure and environmental attributes with reference to spatial distributions of NIFS (Russell et al., 2003; Kiernan and Moyle, 2012), few studies have defined non-indigenous fish assemblages. By investigating the introduction of NIFS, valuable insights can be gained in order to unravel the processes structuring impacted and homogenized river fish assemblages as a result of NIFS introductions (Ross, 1991; Scott and Helfman, 2001). More recent studies, used community measures in order to explore the diversity of riverine fish assemblages in novel ecosystems (sensu Light and Moyle, 2015) through the role of non-indigenous species (Erős, 2007), while others have analyzed the patterns of several European native and non-indigenous species composition turnover in relation to environmental filtering and dispersal limitation (Leprieur et al., 2009). Finally, Lapointe and Light (2012) were the first to assess the ecosystem invasibility of several river drainages through the linkage between non-indigenous fish community composition and ecosystem characteristics. Therefore, it is important to prioritize research on patterns associated with fish invasions at the community-level, while in addition there is a need to apply alternative integrated methods, that can maximize the insights into ecosystem invasibility and invasion processes in order to inform and direct successful managerial actions targeting invasive species (Lapointe and Light, 2012; Aschonitis et al., 2018; Koutsikos et al., 2019a).

Studies using network analyses are constantly increasing in many disciplines (e.g. physics, computer science, biology, economics, finance, sociology etc.) primarily due to the clarity of visualization (graphs), which advances the ability of researchers to unravel network structures in large datasets and extract or predict insightful patterns (Bellard et al., 2017). Within the field of ecology, network analysis has been mainly applied to delineate biogeographical regions (Vilhena and Antonelli, 2015; Bloomfield et al., 2018; Leroy et al., 2019) and to define and/or predict the structure of biotic communities in ecological networks (Brosse et al., 2001; Olesen et al., 2007; Sales-

Pardo et al., 2007; Encinas-Viso et al., 2016). However, while there is an increase of ecological network studies published since the mid 1980s (Heleno et al., 2014), studies targeting non-indigenous species are currently quite limited. Most studies using network analysis methods on non-indigenous species have applied Self-Organizing Maps to assess associations between recipient locations and biological variables, in order to identify occurrence probabilities and spreading potentials in various geographic areas (Céréghino et al., 2005; Gevrey et al., 2006; Paini et al., 2010). These studies focus on the pre-border/pre-invasion phase and evaluate the likelihood of non-indigenous species' introduction and spread, in order to predict potential adverse effects in case of invasion, allowing managers to be proactive rather than reactive (Haak et al., 2017). In contrast, while the use of network analysis concerning introduced non-indigenous species works for pre-invasion stage screening, it also moves the focus to the post-invasion phase, inevitably reshaping potential managerial actions (McGeoch et al., 2016).

This study aims to develop a classification framework to define non-indigenous fish assemblage types (hereafter FATs) in Mediterranean riverine ecosystems and to identify the linkage with various factors, i.e. local and regional environmental parameters, biotic indices, potential pathways of introduction and measures of propagule pressure, that may best explain their structure and distribution at local and regional scales. A network analysis approach was applied in order to extract community level information from NIFS composition data and to explore NIFS assemblage patterns within lotic ecosystems. Within this frame, this study contributes to the design of effective "tailor made" management actions dealing with specific NIFS assemblages rather than focusing on single species. Additionally, this framework can identify priorities within FATs and also help designing specific-type post-invasion actions tackling NIFS, e.g. the application of site/reach or water body-specific management and eradication measures. Finally, our results can provide valuable information for the design of appropriate conservation measures targeting high-priority water bodies at the pre-invasion phase.

MATERIAL AND METHODS

Study area

Greece stands at the crossroads between Europe, Asia and Africa wherein geological and climatic events have generated geographical isolation and conditions conductive for species speciation (Economidis and Banarescu, 1991; Skoulikidis et al., 2009). This resulted in an exceptionally high freshwater fish fauna diversity; Greece hosts many range restricted fish species, including 47 country-specific endemics, while another 42 species are Balkan endemics (Barbieri et al., 2015). The majority of the larger river basins in Greece are located in the western as well as in the northern part of the country. The river network of western Greece flows into the Ionian/Adriatic Sea, while the rivers of northern Greece, which are mainly transboundary rivers shared by Albania, Bulgaria, North Macedonia, Serbia and Turkey, are draining in the Aegean Sea. Greece is divided into eight freshwater ecoregions (Zogaris and Economou, 2017), namely Thrace, Macedonia-Thessaly, Ionian, Southeast Adriatic, Western Aegean, Eastern Aegean, Crete and South Anatolia. Each of the these freshwater ecoregions assembles distinctive freshwater fish fauna, with the exception of islets territory located in the South Anatolian Ecoregion which is fishless and lacking perennial water bodies or streams.

Data acquisition and usage

Fish data were obtained from various research surveys conducted during 2001-2015, covering the entire mainland as well as the major islands of Greece (Fig. 3.1, see Step 01). Samplings were conducted through a standardized electrofishing procedure following the guidelines of the FAME research project (Schmutz et al., 2007) with some modifications; for a detailed description of the sampling procedure see IMBRIW-HCMR (2013) and Economou et al. (2016). Freshwater fish species taxonomy and nomenclature follows Barbieri et al. (2015). NIFS includes alien and translocated species, while the autochthonous ichthyofauna is cited as native fish species. Euryhaline species were excluded from the inventory dataset while only the samples containing NIFS were used in the analyses. In total, data from 270 sites comprising 393 different samples from 51 river basins in Greece were used in our analysis (Fig. A.3.1).

Network analysis and assemblage delineation

In order to properly organize NIFS data and to avoid biases of species' distributions influenced by biogeographical processes or by varying sampling effort, we used NIFS percentage composition for network analysis. NIFS composition was calculated by expressing the contribution of each NIFS relative abundance as a percentage (%) to the total abundance of all species captured at each site.

Bipartite networks were created between two sets of nodes, where the connections between the nodes are referred as edges. Connectivity in bipartite networks is established exclusively through the species they contain (Vilhena and Antonelli, 2015) and in our analysis NIFS composition data were connected through edges with sites and vice versa. Gephi Software (v. 0.9.2) was used in order to create and illustrate the NIFS network (Bastian et al., 2009) and once the network was created, the "Forced Atlas 2" algorithm was applied for the interpretation of the data (Jacomy et al., 2014). In the latter algorithm, nodes repulse each other, while edges attract their nodes, hence this process results in sites that contain common species to be closer in the produced two-dimensional graph. For the discrimination of the different assemblages of the network, modularity optimization was used (Blondel et al., 2008). The algorithm passes repeatedly through two phases, the first allowing only local changes of the community and the other aggregating the founding communities. The process stops when there is no further increase in the modularity, revealing the distinct assemblage type of each class, in our case non-indigenous fish modularity classes (Fig. 3.1, see Step 02). The algorithm was determined with Gephi Software, which also calculates the modularity score. The score receives values from 0 to 1 where the higher score indicates a more sophisticated internal structure.

Subsequently, we conducted a Similarity Percentage analysis (SIMPER) by using Bray-Curtis dissimilarity, for revealing differences between pairs of modularity classes and estimating the average similarity within the modularity classes of each FAT (Fig.

3.1, see Step 03). The same analysis was also applied to identify the contribution of each NIFS within each modularity class and NIFS with the highest contribution of similarities of each FAT were defined as indicator species (i.e. key dominant species). The analysis was conducted using Primer-e software package (Clarke and Gorley, 2006). Finally, the produced FATs were projected into the map in order to reveal their spatial patterns (Fig. 3.1, see Step 04).

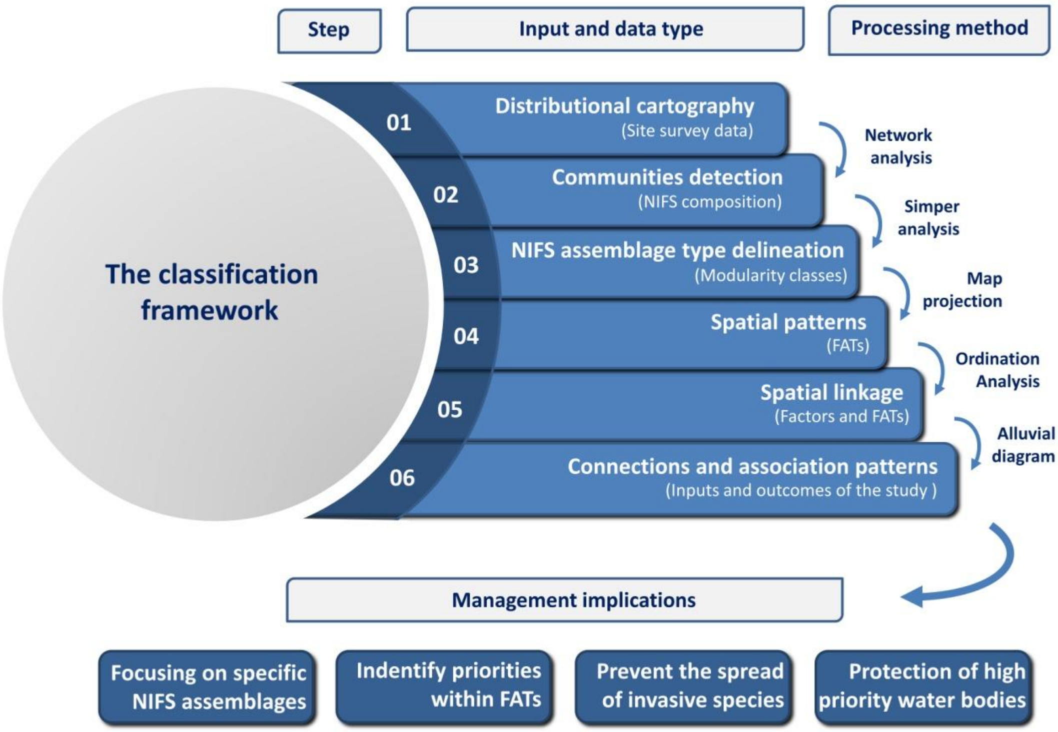

FIGURE 3.1 The classification framework applied in this study: inputs, data type, main steps, processing methods, outputs and management implications of the assessment procedure.

Biotic, environmental and anthropogenic factors

Mean abundance and frequency of occurrence (FO, %) were calculated for each species. The diversity of fish communities at each site was described by native species richness (n) and two biotic indices, i.e. the Shannon Index and the Simpson's Diversity Index. Shannon Index is considered to be biased toward measuring species richness, due to its sensitivity to the number of species per sample, while Simpson's Diversity

Index provides a proportional measure of diversity which is less sensitive to richness, emphasizing species' abundance in its calculation (Peet, 1974; Morris et al., 2014).

Shannon index was computed as:

$$H' = -\sum_{i=1}^{s}\left[\frac{n_i}{N}\left(\ln\frac{n_i}{N}\right)\right],$$

where n_i is the total abundance of each species i, N is the total number of individuals and s is the number of all species. The index increases as both the richness and the evenness of the community increase and values usually range from 1.5 to 3.5 and only rarely exceed 4.5. Simpson's Diversity Index was computed as:

$$Ds = 1 - \frac{\sum_{i=1}^{s} n_i(n_i - 1)}{N(N-1)},$$

where n_i is the total abundance of each species i, N is the total number of individuals and s is the number of all species. The value of D_s ranges between 0 and 1, where 1 represents infinite diversity and 0, no diversity.

For each site, a number of local environmental variables known to determine fish niches were recorded *in situ* using a field protocol modified from FAME (2005). Specifically, habitat parameters, i.e. mean wetted width (m), active channel width (m), mean depth (cm) and shadedness (%) were recorded at 10 transects in each site. Substrate coarseness was defined at each sampling site by using a modified Wentworth scale (Cummins, 1962), i.e. as coarse substrate we defined substrate > 64mm (cobbles and boulders), while as fine substrate we defined substrate ≤ 64mm (pebbles, gravel, sand, etc). Stream habitats, namely, fast flowing habitats, i.e. runs (deep/flowing), riffles (shallow/turbulent), rapids (steep gradient/fast flow), and slow flowing habitats, i.e. pools (deep/still), glides (shallow/flowing), were expressed as percentage coverage of each sampling site. Finally, at each site we recorded the presence of helophytes and bottom vegetation with visual assessment. In duplicate samples that were collected in multiple years at the same site, we used mean values of the factors (e.g. wetted width, depth, etc). Spatial parameters such as elevation (m), distance from source (km), upstream catchment area (km^2), slope, coordinates (DD) and mean air temperatures (^{o}C) were derived from geographical information systems (ESRI - ArcGIS v. 10.4). For the identification and classification of different land use types, CORINE Land Cover inventory (CLC, 2018) was used. Finally, in an effort to investigate potential relationships between the occurrence of FATs and the introduction pathways and propagule pressure, information on the presence of artificial lakes/dams within the basin or the stream corridor, as well as the presence of aquaculture units within the basin was collected, through a survey of published sources. Prior to all analyses, a Spearman correlation coefficient (rho) was applied using Primer-e software package, in order to exclude variables that were strongly correlated (>0.75). All data were log transformed, except for percentage data that were Arcsine transformed prior to use.

A multivariate ordination analysis was applied to detect the biotic and/or environmental factors that best explain the structure and distribution of FATs (Fig. 3.1, see Step 05). For this purpose, primary data in the analyses constituted of sites with the number of species represented in each of the main FAT modules. The gradient length of the first axis in DCA analysis was used for specifying the unimodal or linear response of the primary data. This value was estimated at 2.587 < 3 indicating linear response, concluding in the Redundancy Analysis (RDA) as the appropriate multivariate regression analysis. Prior to the RDA analysis, the Monte Carlo permutation test was applied to test the significance of the explanatory biotic, environmental and anthropogenic factors. The number of permutations was set at 499. To exclude variables that are strongly correlated and therefore have no unique contribution to the regression equation, we additionally considered the variance inflation factor to be estimated at less than 20 (Ter Braak and Smilauer, 2002). For the RDA analysis, Canoco for windows 4.5 (Ter Braak and Smilauer, 2002) was used.

Relationships between procedure elements

Finally, an additional analysis was performed in order to visualize the relationships of NIFS region of origin, NIFS type (i.e. alien or translocated), FATs and invaded riverine ecosystems within basins and freshwater ecoregions (Fig. 3.1, see Step 06). Alluvial diagrams are similar to Sankey diagrams which are typically used to display observation groups as flows across dimensions, time, types of processes or sets of features (Rosvall and Bergstrom, 2010; Muñoz-Mas and García-Berthou, 2020). The alluvial diagram was depicted in R syntax (R Development Core Team, 2017) by applying the function "sankeyNetwork" of the networkD3 package (Gandrud et al., 2015).

RESULTS

NIFS distribution in Greece

Overall, in 51 Greek river basins (Fig. A.3.1), we recorded 30 NIFS of which 10 were categorized as alien, and 20 translocated (Table 3.1). The majority of NIFS (14 species) demonstrated a low frequency of occurrence in river basins (<10%), while six species displayed percentages ranging between 18% and 80%. The five most commonly recorded NIFS within the riverine ecosystems of Greece included only alien species, namely *Gambusia holbrooki* (Eastern mosquitofish), *Carassius gibelio* (Prussian carp), *Lepomis gibbosus* (Pumpkinseed), *Pseudorasbora parva* (Topmouth gudgeon) and *Oncorhynchus mykiss* (Rainbow trout) (Table 3.1). The five most abundant species within the samples included four translocated fish species, i.e. *Squalius orpheus* (Maritza chub), *Barbus sperchiensis* (Sperchios barbel), *Squalius peloponnensis* (Peloponnese chub) and an unidentified chub, *Squalius sp.,* and the alien *G. holbrooki*.

Results indicate a wide distribution of NIFS throughout Greek territory, but notably absent from many upland riverine ecosystems at the Pindus mountain range across the center of mainland Greece, and from small-sized river basins of southern Greece (Fig. 2). Out of all NIFS, *G. holbrooki* was the only species that has been recorded as introduced in lotic ecosystems on both the mainland and the islands (Fig. 3.2a). *Carassius gibelio* and *L. gibbosus* were found within all ecoregions, with the exception of Southeastern Adriatic and Western Aegean ecoregions (Fig. 3.2b, c). *Pseudorasbora parva* distributed in the country's northern and northeastern rivers (Fig. 3.2d), while salmonids (*O. mykiss, O. kisutch* and *S. trutta*) occupied lotic ecosystems mainly at western Greece (Fig. 2e). The majority of the translocated species were distributed in the Ionian and Western Aegean ecoregions, with few exceptions however, such as in some basins within Thrace (Axios/Vardar, Evros/Meric, and Thassos Isl.; Fig. A.3.1) and Macedonia-Thessaly ecoregion (Pinios Thessaly, Fig. 3.2f).

TABLE 3.1. Non-indigenous fish species name, authority, type of NIFS, F.O.% in basins and samples, site occurrence and mean abundance, ±SE.

Species	Authority	Type of NIFS	F.O. (%) in Basins (n=51)	Occurrence in sites (n= 270)	F.O. (%) in samples (n= 393)	mean Abundance	±*SE*
Alburnoides sp.	–	T	1.96	1	0.25	1.00	–
Barbus sperchiensis	Stephanidis, 1950	T	1.96	1	0.25	150.00	–
Carassius auratus	(Linnaeus, 1758)	A	1.96	1	0.25	5.00	–
Carassius gibelio	(Bloch, 1782)	A	47.06	128	47.84	25.92	4.21
Ctenopharyngodon idella	(Valenciennes, 1844)	A	1.96	1	0.25	4.00	–
Cyprinus carpio	(Linnaeus, 1758)	T	17.65	16	5.09	1.63	0.22
Economidichthys pygmaeus	(Holly, 1929)	T	9.80	6	2.54	38.29	16.29
Gambusia holbrooki	Girard, 1851	A	80.39	150	53.94	88.30	27.81
Knipowitschia caucasica	(Berg, 1916)	T	1.96	1	0.25	50.00	–
Lepomis gibbosus	(Linnaeus, 1756)	A	21.57	81	29.52	16.16	2.56
Neogobius fluviatilis	(Pallas, 1811)	A	1.96	1	0.25	8.00	–
Oncorhynchus kisutch	(Walbaum, 1792)	A	1.96	1	0.25	1.00	–
Oncorhynchus mykiss	(Walbaum, 1792)	A	21.57	23	7.89	7.73	3.01
Oxynoemacheilus bureschi	(Drensky, 1928)	T	1.96	8	2.80	25.38	8.06
Pachychilon macedonicum	(Steindachner, 1896)	T	1.96	1	1.02	9.75	–
Pelasgus marathonicus	(Vinciguerra, 1921)	T	1.96	3	1.02	12.67	9.21
Pelasgus stymphalicus	(Valenciennes, 1844)	T	1.96	1	0.25	14.00	–
Pseudorasbora parva	(Temminck & Schlegel, 1846)	A	19.61	76	29.77	22.32	4.88
Rhodeus meridionalis	Karaman, 1924	T	1.96	4	1.78	16.25	5.48
Rutilus panosi	(Bogutskaya & Iliadou, 2006)	T	1.96	1	0.25	4.00	–
Rutilus sp.	–	T	1.96	1	0.25	3.00	–
Salmo fariodes	Karaman, 1924	T	1.96	11	2.80	17.75	6.30
Salmo trutta	Linnaeus, 1758	A	1.96	1	0.25	2.00	–
Silurus aristotelis	Garman, 1890	T	1.96	1	0.25	3.00	–
Silurus glanis	Linnaeus, 1758	T	3.92	5	1.27	3.40	1.91
Squalius orpheus	Kottelat & Economidis, 2006	T	1.96	1	0.25	162.00	–
Squalius peloponnensis	(Valenciennes, 1844)	T	1.96	1	1.02	69.50	–
Squalius sp.	–	T	1.96	1	0.25	66.00	–
Squalius vardarensis	Karaman, 1924	T	1.96	1	0.25	59.00	–
Tinca tinca	(Linnaeus, 1758)	T	3.92	3	1.27	6.67	4.26

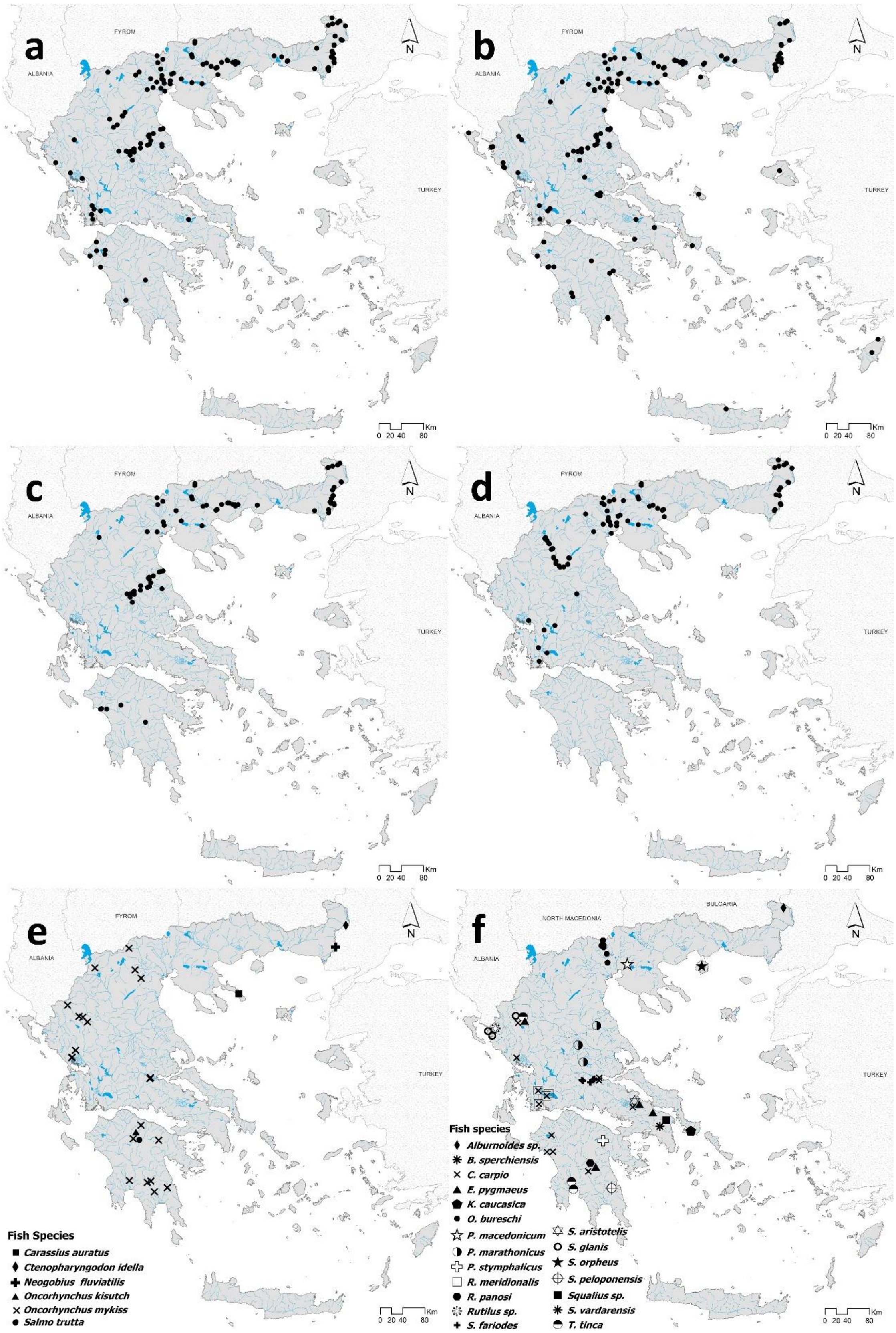

FIGURE 3.2. Distributions of NIFS in Greek riverine ecosystems – a) *G. holbrooki*, b) *C. gibelio*, c) *L. gibbosus*, d) *P. parva*, e) rest of alien species sampled, f) translocated fish species.

Network specialization and FATs

Gephi Software generated the matrix between sites and NIFS composition and the resulting data had 299 nodes and 530 edges. Forced Atlas 2 algorithm applied to the bipartite matrix and the graphical representation of the network illustrated one large interconnected system and six isolated groups around the main structure (Fig. 3). Once the network was created, the implementation of the community detection algorithm (modularity test = 0.412) created 12 modularity classes (Table 3.2, Fig. 3.3). Overall, five of the modularity classes enclosed approximately 95% of the total nodes, while the remaining seven classes displayed percentages below 1%.

TABLE 3.2. Modularity classes and fish assemblage types of NIFS in riverine ecosystems of Greece.

Modularity class	Participation of nodes (%)	Nodes/Edges	Fish Assemblage Types
● **1**	33.11	99/216	*C. gibelio; L. gibbosus; C. idella; P. marathonicus; Alburnoides sp.; N. fluviatilis; P. macedonicum*
● **0**	30.43	91/151	*G. holbrooki; S. aristotelis*
● **2**	14.05	42/84	*P. parva; O. bureschi*
● **4**	12.04	36/36	*O. mykiss; S. farioides; O. kisutch; P. stymphalicus; S. peloponensis*
● **6**	5.36	17/35	*C. carpio; E. pygmaeus; S. glanis; R. panosi; R. meridionalis; T. tinca*
○ **3**	0.99	3/2	*B. sperchiensis; S. vardarensis*
○ **5**	0.67	2/1	*Squalius sp.*
○ **7**	0.67	2/1	*C. auratus*
○ **8**	0.67	2/1	*S. orpheus*
○ **9**	0.67	2/1	*S. trutta*
○ **10**	0.67	2/3	*Rutilus sp.*
● **11**	0.67	2/1	*K. caucasica*

Dashed line denotes modularity classes with at least 1% of nodes

The three largest modularity classes were class 1 (92 sites), class 0 (89 sites) and class 2 (40 sites). Class 1 included seven NIFS with *C. gibelio* and *L. gibbosus* being the most dominant species, class 0 contained two NIFS with *G. holbrooki* being dominant, while class 2 included two NIFS, *P. parva* and *O. bureshi*. The other two largest classes were class 4 (31 sites) and class 6 (11 sites), with five NIFS with *O. mykiss* and *S. farioides* being the most dominant in class 4, and six NIFS with a dominance of *C. carpio* in class 6 (Table 3.2).

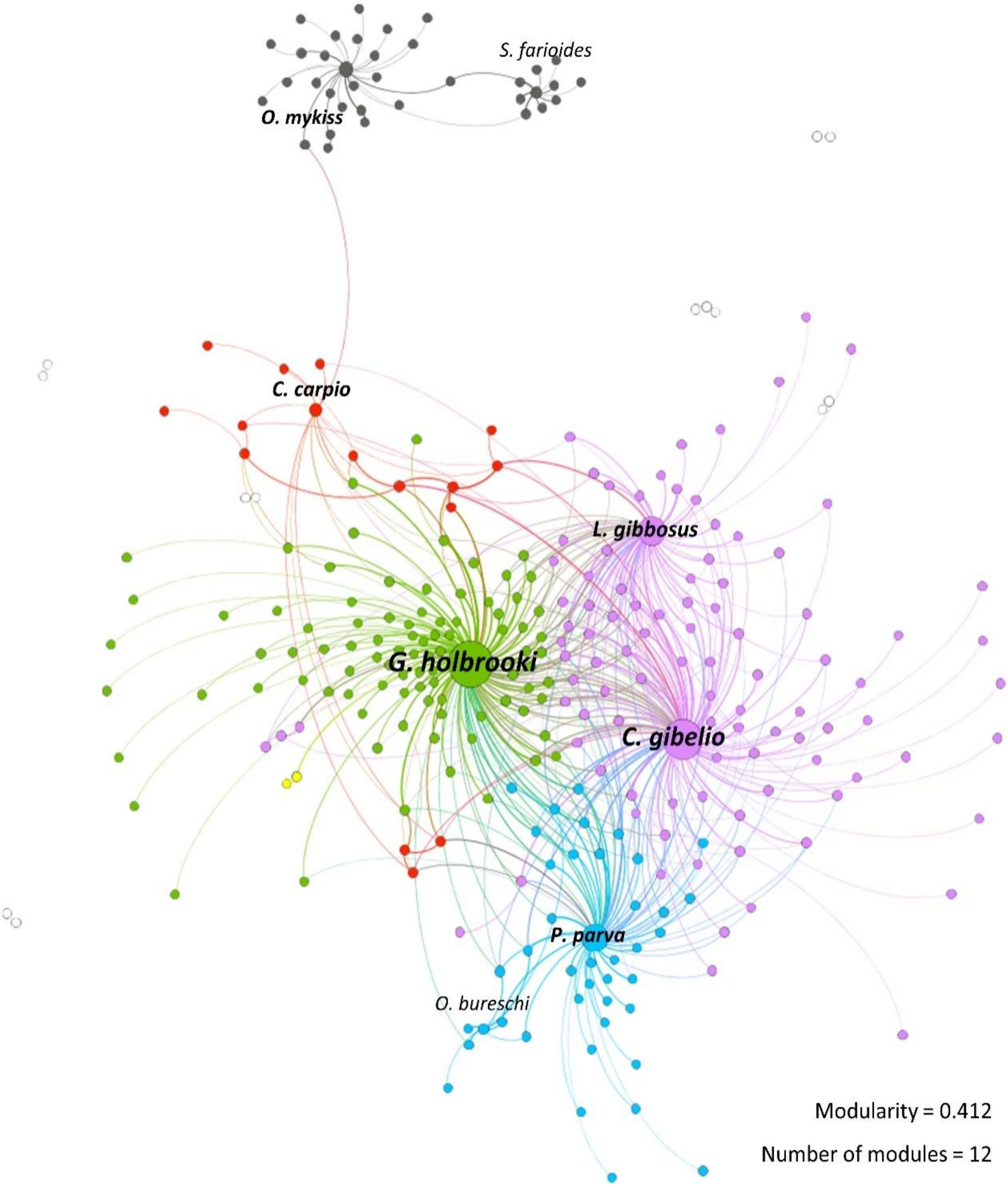

FIGURE 3.3. Bitrate network of NIFS assemblage types in riverine ecosystems of Greece. Distinct module colors display the 12 different classes derived from modularity test, described in detail in Table 3.2.

According to SIMPER analysis, there were differences between modularity classes (Table A.3.1). The lowest percentage of dissimilarity value was between modularity classes 0 and 11 (54.77%), while all other average dissimilarities (shown in Table A.3.1), ranged between 77.18% and 100%, indicating very different FAT among classes. In the majority of the cases examined, the average dissimilarity between modularity classes was 100%, sharing no common species. In contrast, the average similarity values within modularity classes ranged from 19.39% to 59.28% (Table 3.3), though the test did not perform in classes with less than two samples (classes 3, 5, 7, 8, 9, 10 and 11; Table 3.2). The lowest similarity value was detected in class 6 (19.39%) displaying the highest heterogeneity. The only similarity value having a percentage above 50% was detected inside class 0 (59.28%), with a solitary contribution given by *G. holbrooki* (96.45%). In the case of modularity classes 1 and 2 similarity values ranged around 45%, with the greatest contribution within FAT given by *C. gibelio* (63.19%) along with *L. gibbosus* (24.32%) and *P. parva* (81.99%), respectively. Finally, the average similarity of class 4 was 38.88%, and the only contributors were two salmonids, namely *O. mykiss* (83.77%) and *S. farioides* (16.23%).

TABLE 3.3. Average similarity within the main modularity classes and species contribution (%) in each FAT according to Simper analysis. Class ranking follows Table 3.2.

Modularity class	FAT	Average similarity (%)	Species	Contribution (%)
● 1	***Carassius/Lepomis***	44.08	*C. gibelio*†	63.19
			L. gibbosus†	24.32
			G. holbrooki	8.93
● 0	***Gambusia***	59.28	*G. holbrooki*†	96.45
● 2	***Pseudorasbora***	46.65	*P. parva*†	81.99
			C. gibelio	8.86
● 4	***Salmonids***	38.88	*O. mykiss*†	83.77
			S. farioides†	16.23
● 6	***Carp***	19.39	*C. carpio*†	39.39
			C. gibelio	26.19
			G. holbrooki	11.14
			E. pygmaeus†	9.85
			S. glanis†	7.1

The major NIFS contributors of average similarity within each modularity class provided the indicator species for each FAT (Table 3.2, Table 3.3). More specifically, G. holbrooki was the key indicator species for class 0, C. gibelio and L. gibbosus for class 1, P. parva for class 2, O. mykiss and S. farioides for class 4 and C. carpio for class 6. The five main modularity classes (hereafter "indicator species"–FAT), namely, Gambusia–FAT; Carassius/Lepomis–FAT; Pseudorasbora–FAT; Salmonids–FAT and Carp–FAT, were separately projected into the ecoregional map of Greece, exhibiting different spatial patterns, while the remaining modularity classes were sporadically distributed throughout the country (Fig. 3.4).

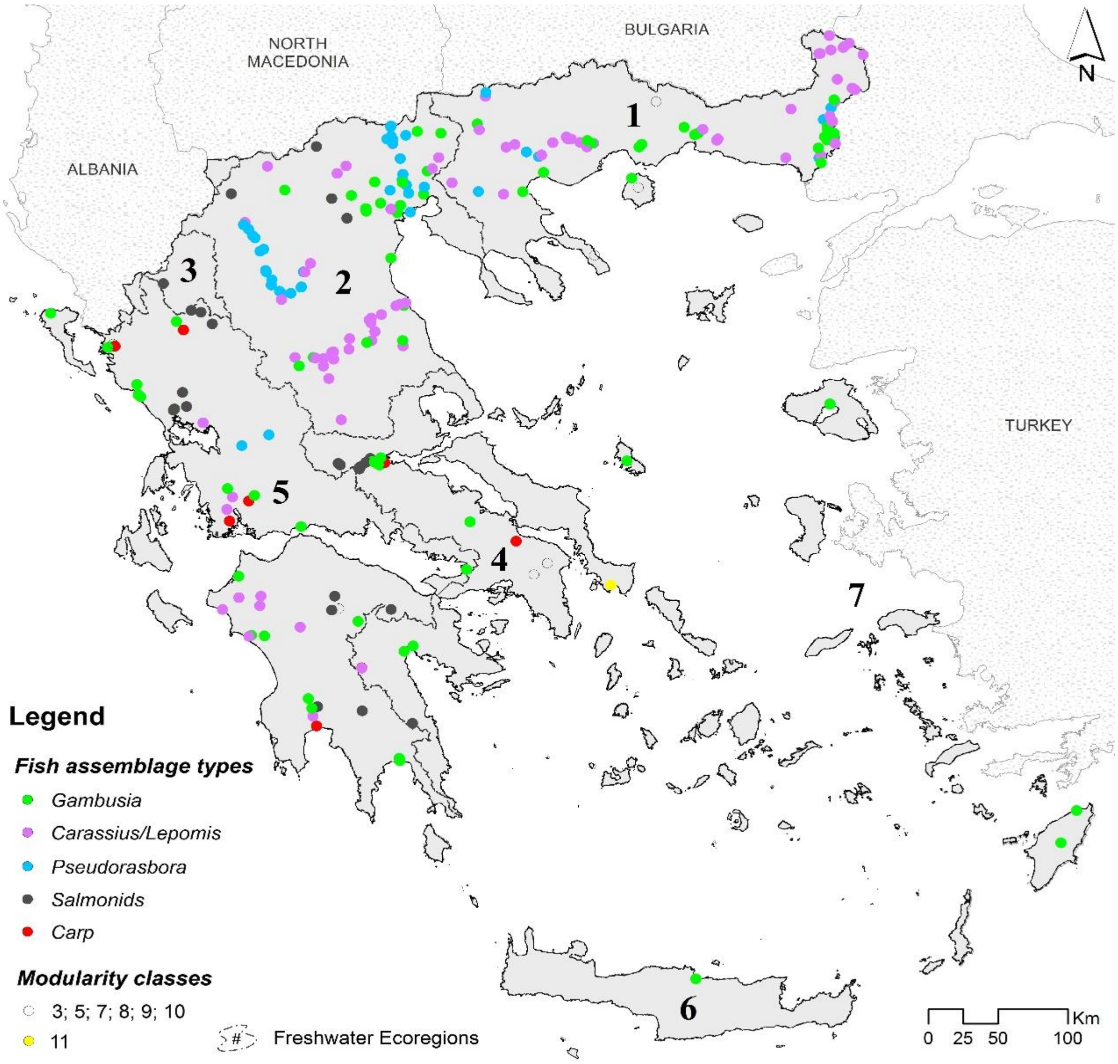

FIGURE 3.4. Spatial patterns of FATs and modularity classes in Greek riverine ecosystems. Numbers in the map indicate the freshwater ecoregions: 1. Thrace; 2. Macedonia-Thessaly, 3. Southeast Adriatic; 4. Western Aegean; 5. Ionian; 6. Crete; 7. Eastern Aegean.

Biotic, environmental and anthropogenic factors matching

According to Spearman correlation, a number of biotic and environmental factors were strongly correlated (mean annual air temperature; upstream catchment area; Shannon Index; agricultural land cover; coarse substrate and slow-flow habitat $r>0.75$, Table A.3.2) and hence were excluded from ordination analysis. Subsequently, the variance inflation factor was computed for the remaining factors and confirmed the independence among them (<20). The final factors that were incorporated in the ordination model are displayed in Table 3.4. The Monte Carlo test indicated that nine factors were statistically significant at 1% level ($p<0.01$) and four at 5% level ($p<0.05$).

TABLE 3.4. Factors used in the ordination model, their abbreviation and the p values of the applied Monte Carlo test. Bold characters indicate statistically significant values.

Factor	Code	Mode Carlo (*p* value)
Elevation (m)	*Alt*	***0.002*****
Slope	*Slope*	*0.190*
Distance from source (km)	*Dist. source*	*0.072*
Longitude (DD)	*Lon*	***0.018****
Latitude (DD)	*Lat*	*0.342*
Wetted width (m)	*Wet. width*	***0.002*****
Mean depth (m)	*Depth*	*0.198*
Fine substrate (< 63 mm)	*Fine Sub*	*0.376*
Fast-flowing habitats *runs (deep/flowing), riffles (shallow/turbulent), rapids (steep gradient/fast flow)* (%)	*Fast flow*	***0.008*****
Shadedness (%)	*Shade*	*0.322*
Mean air temperature of January (^{o}C)	*Jan Temp*	***0.002*****
Mean air temperature of July (^{o}C)	*Jul Temp*	***0.032****
Presence of helophytes *missing, sparce, intermediate, rich*	*Heloph*	***0.026****
Presence of bottom vegetation *missing, sparce, intermediate, rich*	*Bottom Veg*	*0.330*
Native species richness (n)	*Native Rich*	***0.002*****
Native species composition (%)	*Native Comp*	***0.014****
Simpson's Diversity index	*Ds*	*0.320*
Land covered by artificial surfaces (%)	*Artificial*	*0.156*
Land covered by forest / semi natural areas (%)	*Forest*	***0.002*****
Land covered by wetlands (%)	*Wetlands*	*0. 846*
Land covered by water bodies (%)	*Water bodies*	***0.004*****
Presence of artificial lake / dam within the basin (P/A)	*Dam basin*	***0.008*****
Presence of artificial lake / dam within the stream (P/A)	*Dam stream*	*0.329*
Presence of aquaculture within the basin (P/A)	*AQ basin*	***0.002*****

** Significant at 0.05 level; ** Significant at 0.01 level*

RDA results revealed differences in site-specific factors among the five FATs (Fig. 3.5). The ordination model was significant in the first (p=0.002) and in all canonical axes (p=0.002). The first axis explained 60% and the second 82.6% of the cumulative percentage variance of FAT–environmental factors relations.

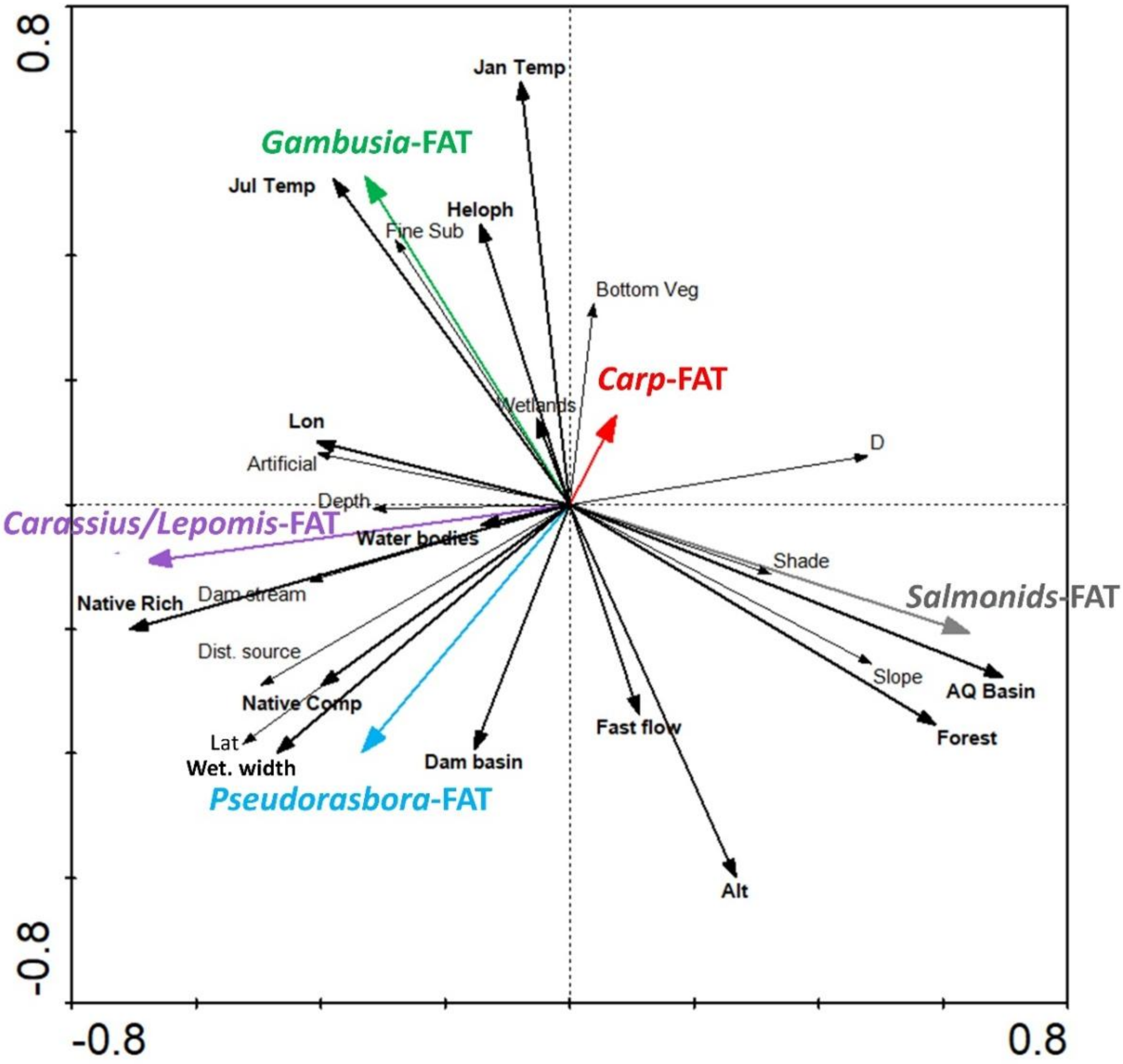

FIGURE 3.5. Ordination analyses (RDA; Canoco) among environmental factors and the five main fish assemblage types (FAT). Bold arrows and characters indicate the statistically significant factors (for significant levels and abbreviations see Table 3.4).

Gambusia–FAT was positively correlated with presence of helophytes, fine substrate, mean July air temperature and land covered by wetlands, and negatively correlated with fast-flowing habitats and altitude. Carassius/Lepomis–FAT was positively correlated with native species richness, presence of artificial lake/dam, mean depth and land covered by water bodies, and presented a strongly negative correlation with Simpson's index. Pseudorasbora–FAT showed a positive correlation with wetted width, native composition, artificial lake/dam, and distance from source, as well as latitude, and to some extent a negative correlation with the presence of Carp–FAT. A different pattern emerged for Salmonids–FAT where a positive correlation with the presence of aquaculture within the basin, forest/semi natural areas, slope and shadedness, and a negative correlation with longitude and with artificial surfaces was indicated. Finally, Carp–FAT was mainly negatively correlated with the presence of Pseudorasbora–FAT, wetted width, native species composition and latitude.

Connections and association patterns of the main procedure elements

According to the alluvial diagrams, half of the alien species that originated from North America and Asia have established reproducing populations; while as expected, all translocated species are already naturalized in Greece for a long period (Fig. 3.6). Only two species with European origin were detected (N. fluviatilis and S. trutta), but both are not yet considered naturalized. In terms of species richness, alien species dominated the invaded sites as well as sites within the formed assemblage types. The largest proportion of FATs was primarily located in transboundary basins of Northern Greece (Evros/Meric, Aliakmonas, Strymon/Struma, etc; Fig. A.3.1) as well as in the largest rivers basins located in Central Greece (Pinios (Thessaly), Sperchios, Acheloos, etc). Finally, Translocated and Salmonids FATs were mainly distributed within basins of the Ionian ecoregion (Arachthos, Louros, Pamisos etc), S.E. Adriatic ecoregion (Aoos/Vjose) and W. Aegean ecoregion (Sperchios, Asopos Boeotia etc.; Fig. A.3.1).

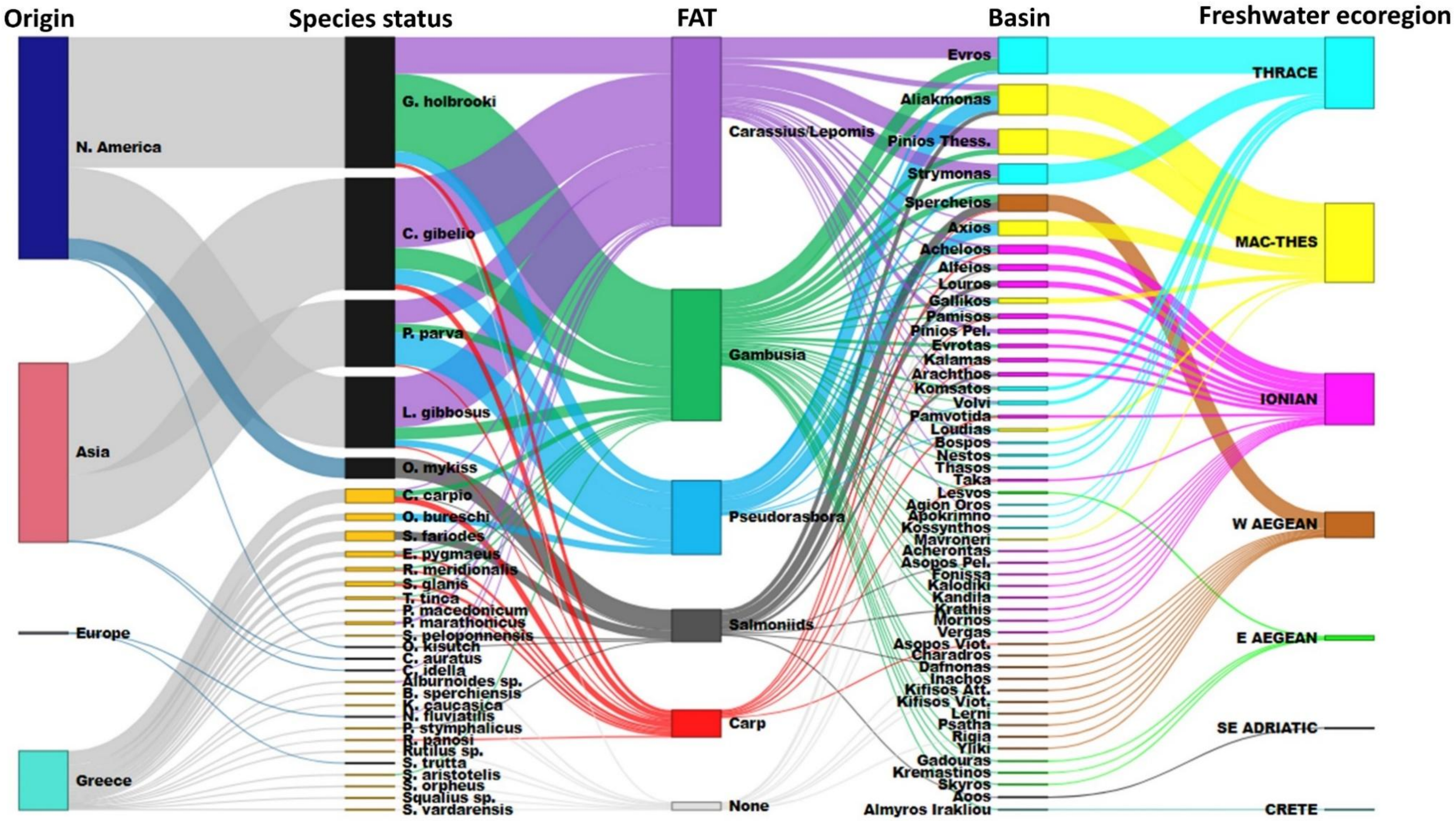

FIGURE 3.6. Alluvial diagram relating NIFS' origin, NIFS status (alien; black, translocated; orange), FATs and invaded riverine ecosystems within basins and freshwater ecoregions in Greece. Different colors on the arrows connecting the origin with species status denote NIFS current establishment status: light grey denotes naturalized; sapphire blue denotes acclimatized.

DISCUSSION

Distributional patterns of FATs and key indicator species

Overall, five modularity classes enclosing the largest percentage of nodes (95%) were generated through our analysis, denoting five FATS with key indicator species. The *Gambusia*–FAT was the most widespread group distributed in almost all freshwater ecoregions throughout the Greek peninsula with the exception of Southeastern Adriatic, while it was the only FAT covering the Aegean and Ionian Islands. Lowland vegetated riverine sections with high air temperatures, slow flow habitats, fine substrate, often in close proximity with wetlands and usually away from unmodified areas (e.g. forests), were mainly associated with the distribution of the *Gambusia*–FAT. The successful invasion of *G. holbrooki* indicated by our study is attributed to the species' advantageous adaptabilities in shallow inland waters in Mediterranean-climate conditions (i.e., high environmental tolerance, high fecundity, high survival of juveniles and rapid population growth, see Pen et al., 1993; Vargas and De Sostoa, 1996). This distribution pattern of *Gambusia*–FAT is expected as a result of the anti-malaria campaigns prompted worldwide by the Hygiene Commission of the League of Nations (Malaria Commission) after the First World War (Livadas and Sphangos, 1941; Gachelin and Opinel, 2011). Following the successful introduction of mosquitofish on a global scale, many studies have associated *G. holbrooki* with the decline of several native species in the Mediterranean basin (Rincón et al., 2002; Caiola and De Sostoa, 2005).

Similarly with *Gambusia*–FAT, the spatial pattern of *Carassius/Lepomis*–FAT was widespread throughout the mainland, not extending however to Eastern Aegean, Southeastern Adriatic and Crete. The distribution pattern of this FAT encompassed mainly the middle and lower sections of large-sized river basins. In addition, this FAT was associated with high water depth and proximity with artificial lakes. Disturbance and altered environments (e.g. urbanization, artificial surfaces, water development etc.) play an important role in the establishment and spread of NIFS in Mediterranean-climate streams and rivers (Clavero et al., 2004; Marchetti et al., 2004). *Carassius gibelio* and *L. gibbosus* are primarily associated with lacustrine habitats (Copp and Fox, 2007; Cucherousset et al., 2009); however it has been shown that they are capable of establishing self-sustaining populations in a variety of lowland river reaches, segments with regular flooding and regulated rivers of western Mediterranean ecosystems (Ferreira et al., 2007; Kottelat and Freyhof, 2007; Hermoso et al., 2008). Both key indicator species of this FAT are considered as extremely invasive and they are widely introduced throughout European freshwater ecosystems (Copp and Fox, 2007; Kottelat and Freyhof, 2007) with significant negative ecological impacts on native biota (García-Berthou and Moreno-Amich, 2000; Kottelat and Freyhof, 2007; van Kleef et al., 2008; Copp et al., 2010). *Carassius/Lepomis*–FAT occupied sites that are generally characterized by high native species richness, and both key indicator species typically occurred in low abundances relative to native species within natural streams (Clavero et al., 2004; Mesquita et al., 2006; Almeida et al., 2009; Koutsikos et al., 2019a).

The *Pseudorasbora*–FAT was mainly distributed in the country's northern and northeastern rivers, occupying mostly the middle segments of large-sized river basins. This assemblage type occupied sites far from the river source, often impounded and with artificial lakes. Within 50 years of spread, since the species first introduction in European ecosystems as a contaminant of herbivorous fishes imported from China (*C. idella, Aristichthys nobilis, Hypophthalmichthys molitrix*), *P. parva* has managed to colonize almost the entire European continent (Gozlan et al., 2010) and adjoining parts of Asia (e.g. asiatic Turkey, Copp et al., 2005). In Central and Eastern European lowland rivers, the species is now a common component of the local ichthyofauna, often being the dominant species (Witkowski, 2011). The key indicator species is usually found in a wide variety of habitats and while in its native range it is generally considered as rheophilic (Asaeda and Manatunge, 2005), in its introduced range it displays highest abundances in still waters (e.g. floodplain water bodies, ponds, small lakes, (see Pollux and Korosi, 2006; Kottelat and Freyhof, 2007). The significant positive correlation observed between *Pseudorasbora*–FAT with native species composition, may suggest the potential for adverse impacts through competition and further indicate either shared resource use or a possible biological resistance by native species (Beyer et al., 2007).

In contrast with the previous FATs, where eurytopic species with dominant limnophilic characteristics prevailed, a different pattern emerged for the *Salmonids*–FAT. This assemblage type included two key indicator species, the alien *O. mykiss* and the translocated *S. fariodes*, the two most rheophilic NIFS in Greek lotic ecosystems. As expected, *Salmonids*–FAT was located mainly in the upper sites of medium-sized rivers and, frequently, in their upland tributaries. Since the *Salmonids*–FAT was mainly distributed in cold-water streams, the environmental factors controlling the indicator species' distribution were coarse substrate, steep slope, dense canopy cover, proximity to forests and semi natural areas, typically away from artificial surfaces. This assemblage type revealed a strong positive correlation with the presence of aquaculture units within the river basin, identifying them as possible pathways for their introduction (Cook et al., 2008). Though, *O. mykiss* has been introduced worldwide after a century of intensive stocking, the extent of its establishment in Europe remains limited. Lack of suitable habitats or insufficient propagule pressure do not appear to be the main factors of establishment failures. Outbreeding depression seems to be a possible cause of poor establishment, at least for the Greek and other European populations of rainbow trout (Koutsikos et al., 2019b). On the other hand, *S. farioides,* as a translocated species, has easily established reproductive populations, since environmental conditions between the source and receiving areas match, due to their geographic proximity (Ribeiro et al., 2008). Such translocations may have considerable negative effects on many endemic invertebrate and threatened fish species (e.g. through predation, competition, hybridization, etc.) and other indirect ecological impacts (e.g. through diseases/parasites, food-web alterations, habitat degradation, etc.) that may exceed those induced by alien introductions (Buoro et al., 2016; Koutsikos et al., 2019a,b).

Finally, the *Carp* assemblage type included only translocated species. *Carp*–FAT displayed a restricted distribution within the river basins of the two most depauperate ecoregions of Greece, in terms of native species richness and often in biodiversity hotspots, namely, the Ionian and the Western Aegean (Economou et al., 2016; Zogaris et al., 2018). According to Koutsikos et al. (2019b), these ecoregions are identified as the main recipient regions of carp. *Cyprinus carpio* is the most widely distributed freshwater fish globally, being used in aquaculture since at least the middle and late Roman period (Balon, 1995). In Greece, extensive translocations have occurred in various freshwater systems since at least the mid-1930s (Economidis et al., 2000; Perdikaris et al., 2010), although the majority of these introductions, and in particular the more recent ones, belong to domesticated and/or non-indigenous strains (Barbieri et al., 2015).

Network analysis as a classification tool for NIFS

Network analysis has the potential to document ecological processes, identify and describe fish community structure, provide general patterns, and explore ecosystem functioning (Heleno et al., 2014; Delmas et al., 2019). By building a bipartite network, sampling sites are connected only through the shared species (Bastian et al., 2009), while the produced groups are identified by associated modules (Blondel et al., 2008). This concept of grouping is highly applicable as a more sensitive method (e.g. in biogeographical studies) compared to conventional clustering classification methods (Vilhena and Antonelli, 2015). The main advantages of this procedure are: a) species' identities are not lost, as opposed to being abstracted into dissimilarity matrices; b) each species is assigned within specific modules enabling species level description; and c) grey zones between different modules can be detected (Vilhena and Antonelli, 2015; Bloomfield et al., 2018; Leroy et al., 2019). Network-oriented analysis has been further applied in detecting possible interactions between invasive and threatened native species, in order to provide information for the implementation of effective eradication strategies (Bellard et al., 2017).

Current management actions to tackle species invasions are now focusing more on the pre-invasion phase (Cook et al., 2010; Simberloff et al., 2013), by assessing the potential invasiveness of individual non-indigenous species through risk analysis tools (Copp et al., 2005; 2016). Tracking non-indigenous species distribution and the factors that regulate their introduction and spread are crucial steps in determining establishment success, interpreting the stage of invasion process and defining key indicators for strategic decision-making purposes (Fonseca et al., 2019; Koutsikos et al., 2019a). Within the frame of this approach, network analysis can be a helpful tool.

Studies focusing on non-indigenous fish assemblages, rather than single NIFS species, are quite limited, while rarely translocated non-indigenous species have been given equal standing to alien species (Scott and Helfman, 2001; Russell et al., 2003; Sommerwerk et al., 2017). By using network analysis, we provide a straightforward approach to define the most distinct non-indigenous fish assemblages and identify their key indicator species, both alien and translocated. Our approach can be utilized to describe emerging invasive species assemblages and explore their interactions in local riverine ecosystems. This method also allows the spatial representation of these FATs and sets the critical priorities for conservation, by providing emerging information on the establishment of alien fish assemblage types. Results of cross-taxonomic methods offer important advantages in ecological studies, as compared to those that focus on single taxa (Vilhena and Antonelli, 2015). This is especially important in areas such as Mediterranean lotic systems with varied habitats patterns and more diverse fish assemblages than other European systems (Ferreira et al., 2007). In this sense, this study contributes to the design of “tailor made” management actions dealing with specific NIFS assemblages.

Data limitations and uncertainties

Despite the fact that our study utilizes broad scale quantitative data of NIFS inhabiting lotic ecosystems, we acknowledge its spatial limitations by not incorporating NIFS in lentic environments. However, quantitative fish fauna data for Greek lakes are scarce and only available for the largest lakes. In addition, our study lacks temporal variations of NIFS that could provide trends concerning their interannual abundance and distributional expansion, or define the influence of various climatic variables on the structure of the defined fish assemblages (Kiernan and Moyle, 2012; Wedderburn et al., 2014). Finally, taxonomic ambiguities of NIFS may cause additional uncertainties by concealing fish species (e.g. cryptogenic species sensu Essl et al., 2018) or creating identification difficulties (e.g. translocated species; hybrids) especially in field survey conditions. Apparently, our contribution, along with other methods of identifying and interpreting community classifications as well as tracking xenodiversity hotspots (e.g. NIFS genetic screening; eDNA methods), needs to be further refined and integrated for wider use.

The interface between scientific research and management implications

Ecological networks serve as locus for engaging scientific research and policy making, with even greater potential than has currently been realized by both the scientific community and the relevant stakeholders (Pocock et al., 2016). Overall, network theory as a framework supports ecologists with ways of exploring nature's complex web of interactions (Heleno et al., 2014) and provides important approaches for summarizing different kinds of ecological information that can be used to answer several types of ecological questions, from local to global scales (Delmas et al., 2019). The whole effort of the present study is essential for identifying and prioritizing entire assemblages in order to improve strategies dealing with NIFS at the national and regional scales, primarily in the post-invasive phase and equally important for a pre-invasion stage screening.

The implications of FATs illustrated through the alluvial diagram, summarize the connections and the association patterns of all the outcomes of the study providing insights after the invasion events across and within different spatial scales. Thus, the match between ecosystem invasibility and FATs patterns can be assessed both at larger scales (e.g. freshwater ecoregions) and at finer scales (meso or microhabitats; e.g. river reach, riffle) in order to direct specific-type and effective management actions on biological invasions, to predict and prevent further species invasions and spread, or to provide information in designing protected areas for species conservation (Saunders et al., 2002; Hermoso and Clavero, 2011; Lapointe and Light, 2012). Based on species region of origin, nine out of ten alien species originated from North America, Asia and Eastern Europe following a similar origin pattern observed in many freshwater and terrestrial ecosystems all over Europe (Piria et al., 2018; Muñoz-Mas and García-Berthou, 2020), while the remaining continents typically display incidental presences. The vast majority of the alien key indicator species fell within the category of the most common invasive fish species in Europe as well as worldwide (Koehn and MacKenzie, 2004; Savini et al., 2010; Nunes et al., 2015; Toussaint et al., 2016). On the contrary, while high establishment rates have been reported from other Mediterranean countries (García-Berthou, 2007; Ribeiro et al., 2009; Maceda-Veiga, 2013; Bianco, 2014), only four aliens are fully naturalized within the Greek lotic waters. Several other authors have noted that only a minority of the introduced species appeared to be invasive in the Mediterranean basins, and highlighted the necessity of developing additional criteria for prioritizing future intervention measures, mainly through further research on the spatial distribution of non-indigenous species (Beisel et al., 2017; Nocita et al., 2017; Teletchea and Beisel, 2018; Koutsikos et al., 2019a)

In a more general ecoregional context, the largest proportion of FATs were primarily located in Northern Greece within Thrace and Macedonia-Thessaly freshwater ecoregions, mainly due to a plethora of transboundary water courses. Large-sized river basins that cross international political boundaries typically support rich fish faunas and are commonly considered as emerging hotspots for NIFS introductions (Hulme, 2015; Piria et al., 2018). In contrast, Greece's relatively small/medium-sized river basins located in the western and southern parts of the country display a capacity to resist alien species' intrusion due to their highly variable hydrological conditions and their fragmented hydrographic network (Koutsikos et al., 2019a). Usually, most of temperate lotic and lacustrine alien species spread in more stable and large European temperate/northern lotic waters (Irz et al., 2004; Marr et al., 2010). Likewise, FATs which mostly consisted of translocated species (*Carp* and *Salmonids*) were mainly distributed within basins with similar characteristics in the Ionian, S.E. Adriatic and W. Aegean freshwater ecoregions.

Even though translocated species are widely considered as an important element of the issue of NIFS introductions, often fish species' translocations are overlooked as an invasive species issue (Helfman, 2007; Koutsikos et al., 2019a, b). In comparison to the potential invasive success of non-indigenous aliens, the invasion ability of translocated species is enhanced by both the proximity between recipient and receiving areas and the country's general natural flow regimes and climatic conditions

(Ribeiro et al., 2008). Approximately, two thirds of the translocated species that have been introduced into Greek lotic ecosystems were until now locally restricted and rare, however they occupy freshwater biodiversity hotspots with several Greek native fish species of particular importance (Koutsikos et al., 2019a). Similarly, while *Carp* and *Salmonids* FATs are the two lesser main assemblage types, both primarily occupy areas across freshwater ecoregions that host many threatened fish species and range restricted endemics. Finally, the impacts of native species' translocations can be twofold: (i) on species level, such as interbreeding on sister species or inbreeding on wild conspecifics; and (ii) on ecosystem levels, such as transform directly or indirectly the structure and species' composition (Helfman, 2007; Moyle et al., 2013), having noxious effects as those induced by non-indigenous aliens (Buoro et al., 2016). To this end, effective management actions should be implemented mitigating the spread of translocated species within high-priority water bodies. Moreover, as important biodiversity hotspots, targeted conservation measures are required in order to prevent further species invasions in these areas.

Calls for assessing the integrity of natural fish assemblages and identifying "artificial diversity" (sensu Angermeier, 1994) have been persistent, but few studies have focused on surveying, defining, monitoring and management of non-indigenous fishes as assemblage units (Lanzoni et al., 2018). In this study, we developed a classification framework by integrating a network analysis approach based on sampling data, and identified key factors which relate to the structure and distribution of specific FAT. The dominant FATs varied spatially within the study area, indicating different community structures, mainly based on the diverse habitat preferences and life-history traits of the indicator NIFS. Apart from the NIFS ecological preferences and traits within each FAT, biotic interactions and modes of propagation appear to be crucial in determining both community structure and spatial distribution. Overall, this study provides a useful method which systematizes sampling data, and the resulting classifications, identifies the priorities within FATs, provides valuable information for the protection of high-priority water bodies and, finally, can be utilized in country-wide pre- and/or post-invasion actions to manage NIFS.

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Chapter 4

GLOBAL DISTRIBUTION

AND CLIMATIC MATCH

OF A HIGHLY TRADED ORNAMENTAL FRESHWATER FISH: THE SAILFIN MOLLY *POECILIA LATIPINNA* (LESUEUR, 1821)

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INTRODUCTION

Species invasions is a composite outcome in which human drivers determine dispersal possibilities and extent, while establishment probabilities and rates are determined by natural drivers (Marchetti et al., 2004; Lockwood et al., 2005; Ribeiro et al., 2008; Blanchet et al., 2009). There is now a general consensus that human activity is a major vector of the invasion process and the best predictor of invasion success (Lockwood et al., 2005; Colautti et al., 2006; Korsu and Huusko, 2009).

At different stages of the invasion process (Moyle and Marchetti, 2006), non-indigenous species have to overcome several barriers (biotic and abiotic) in order to invade successfully a new area. The first two steps of this process are "the arrival stage" of the species to the invading ecosystem through anthropogenic transport, and its" survival" throughout it (Sakai et al., 2001; Moyle and Marchetti, 2006). Thus, when suitable ecological conditions and habitats for a species do exist at a large scale, non-native range expansion may be constrained by the limited possibility of anthropogenic transport and thus entry into new aquatic systems. Yet, the quantification of human influences on freshwater fish invasions remains a challenge, due to the lack of effective indicators to express the degree of human activity (García-Berthou, 2007). In order for a non-indigenous species to become resident into a novel environment, propagule pressure is critical in determining which introductions are going to lead to establishment (Marchetti et al., 2004). High propagule pressure usually, but not always, leads to high success rates of colonization (Moyle and Marchetti, 2006) and the establishment of a viable, self-sustaining population (Sakai et al., 2001). Finally, spread and integration are local processes, conditioned by the interplay between abiotic and biotic factors (Moyle and Marchetti, 2006).

Mollies, members of the genus *Poecilia* (family Poecilidae), are small and short-lived livebearing fishes of the New World with a distribution range from the southern United States to Central America (Meffe and Snelson, 1989; Simpson et al., 2015). They occupy temperate and tropical zones and exploit a broad array of aquatic habitats (Meffe and Snelson, 1989). Some species or their hybrids are popular ornamental fish, and their association with human mechanisms of transport has resulted in numerous introductions worldwide (FAO, 2010; Froese and Pauly, 2014). The sailfin molly *Poecilia latipinna* (Lesueur, 1821) is the species with the northernmost native distribution range of all species of the genus. It is endemic to the eastern coastline of North America, from North Carolina, through the east coast of Florida to the Gulf of Mexico (Meffe and Snelson, 1989). Due to its popularity as an ornamental fish, but also in the assumption that it is an effective biocontrol agent for preventing malaria by preying on mosquito larvae, the sailfin molly has been introduced throughout the world (Courtenay and Meffe, 1989) with 13 country-level introductions reported in the DIAS database (FAO, 2010) and 17 introductions reported in the Fishbase (Froese and Pauly, 2014). In Europe, and the wider Mediterranean area, the only known population of the sailfin molly has been reported in Greece (Lake Vouliagmeni), originally attributed mistakenly to *P. sphenops* (Chintiroglou et al., 1996). Chintiroglou et al. (2004, 2008) refrained from naming this taxon and referred to it as a member of the subgenus *Mollienesia* (*Mollienesia* sp.). In subsequent publications, Koutsikos *et al.* (2012) and Barbieri *et al.* (2015) referred to this taxon as a sailfin molly, *P. latipinna*. A recent study (Koutsikos et al., 2017) provided a definite taxonomic identification of this molly population as sailfin molly, based on morphological characters.

Aspects of the life-history, biology and ecology of the sailfin molly in its native habitats have been described in several studies (Snelson, 1980; Felley and Daniels, 1992; Trexler et al., 1992; Nordlie, 2006). Briefly, it is a small ovoviviparous species (maximum size 12.5 cm) that feeds principally on algal material (Chick and Mlvor, 1997) and typically lives up to three years. Its short generation time promotes resilience, with minimum population doubling time being less than 15 months (Froese and Pauly, 2014). It prefers hard, alkaline waters and thrives in warm brackish wetlands; however, it is euryhaline and can tolerate salinities from freshwater to 80‰ (Nordlie et al., 1992). It is extremely tolerant to oxygen deprivation (Timmerman and Chapman, 2004; Nordlie, 2006) with an ability to withstand pollution, frequently becoming the dominant species when introduced in very disturbed aquatic habitats (Gonzales and Moran, 2005; Felley and Daniels, 1992). It is among the few species that has been adapted to, and even thrive in, sulfidic habitats which are toxic for most metazoans (Riesch et al., 2015). Though sailfin molly is typically considered a warm-water species (Froese and Pauly, 2014; Corfield et al., 2008), it exhibits a fairly broad thermal tolerance, ranging from 4.0 to 40.0 *°C* (Nordlie, 2006; Fischer and Schlupp, 2009). However it appears to be stenothermic in respect to breeding temperature requirements (above 22 °C; Dawes, 1991). Its native distribution extends to an area that spans subtropical latitudes (such as Florida, with *Cfa* type in Köppen-Geiger climate classification, Peel et al., 2007); consequently, it has been suggested that temperature is the limiting factor that largely determines its native geographic range (Dill and Cordone, 1997). However, established populations occur also in California that has a Mediterranean-type climate (Dill and Cordone, 1997). This raises some doubt about the role of temperature as the main limiting factor to its invasions. Temperature alone cannot explain the poor representation of the sailfin molly in European waters, especially around the Mediterranean, where summer temperature in some areas would allow at least a seasonal reproduction (Kalous et al., 2015; Perdikaris et al., 2016). Moreover, photoperiod and salinity are critical environmental factors affecting the life and reproductive traits of poeciliids (Baird, 1974; Meffe and Snelson, 1989; Martin et al., 2009). They are also affected by the synergetic effects of temperature and photoperiod, which in general change concurrently (Vinagre et al., 2009). The relationship between photoperiod and latitude is well illustrated for the biological cycles of various organisms (Lee, 1970). Finally, seasonality, as well as overcast, also interact with photoperiodic cycles, varying latitudinally (Francis, 1970; Lehner, 1987).

An important question in invasion biology is thus whether large-scale climatic factors constitute the main determinant of establishment success (and therefore can provide a good indication of potential establishment in a new area) or local habitat characteristics are of greater importance. Relevant literature shows that in general, established sailfin molly populations show consistent associations with warm lentic or slow-flowing lotic habitats that provide an abundant vegetative food resource (salt marshes, estuaries and nearby marine areas, slow-moving portions of rivers and quiet lowland streams, canals and backwaters, and geothermally-heated wetlands). For example, the sailfin molly is extremely abundant and by far the dominant fish species in the warm, brackish and weedy Al-Hammar marsh of Iraq (Hussain et al., 2009), in the heavily polluted by sewage and agriculture effluents Wadi Haneefah Stream in Saudi Arabia (Al-kahem et al., 2007), in ponds and other stillwater habitats in Hawaii (Englund 2000), and hotspring-fed pools and wetlands in Nevada (Scoppettone et al., 2005), New Zealand (McDowal 1999, 2006), Canada (Nelson and Paetz 1992) and Greece (Koutsikos et al., 2017). These examples indicate that the sailfin molly is conservative with respect to its ecological requirements and can only tolerate conditions for which it is evolutionarily experienced.

The aim of this study was to evaluate the current distribution and potential establishment of the sailfin molly worldwide, with emphasis on Europe and the Mediterranean, by exploring climatically suitable areas available to the sailfin molly in different target regions, through the use of climate matching. The concentration (albeit fragmented) of its established populations in tropical and subtropical zones and the pattern of their spatial clustering are discussed, as well as possible agents impeding its establishment in Europe and the Mediterranean, such as low introduction effort and genetic constraints associated with domestication. The role of favorable local environmental conditions that may enable the species' establishment, despite climate mismatch, is also explored.

MATERIALS AND METHODS

To map the distributional range of the sailfin molly and to evaluate the relative contribution of the invasive spread drivers, worldwide occurrence records of the species were obtained from two sources. The first source of information was scientific publications (scientific articles, books, conference proceedings, doctoral and master theses); however, a common and significant obstacle for obtaining distributional data of alien fish is that they are not easily accessible, they are either poorly known or when they are reported, the data are not always available. To overcome these limitations we extended our publication review to include references from technical reports. Another source of information was online databases, namely, CABI - Invasive Species Compendium (CABI, 2014), DAISIE - Delivering Alien Invasive Species Inventories for Europe (DAISIE, 2008), DIAS - Database on Introductions of Aquatic Species (FAO, 2010), FishBase (Froese and Pauly, 2014), IUCN/SSC Invasive Species Specialist Group (Global Invasive Species Database, 2015), and NAS - Nonindigenous Aquatic Species USGS (Nico et al., 2014). In order to assess the establishment status of a sailfin molly population in the current study the following criteria were used: a population was recorded as “established” when this population was explicitly registered as such in at least one source. A population was recorded of “unknown status” when there was only a single reference to the presence of the species. A population was recorded as “probably established” when it was referred as such in at least one scientific source and/or there were multiple references to its presence at a specific location. When comparing the establishment status of the three Poecilia species in Europe, a fourth category “Probably not established” is used, when it was referred as such in at least one scientific source.

We applied the Climatch v.1.0 (Bureau of Rural Sciences, 2008) in order to compare the climate match between the native geographic range of the sailfin molly (source area) and four different target areas: a) validated introductions of the target species on a global scale, b) potential areas of invasion worldwide, c) potential areas of invasion in the European continent and d) potential areas of invasion in the Mediterranean region. For the imported occurrence records of validated introductions, Climatch automatically selected 100 of the nearest available meteorological stations to those listed in the location file. The other three target areas utilised data from 19,000, 1,753 and 240 climatic stations respectively, of the WordClim project database (Hijmans et al., 2005). The ecological requirements of freshwater fishes are primarily related to temperature and hydrology (Matthews, 1998). Hence, climatic matching conditions were represented in the current study by the following variables: “Annual mean temperature”, “Temperature during the coldest quarter of the year”, and “Mean annual rainfall” for the accurate investigation of the potential spread of the evaluated species within the target areas (Costa and Schlupp 2010; Kalous et al., 2015; Kotovska et al., 2016; Patoka et al., 2017), with an Euclidean algorithm. The first two variables were used in order to represent temperature requirements, while the third one was applied as a key component of the hydrological cycle, as well as for its correlation to salinity and photoperiod, tightening up further the climate-matching function. Climate-match values range from 10 to 0, wherein 10 stands for the highest-level match and 0 for the poorest match. Values that equal or are higher than 7.0 indicate that temperature is not an environmental barrier to survival.

RESULTS

Current distribution

The non-indigenous distribution of the sailfin molly can be characterised as global but highly fragmented (Fig. 4.1). We positively validated 100 non-indigenous occurrence records worldwide (see Appendix Table A.4.1 and Table A.4.2 for relevant information on non-indigenous occurrences by continent and site details respectively). Of the 100 validated introductions worldwide, 80 populations are confirmed "established" and 14 "probably established", while the status of the remaining six is unknown (Fig. 4.1).

TABLE 4.1 Climatch scores for globally validated introductions, and for areas with probability of sailfin molly establishment worldwide, European and peri-Mediterranean scale. Scores of ≥7.0 (marked in grey) are interpreted as indicating no environmental barrier to survival.

Climatch Score	Global introductions	Worldwide	European continent	Mediterranean region
0	8	9386	1106	2
1	1	372	114	9
2	0	381	128	5
3	0	437	141	9
4	4	550	89	7
5	2	870	64	28
6	22	1730	32	40
7	34	1875	28	69
8	18	2308	43	57
9	10	1091	8	14
10	1	0	0	0
Total	**100**	**19,000**	**1,753**	**240**

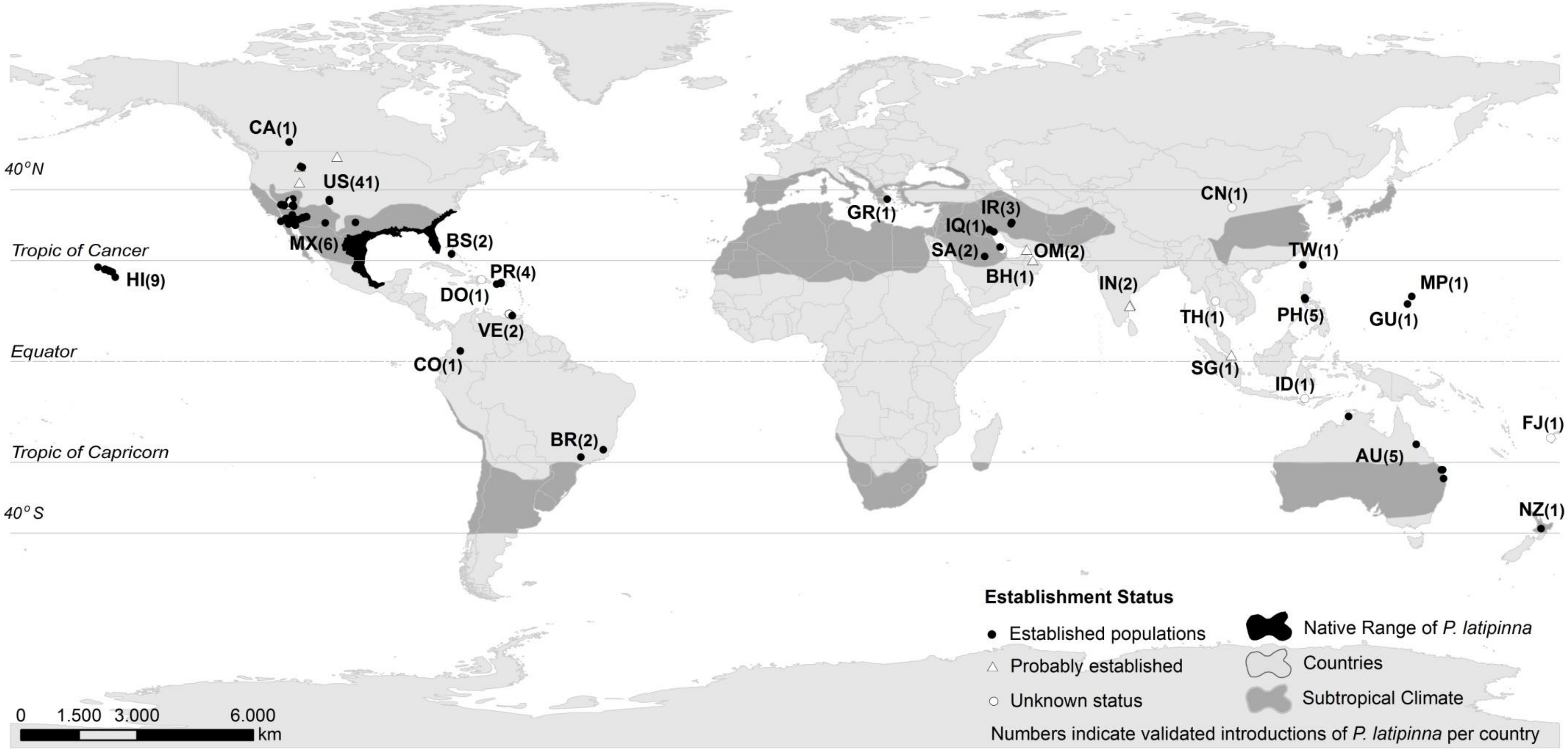

FIGURE 4.1 The global distribution of validated introductions of the sailfin molly (*P. latipinna*) with establishment status noted. AU: Australia, BS: Bahamas, BH: Bahrain, BR: Brazil, CA: Canada, CN: China, MP(US): Commonwealth of the Northern Mariana Islands, CO: Colombia, DO: Dominican Republic, FJ: Fiji, GR: Greece, GU: Guam, HI(US): Hawaii, IN: India, ID: Indonesia, IQ: Iraq, IR: Iran, MX: Mexico, NZ: New Zealand, OM: Oman, PK: Pakistan, PH: Philippines, PR: Puerto Rico, SA: Saudi Arabia, SG: Singapore, TH: Thailand, TW: Taiwan, US: USA, VZ: Venezuela.

TABLE 4.2 Reported occurrences of *Poecilia latipinna*, *P. reticulata* and *P. sphenops* in European continent

Countries	*P. latipinna*	*P. reticulata*	*P. sphenops*	Reference
Albania		U		[4, 7, 9, 13, 20]
Austria		–		[5, 10]
Bulgaria		U		[20]
Czech		+ / –	–	[7, 9]
France		U		[12]
Germany		●	U	[11, 15]
Greece	●			[16]
Hungary		+	●	[7, 9]
Ireland		U		[12]
Italy		–	+	[7, 9, 19]
Netherlands		●		[5, 7, 9, 15]
Poland		–		[17]
Romania		●	●	[5, 13, 18]
Russia		●		[2, 9, 13, 15]
Serbia		●		[20]
Slovakia		●	–	[7, 9, 13, 14]
Spain		+		[3, 6, 7, 8, 15]
UK		●		[5, 7, 9, 21]
Ukraine		–		[1]
Occurrence	**1**	**18**	**6**	

● = Established, + = Probably established, – = Probably not established, U = Unknown

(1) Alexandrov et al., 2007, (2) Budaev, 1997, (3) Cobo et al., 2010, (4) Crivelli, 1995, (5) DAISIE, 2008, (6) Dhora, 2010, (7) Elvira, 2001, (8) Elvira and Almodovar, 2001, (9) Froese and Pauly, 2014, (10) Füreder and Pöckl, 2007, (11) Geiter et al., 2002, (12) Global Invasive Species Database 2015, (13) IMPASSE, 2007, (14) Koščo et al., 2010, (15) Kottelat and Freyhof, (16) Koutsikos et al., 2017, (17) Nowak et al., 2008, (18) Petrescu-Mag et al., 2008, (19) Piazzini et al., 2010, (20) Piria et al., 2018, (21) Zięba et al., 2010.

More than half (60 occurrence records) are located in the American continent (51 established populations). Most validated introductions in this continent fall within the south-western part of the United States (41 occurrences), characterised by Mediterranean-type climate conditions (Fig. 4.1; and Tables A.4.1, A.4.2 in Appendix).

In the other continents, the occurrence of the species is sporadic with the introduced populations sparsely distributed and highly fragmented. There have been 23 validated introductions (13 established populations) in Asia (with several around the Persian Gulf) and 16 introductions (15 established populations) in Oceania (Fig. 4.1, Tables A.4.1, A.4.2 in Appendix). There are no validated introductions in Africa. Three previous reports in Kenya were proved misidentifications (see Seegers et al., 2003). Finally, there is only one validated introduction in Europe, of an established population at the geothermal Lake Vouliagmeni (Attica, southern Greece, Fig. 4.1, Tables A.4.1, A.4.2 in Appendix). The absence of established sailfin molly populations in Europe, except the one in Greece, contrasts sharply with the occurrence of two other closely related and ecologically similar poeciliids, the guppy *P. reticulata* Peters, 1859 and the shortfin molly *P. sphenops* Valenciennes, 1846, which have been reported from 18 and six European localities respectively (Table 4.2).

Potential establishment of the sailfin molly through climate match

The climate match map of validated introductions of the sailfin molly on a global scale (Fig. 4.2a, Table 4.1) showed that 62% of the meteorological sites matching validated populations scored ≥7.0, indicating no environmental barrier to establishment. A small proportion (9%) indicated a very low probability of establishment of the species (climatch scores 0-1). The respective localities, all in Northern US and Canada, are all wetland habitats associated with hot springs in areas with otherwise adverse climatic conditions for the species' survival (Fig. 4.2a), with the favorable local conditions possibly enabling the support of populations despite climate mismatch. Worldwide, the climate match map indicated no environmental barrier to survival for 28% of the localities assessed (score ≥7.0, Table 4.1, Fig. 4.2b).

In the European continent, the climate match maps indicated low probabilities of sailfin molly establishment (Fig. 4.3) but much higher probabilities at localities around the Mediterranean (Fig. 4.4). Specifically, on the European continent, the probability of establishment of the sailfin molly was 5% (Climatch scores ≥7.0). Conversely, in the Mediterranean Basin, climatic conditions were more suitable for its establishment, with 60% of the meteorological sites indicating scores ≥7 of the Climatch scores (Table 4.1).

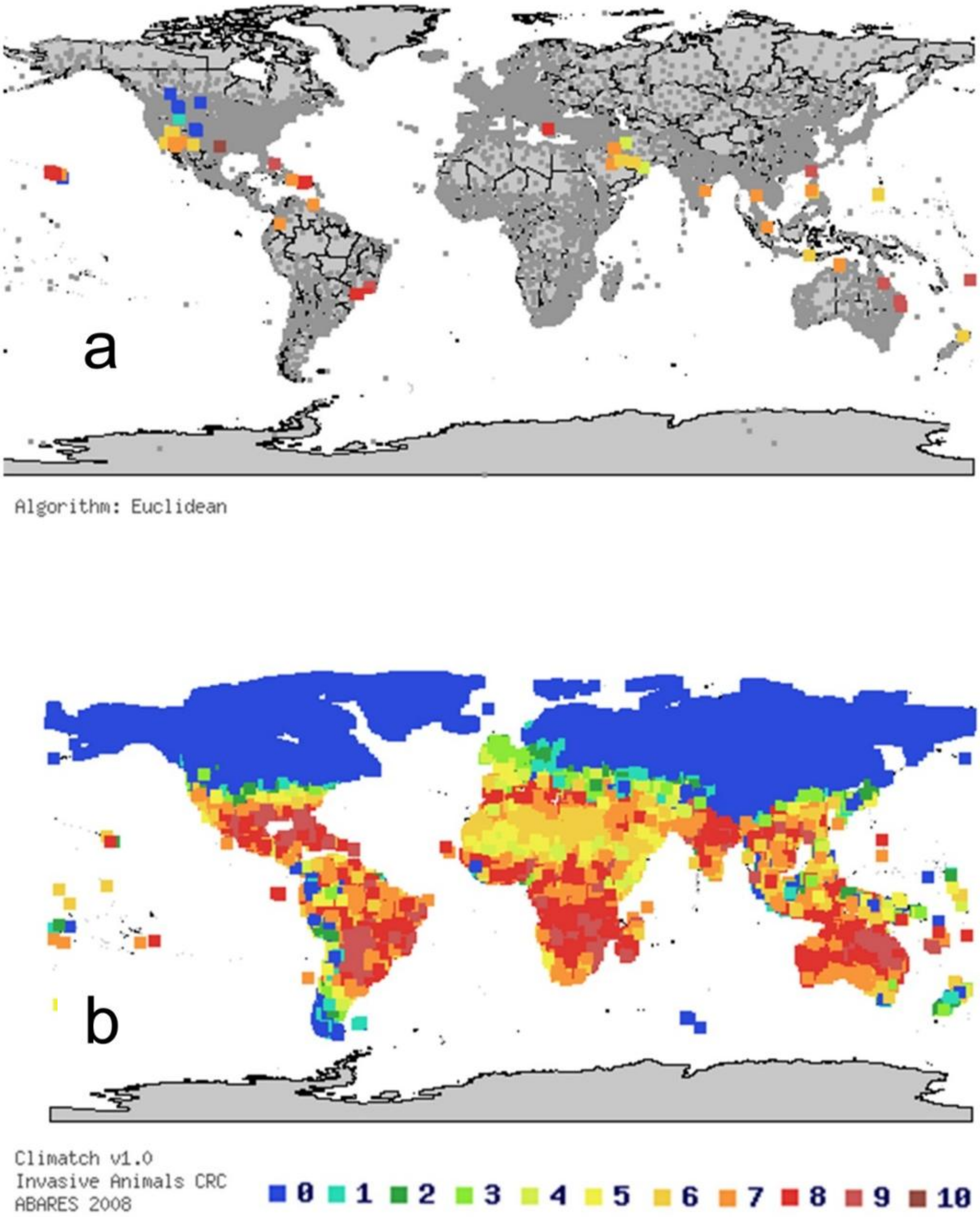

FIGURE 4.2 (a) Climate match map of validated introductions of the sailfin molly (*Poecilia latipinna*) on a global range; (b) Climate match map of areas with probability of establishment for the sailfin molly worldwide. Scores of ≥7.0 are interpreted as indicating no environmental barrier to survival.

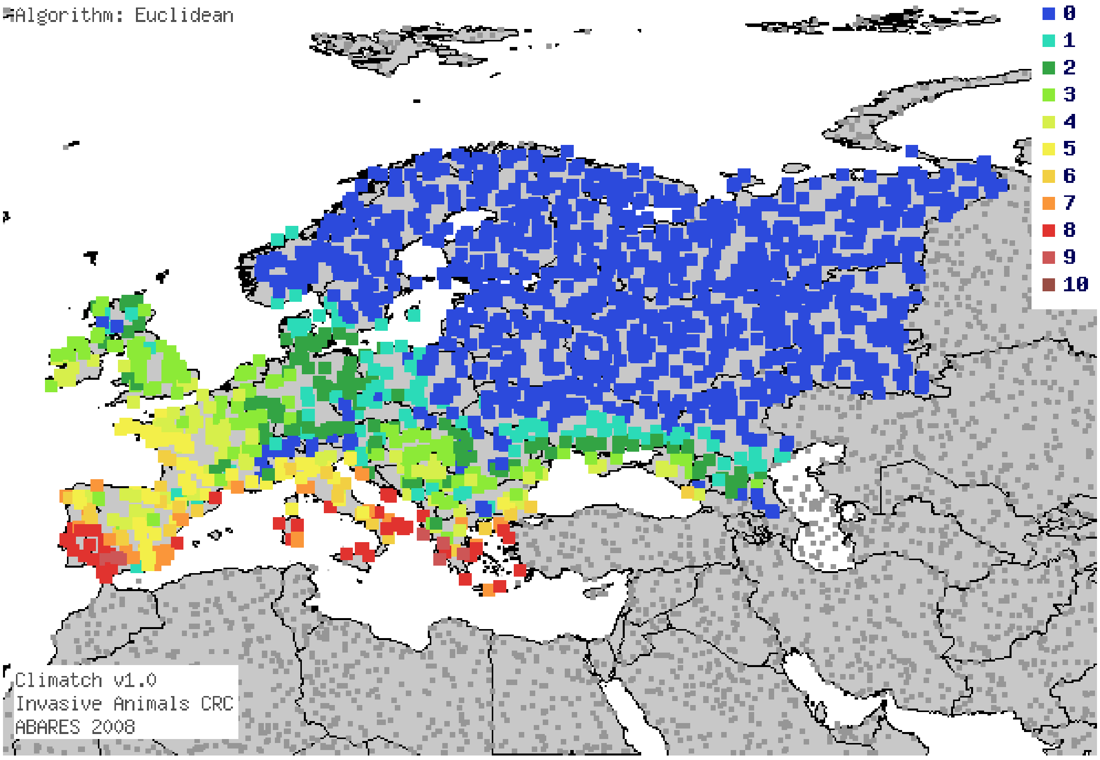

FIGURE 4.3 Climate match map showing areas with probability of establishment for the sailfin molly (*Poecilia latipinna*) in Europe.

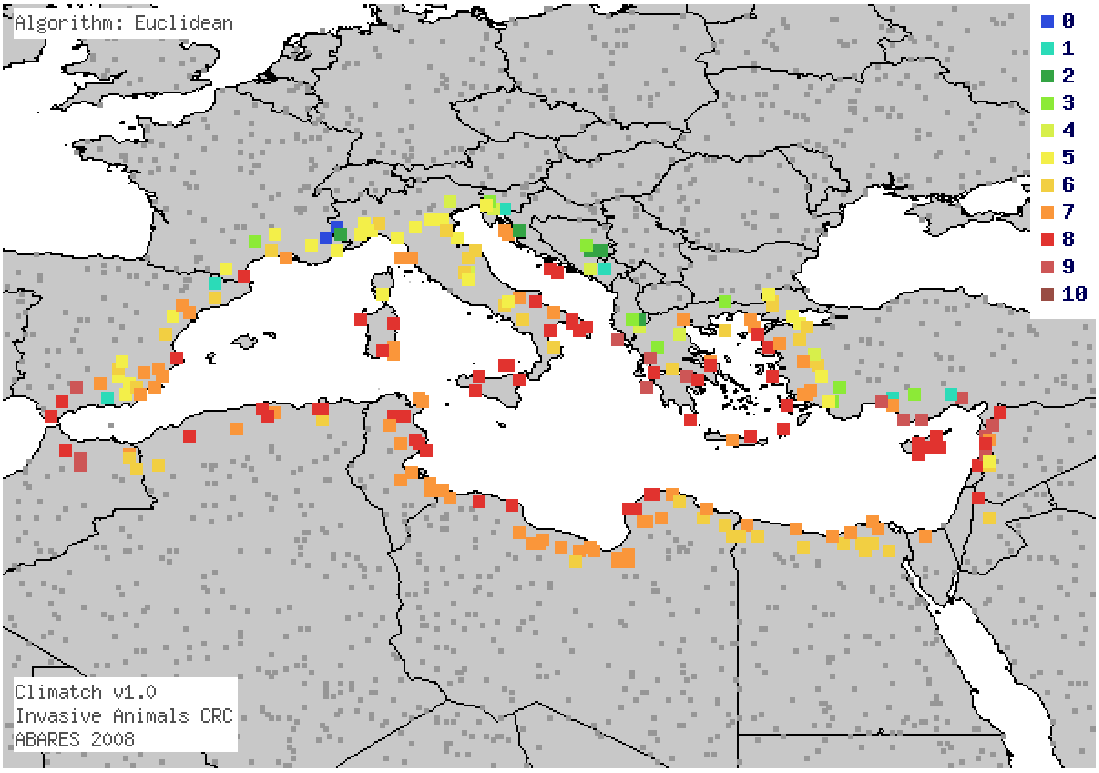

FIGURE 4.4 Climate match map showing areas with probability of establishment for the sailfin molly (*Poecilia latipinna*) in the peri-mediterranean region.

DISCUSSION

Non-indigenous distribution patterns of sailfin molly

The global distribution pattern of the sailfin molly outside its native range points to temperature as the dominant ecological factor influencing the probability of establishment. Indeed, most validated introductions were recorded from tropical and subtropical regions. In temperate climatic zones, the occurrence of the species appears to be rare and highly localized. In fact, the majority of recorded sailfin molly occurrences outside the subtropical belt are in small geothermally heated water bodies. Evidently, specific local conditions may create pockets of environmental suitability, allowing the species to overcome the climate barrier to establishment (Scoppettone et al., 2005). Costa and Schlupp (2010) used abiotic variables from known occurrence localities of the sailfin molly to model its potential distribution in South and East United States. They asserted that minimum temperature of the coldest month is the factor best explaining establishment success; however, they noted that large-scale climatic features may not sufficiently explain the presence of the species at some locations (e.g. in central Texas).

Another distribution pattern evident from our data is the spatial clustering of many introduced sailfin molly populations with a human relevance. More specifically, higher occurrence frequencies are observed in regions close to the native area of the species, such as in the southwestern US, where it has been established in five states (CABI, 2014), as well as in certain Caribbean islands. This clustering seems to be human-mediated, rather than having an ecological basis, and it is likely to reflect differences in introduction rates. Ornamental fish trade may be a major vector of introduction within the more urbanized southwestern US, since there is a flourishing ornamental fish production industry in the state of Florida which accounts for approximately 95% of all ornamental fish produced in the U.S.A. (Hill and Yanong, 2002). Outside the US, relatively high occurrence frequencies were noted around major centers of ornamental fish production and trade, as in the islands of the south-western Pacific. Some non-indigenous occurrences have been attributed to its intentional release as a biocontrol agent for malaria, such as in the islands of the central Pacific (for Hawaii see Englund, 1999) and possibly in countries around the Persian Gulf, though at a much smaller scale than mosquitofishes (Arthington and McKenzie, 1997; Lintermans, 2004). However, the release of poeciliids by hobbyists as a vector of introduction in rapidly developing countries bordering the Persian Gulf cannot be excluded (Esmaeli et al., 2017).

Potential agents impeding sailfin molly establishment in Europe and the Mediterranean

The results from the current study indicate that temperature is the dominant ecological factor influencing the probability of sailfin molly establishment globally. The low representation of the sailfin molly in the Mediterranean area cannot, however, be solely attributed to climatic constraints, as it runs contrary to the predictions of the Climatch tool, which indicated a 60% suitability of the climatic conditions for establishment in this region. It is well established that the sailfin molly is a tolerant species with broad limits to a number of habitat factors (Marchetti et al., 2004). Indeed, habitat descriptions in its native distributional range indicate strong preference to alkaline and brackish waters and rich submerged vegetation (Nordlie *et* al., 1992). In the course of the current study, a bibliographic search was conducted to retrieve data on additional environmental factors (e.g. vegetation cover, flow values, alkalinity) that possibly affect the establishment of sailfin molly; however, available data on habitat conditions in the introduced areas were extremely limited. Nevertheless, the specific habitat requirements of the sailfin molly appear to limit the amount of habitat that is suitable for the species, even in thermally-suitable areas. Thus, scarcity of suitable habitat (e.g. lentic systems) and trophic conditions (e.g. vegetative food supply), could account, at least partially, for the unexpectedly low occurrence frequency of the sailfin molly in Europe and the Meditteranean.

Overall, cold intolerance and release to unfavorable habitat environments may preclude widespread establishment of the sailfin molly, as well as of the shortfin molly and the guppy, in European waters. However, it is reasonable to expect the establishment probabilities of the sailfin molly to be higher than that of the other two species, as the former occurs naturally at higher latitudes and has lower thermal tolerance ranges than the other two poeciliids (Dill and Cordone, 1997).

Apart from ecological factors, propagule pressure and various other types of human involvement (e.g. breeding practices) may affect the invasion process or may confound interpretations of invasion patterns. Holčík (1991) and Maceda-Veiga et al., (2013) indicated that the guppy, and to a lesser degree the shortfin molly, are more commonly traded as ornamental fish in Europe, compared to the sailfin molly. Indeed, a recent study assessing the availability of ornamental species in Greece (Papavlasopoulou et al., 2014) indicated that *P. reticulata* had a >50% frequency of presence in Greek aquarium stores, while the presence of sailfin molly in pet stores was lower than 50%. Thus, a "lower release rate" explanation, i.e. that the frequency differences in establishment success stem from introduction effort differences rather than from ecological constraints, is also likely. It is known that releasing unwanted pet fishes is the main invasion pathway of ornamental fish (Semmens et al., 2004; Copp et al., 2007; Krishnakumar et al., 2009). Under the assumption that the most popular and frequently traded fish have more opportunities to be released, trade data may be used as a surrogate for introduction effort (Rixon et al., 2005; Gertzen et al., 2008). Rixon *et al.* (2005) measured the frequency of occurrence of ornamental fish species in a number of pet stores in Canada and the US and showed that, among poeciliids, the guppy had a higher frequency (95%) than the sailfin molly (80%) and the shortfin molly (75%). These may explain, at least partially, the highest number of European localities hosting feral guppy rather than sailfin and shortfin molly populations.

Other explanations for the poor establishment rate of the sailfin molly in Europe may be related to taxonomic ambiguities. This is evident in previous misidentifications of *Poecilia* species, as in the case of the Lake Vouliagmeni sailfin molly in Greece (Chintiroglou et al., 1996; see also Dill and Cordone 1997 for *P. sphenops* confused with *P. latipinna*). Poeciliids have a complex taxonomy (Breden et al., 1999), which is further complicated by the ease of interspecific hybridization (Kittell et al., 2005).

The differential vulnerability of colour morphs to several predators (carnivorous fishes, water snakes, birds etc.) can be invoked as an additional explanation for its poor establishment success in Europe. Finally, reduced reproductive or physical fitness due to breeding manipulations, and/or of long-fin males’ gonopodium, may also explain the low establishment rate of the sailfin molly in Europe. Commercial strains of sailfin molly are becoming progressively a fusion of various origins that include artificially selected breeds and crosses with other molly species (Fossa, 2004; Koutsikos et al., 2017) that may be sterile, or have offspring with reduced capacity for survival and sustained reproduction (Rodionova et al., 1996; Lampert et al., 2007). Artificial selection aims for improved ornamental traits, and often disrupts the stabilised biological systems that ensure normal development or the ability to survive and reproduce under harsh environmental conditions. It is notable in this context that the Vouliagmeni sailfin molly population in Greece represents a relatively old introduction (early 1960's; Koutsikos et al., 2017) before hybridisation became a widespread practice in ornamental fish production. The same is also evident for the establishments in Iraq, Hawaii and Texas (US) in the early 20th century (Kennedy, 1937; Englund, 1999; Costa and Schlupp 2010), as well as in Australia, Canada, Nevada and California's Salton Sea (US) in the late 1960's (Nelson and Paetz, 1992; Scoppettone et al., 2005; Corfield et al., 2008; Martin and Saiki, 2009).

Potential establishment and impacts on native biodiversity

The current study has shown that the probability of establishment of the sailfin molly globally was moderate (~30%) and confined to the subtropics, where currently most of the species' established populations are located. Bomford and Glover (2004) and Bomford (2008) asserted "very high" and "extreme" establishment risks for the sailfin molly in Australian and New Zealand waters, using a combination of variables, including climate match and history of establishment elsewhere. One of the most commonly used variables is the "previous establishment success rate", estimated as a proportion of successful introductions to the total number of introductions made in other regions. However, the use of such a variable may overestimate the invasion risk, when establishment failures are poorly documented. This could be the case for the sailfin molly, when assessed with this method, as there are practically no records of failed introductions for this species, except from the USA and New Zealand (Nico et al., 2014; McDowall, 1999).

According to Gozlan et al., (2010), the majority of ornamental fishes have very low probabilities of invading Europe due to their ecological and physiological requirements. However, a recent Fish Invasiveness Screening Kit (FISK) evaluation for Europe of several ornamental fishes (Kalous et al., 2015), showed that the climate of a number of locations within southern Europe matches that of their native areas. In the current study, the probability of establishment of the sailfin molly on the European continent was generally very low (below 7%). It is highly possible that failed sailfin introductions have repeatedly occurred in Europe, mostly pet releases in unsuitable habitats. However, the data are anecdotal, or the introductions remain unrecorded. Overall, the risk of invasion spread of the sailfin molly in Europe is assessed to be limited due to various reasons, discussed above, as also evident by our Climatch data, despite probably high release rates. In the Mediterranean Basin, in contrast, our data indicated that climatic conditions are more suitable for the species' establishment, with no environmental barrier to survival to almost 60% of the assessed sites in the peri-Mediterranean countries. Indeed, Perdikaris et al. (2016) ranked the invasion risk of the sailfin molly in Greece as being moderately high by applying FISK. These results highlight the importance of rigorous surveying of those lentic habitats that fulfill the species' habitat requirements, with special focus on urban ponds and lakes, as potential release sites by hobbyists.

Ornamental poeciliids have been repeatedly blamed for adverse impacts on native fish communities and other biota (NACA, 2005; Corfield et al., 2008; Englund et al., 2000). On the basis of published data reporting adverse ecological impacts of introduced species, the sailfin molly has been identified as one of the top 18 species with adverse ecological effects (Casal, 2006), and is classified among the 14 most invasive ornamental fish species (Froese and Pauly, 2014). Possible effects of the sailfin molly on other native fauna and other elements of the biota are expected to be mainly indirect, e.g. effected through food web alterations or non-native disease transmission (Arthur and Lumanlan-Mayo, 1997), as the species is a non-aggressive fish, feeding primarily upon algae and detritus and thus apparently not interacting trophically with conspecifics. For instance, Kryss et al. (2008) have assessed that *Poecilia* sp. hybrids introduced in Hawaiian waters have been the source of parasites that now infest native gobies and have also impacted the water quality of coastal ecosystems. It should be the aim of future studies to evaluate the nature and magnitude of environmental impacts caused by sailfin molly introductions.

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Chapter 5

DOES RAINBOW TROUT JUSTIFY ITS HIGH RANK

AMONG ALIEN INVASIVE SPECIES?

INSIGHTS FROM A NATIONWIDE SURVEY IN GREECE

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INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) is an important species for aquaculture and inland fisheries. It occupies the second position in the list of the most frequently introduced species in the world, having been spread to more than 100 countries for farming and stocking (Crawford and Muir, 2008; Hutchings, 2014; Jönsson et al., 2010). In Europe, it ranks as the most frequently introduced species with reported entry to at least 30 countries (Gherardi et al., 2009).

Given the enormous global scale of rainbow trout introductions, concerns have been raised about negative impacts on local biotas. The literature reports many adverse impacts, especially on other salmonid species through mechanisms such as predation, resource competition, hybridisation, behavioural disruption, disease transmission and food web alteration (Kerr and Lasenby, 2000). Impacts have been well documented in countries where rainbow trout is well established and widespread [e.g. US (Hitt et al., 2003), Canada (Van Zyll de Jon et al., 2004), Australasia (Jackson et al., 2004), S. Africa (Shelton et al., 2015), Argentina (Pascual et al., 2007), Chile (Arismendi et al., 2014) and Japan (Sahashi and Morita, 2016)]. Through such documentation, rainbow trout has gained a reputation as an exceptionally harmful invasive species. It is listed as one of the 100 'worst invasive alien species' identified globally by the IUCN (Lowe et al., 2000) and ranks high in the list of top 18 fish species with adverse ecological impacts compiled from establishment records and impact assessments data contained in the FISHBASE (Casal, 2006).

In Europe, ecological impacts of rainbow trout are less well documented and have often been inferred from indirect evidence. Some of this evidence comes from laboratory and small-scale field experiments that have highlighted potential competition with, and predation on, native fishes (Blanchet et al., 2007; Landergren 1999; Nellen and Plate, 1997). However, a meta-analysis by Korsu et al. (2010) raised the possibility that such experimental results may reflect a laboratory or scale artifact due to intensified species interactions under conditions of confinement. Only a few studies have investigated impacts using field data on species distributions and demographic structure. The most substantive evidence of this kind has been produced for the alpine streams of Rhine and the Lake Constance, where large increases in the range and abundance of rainbow trout in recent decades coincided with the decline or collapse of several native brown trout populations (Bassi et al., 2001; Burkhardt-Holm et al., 2002). There remains a minority of studies which have indicated weak or negligible impacts of rainbow trout on native fishes (Musseau et al., 2016; Musseau et al., 2017; Vincenzi et al., 2010).

In the absence of sufficient documentation of ecological effects of rainbow trout introductions from most European regions, impacts are sometimes speculated rather than demonstrated, on the basis of literature data on species feeding habits and habitat use patterns, pathogen spillover and adverse effects elsewhere (e.g. Almeida and Grossman, 2012; Candiotto et al., 2011; Larios-Lópezet al., 2015; Leunda, 2010; Oscoz et al., 2005). Broader reviews on this subject are dominated by evidence from other regions of the world and cite only few studies providing evidence from Europe (Cowx et al., 2012; Fausch, 2007; Korsu et al., 2008; Stanković et al., 2015). With few exceptions, the studies cited for Europe were conducted in areas where rainbow trout is not known to have become established. Establishment is a key issue to consider when assessing environmental impacts of biological invasions. Through its control over the recruitment process, establishment exerts dominant influence on the invader's abundance which, along with the total area occupied and the per capita impact, is a major determinant of the overall impact of the invader (Parker et al., 1999). If a species fails to establish self-sustaining populations, the impacts are localised, variable (depending on stocking densities), temporary and possibly reversible.

So far, risk assessments of rainbow trout introductions in Europe have been based on general considerations about impacts on biodiversity but absence of sufficient information on establishment rates or possibilities. Rainbow trout has been listed among the worst invasive alien species in the Continent (van der Veer and Nentwig, 2015) and has been included in the Black Lists of some countries (Essl et al., 2011; Gederaas et al., 2012; Pergl et al., 2016). Recently it was proposed as a candidate for inclusion in the list of invasive alien species of Union concern (the Union List), according to the EU Regulation 1143/2014 (Nentwing et al., 2017). Risk assessment models developed to identify potential invaders have generated variable but mostly high risk scores for European countries. In Luxemburg it was assessed to be a species of "low" invasion risk (Ries et al., 2014). "Medium" risk assessments were made for Finland (Puntila et al., 2013) and Hungary (Ferincz et al., 2016). For Bosnia and Herzegovina, Croatia and Slovenia, Greece and the Iberian peninsula the risk scores range from "medium" to "moderately high" (Almeida et al., 2013; Glamuzina et al., 2017; Piria et al., 2016; Perdikaris et al., 2016). Finally, "high" or "very high" risk scores were assessed for the UK (Copp et al., 2005), Belarus (Mastitsky et al., 2010) and Serbia (Simonović et al., 2015).

Is rainbow trout an invasive species in Greece? Does this species justify its generally high rank among the invasive alien species of Europe? An answer to these questions requires an understanding of what is meant by the term "invasive alien species". Several definitions have been proposed (reviewed by Heger et al., 2013; Pereyra, 2016), but two groups of definitions prevail in scientific literature: the "ecological definitions" and the "policy definitions" (Heink et al., 2018). Both groups include establishment and spread as necessary invasiveness criteria but differ over whether ecological or other (e.g. economic, societal) impacts should be included (Lockwood et al., 2013; Young and Larson, 2011). Ecological definitions emphasise on the ecological aspects of species invasions and do not include any connotations to impacts (Blackburn et al., 2011; Colautti and Richardson, 2009; Ricciardi and Cohen, 2007). Policy definitions largely follow the definition of an invasive alien species provided by the World Conservation Union (IUCN): *"a species which becomes established in natural or semi-natural ecosystems or habitats, is an agent of change, and threatens native biological diversity*" (IUCN, 2000). This definition, and conceptually similar definitions adopted by the Convention of Biological Diversity (CBD, 2002) and the EU Regulation 1143/2014 on Invasive Alien Species (EU, 2014), implicitly draw a connection between 'invasiveness' and 'impacts" and require an evaluation of harmfulness. Only those alien species that have a demonstrable ecological or economic impact should be considered as invasive, based on a comprehensive risk assessment.

What definition for invasive species will be adopted is largely a matter of research focus and motivation (Heger et al., 2013). Although we lean toward the first (ecological) definition of invasiveness, i.e. based solely on establishment and spread criteria, for the purpose of the present article we follow the second (policy) definition in order to keep consistency with European policy for alien invasive species, as reflected in the EU Regulation 1143/2014. From the perspective of this definition, three criteria must be satisfied for an alien species to be regarded as invasive: (a) transfer and introduction mechanisms to new systems exist, (b) establishment into new systems is successful and an expansion of range is observed, and (c) negative impacts on the native biota are documented or can reasonably be expected. Due to the widespread stocking and farming of rainbow trout, the first criterion is not difficult to be compiled with. The third criterion is met in various extents in some European regions. Hence, establishment (actual or potential) becomes the decisive invasion criterion.

The present study was undertaken with a two-fold objective: (a) to describe the degree of rainbow trout establishment in Greek freshwaters, and (b) to explore possible factors that may account for establishment success or failure. We address the first objective through an analysis of field survey data from the freshwaters of Greece. So far, no clear and consistent views have emerged as to which extent rainbow trout is established in this country. With regards to Greece, rainbow trout is referred to as "established" by the IUCN (GISD, 2018), as "probably established" by the FISHBASE (Froese and Pauly, 2017), and as "not established" by the DAISIE (2018). Scientists within Greece have asserted this species to be not generally established in the country (Economidis et al., 2000; Economou et al., 2007a). Few breeding populations have been reported by previous studies (Koutsikos et al., 2012; Stoumboudi et al., 2017). However, the establishment status of this species over the entire territory of Greece has not been rigorously surveyed with country-wide distributional and demographic data. We pursue the second objective by analyzing fish assemblage data together with environmental data from the sampling locations. Possible mechanisms that may account for impacts of hatchery practices and stocking operations are discussed. Finally, some of the wider implications of the results of the current study for the conservation of native trout are highlighted.

MATERIALS AND METHODS

Sampling procedures

Fish data were obtained from various research surveys conducted over the past 18 years (2001-2017) covering the entire mainland as well as the major islands of Greece. Fish samplings were typically conducted during spring and summer periods (March-October). In total, 956 samples (665 sites from 76 different drainage basins) collected through electrofishing surveys of the Hellenic Centre for Marine Research (HCMR) were utilised for the present study.

Fish sampling and environmental data collection was conducted using standardized procedures developed under the European research project FAME (Schmutz et al., 2007) with some modifications (IMBRIW-HCMR, 2013). Briefly, a single electrofishing pass was conducted at a stream section about 100 m, while no stop nets were used; however, in each occasion attention was devoted by the crew members to sample a river stretch demarcated by physical barriers (e.g. shallow riffles) in order to minimize fish escape in either direction. In small rivers (<10m width), the entire river channel was surveyed. When the active channel exceeded 20-30 m width, or when the water column exceeded waist-depth, sampling was conducted partially from one river bank. Throughout the surveys, two main types of electrofishing devices were used: a) a Hans-Grassl GmbH battery-powered backpack electrofisher (Model IG200-2, DC pulsed, 1,5 KW output power, 35-100 Hz, max. 850) which was routinely used to sample fish in small streams and b) a generator powered unit EFKO Elektrofischereigeräte GmbH, Model FEG 6000 (DC unpulsed, 7,0 KW output power, 150-600 V), which was used in deeper streams and rivers. Fish were identified to species level following Barbieri et al. (2015) as the main taxonomic reference. All fish were measured (TL), grouped in 5 cm length class intervals, and returned alive to the river.

Site characteristics, landscape features and key habitat parameters were recorded in a field protocol modified from FAME (2005). The protocol accommodates fields for sampling details, topographic parameters, physicochemical variables, hydrological characteristics, habitat variables, substrate composition and important anthropogenic pressures affecting the river segment where electrofishing was conducted.

Data analysis

A literature review was conducted in order to document the historical occurrence of rainbow trout at the river basin scale in Greece. By using standardized sampling data, a nationwide distributional database was developed and was used to assess the extent of occurrence of native salmonids and rainbow trout. Native salmonids, which are a part of the ubiquitous brown trout (*Salmo trutta* complex) (Kottelat and Freyhof, 2007), were included in the analyses on the ground that these are ecologically similar taxa that share similar environmental requirements to rainbow trout (Molony, 2001; Moyle et al., 2003).

By examining the spatial distribution and demographic structure of native trout, insight into the ecological conditions and processes that influence population persistence and responses to environmental conditions in rainbow trout, may be provided. Introduced species are typically considered as established when they found self-sustaining populations in their novel habitats (Lockwood et al., 2013). The notion of self-sustainability implies that individuals survive and reproduce at sufficient rates, and the population is maintained through time without the need of additional introductions. Therefore, we used demographic criteria (overall abundance, mean abundance at sampling sites, areal densities, length frequency distribution and proportions of juveniles) to explore evidence of natural reproduction and its contribution to recruitment and to infer demographic viability. Separate analyses were conducted for individuals smaller than 10 cm (categories “fry”, <5 and fingerlings “6-10” in total length size-classes) and for larger individuals (all categories >10 cm), roughly corresponding to juveniles and pre-adults/adults respectively.

Spatial variability in species densities and size-related parameters were examined for native species and rainbow trout with the prospect of exploring the possible sources of recruitment. Specifically, length-frequency distributions were developed for a) native trout and b) rainbow trout, c) a rainbow trout population at a remote spring-fed stream in S. Peloponnese (Vlisidia stream), which appears to be established (Koutsikos et al., 2012) and d) a rainbow trout population at a stream in Central Greece, Macedonia (Arapitsa stream), where stocking is performed regularly while fishing is forbidden. In addition, rank density diagrams were created to graphically display the site-specific (local) densities of the two species, with the sites ordered by decreasing densities.

Canoco 4.5 software (ter Braak and Smilauer, 1998) was used to analyze biotic and environmental data and assessing the differences in environmental features between native trout and rainbow trout sites. The environmental parameters included in the analyses were (a) physicochemical, i.e. conductivity ($\mu S \cdot cm^{-1}$), dissolved oxygen ($mg \cdot L^{-1}$), and water temperature (°C) and, (b) habitat attributes; i.e. mean active channel width (m), mean wetted width (m), mean depth (cm) and substrate coarseness (coarse substrate >63mm (including cobbles and boulders), while as fine substrate we defined substrate <63mm (pebbles, gravel, sand, etc.). Additionally, instream generic habitats were also included; i.e. pools (deep/still), glides (shallow/flowing), runs (deep/flowing), riffles (shallow/turbulent) and rapids (steep gradient/fast flow). Wider environmental parameters such as site elevation (m), distance from source (m) and slope were derived from geographical information systems (ESRI - ArcGIS v. 10.4).

For the same analyses, pools and glides were counted as slow flowing habitats while runs, riffles and rapids as fast flowing habitat. Prior to all multivariate analyses, fish densities and abiotic data were log (x+1) transformed, except of those variables (coarse/fine substrate, slow/fast habitat) that were presented as % percentages and were arcsine transformed. Correlations with abiotic variables were conducted for: a) densities of all fish species sampled within native trout and rainbow trout sites and b) juveniles and pre-adults/adults densities for native and rainbow trout respectively. A detrended correspondence analysis (DCA) was conducted, to test the heterogeneity of trout's community data composition. In all cases, the lengths of gradients for the first axis was <3 revealing a linear structure of the data and indicating Redundancy Analysis (RDA) as the most appropriate responded method for multivariate multiple regression analysis (ter Braak and Smilauer, 1998). The Monte Carlo test was further applied with

499 permutations, in order to test the significance of abiotic variables in the ordination model.

Fish abundance data at each site (numbers per single run fishery) were converted to areal densities (dividing numbers by the wetted surface area sampled). The surface area sampled at each site was estimated from its geometrical characteristics (fished length and cross-sectional width). Due to a significant positive correlation (R^2=0.621, Pearson $p<0.001$) between abundance (number of individuals per site) and fish density (number of individuals per m^2 per site), these both population indices were used interchangeably depending on our analyses.

Finally, in an effort to determine the influence of anthropogenic factors on establishment success we collected information on past stocking activities and depicted the location of rainbow trout fish farms in the investigated river basins were rainbow trout individuals were caught during this study, through a survey of accessible sources.

RESULTS

Salmonids in Greece: overview of species in freshwater ecoregions / drainages

Five salmonids are native to Greece: *Salmo farioides* (Karaman, 1938); *Salmo lourosensis* Delling, 2011; *Salmo macedonicus* Karaman, 1924; *Salmo pelagonicus* Karaman, 1938 and *Salmo peristericus* Karaman, 1938 (Table 5.1). All of these species have restricted distributions

Hereafter, these species are collectively referred to as “native trout”. Native trout has been reported in total from 20 drainages within the Greek territory. Another six salmonids have been reported as alien (*Oncorhynchus kisutch* (Walbaum, 1792); *O. mykiss*; *Salmo letnica* **(**Karaman, 1924); *Salmo salar* Linnaeus, 1758; *Salmo trutta* Linnaeus, 1758 and *Salvelinus fontinalis* (Mitchill, 1814). Rainbow trout is by far the most widely introduced of these aliens; the literature review documents its introduction or occurrence in 29 drainages (Table 5.1). From the 16 river basins where rainbow trout individuals were caught during this study, only in two river basins

Dafnon and Assopos Pel.) there is no presence of a rainbow trout aquaculture facility (Fig. 5.1). and have been assessed for their threat status (Table 5.1).

In addition, based on the only officially available data (Ministry of Agriculture, 2000) over a 13 year period (1988-2000), approximately 2,600,000 rainbow trout fingerlings were stocked in seven river basins (Aliakmon, Acheloos, Alfios, Aracthos, Louros, Axios and Aoos). From the 956 different samples collected, only 216 samples (163 sites from 17 river basins) contained at least one salmonid species (Fig. 5.1). Native trout were the most frequently recorded taxa of all samples containing salmonids, found in 147 sites (57 courses of 12 river basins) (Table 5.2).

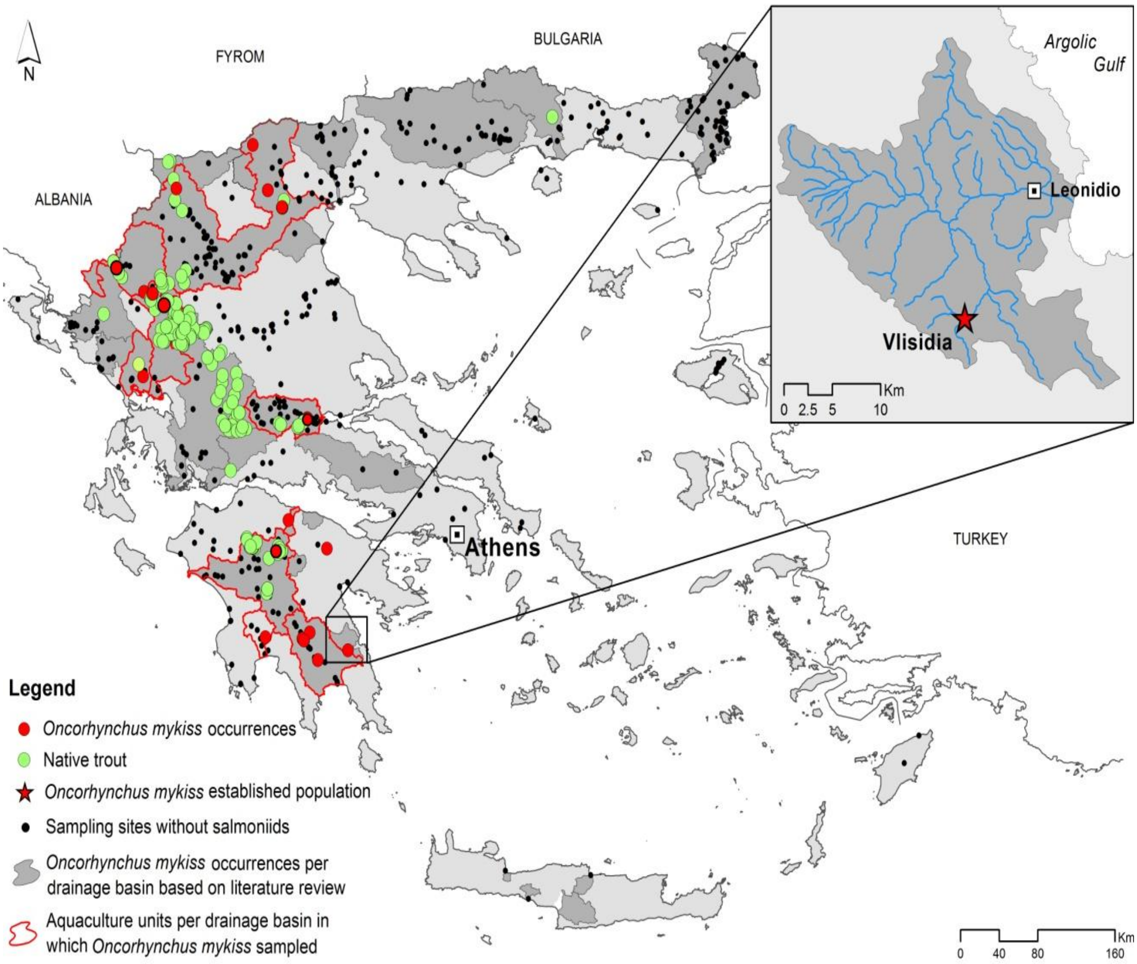

FIGURE 5.1 Occurrence of trout in the freshwaters of Greece based on sampling surveys (216 samples of 163 sites from 956 different ichthyological samples), the presence of rainbow trout based on literature (in 29 river basins) and the presence of aquaculture units per drainage in which rainbow trout individuals were caught.

TABLE 5.1 Basin spatial distribution within the given freshwater ecoregion of native and alien salmonid species in Greece based on ichthyological surveys and the literature. Freshwater ecoregions defined by Zogaris and Economou (2017).

Salmonid species	IUCN Red List	Greek Red List	Freshwater Ecoregions						Total
			Crete	Ionian	Macedonia – Thessaly	SE Adriatic	Thrace	W Aegean	
Native									
Salmo farioides Karaman, 1938	-	VU		7	2 †	1	1 †	1 †	**12**
Salmo lourosensis Delling, 2011	-	EN		1					**1**
Salmo macedonicus (Karaman, 1924)	DD	DD			1 ‡		3		**4**
Salmo pelagonicus Karaman, 1938	VU	VU			2				**2**
Salmo peristericus Karaman, 1938	EN	EN				1			**1**
Alien									
Oncorhynchus kisutch (Walbaum, 1792)	-	-	1	2	1			1	**5**
Oncorhynchus mykiss (Walbaum, 1792)	-	-	4	12	5	2	3	3	**29**
Salmo letnica (Karaman, 1924)	[DD]	-				1			**1**
Salmo salar Linnaeus, 1758	[LC]	-	1					1 ‡	**2**
Salmo trutta Linnaeus, 1758	[LC]	-		1	1				**2**
Salvelinus fontinalis (Mitchill, 1814)	-	-		1	1		1		**3**

IUCN abbreviations: EN, Endangered; VU, Vulnerable; DD, Data deficient. Abbreviations in brackets indicate species included in IUCN categories, whose Greek populations however are introduced.

†indicates translocated population

‡ indicates a doubtful species presence.

Rainbow trout was by far less common, appearing in only 25 sites (19 courses of 11 river basins) (Table 5.2). Both taxa were found together in a total of 9 sites (8 courses of 5 river basins) occupied by salmonids with a degree of samples co-occurrence at 5.56%. From the 956 different samples collected, only 216 samples (163 sites from 17 river basins) contained at least one salmonid species (Fig. 5.1). Native trout were the most frequently recorded taxa of all samples containing salmonids, found in 147 sites (57 courses of 12 river basins) (Table 5.2). Rainbow trout was by far less common, appearing in only 25 sites (19 courses of 11 river basins). Both taxa were found together in a total of 9 sites (8 courses of 5 river basins) occupied by salmonids with a degree of samples co-occurrence at 5.56%.

The site occupancy map (Fig. 5.1) indicates the known distributions of the native trout and the rainbow trout providing a large-scale picture of the current distributions and their actual and nominal ranges. With few exceptions, which mostly pertain to spring-fed rivers, native trout sites are located mainly in high altitude (mean 702.66 m ±21.12) and in streams with steep slopes (mean 3.26 ±0.25). The spatial range of rainbow trout is narrower than the range of native trout, with a far less mean altitude (approx. 408.06 m ±41.08) and in streams with gentle to moderate slopes (mean 2.28 ±0.46). Rainbow trout has been reported from drainages of different sizes in both mainland and insular Greece (i.e. Crete), while native trout occur mainly in upper catchments of large drainage systems of mainland Greece.

Population structure: size distribution, abundance and juveniles

The dominant size class of rainbow trout (21-25 cm) consisted mainly of adults (Fig. 5.2b). On the contrary, the populations of native trout were mainly structured by the dominance of juveniles (6-10 cm) followed by older classes (Fig. 5.2a). Due to stocking activities, it is not clear whether and to which extent recruitment in rainbow trout, arises from natural reproduction, stocking or escapes. This is particularly evident by comparing two sites were at the first no stocking actions have been conducted for the last 20 years (Vlisidia population, Fig. 5.2c) in contrast with the other were stocking is performed regularly (Arapitsa population, Fig. 5.2d).

Table 5.2 Spatial occurrence of native trout species and rainbow trout in Greece based on sampling data (216 samples of 163 sites during 2001-2017).

	Native Trout				Rainbow Trout				Co-occurrence			
Basins	**Presence**	**Streams**	**Sites**	**Samples**	**Presence**	**Streams**	**Sites**	**Samples**	**Presence**	**Streams**	**Sites**	**Samples**
Acheloos	●	21	59	87		-	-	-		-	-	-
Alfeios	●	7	22	31	●	2	2	3	●	1	1	1
Aliakmonas	●	6	12	16	●	4	5	7	●	3	4	6
Aoos	●	4	11	14	●	1	1	1	●	1	1	1
Arachthos	●	10	26	29	●	3	3	3	●	1	1	1
Asopos Pel.		-	-	-	●	1	1	1		-	-	-
Dafnonas		-	-	-	●	1	1	4		-	-	-
Evinos	●	1	1	1		-	-	-		-	-	-
Evrotas		-	-	-	●	2	6	7		-	-	-
Kalamas	●	1	1	1		-	-	-		-	-	-
Krathis		-	-	-	●	1	1	1		-	-	-
Louros	●	1	1	1	●	1	2	2		-	-	-
Nestos	●	1	1	1		-	-	-		-	-	-
Pamisos		-	-	-	●	1	1	1		-	-	-
Prespes	●	1	2	3		-	-	-		-	-	-
Pinios The	●	1	1	1								
Sperchios	●	3	10	12	●	2	2	3	●	2	2	3
Total	**12**	**57**	**147**	**197**	**11**	**19**	**25**	**33**	**5**	**8**	**9**	**12**

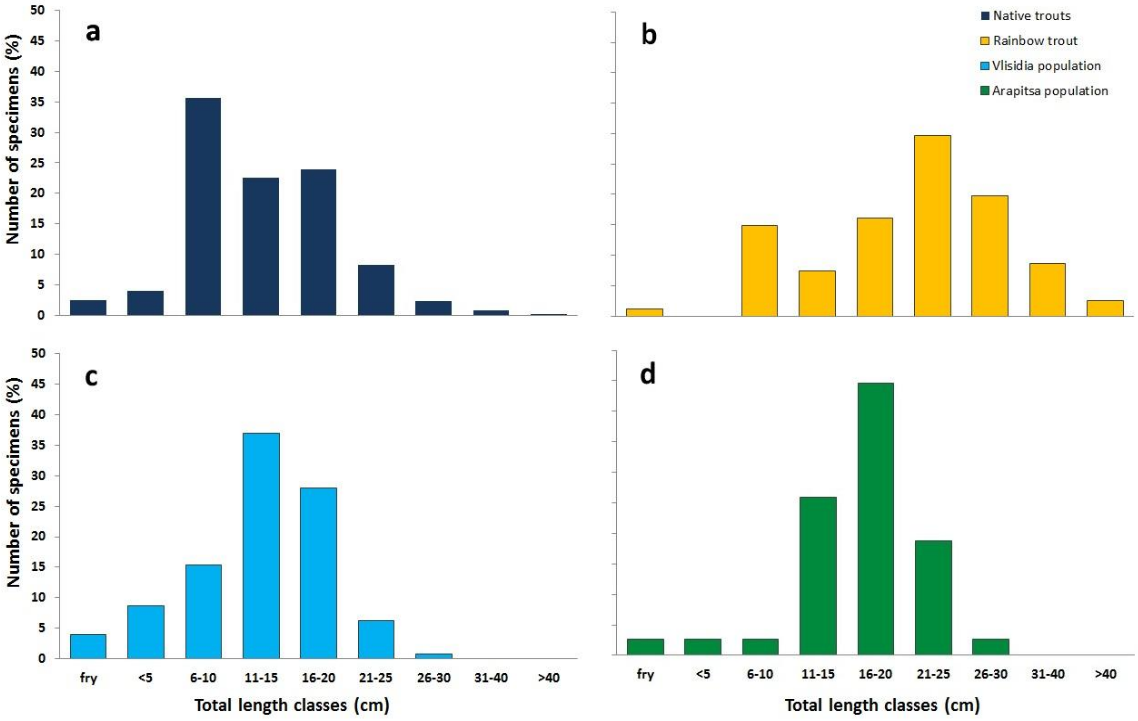

FIGURE 5.2 Length frequency distributions of a) native trout and b) rainbow trout, in freshwaters of Greece, c) rainbow trout at a remote spring-fed stream in S. Peloponnese (Vlisidia stream) and d) rainbow trout at a stream that stocking is performed regularly while fishing is forbidden (Arapitsa stream). Data were averaged over all salmonid sites and sampling periods, respectively.

Differences among taxa were particularly evident in abundance, since native trout substantially exceeded rainbow trout both in total (overall abundance) and per site (mean local abundance) values (Table 5.3). Abundance of juveniles was also substantially higher for native trout (Table 5.3, Fig. 5.3). The two taxa were similar in size range, however, they differed substantially in the proportion of juveniles to older fish, which was much lower in rainbow trout than in native trout.

For native trout, juveniles comprised 41.98 % of the total number of individuals captured, while for rainbow trout the corresponding value was 20.81 % (Table 5.3). The juvenile to adult ratio for rainbow trout was 0.19, whereas the ratio for native trout was 0.62.

Table 5.3. Abundance of salmonid species collected in freshwaters of Greece based on sampling data .

Data	Species-specific samples of native trouts					Native trout	*O. mykiss*
	S. farioides	*S. macedonicus*	*S. lourosensis*	*S. pelagonicus*	*S. peristericus*		
N. of samples	176	1	2	16	2	197	33
N. of sites	131	1	1	13	1	147	25
N. of specimens	4103	3	4	424	6	4538	447
Mean local abundance	23.45	3.00	4.00	24.94	3.00	23.15	13.55
N. of juveniles	1748	2	0	153	2	1905	93
Juveniles %	42.60	66.67	0.00	36.08	33.33	41.98	20.81

Mean local abundance is the product of N of specimens divided by N of samples.

YOY indicates young of the year individuals.

*fish <10 cm in length were considered YOY

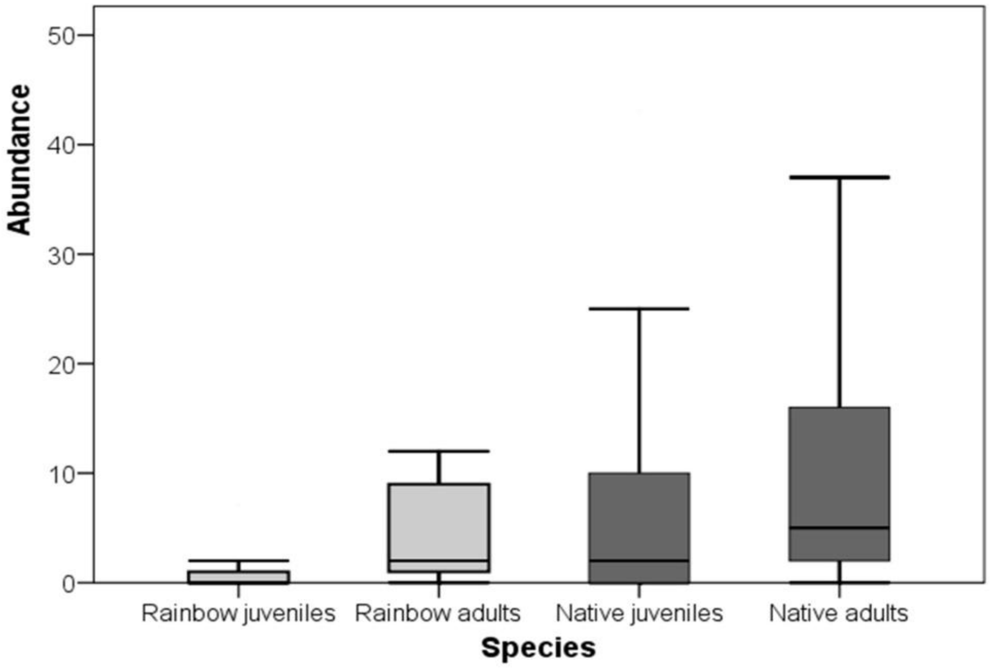

FIGURE 5.3 Abundances (inds) of adult and juvenile individuals caught (rainbow and native trout species).

Rank densities diagrams showed that the curves of local density, for both taxa, were strongly concave, indicating that a large proportion of the overall densities was due to the contribution of relatively few sites (Fig. 5.4 a,b). However, juveniles of rainbow trout were low in numbers and collected from extremely limited sampling sites (Fig. 5.4b), compared to the frequent presence of juveniles of native trout (Fig. 5.4a). This observation is further confirmed by the comparison of the regressions generated for juvenile abundance against total abundance in both taxa (Fig. 5.4 c,d). For native trout, a strong relationship between total and juvenile local abundances was observed (R^2 = 0.81; Fig. 5.4c). For rainbow trout, the data indicate that the samples consisted of relatively few, often one or two, individuals. The proportion of juveniles was generally small and varied considerably among sites. By excluding from the analysis the only established rainbow trout population in Greece (Vlisidia population), the relationship between total and juvenile abundances was found to be weak and not significant (R^2 = 0.05; Fig. 5.4d), and the slope of the relationship was much lower than the slope for native trout. In fact, juveniles were absent from most sites and the slope was heavily influenced by data from only few sites in which juveniles were relatively abundant.

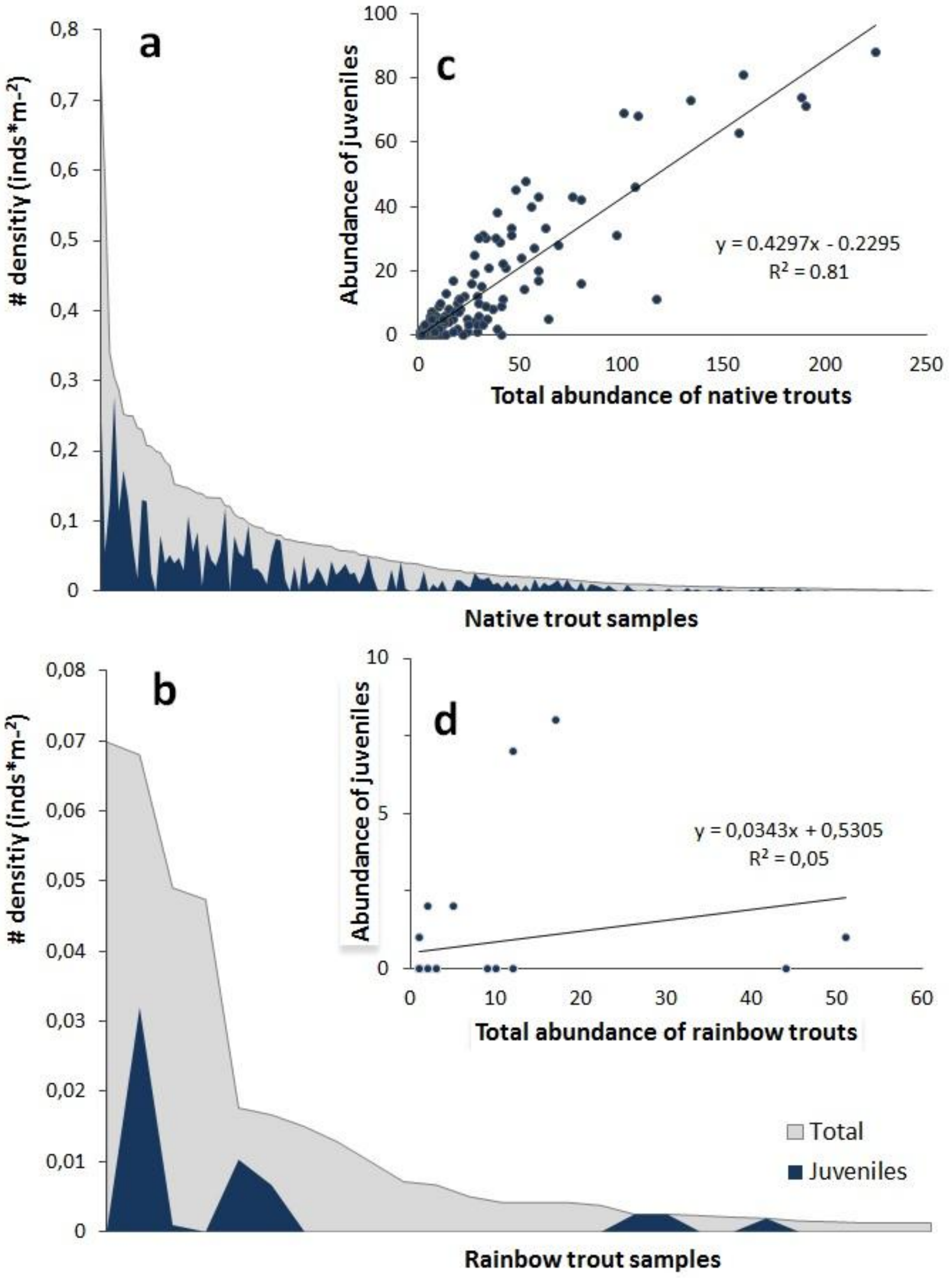

FIGURE 5.4 Ranked total (grey lines) and juveniles (black lines) densities (inds m^{-2}) of (a) native trout and (b) rainbow trout at decreasing order for each sample, and regression between total abundance (inds) and juvenile abundance (inds) in (c) native trout and (d) rainbow trout. (Note that scales vary between figures. In addition, the rainbow trout population of Vlisidia stream is excluded from figures b and d).

Environmental matching

Monte Carlo test indicated that dissolved oxygen (D.O.), coarse substrate and active channel width, were the statistical significant environmental variables ($p<0.05$). RDA results revealed differences in environmental variables between native, rainbow trout and all the other species (Fig. 5.5a; and Table A.5.1 in Appendix).

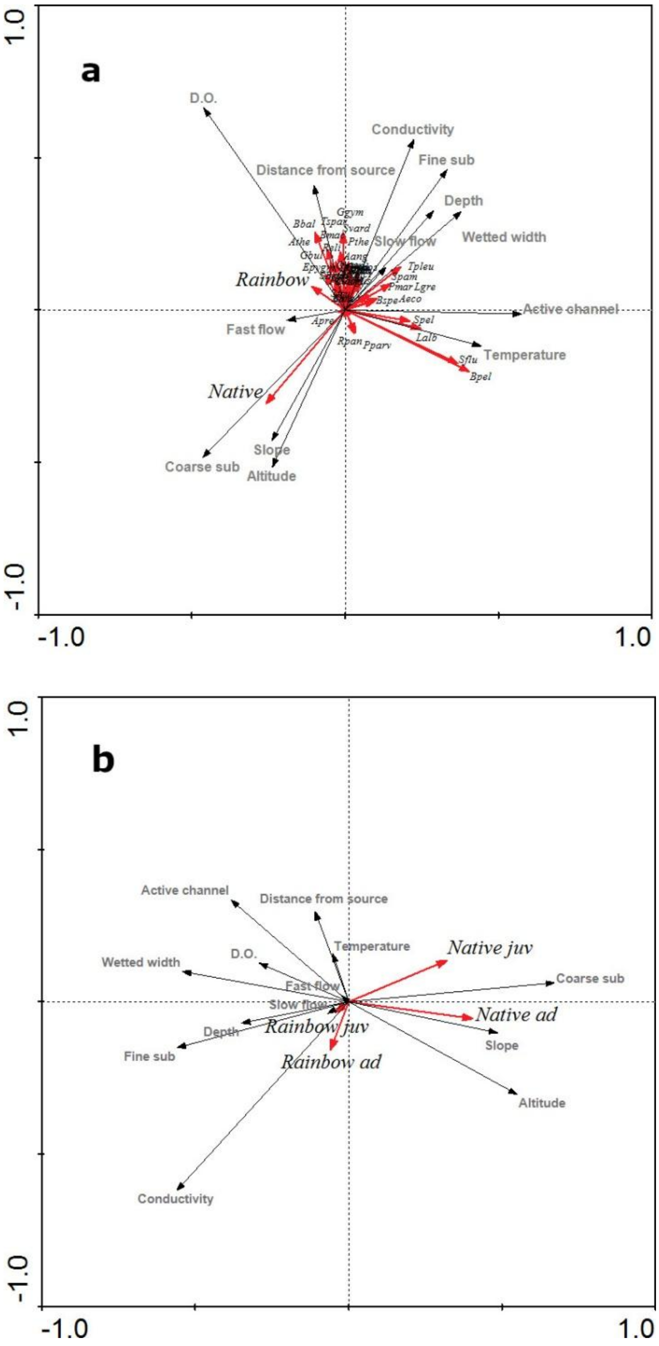

FIGURE 5.5 Ordination analyses (Canoco) among various environmental parameters: a) all fish species sampled (for species abbrevations see Table A.5.1 in Appendix); b) adult native (*Native ad*) and adult rainbow trout (*Rainbow ad*) and native (*Native juv*) and rainbow trout (*Rainbow juv*) juveniles.

Native trout had a positive correlation with coarse substrate, slope and altitude but negative with conductivity, wetted width, active channel width, depth and distance from source. Densities of rainbow trout had a positive correlation with dissolved oxygen and negative with temperature and active channel width. The ordination model was significant in all canonical axes, with the second axis explaining 64.3% of the fish densities data variance and the fourth 85.6%.

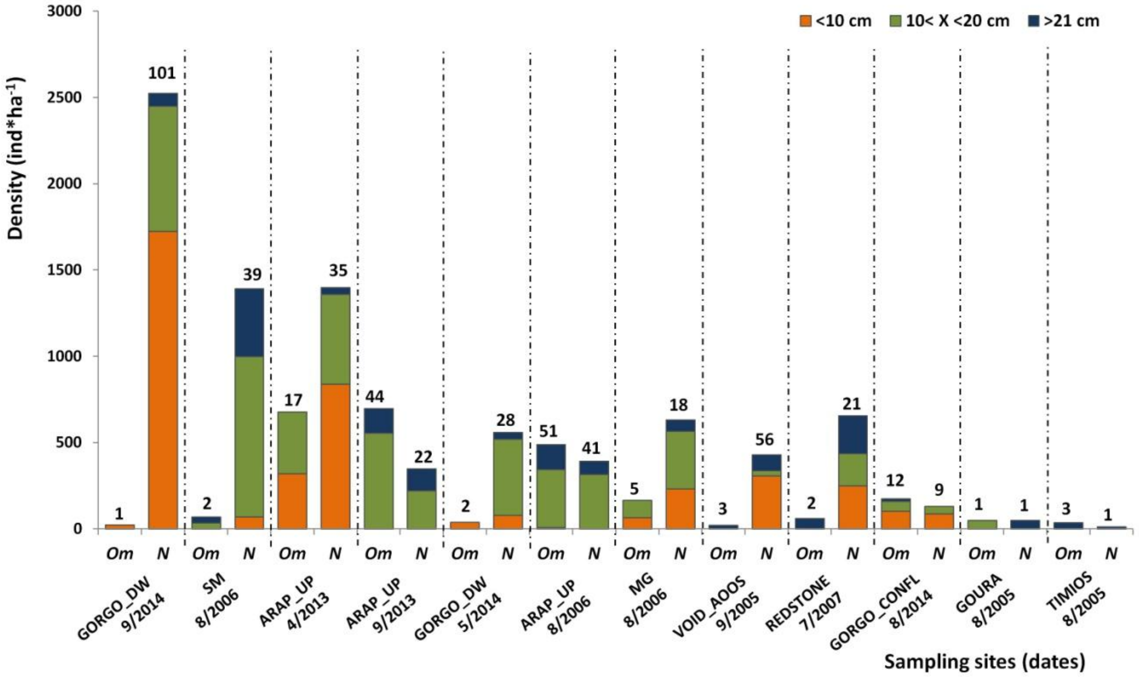

FIGURE 5.6 Ranked densities of total recorded at instances of co-occurrence with both rainbow trout (*Om*) and native trout species (*N*). Numbers upon bars denote total species abundances for each site.

A similar pattern appeared when the two trout species were separated into juveniles and adults. RDA results indicated differences in environmental variables between native and rainbow trout (Fig. 5.5b). The ordination model was significant for all axes, with the first axis explaining 97.9% of the fish densities data variance, while the explained variance in the second axis, between fish densities and environmental variables was 98.0%.

The co-occurrence of rainbow and native trout was rare (Table 5.2; and Table A.5.2 in Appendix). Both species were collected in only five basins (out of the 76 surveyed).

Only in four cases (out of 12 samples of co-occurrence) are native trout numerically outnumbered by the rainbow trout (Fig. 5.6).

DISCUSSION

Status of rainbow trout populations in Greece

Rainbow trout has been intensively farmed and stocked in Greece for almost six decades, following an initial importation of fertilized eggs from Switzerland in 1951 for aquaculture production (Economidis et al., 2000). In the following years, more but not well-documented imports, took place from Denmark, Poland, Spain and USA, particularly by private trout farms and several new mainly small in-farm hatcheries were established. Around 80 small-to-medium scale trout farms have been established in rivers, streams and springs, mostly in the north-western part of Greece (Piria et al., 2018) being a key introduction vector of rainbow trout into natural systems (Liasko et al., 2012). In addition, intensive stocking programs have been put in place (and continue to date) by government agencies, and there is no doubt that many unrecorded introductions of rainbow trout in natural waters have taken place by local authorities and anglers. However, the vast majority of stocking activities are undocumented (Ministry of Agriculture, 2000).

Recent compilations of the Greek ichthyofauna based on published sources and survey results, rank rainbow trout as the second most widespread alien species in the Greek freshwaters with occurrence in 29 river basins (Economou et al., 2007a; Koutsikos et al., 2012). Despite its rather widespread occurrences throughout the country (Economou et al., 2007a), no documented evidence of establishment in the wild had been provided until recently. Koutsikos et al. (2012) and Stoumboudi et al. (2017) reported evidence of natural reproduction of the species within Greek freshwaters, in southeastern Peloponnese and in the island of Crete, respectively.

Historical information on drainage-specific native species occurrences and alien species introductions indicate a much wider spatial distribution of rainbow trout (29 drainages) than of native trout (20 drainages). According to the site-specific catch data presented here, rainbow trout was missing from many drainages in which it was historically recorded, and appeared to be far less common than native trout, both spatially and numerically. Indeed, rainbow trout was sampled in only 25 sites (compared to the 147 sites where native trout were sampled) and the catches consisted of relatively few specimens, often single individuals. Moreover, juveniles were either absent or made a very small portion of the catch in most sites. From this demographic profile, it can be inferred that the contribution of wild spawning to recruitment is small and probably insignificant in the majority of examined locations. Eventually, natural recruitment is spatially restricted and not quantitatively important to support viable populations. We allow ourselves the speculation that most populations would not persist in the absence of stocking. The picture emerging from this study is that rainbow trout is not currently established in the greatest part of Greece, and the reason for this appears to be failure of natural reproduction.

The comparisons of the distributional, demographic and habitat data of rainbow trout with those of native trout indicated broadly overlapping distributions, occupying sites with similar environmental conditions as both species did not differ appreciably in the range of most environmental variables. Other studies involving comparisons of rainbow trout with the brown trout (*S. trutta*) have indicated similar habitat preferences and tolerance ranges to a variety of environmental factors (Kerr and Lasenby, 2001; Moyle et al., 2003; Shirvell and Dungey, 1983). The only difference noticed by Molony (2001) is that rainbow trout can tolerate slightly higher temperature than brown trout (see also Beitinger et al., 2000). These ecological similarities between rainbow trout and European trout species indicate considerable niche overlap and imply that the amount and quality of habitat available to native trout species may provide an at least minimum estimate of the extent and suitability of habitat available to rainbow trout.

Concordance with the geographic distributions of native trout and rainbow trout in Greek freshwaters, as well as their similar demographic responses to environmental conditions, suggest that the availability of suitable habitat is not a limiting factor for rainbow trout survival and reproduction. On the assumption that conditions that are favourable for the reproduction of native trout species are also appropriate for the reproduction of rainbow trout, these data provide another piece of evidence that lack of suitable breeding habitat is not the reason for the difficulty of rainbow trout to become established in Greece. The rainbow trout population in the remote spring-fed stream of Vlisidia (Dafnon river basin) on Mount Parnon in the southeastern Peloponnese, stands out as a notable exception to this pattern of the demographic dynamics. This population has a robust population structure consisting of multiple year-classes and appears to be successfully reproducing in the absence of stocking. Moreover, there are no trout farms in the area and the nearest farm is located in a different river basin. Apparently, the small individuals recorded during the surveys were the product of recent natural spawning activity. In addition, the overall proportion of juveniles was well above the country-wide percentage for the species. These demographic characteristics provide evidence of successful reproduction and sufficient natural recruitment. We therefore assert that this population is established and persists without any apparent human intervention.

Factors influencing establishment success

Recent evaluated literature shows that low establishment success of rainbow trout is a general phenomenon across Europe. Rainbow trout appears to be firmly established and widespread in alpine streams of Austria (Füreder and Pöckl, 2007), Liechtenstein (Peter et al., 1998), Slovenia (Povž, 2017) and Switzerland (Wittenberg, 2005). Instances of localised establishment (single or few isolated populations) have been reported from a number of other countries:, Greece (Koutsikos et al., 2012; Stoumboudi et al., 2017), Italy (Candiotto et al., 2011), Norway (Hesthagen and Sandlund, 2007), Slovakia (Koščo et al., 2010), UK (ICES, 2013), in France (Pascal et al., 2003) and possibly in Cyprus (Zogaris et al., 2012) and Czech Republic (Musil et al., 2010).

The reasons impeding the establishment of rainbow trout in Europe have been debated for many years and are still not fully understood (Fausch et al., 2001, Fausch, 2007; Hindar et al., 1996; Korsu and Huusko, 2010). In the search for explanations, various hypotheses have been developed. Most link establishment success with three sets of causative agents: ecological conditions, propagule pressure, and genetic factors.

Ecological constraints

The following factors, or combinations of them, have widely been considered as key ecological constraints on the establishment process of rainbow trout: unsuitable thermal regimes, low levels of oxygen saturation, adverse water flows, lack of appropriate reproductive substrate, barriers preventing access to spawning grounds, angling pressure, and competition from native salmonids (reviewed by Fausch, 2007; Fausch et al., 2001, 2009; Kerr and Lasenby, 2000; Korsu and Huusko, 2010).

In other parts of the world, ecological hypotheses have been successful in explaining patterns and rates of rainbow trout establishments (Fausch et al., 2001; Lapointe and Light, 2012). In Europe, ecologically-based hypotheses have fared poorly in explaining why establishment has succeeded or failed. Each explanation can account for particular cases of establishment success or failure; none has sufficient generality and predictive power. Hindar et al. (1996) remarked that rainbow trout is a highly flexible and adaptable species having overlapping habitat requirements with brown trout and salmon. He asserted that there is plenty of good habitat for this species in Norway and implied that there is no obvious environmental constraint on establishment. Other researchers have similarly asserted that lack of suitable environmental or habitat conditions is not the main limiting factor for rainbow trout reproduction and establishment in Europe (Fausch, 2007; Korsu and Huusko, 2010; Landergren, 1999; Welton et al., 1997). In our study, rainbow trout was typically encountered in a broad range of altitudes (from lowlands close to the sea level to up to 825 m mountain tributaries) with water temperature above 10°C, fast moving water, hard bottom substrate and high levels of dissolved oxygen. The values measured for these variables were within the limits reported as favourable for this species in other works (Fausch, 2007; Montgomery et al., 1999; Moyle et al., 2003; Raleigh, 1984; Shelton et al., 2015). Although not all variables were found at optimal values in all sites, at least those variables considered as being critical for successful ovulation and spawning, namely temperature, flow regime, oxygen saturation levels and availability of gravel substrate (Montgomery et al., 1999) were within appropriate ranges in most sites and broadly match those in native habitats of rainbow trout.

A highly speculative hypothesis links reproductive failure of rainbow trout in Europe with high susceptibility to the whirling disease caused by the myxozoan parasite *Myxobolus cerebralis*. Hindar et al. (1996) have put forward the hypothesis that the high susceptibility of rainbow trout to the whirling disease can potentially account for the difficulty of this species to become established in Europe. Some authors have accepted this hypothesis partially (Fausch, 2007; Jonsson et al., 1993; Jönsson et al., 2010; Landergren, 1999) while others are skeptical (Walker, 2003). To our knowledge, whirling disease has not yet been reported from Greek freshwaters probably due to lack of research targeting this issue. However, the fact that the disease has not been yet reported from the rainbow trout farming sector (Savvidis G., pers. comm.) leads to the suggestion that it may be also at least uncommon in the wild. Nevertheless, the presence and prevalence of this and other diseases need verification in Greece and its possible impact on recruitment must be evaluated against other probable causes.

Propagule pressure

The propagule pressure for rainbow trout is undoubtedly among the highest for alien vertebrate taxa (Fausch, 2007). A positive relationship between propagule pressure and rainbow trout establishment success has been reported from some environments (Consuegra et al., 2011; Monzon-Arguello et al., 2014). In Europe high and constantly increasing stocking rates over the past 100 years (MacCrimmon, 1971) have resulted in a very small number of established populations in European waters (Stanković et al., 2015), and there is evidence suggesting that this number is declining through time (e.g. Britain and Ireland (Frost, 1974; Welton et al., 1997), Norway (Hindar et al., 1996; Sandlund and Hesthagen, 2011). This evidence runs contrary to the expectations from the propagule pressure invasion hypothesis, which posits that the probability of establishment increases with introduction events and the number of individuals introduced (Lockwood et al., 2005). We do not mean to imply that propagule pressure per se impedes establishment. Rather, we explore below the probable influence of propagule-driven genetic influences, which may be responsible for both poor establishment success and for loss of previously established populations.

Genetic effects of hatchery propagation

While there is substantial evidence that genetic change is occurring during hatchery propagation in salmonid species, the nature of this change and the impact of hatchery effects both on wild trout conspecific populations and on establishment success have long been debated (Naish et al., 2008; Scott and Gill, 2008). Three major and not mutually exclusive mechanisms for adverse hatchery effects have been postulated:

- Domestication selection and artificial selection imposed by breeders with the intention to enhance desired traits but possibly maladaptive in the wild (e.g. Araki et al., 2008).
- Inbreeding depression which leads to a decrease in heterozygosity with a concomitant reduction of fitness through either or both of two mechanisms: increased expression (unmasking) of deleterious recessive alleles that otherwise would remain at low frequency, and reduced frequency of beneficial allelic combinations (Keller and Waller, 2002; Naish et al., 2013).
- Outbreeding depression by mingling of previously allopatric lineages which can result in harmful hybridizations with detrimental effects on offspring fitness and particularly their ability to spawn in natural conditions, mainly through the loss of adaptive capacity to local conditions (Allendorf et al., 2010; McClelland and Naish, 2007; Tymchuk et al., 2006).

Inbreeding depression and outbreeding depression are widely accepted as explanations for the reduction of fitness in introduced fish, but their relative importance and contribution to the dynamics of the invasion process are not clearly understood (Blanchet, 2012; Roman and Darling, 2007; Salmenkova, 2008;). By far, the risk of inbreeding depression has received most research attention (Edmands, 2007). However, successfully established populations of rainbow trout in the southern hemisphere (e.g. New Zealand: Scott, et al., 1978; Argentina: Riva Rossi et al., 2004) and in some European locations (Italy, Lemme Creek in River Orba: Candiotto et al., 2011; Slovenia, Idrijca stream in River Soca: Vincenzi et al., 2010) originated from small founding populations and persist to date, despite their probably reduced genetic diversity. The established rainbow trout population of Vlisidia stream (present study) also originated from a single introduction event and has persisted with no further stocking thereafter. On such evidence it is reasonable to speculate that reduced genetic variability due to founder effects and low introduction effort, and the resulting inbreeding depression, is not the reason of most establishment failures of rainbow trout in Europe (see Valiente et al., 2007 for a further discussion on this topic). It is therefore tempting to suggest that outbreeding depression is a possible cause of poor establishment success of rainbow in the Continent. Outbreeding depression also has the capacity to explain the decline of established populations in several European countries which occurred because of (rather than despite of) increasing propagule pressure. This speculation leads to the hypothesis that rainbow trout stocking may impede, rather than facilitate establishment, and fits in with the findings of Miller et al. (2004), who showed that the viability of naturalised rainbow trout populations in streams of Minnesota can be compromised by continued stocking of hatchery propagated fish from unrelated sources. We conclude that, genetic factors affecting the reproductive process, possibly through a combination of outbreeding depression resulting from the admixture of unrelated intraspecific lineages and maladaptive behaviours resulting from domestication selection acting in captivity, remain probable causes of poor establishment for the Greek populations of rainbow trout.

Conservation implications

Rainbow trout stocking in Greece is often undocumented and more crucially without any scientific supervision or any justification that stocking actions are needed to enhance salmonid populations for particular targets. Increased stocking of rainbow trout may have negative impacts through agonistic behaviour on native salmonids due to predation, competition for space and food and rarely by redd superimposition (Scott and Irvine, 2000; Seiler and Keeley, 2009; Van Zwol et al., 2012). The impacts could be severe on a local scale such as in certain enclosed aquatic habitat types such as cold-water springs which may be inhabited by local endemic aquatic species. However, based on the current knowledge gained by this study, the potential for widespread establishment or future spread of rainbow trout in Greece seems to be highly unlikely. Even changing conditions, such as climate change impacts, should affect all cold-water salmonid species negatively (Papadaki et al., 2016). Although some aquacultural strains or populations of rainbow trout could be able to survive in slightly warmer conditions than what is documented for the *S. trutta* complex, there is no evidence that rainbow trout may benefit over native trout by climate-change warming in Greece.

Similarly, Greek native salmonids are often translocated and stocked in areas outside their historical native range, in order to increase recreational fishery potential. We assert that the impact of native salmonid translocations on genetic diversity may create irreversible negative impacts to native trout due to the high possibility of introgressive hybridization between populations or closely related *Salmo* species (Berrebi et al., 2017; Jug et al., 2005). Evidence for this hybridization among translocated trout species and native forms has already been documented in Greece (Apostolidis et al, 2008) and it has been seen in many other Mediterranean catchments (Vincenzi et al., 2010). The widespread threat of this kind of indiscriminant stocking on intraspecific and intragenus negative impacts on native *Salmo* species has also been widely voiced (Buoro et al., 2016) but not widely referred as a conservation problem in Greece. Thus, conservation efforts in Greek trout streams should concentrate towards controlling translocations of native or related "brown trout" clones and monitoring stocking practices and fish farming disease outbreaks.

This study provides evidence that the establishment of rainbow trout is geographically limited in Greek streams and rivers mainly due to spawning failure in the wild, possibly attributed to genetic factors, which is also supported from observational evidence in other European countries. Rainbow trout stands out as an example where the risk assessment tools may promote an artificially increased risk status since they cannot appreciate the idiosyncrasies of the problem (i.e. genetic issues and limitations of establishment). Hence, rainbow trout should not be considered at present as a primary conservation threat in Greece, taking into account that stocking actions must be properly managed. The perceived problem with rainbow trout as an invasive species may actually mask other serious conservation issues that plague cold water lotic conservation, a prominent one being fish farming pressures and hatchery-based stocking translocations on native trout streams. It is widely acknowledged that the management rational and implications of stocking activities have not received the attention they require (Cowx, 1999) and in the case of Greece this issue may produce multiple adverse effects on trout streams.

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Chapter 6

POTENTIAL USE OF

INTRODUCED SPECIES

INTO SCIENTIFIC RESEARCH: INVESTIGATING MICROPLASTICS IN A GREEK URBAN RIVER, VIA TRANSLOCATED FISH SPECIES AS BIOINDICATORS

INTRODUCTION

For decades now, the use of plastics has provided a plethora of applications and socio-economic benefits, where in their absence human societies would have been far more different than today (Andrady and Neal, 2009). However, huge amounts of plastics are used on a daily basis all over the world, while the vast majority will be disposed after a few minutes of use fulfilling shortly their purpose; within the frame of “use-and-dispose culture”. Plastic production worldwide increased from 1.7 million tonnes/year in the 1950s to almost 369 million tonnes/year in 2018, while plastics production in Europe reached 17% of world's plastics production (61.8 million tonnes; PlasticsEurope, 2019). The unsustainable use of substantial amounts of plastic combined with their inappropriate waste management have created an emerging, harmful contaminant for natural ecosystems and their biota called microplastics (Barnes et al., 2009).

Microplastics are synthetic polymers smaller than 5 mm and are divided into two broad categories, the primary and secondary microplastics. Primary microplastics are those that are manufactured from the beginning to have a size less than 5 mm, while secondary microplastics, are created by fragments of larger plastics due to processes such as photo-degradation, physical, chemical or biological interactions (Thompson et al., 2009; Galgani et al., 2013). The latter consist also the majority of microplastics found in nature (Eriksen et al., 2013) and vastly in aquatic environments (Wagner et al., 2014; Auta et al., 2017). The understanding of microplastic pollution in the environment is rapidly developing, yet there are important questions to be answered and key knowledge gaps to be identified (Horton et al., 2017). So far, most research efforts on microplastics have been focused on the marine environment and their impacts on marine organisms (Lambert and Wagner, 2018). Studies of plastic contamination in freshwater ecosystems and their biota remain quite scarce; despite of the fact that the vast majority of plastic waste (~80%) derive from terrestrial sources and pass initially through freshwater lotic ecosystems (Bowmer and Kershaw, 2010; GESAMP, 2010), which may act as natural filters for the marine environment.

Moreover, the majority of the latter studies typically investigate the presence and the abundance of microplastics in freshwater ecosystems, however the dynamics of microplastic distribution, sources and transport, habitat influence as well as the implications of microplastics in aquatic community components are still very limited (Adeogun et al., 2020).

Fish species are one of the most important and valuable biological elements of the aquatic environment as they generate many ecosystem-services (Pinheiro et al., 2017), and have been extensively used as ecological indicators of riverine ecosystems health for a long time (Fausch et al., 1990; Simon, 1991). Specifically, several freshwater fish species have been applied as bio-indicators due to their i) excellent response to different contaminates in water; ii) diverse variety of habitat use that make them suitable for assessing different special scales; iii) long lifespans that can both reflect current and long-term water quality and iv) highly visibility to the public among the rest aquatic biota, that renders communication easier (Bartram and Balance, 1996; Benejam et al., 2015). Studies investigating the occurrence of microplastics in fish species that inhabit lentic and lotic ecosystems, have increased from barely three in 2016 to 24 within the first quarter of 2020 (Table A.6.1). Additionally, during the last few years laboratory-based studies on accumulation, functional responses, biological effects, toxicity and other adverse effects of microplastics in freshwater fishes have become an emerging area of research (Grigorakis et al., 2017; Ding et al., 2018; Lei et al., 2018; Mbedzi et al., 2020).

Monitoring rare native species is important as they can reflect relict or undisturbed conditions of distinctive habitat types (Eyre et al., 1986), yet the risks associated with such choices include the threat of sampling endangered species or individuals from non-viable and sink populations (Vane-Wright, 1996). On the other hand, monitoring the abundant species of an ecosystem can be important for the early detection of disturbance signs (Hawking and New, 2002). Non-indigenous species population may generally lack high specificity of a unique system, nevertheless by being the most abundant and wide-spread species within the assemblages could have high information contents and high probabilities of being sampled (McGeoch and Chown, 1998). Consequently, non-indigenous fish species with widespread distribution, high level of biomass and dominance, ease of capture and ability to adapt laboratory settings could be indicative of reflecting the abiotic and biotic state of a freshwater environment (Pyke, 2005; Rabee and Turki, 2014). Furthermore alien and translocated fish species are often found inhabiting urban rivers, novel environments and polluted ecosystems where native species typically do not occur (Hopkins et al., 2003; Su et al., 2019). Thus, biomonitoring investigations based on translocated fish species of a given area may provide valuable information regarding pollution and its impacts on native fish species and/or their sister species populations within the country.

The aim of this chapter is to explore the potential use of introduced species into scientific research, by utilizing translocated fish species as bio-indicators in order to detect the occurrence and the abundance of microplastics in a heavily modified urban river (Kiffisos River, Attica) in Greece.

MATERIALS AND METHODS

Study area

The Kifissos River is located in the Attica plain in central Greece. The river originates between the mountains of Parnitha (a.s.l 1.413m) and Penteli (a.s.l 1.109m) and flows southwest trajecting through the largest part of the city of Athens and most of the city's suburbs; an area with a population of approximately 3.8 million inhabitants (in 2011; ELSTAT, 2012) where after 25km discharges into the Saronic Gulf.

The catchment area of the river extends to 361 km^2, while its flow regime is intermittent mainly due to human interventions, following however a seasonal pattern with low or zero flows during the summer and high flash floods during the winter. Discharges fluctuate from 0 up to 1,400 m^3/s, mainly depending on past hydrometeorological conditions (Panagiotopoulos et al., 2010; Partsinevelou and Evrenoglou, 2016). The upper sections of the basin are within the protected areas of the National Forest or included in the Natura 2000 network, whereas cultivated and industrial areas are also present (Evelpidou et al., 2009). Apart from these upper sections of the basin, the greater part of Kifissos River catchment is currently a built-up urban area (approximately 70% its total extent) (Evrenoglou et al., 2013). On both river banks, major roads have been built to serve the capital city, as well as a part of the national road connecting Athens with the northern adjacent prefecture. Hence, Kifissos river is considered as heavily modified river according to the WDF guidelines (WFD Greece, 2016) since the largest section of the main channel and most of its tributaries have suffered serious hydro-morphological alterations. Finally, the last 10 km of the main channel has undergone major flood-prevention construction works has transform to a drainage channel that transfers rainwater, industrial and domestic wastewater to the sea.

The freshwater ichthyofauna of Kifissos Att. basin consists of five freshwater fish species: the endangered Marathon minnow, *Pelasgus marathonicus* (Vinciguerra, 1921), the native European eel, *Anguilla anguilla* (Linnaeus, 1758) and three introduced fish species, namely, the Vardar chab, *Squalius vardarensis* Karaman, 1928, the Sperchios barbell *Barbus sperchiensis* Stephanidis, 1950, probably both translocated from the adjacent Sperchios river basin (Koutsikos et al., 2012) and the alien eastern mosquitofish, *Gambusia holbrooki* Girard, 1859, native to Atlantic and Gulf Slope drainages of the United States of America.

Squalius vardarensis inhabits rivers and streams usually with moderate flow; although is common in reservoirs and sometimes in lakes and spring-fed marshes (Barbieri et al., 2015) and is an omnivorous fish species that feeds from the water column on a variety of plant and animal food (Zogaris et al., 2018).

Sampling site

Due to the fact that freshwater fish species of Kifissos river are restricted in the upper part of the basin while in addition the lower part is heavily modified and accessibility due to the built-up urban area is limited, we were forced to unravel a location that would fulfill the purposes of this study. Hence, our sampling site was finally found to a location where the three later criteria met: the main stem of the river corridor was accessible, the site drains the lower part of the upstream area which is representative of urban land uses of the greater area and last but not least the fish species were present (Fig. 6.1).

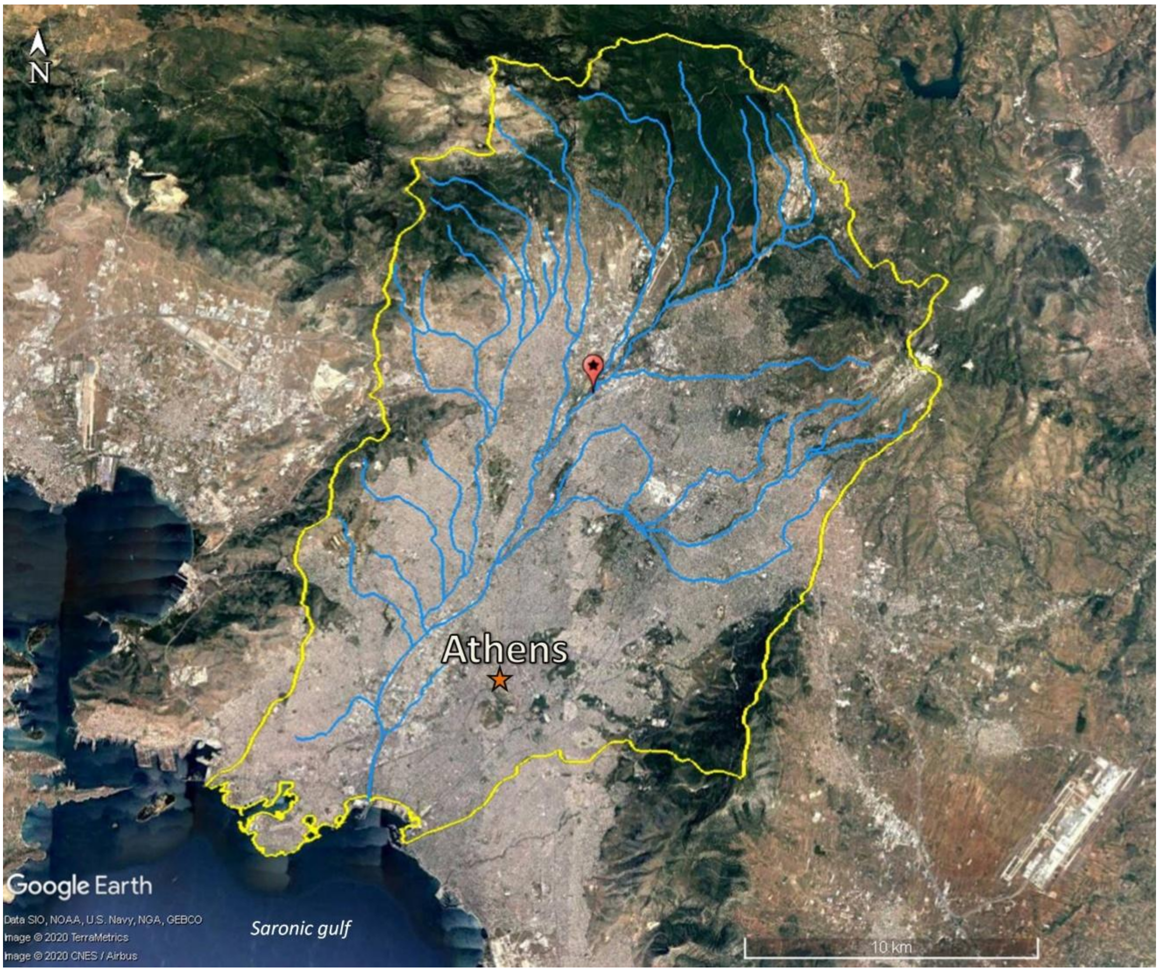


FIGURE 6.1. City of Athens and its suburbs, Kifissos Att. river basin and sampling site.

Data sampling and analyses

Fish sampling was conducted in November of 2018 through the use of a shore-based electrofishing unit (EFKO Elektrofischereigeräte GmbH, Model FEG 6000; DC unpulsed, 7,0 KW output power, 150-600 V), using standardized procedures developed under the European research project FAME (Schmutz et al., 2007) with some modifications (IMBRIW-HCMR, 2013); for method description see Economou et al. (2016). In total three species have been sampled, namely, *S. vardarensis*, *B. sperchiensis* and *A. anguilla*. Target fish species (*S. vardarensis*) were first anesthetized in 1% clove oil, preserved with ice in the field and when transported to the laboratory fish s were stored at −20 °C. In addition, the total length (*TL*, cm), the total weight (*TW*, g) and the net weight of the gastrointestinal content (NetGI Weight, g) of the fish specimens were recorded, prior to microplastic detection.

Length–weight relationships were calculated using the equation:

$$TW = \quad a * TL^{b},$$

where TW is the total weight (expressed in grams), TL is the total weight (expressed in centimeters) and a and b are the parameters of the equation.

Water sample collected at same fishing sampling site by using a manta net during July of 2019. The manta net (300 μm mesh size) held in the center of the river channel across the two banks in the upper 25 cm of the water column. This method of sampling was chosen as it ensures that water sample is taken from the same point of the river column. Water flows were measured concurrently using a flow meter (Global Water FP101). The particulates accumulated on the net were rinsed off with creek water into a glass jar and stored at 4 °C until laboratory analysis.Finally, the land use types of the basin were derived from geographical information systems (ESRI - ArcGIS v. 10.4) with the use of CORINE Land Cover inventory (CLC, 2018).

Microplastic observation and quantification

Tissue from fish individuals (stomach and intestine) was removed, weighted, and digested by hydrogen peroxide to enable microplastic detection (Mathalon and Hill, 2014). Tissue samples were placed into glass beakers in 1:20 (w/v) H_2O_2 (30% H_2O_2, Chem-Lab, Germany) and heated at 55–65 °C until H_2O_2 was evaporated. Samples were diluted with 100 ml of purified water (Milli-Q), stirred, and filtered under vacuum on fiberglass filters (Whatmann, GE Healthcare, UK), which were placed in petri dishes and dried. Filters were examined under a stereomicroscope (Olympus, SZE and SZX7) for all items resembling microplastics. Moreover, the water sample was sieved to separate microplastics into small (0.3–1 mm) and large microplastics (1–5 mm) and then dried for 24 h at 90 °C. Large microplastics were visually sorted, while small microplastics were segregated by digestion in hydrogen peroxide (Mathalon and Hill, 2014), filtration (pore size 1.2 lm), as well as observation under stereoscope.

FT-IR analysis

Microplastics were analyzed by the use of Fourier transform infrared spectroscopy (FT-IR) for confirming the synthetic polymer origin of the possible microplastic items. FT-IR analysis was carried out on an Agilent Cary 630 FTIR spectrometer using a self-generated polymer library (i.e. spectra of reference polymer types provided by industry). The level of certainty when comparing sample spectrum to that of the self-generated library database was set up to 70% (Digka et al., 2018). All items (fragments, fibers, films and foam), were further analysed by FT-IR as suggested by the guidelines produced by the MSFD technical group on marine litter (Galgani et al., 2013).

Contamination precautions

Glassware was rinsed in the laboratory thoroughly with purified water, while for the examination of fish gastrointestinal content, the stereomicroscope observation area was isolated by the use of a plastic cover (Torre et al., 2016). Fish speciemens were covered by foil paper during digestion procedure and when not in use. A glove bag was used as working area for sample rinsing and filtration. Filters were also covered with glass lids during observation under a stereomicroscope. Finally, procedural blank samples were used in all steps and every item similar to those found in blank samples were excluded, as they were considered airborne contamination. Procedural contamination was less than 10% of the mean microplastic number in the samples (Galgani et al., 2013).

Statistical analysis

Descriptive statistics were applied for length and weight as well as for the estimated parameters of the length–weight relationship. In order to confirm whether b value obtained in the linear regression was significantly different from the isometric value (b=3), t-test with appropriate degrees of freedom were used. Moreover, in order to test if the occurrence of microplastics in fish were correlated with biometric variables (total weight, total length) Kruskal Wallis test have been applied, since our data, didn’t follow normal distribution (Shapiro – Wilk test).

RESULTS

In total, 32 specimens of *S. vardarensis* were sampled with a 16.98 cm mean *TL* and 67.39 cm mean *TW*, while the *WetGI* Weight ranged from 0.91 g to 37 g (Table 6.1). The largest individual sampled was 39.2 cm with a 625.3 g of *TW*. Length−weight relationship displayed a high correlation coefficient (R^2=0.98, *p*<0.01) and the value of parameter *b* computed to 3.069 ±0.089 (Fig. 6.2). In the present study, *t-test* showed a statistical difference between the estimated value of parameter *b* and the theoretical value 3, indicating a positive allometric growth (*b*>3; *t*=0.775, *p*<0.05) of the species. Relationships between TW, NW and *WetGI* Weight displayed also high correlation coefficients (Fig. 6.3).

TABLE 6.1. Descriptive statistics for total length (*TL*), total weight (*TW*) and gastrointestinal content weight (WetGI W) *Squalius vardarensis* from Kifissos Att. river.

Species	n	*TL* range (cm)	mean *TL* (±SE)	*TW* range (g)	mean *TW* (±SE)	*WetGI* W range (g)	mean *WetGI* W (±SE)
S. vardarensis	32	10.5−39.2	16.98 (±0.92)	10.6−625.3	67.4 (±18.99)	0.9−37.0	5.60 (±1.16)

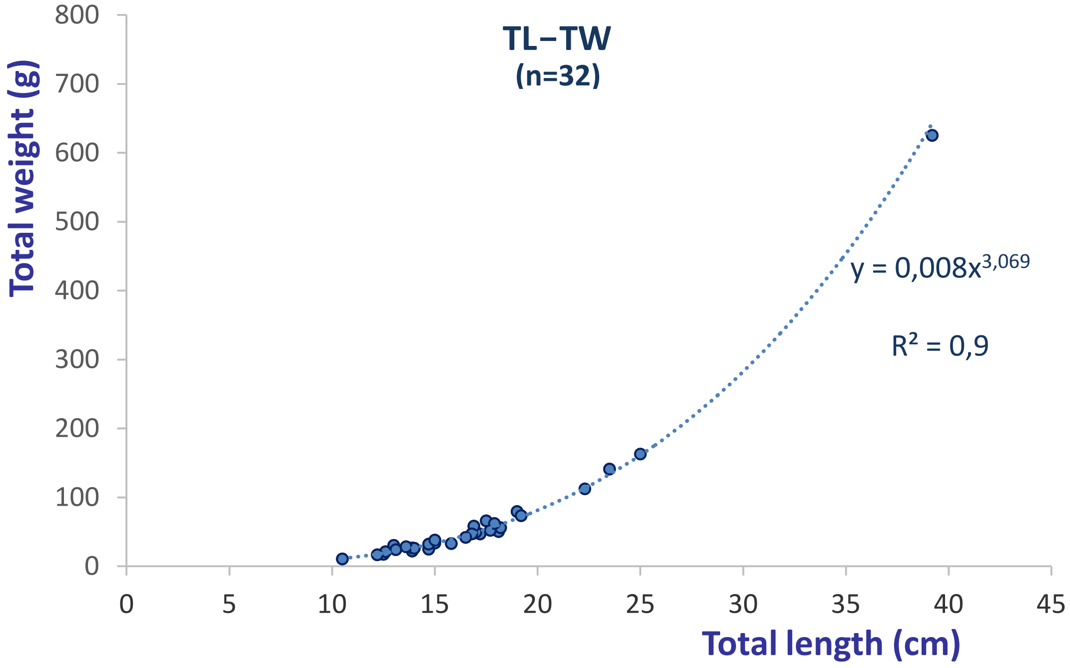
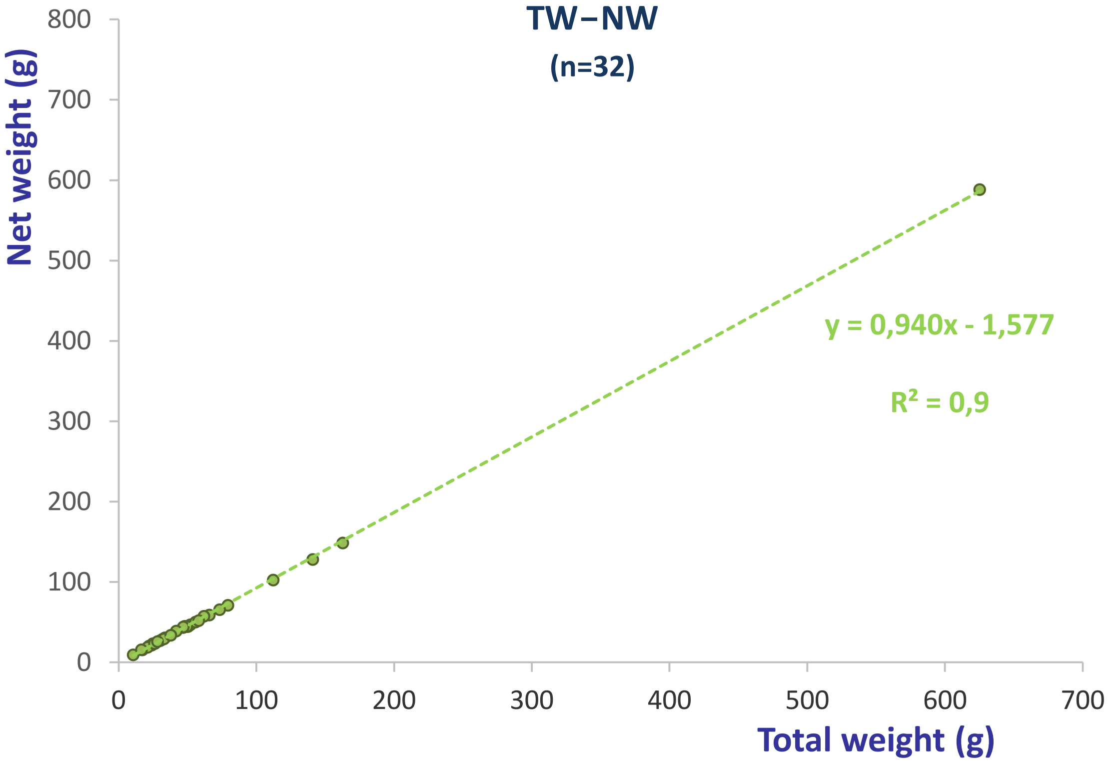


FIGURE 6.2. Length-weight relationship of *Squalius vardarensis* from Kifissos Att. river basin.



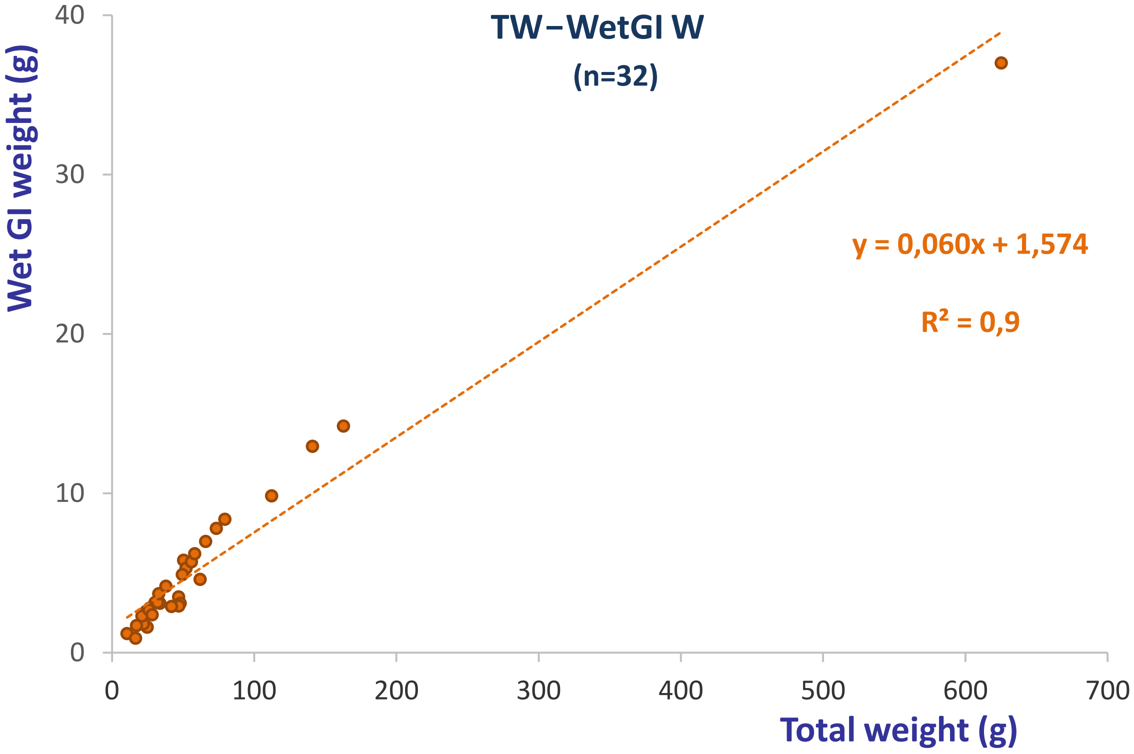


FIGURE 6.3. Total weight – net weight and total weight – *WetGI* weight relationships of *Squalius vardarensis* from Kifissos Att. river basin.

Microplastic ingestion

Microplastics (19 items) were found in 11 *S. vardarensis* (Table 6.2). The prevelance (%) of ingested microplastics in all specimens was approximately 35%. Kruskal Wallis test indicated no significant correlations with biometric variables (total weight, total length) between fishes with and without microplastics (*p* >0.05). Average number of microplastics per individual (the total the number of microplastics in examined tissues) in specimens containing microplastics was 1.7 ±0.2 items/individual (Table 6.2).

TABLE 6.2. Abundance (mean ±*SE*) and frequency of occurrence (%) of ingested microplastics (in specimens containing microplastics, MPs) detected in *Squalius vardarensis* from Kifissos Att. river basin.

Species	*S. vardarensis*
Number of specimens examined	32
Number of specimens containing microplastics	11
MPs number	19
MPs prevelance (%)	34.4
MPs mean dimension length range (mm)	2.1 ±0.3
MPs min dimension length range (mm)	0.3
MPs max dimension length range (mm)	4.7
MPs abundance:	
a) mean number of items per individual in all specimens examined	0.6 ±0.1
b) mean number of items per individual in specimens containing MPs	1.7 ±0.2
c) mean number of items per gram wet *GI* weight* in specimens containing MPs	0.7 ±0.2

† *Based onall items in full stomach and intestine.*
* *Wet weight of fish gastrointestinal content.*

In all specimens, only one mesoplastic was found (5 mm to 2 cm) and excluded from analysis, while ingested microplastic ranged from 0 to 3 items per individual. Among the specimens with a plastic item, 6 individuals (54.5%) contained at least 2 items, and the maximum number of ingested items per specimens detected in a 14.7 cm *TL* individual, consisting of 3 fibers. The dominant size class of *S. vardarensis* was 12−15 cm, followed by the class 18−21 cm (Fig. 6.3). Additionally, the latter two classes contained the most specimens with a plastic item ingested, 38.5% of the class 12−15 cm (5 specimens) and 20% of the class 18−21 cm (4 specimens), as well the highest amount of items, 11 and 7 microplastics, respectively. The biggest specimen with a plastic item, was also the biggest individual of the introduced population of *S. vardarensis* sampled, in class 39−42 cm (Table 6.2; Fig. 6.3). On the contrary, only two classes were found without specimens containing microplastics namely class 9−12 cm and 24−27 cm, with one individual respectively.

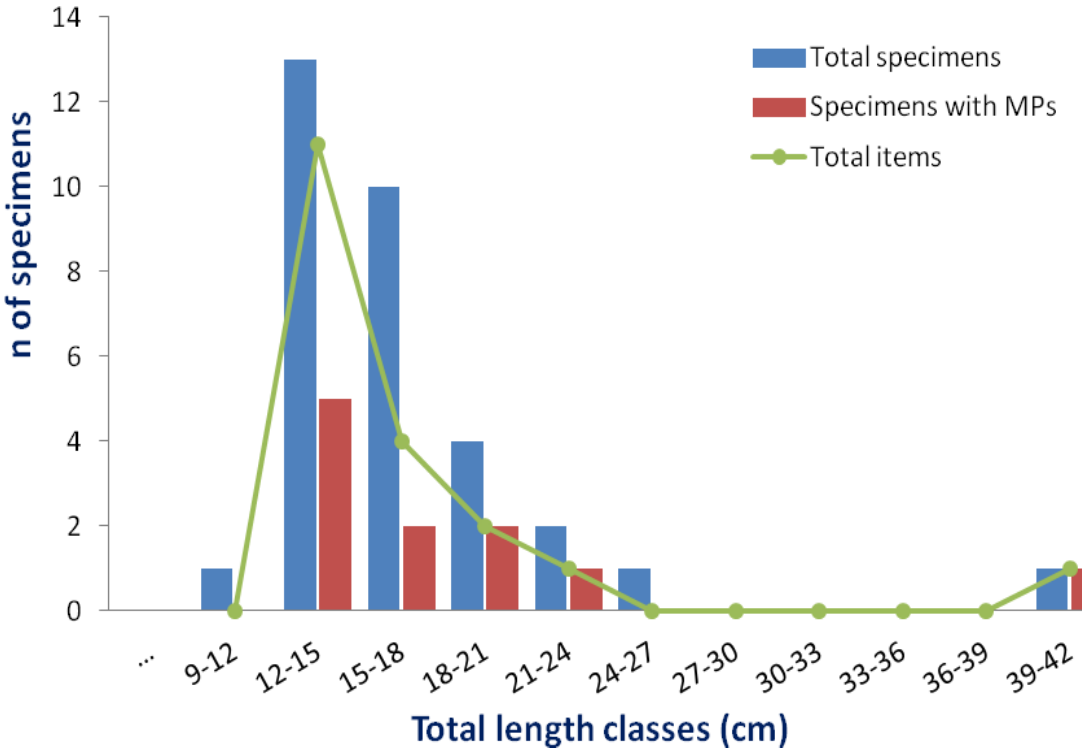


Figure 6.3. Length frequency distribution in total specimens, in specimens containing microplastics (MPs) and the abundance of items per each total length class detected in *Squalius vardarensis* from Kifissos Att. river basin.

Morphological characteristics and types of microplastics in fish

Microplastics detected in all *S. vardarensis* specimens were classified in four categories based on their a) shape, b) size, c) color and d) polymer type. Examples of the items detected in fish stomachs and intestines as well as their FT-IR spectrum were presented in Fig. A.6.1. In shape classification, the detected items categorized as fibers and fragments. The dominant type was fibers (89.5%), while fragments had a small contribution (10.5%) to the total amount of the ingested items (Fig. 6.4a). Regarding the size characterization, since small microplastics (<0.3 mm) did not occur in fish specimens, items were categorized into 2 length classes: i) 0.3-1.0 mm class, accounted for 26.3%; and ii) 1.0-5.0 mm class which was the dominant one with 73.7% (Fig. 6.4b).

FT-IR analysis identified the plastic types of the 11 out of the 19 items (52.4%), to the following four polymer types of microplastics: polyethylene (PE), polypropylene (PP), polyvinyl alcohol (PVA) and polyvinyl chloride (PVC). More specifically, 23.8% of the ingested microplastic identified as PE, 14.3% as PVA, 9.5% as PP and 4.8 as PVC, although there was a 47.6% that couldn’t be characterized hence, it was classified as unidentified plastic (Fig. 6.4c). Finally, the most common colors of microplastics in fish specimens were black (26.3%), followed by blue and transparent (both with 21.1%), some were red (15.8%) and grey (10.5%), while the color with the smallest percentage was brown (5.3%) (Fig. 6.4d).

Microplastic pollution on freshwater column

Manta net held on the water over a 20 min period and a total of 39.6 m^3 of river water of Kifissos Att. were sampled (Table 6.3). Overall 321 microplastic were detected within the sample, while 16 mesoplastic (5 mm to 2 cm) were also identified and excluded from further analyses. The abundance of microplastics in the freshwater column calculated at 8.1 items/m^3. The mean length of microplastics estimated at 2.3 ±0.1mm, while the smallest item detected was 0.32 mm (Table 6.4).

TABLE 6.3. Manta net dimensions, velocity (U) and (Q) of river, time of sampling and volume of sampled water in Kifissos Att. river basin.

Manta height (m)	Manta length (m)	Sampled area (m^2)	U (m/s)	Q (m^3/s)	Time (sec)	Volume of water sampled (m^3)
0,25	0,60	0,15	0,220	0,033	1200	39.6

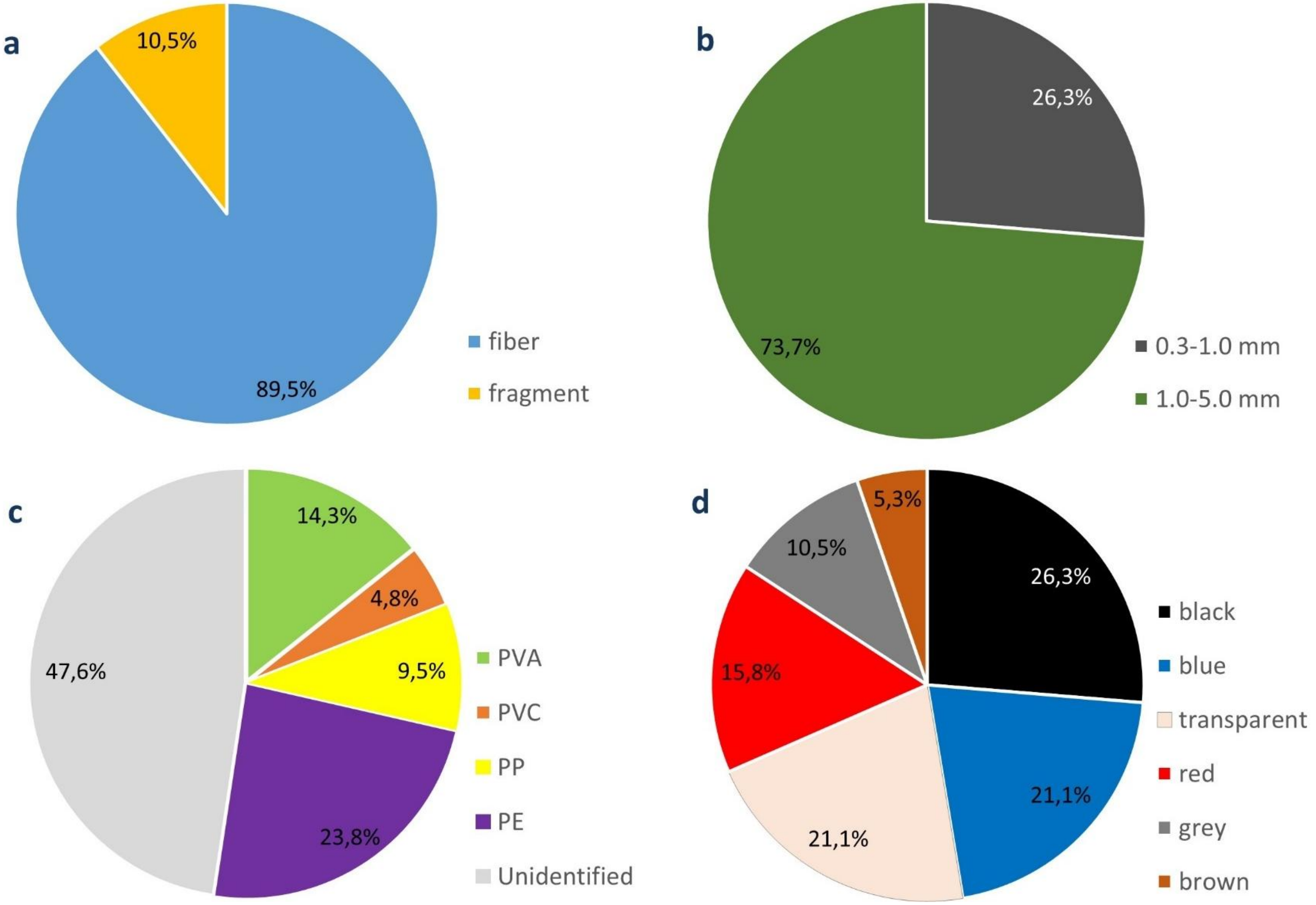


FIGURE 6.4. Shape, size, color and polymer type of microplastics detected in *Squalius vardarensis* from Kifissos Att. river basin.

The detected microplastics were categorized based on their size into two classes: i) small microplastics (0.3−1.0 mm) and ii) large microplastics (1–5 mm). Small microplastics were the most abundant size class with 81.3% (Fig. 6.5b, Table 6.4). In addition, all items were categorized based on their shape into fibers, films, fragments and foams. The dominant type in all items was films (55.8%), followed by fibers (30.5%) and fragments (13.1%), while foams had a very little contribution (0.6%) to the water sample (Table 6.5; Fig. 6.5a). Both small and large microplastics were further classified according their shape (Table 6.5). The dominant shape in small microplastics class, was films (48.3%), followed by fibers (36.8%), fragments (14.6%) and foam (0.4%). Finally, in large microplastics films were once again the dominant shape (88.3%), yet the remaining three shapes displayed small percentages (Table 6.5).

TABLE 6.4. Total numbers, abundance and size categories and dimension lengths (mean length ±SE, range) of microplastics (MPs) detected in freshwater column from Kifissos Att. river basin.

Water sample	
Total number of MPs	321
Mean abundance of MPs (m^3)	8.1
Number of small items (0.3-1.0 mm)	261
Number of small items (1.0-5.0 mm)	60
MPs mean dimension length (mm)	2.3 ±0.1
MP min dimension length range (mm)	0.32
MP max dimension length range (mm)	4.97

The major polymer types of microplastics identified by FT-IR analysis were: polyethylene (PE, with 59.5%), polyvinyl alcohol (PVA, with 18.1), polypropylene (PP, with 13.8%), polyurethane (PU, with 1.9%), polyvinyl chloride (PVC, with 1.4%), and a sum of 5.3% by few other polymers, namely, ethylene-vinyl acetate, nylon, PDO, PET, rubber/chlorinated and TPE (Fig. 6.5c). Finally, the most common colors of microplastics in freshwater column were transparent (42.4%), followed by blue (18.4%), many were white (13.4%), black (11.5%) and red (7.5%), while a few green and yellow items had a little contribution (4.3% and 2.5%, respectively) (Fig. 6.4d).

TABLE 6.5. Distribution of microplastic (MPs) type among size classes detected in freshwater column from Kifissos Att. river basin.

Type	0.3-1 (mm)	1-5 (mm)	Total MPs
Fibers	96	2	98
Films	126	53	179
Fragments	38	4	42
Foam	1	1	2
Total	**261**	**60**	**321**

Land use types in Kifissos Att. river

According to Corine Land Cover database, the main land use classes of the entire river basin of Kifissos Att. were: “Artificial surfaces” in approximately 70%, followed by “Forest and seminatural areas” with nearly a 26 % and “Agricultural areas” with 4%, while the class “Water bodies” displayed the smallest precentage (0.05%).

Furthermore, in an effort to investigate the land cover specifically in the upstream area of the sampling site, the detail land use classes (in two-level hierarchy) of the upstream catchment area were calculated. The dominant type was “urban fabric” (31.5%) followed by “Forest” (17.2%) and “Shrubs and/or herbaceous vegetation associations” (11.5%), whereas the three categories: “Heterogeneous agricultural areas”, “Industrial, commercial units and transport units” and “Artificial, non-agricultural vegetated areas” displayed percentages under 10%, namely, 9.4%, 4.4% and 1.3%.

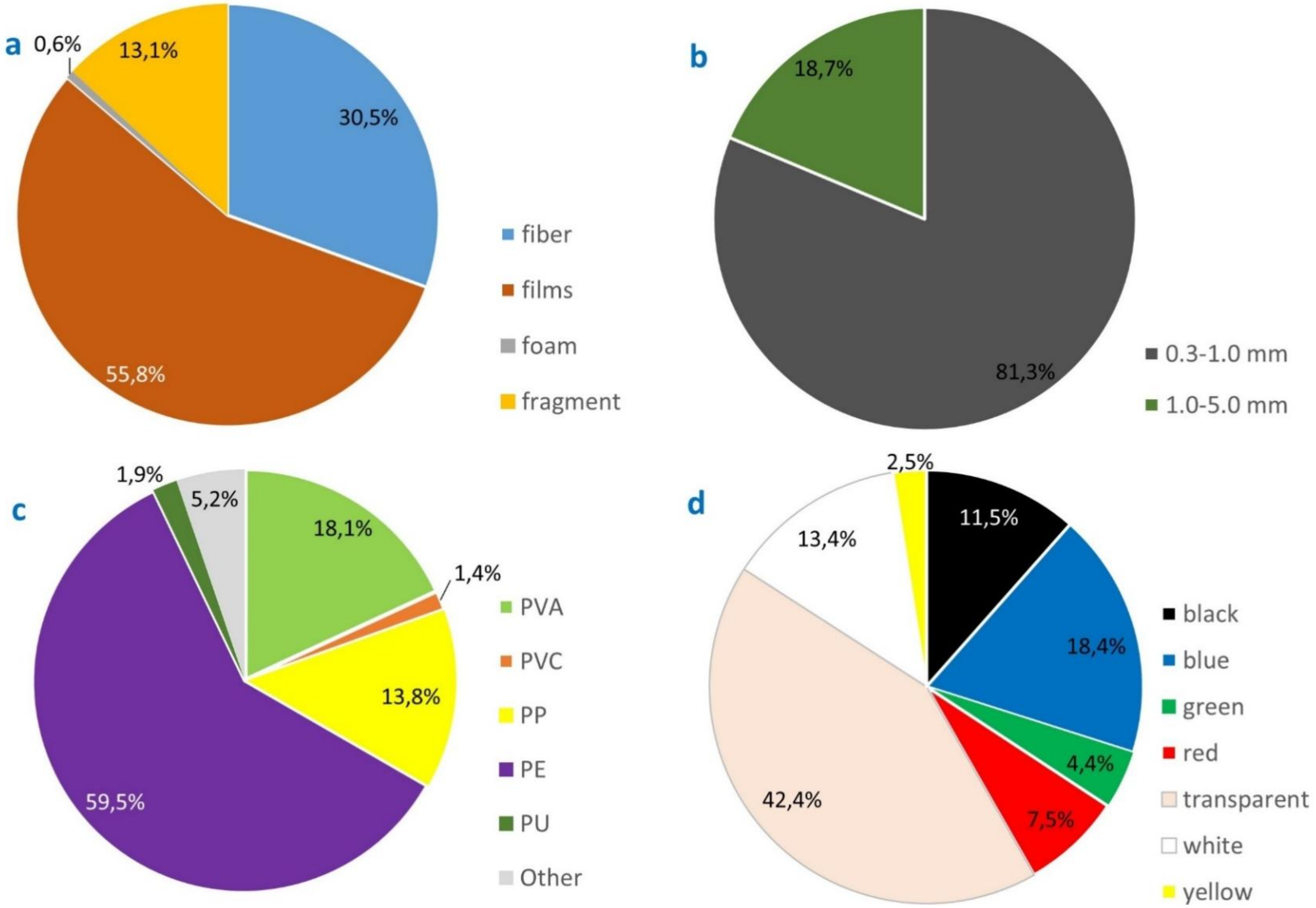


FIGURE 6.5. Shape, size, color and polymer type of microplastics detected on freshwater column from Kifissos Att. river basin.

DISCUSSION

In this chapter we explored the potential of using NIFS into scientific research as bio-indicators, by investigating the presence and abundance of microplastics into a Greek river. Our results confirmed the presence of microplastic pollution in the study area, indicating that both water column and fish species of Kifissos Att. River are contaminated by microplastics.

Research of microplastics in freshwater ecosystems

Microplastic particles have been observed in the marine environment all over the world for three decades now, from beaches of New Zealand and islands of the equatorial Western Atlantic to the Mediterranean Sea and shores of Greek islands (Gregory 1978; Shiber 1987; Ivar do Sul et al., 2009; Digka et al., 2018). However, microplastics in freshwater ecosystems have attracted less attention until recently, where the first studies were initiated in 2010 (Wagner and Lambert, 2018). Studies on microplastic pollution in inland waters typically concern lentic ecosystems, both in Europe and worldwide (Faure et al., 2012; Imhof et al., 2013; Fischer et al., 2016), while research in lotic ecosystems is scarce and mainly focused on central and northern European river basins (Gasperi et al., 2014; Lechner et al., 2014; Klein et al., 2015; Horton et al., 2017). Our study is the first dealing with microplastic pollution in a river basin located in Eastern Mediterranean. The study area was selected due to the fact that Kifissos Att. River is considered as a vastly impacted river due to the insertion of high loads of point and non-point source wastes since the river flows through the largest part of the Metropolitan area of Athens and thus we expected to uncover pollution from microplastics.

Our results had similar mean abundances of microplastics concentration in the freshwater column with a study conducted in Switzerland, where microplastics in several rivers ranged between 0.10 and 64 items/m^3 (from 0.36 to 7 items/m^3; Faure et al., 2015). However, other studies have reported higher mean abundance of microplastics in water samples as the study that was conducted in Seine River in an urban area of Paris were 30 items/m^3 were observed (Dris et al., 2015). High abundances have been detected in Asian counties, with an extreme case in a Chinese urban area, where three river estuaries contained from 100 to 4100 items/m^3 (Zhao et al., 2015). High microplastic abundances have been also reported from other parts of China mainly attributed to the lack of wastewater treatment plants, poor recycling and waste disposal management (Wagner and Lambert, 2018). As it concerns the concentration levels between the different types of microplastics in our water sample and fish specimens large differences were observed. Specifically, films and fibers were dominant in comparison to fragments. Similar trends of higher fiber concentrations in contrast to fragment abundances were also detected in Marne River (France, Paris) as well as in an urban river in Canada, indicating the prevalence and the importance of the category of fibers near large urban areas (Campbell et al., 2017; Wagner and Lambert, 2018). Finally, in some studies, fibers have been suggested to originate from domestic sources such as washing machine effluents (Su et al., 2016).

Research of microplastics in freshwater fish

In 2014, Sanchez et al. provided the first evidence of microplastic ingestion from a freshwater fish (*G. gobio;* France). Since then, there has been a tremendous increase of scientific papers addressing the issue (Fig. 6.6). During the first six months of 2020, 24 studies on microplastics in freshwater fish fauna have been published. Specifically, 84 fish species belonging to 29 families from lentic and lotic ecosystems of 17 different countries have been studied, and the presence of microplastics in gastrointestinal content (stomach and/or gut), gills, liver and flesh of fish species were investigated (Table 6.6 and for additional information, such as species, families and freshwater ecosystem types of the studies see Table A.6.1 in Appendix A).

High prevalence of microplastics in freshwater fish has been reported in several studies varying from 52 to 95.7 % (Table 6.6). The largest proportion in high category has been found in China (90.9 and 95.7; Yuan et al., 2019 and Jabeen et al., 2017, respectively), in several rivers in the U.S.A. (85%; McNeish et al. 2018; 45%; Peters and Bratton 2016), Egypt (75%; Khan et al., 2020), Canada (73%; Campbell et al. 2017), as well as in urban rivers in South America (83%; Silva-Cavalcanti et al. 2017).

Furthermore, three studies in a riverine ecosystem of South Korea (Park et al., 2020), in an Argentinean river estuary (Pazos et al., 2017) and in two artificial reservoirs in USA (Hurt et al., 2020) indicated absolute prevalence of ingested microplastics in examined fishes (100%). On the contrary, relatively low microplastic prevalence showed a number of studies in Europe (9% in Flanders, Belgium Slootmaekers et al., 2019; 12% in France, Sanches et al., 2014) in USA (8.2%; Phillips and Bonner, 2015) in Australia (19.4%; Su et al., 2019) and in Tanzania (20%; Biginagwa et al., 2016). Despite the high variance it is yet unclear whether the wide range reported for the prevalence of microplastics is due to ecological differences of lentic vs lotic freshwater ecosystems types (Hurt et al., 2020).

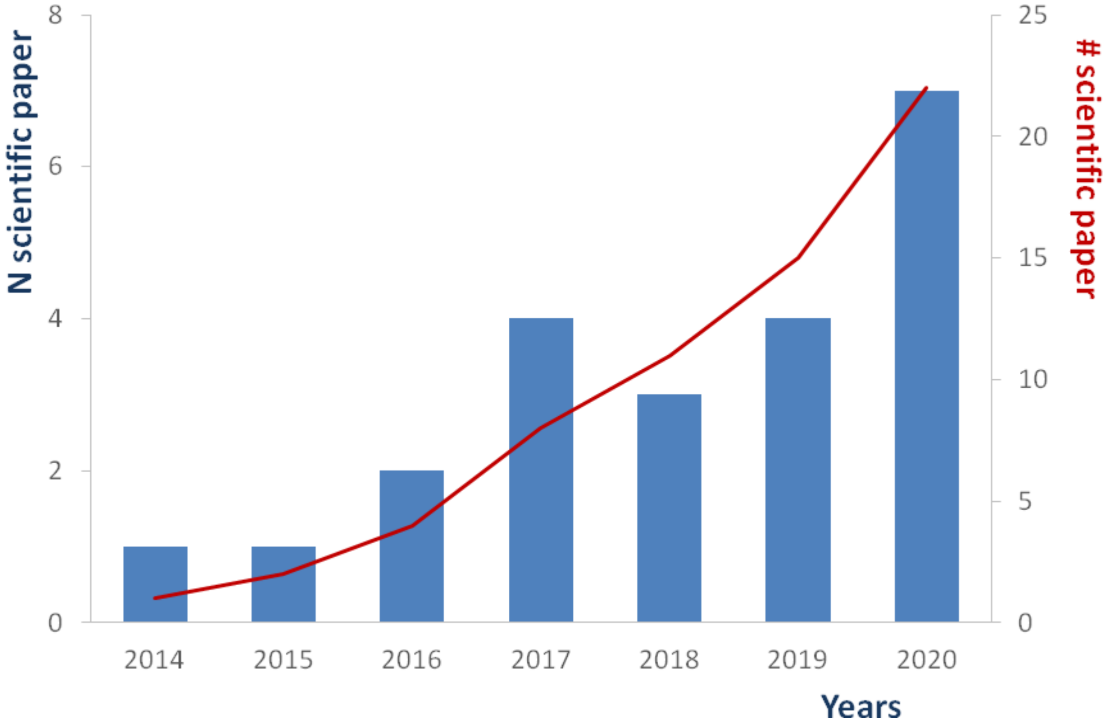


FIGURE 6.6. The number (N) and the cumulative number (#) of scientific papers published per year on microplastics in freshwater fish species.

Our results indicated moderate prevalence and abundance of microplastics in *S. vardarensis* specimens, which could be partially explained due to feeding habits of the species; Vardar chab is a column omnivorous feeder instead of bottom feeder or a predator which generally present higher consecrations of ingested microplastics (Silva-Cavalcanti et al., 2017). Almost one third of our fish specimens (35%) contained microplastics. Similar results have been reported also in the UK (33%; Horton et al., 2018), Malaysia (40%; Sarijan et al., 2019) and in the USA (45%; Peters and Bratton, 2016). In the pelagic habitats of both urbanized and non-urbanized streams, have been reported moderate percentages of microplastics in the herbivore-omnivore species trophic guild (Phillips and Bonner, 2015).

TABLE 6.6. Prevalence of microplastics (MPs) in wild freshwater fish species (excluding marine origin) per country in global scale.

Prevalence category	Country	Species number (abundance)	Prevalence (%)	Organs	References
Low	Belgium	1 (78)	9%	*gastrointestinal tract*	Slootmaekers et al., 2019
	USA	44 (419)	8.2	*gut*	Phillips and Bonner, 2015
	France	1 (186)	12	*gut*	Sanches et al., 2014
	Australia	1 (180)	19.4 ‡	*gut, gills*	Su et al., 2019
	Tanzania	2 (40)	20	*gastrointestinal tract*	Biginagwa et al., 2016
Medium	France	1 (60)	25	*gut, liver*	Collard et al., 2018
	UK	1 (64)	33	*gastrointestinal tract*	Hotron et al., 2018
	Greece	1 (32)	35	*gastrointestinal tract*	*Present study*
	Malaysia	6 (60)	40	*gastrointestinal tract*	Sarijan et al., 2019
	USA	1 (436)	44.9	*gut*	Peters and Bratton, 2016
High	South Africa	4 (174)	52	*whole fish*	Naidoo et al., 2020
	Poland	2 (389)	~54	*gut*	Kuśmierek and Popiołek, 2020
	Nigeria	8 (109)	69.7	*stomach*	Adeogun et al., 2020
	Thailand	8 (107)	72.9	*gastrointestinal tract*	Kasamesiri and Thaimuangphol, 2020
	Canada	5 (181)	73.5	*gut*	Campbell et al., 2017
	Egypt	2 (43)	75	*gastrointestinal tract*	Khan et al., 2020
	Brazil	1 (48)	83	*gut*	Silva-Cavalcanti et al., 2017
	USA	11* (74)	85	*gastrointestinal tract*	McNeish et al., 2018
	China	1 (11)	90.9	*gastrointestinal tract*	Yuan et al., 2019
	China	6 (20-40)	95.7	*gut*	Jabeen et al., 2017
Absolute	USA	2 (96)	100	*gut, gills*	Hurt et al., 2020
	South Korea	6 (6)	100	*gut,, gills, flesh*	Park et al., 2020
	Argentina	11 (87)	100	*gut*	Pazos et al., 2017

** fish taxa*

‡ plastic debris (MPs and items > 5mm)

In total 19 microplastics were found in 11 specimens of *S. vardarensis*. A research on a sister species of *S. vardarensis*, namely, *Squalius cephalus*, the European chub (Linnaeus, 1758), inhabiting a river near Paris, showed a similar microplastic prevalence of 25%, in fish gut content and liver (Collard et al. 2018). The European chub is already used as a common bio-indicator in European freshwaters for other pollutants, such as musk (Hájková et al., 2007), metals (Dragun et al., 2016), dioxins and polychlorobiphenyls (Pacini et al., 2013). The latter studies confirm that chub species can be generally used as bioindicators for many type of pollution as well as for microplastics (Collard et al. 2018).

In general, previous studies have found significant correlations between the concentrations of ingested microplastics and fish length (Peters and Bratton, 2016). According to Horton et al., (2018) fish individuals with larger length have higher energy demand and, thus, higher food intake, which results in a greater chance of direct and indirect ingestion of microplastics (Park et al., 2020). However, our results showed that there was no difference in total length as well as in total weight between specimens with and without microplastics ($p>0.05$). While the size of the sampled fish did not seem to be related to the concentration of microplastics which is also true in the Han River of South Korea, this could be similarly an effect of the limited and small number of the samples (Park et al., 2020).

The average number of microplastics per individual (the total the number of microplastics in examined tissues) in our specimens containing microplastics was relatively low compared to those reported in other freshwater research (e.g., up to 22 items/fish in Park et al. 2020 or 20 items/fish in McNeish et al. 2018 etc). However, fish species traits may play an important role in the ingestion of plastic debris (Jabeen et al., 2017; Pinheiro et al., 2017). Fish species inhabiting the benthic zone are related with higher ingestion of microplastic (Sanches et al., 2014; Silva-Cavalcanti et al., 2017), which could be explain partially the relative low abundance of microplastics in the gastrointestinal tracts of the omnivorous water column feeders of the *Squalius* genus.

The large “artificial surfaces” category of Kifssos Att. basin mainly includes urban fabric, which refers to the physical urban environment (building types, streetscapes, density and networks). A study of an urban river basin in Texas positively correlated the mean number of ingested microplastics by sunfish with the area of major roadways (Peters and Bratton, 2016). Hence, surface runoff, via motorways and major road network, could also be a contributing factor to the reported microplastic characteristic results.

The pollution of plastics (micro, meso and larger plastic debris) has been included in the European Marine Strategy Framework Directive (MSFD, 2008/56/EC), under the broader issue of marine liters, which includes plastics. In contrast, the Water Framework Directive (WFD, 20/60/EC) of inland water, refers abstract to anthropogenic pressures without to specifically denoting plastic pollution. On the other side of the coin, several other European Directives relate to sources of freshwater microplastic contamination, namely, the Directives on packaging waste (2004/12/EC), waste (2008/98/EC), landfills (1999/31/EC), urban wastewater (91/271/EEC), sewage sludge (86/278/EEC), and ship-source pollution (2005/35/ EC). Finally, European Commission addressed the issue of plastic waste as part of a wider review of its waste legislation (Wagner et al., 2014) focusing on potential mitigation strategies for plastic litter at the source, expressing also “particular concern” about microplastics in the environment (European Commission, 2013).

In terms of proportional abundance, the representation of non-indigenous fish species according to Koutsikos et al., (2018b) is low, although with the exception of some being incidentally adjacent to urban centers (e.g. cites of Athens, Thessaloniki, Ioannina etc.). Thus, we suggest the gastrointestinal tract of non-indigenous fish species could be used as a tool for the qualitative assessment of microplastics pollution, as they provide a suitable bio-indicator for freshwaters, especially for those ecosystems which are adjacent to large urban areas. Although more thorough research is needed to assess the microplastic contamination of river sediments and whether the microplastic ingestion could compromise the health of fish species or whether these effects are dependent on species traits, feeding habits and/or plastic type.

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Glossary

GLOSSARY

Acclimatized species: individuals of a species optimally adjusted to their new environment or physically/behaviorally sufficient for the new environment but incapable of reproducing or reproduction is occurring, but population not self-sustaining

Alien fish species: introduced species whose native distributional range did not include any river basin within inland waters of a given counrty

Alluvial/Sankey diagram: diagram which is typically used to display observation groups as flows across dimensions, time, types of processes or sets of features

Bioindicator: species, groups of species or biological communities whose presence, abundance and biological conditions are utilized to make inferences about the quality of the environment

Climate matching: the proccess that identifies suitable extralimital destinations that could be colonized by a potential non-indigenous species on the basis of similarity to climates found in the species' native range

Co-occurrence: the state in which two or more species co-occur among a set of spatial locations

Density: the number of individuals per unit area or volume

Ecoregion: an area defined in terms of its natural features and environment

Endemism: the ecological state of a species being unique to a defined geographic location

Fish assemblage: the variety of species and the abundance of these fish species in a given location/area

Frequency of occurrence: records of a species presence as percentage in a given unit

Habitat: the place in which a particular species of organism lives, derives its food, shelter, and mate for reproduction; including all living and nonliving factors or conditions of the surrounding environment

Inbreeding depression: the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals

Invasion phases/process: a series of stages (transport, introduction, establishment and spread), in each of which there are "barriers" that need to be overcome for a species or population to pass on to the next stage of a biological invasion

Invasive species: self-sustaining population of a species in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction, and an agent of change that threatens native biodiversity

Key indicator species: a species whose presence, absence or abundance demonstrates some aspect of the character or quality of an environment and reflects a specific environmental condition

Mean abundance: the mean composition of an organism of a particular kind per unit relative to the mean total number of organisms in the area

Mediterranean climate: the climate usually characterized by rainy winters and dry, warm to hot summers

Microplastics (MPs): plastic debris that are less than 5 mm in length from a variety of sources

Naturalized/Established species: a self-sustaining population of a species in the wild

Non-indigenous/Non-native fish species : alien and translocated fish species that have been introduced within inland waters of a given counrty

Outbreeding depression: the reduced biological fitness in a given population as a result of breeding between individuals from different populations, subspecies, or species

Pathways and vectors of introduction: the ways/routes (source) and transfer mechanisms (mode) responsible for the introduction and spread of non-indigenous species into new environments

Propagule pressure: a composite measure of introduction effort consisting of: (i) the number of individuals introduced per introduction event (propagule size); and (ii) the frequency of introduction events (propagule frequency or number)

Range restricted species: Species with a geographically restricted area of distribution

Rheophilic species: a species that prefers living in fast flowing waters

River basin: any area of land where precipitation collects and drains off into a common outlet, such as into a river, lake, or other water body

Species composition: the precent contribution of each species relative abundance to the total abundance of all species captured in the area

Species richness: the total number of species in a given location/area

Total abundance: the total number of a population in a given location/area

Translocated fish species: native fish species that have been introduced into a given catchment, within a given country, but outside their historical native range

Appendix A

TABLE A.I.1 Consolidated list of Invasive Alien Species (IAS) of Union concern; A. Plants and B. Animal. Bold denotes freshwater fish species.

A. Plants **Scientific name**	**Common name**	**Entry into force**
Acacia saligna (Acacia cyanophylla)	Golden wreath wattle	15 Aug. 2019
Ailanthus altissima	Tree of heaven	15 Aug. 2019
Alternanthera philoxeroides	Alligator weed	2 Aug. 2017
Andropogon virginicus	Broomsedge bluestem	15 Aug. 2019
Asclepias syriaca	Common milkweed	2 Aug. 2017
Baccharis halimifolia	Eastern baccharis	3 Aug. 2016
Cabomba caroliniana	Fanwort	3 Aug. 2016
Cardiospermum grandiflorum	Balloon vine	15 Aug. 2019
Cortaderia jubata	Purple pampas grass	15 Aug. 2019
Eichhornia crassipes	Water hyacinth	3 Aug. 2016
Elodea nuttallii	Nuttall's waterweed	2 Aug. 2017
Ehrharta calycina	Perrenial veldt grass	15 Aug. 2019
Gunnera tinctoria	Chilean rhubarb	2 Aug. 2017
Gymnocoronis spilanthoides	Senegal tea plant	15 Aug. 2019
Heracleum mantegazzianum	Giant hogweed	2 Aug. 2017
Heracleum persicum	Persian hogweed	3 Aug. 2016
Heracleum sosnowskyi	Sosnowsky's hogweed	3 Aug. 2016
Humulus scandens	Japanese hop	15 Aug. 2019
Hydrocotyle ranunculoides	Floating pennywort	3 Aug. 2016
Impatiens glandulifera	Indian balsam	2 Aug. 2017
Lagarosiphon major	Curly waterweed	3 Aug. 2016
Lespedeza cuneata	Chinese bushclover	15 Aug. 2019
Ludwigia grandiflora	Water-primrose	3 Aug. 2016
Ludwigia peploides	Floating primrose-willow	3 Aug. 2016
Lygodium japonicum	Vine-like fern	15 Aug. 2019
Lysichiton americanus	American skunk cabbage	3 Aug. 2016
Microstegium vimineum	Japanese stiltgrass	2 Aug. 2017
Myriophyllum aquaticum	Parrot's feather	3 Aug. 2016
Myriophyllum heterophyllum	Broadleaf watermilfoil	2 Aug. 2017
Parthenium hysterophorus	Whitetop weed	3 Aug. 2016
Pennisetum setaceum	Crimson fountaingrass	2 Aug. 2017
Persicaria perfoliata	Asiatic tearthumb	3 Aug. 2016
Prosopis juliflora	Mesquite	15 Aug. 2019
Pueraria lobata	Kudzu vine	3 Aug. 2016
Salvinia molesta (Salvinia adnata)	Salvinia moss	15 Aug. 2019
Triadica sebifera (Sapium sebiferum)	Chinese tallow	15 Aug. 2019

B. Animals **Scientific name**	**English name**	**Entry into force**
Acridotheres tristis	Common myna	15 Aug. 2019
Alopochen aegyptiacus	Egyptian goose	2 Aug. 2017
Arthurdendyus triangulates	New Zealand flatworm	15 Aug. 2019
Callosciurus erythraeus	Pallas' squirrel	3 Aug. 2016
Corvus splendens	Indian house crow	3 Aug. 2016
Eriocheir sinensis	Chinese mittencrab	3 Aug. 2016
Herpestes javanicus	Small Asian mongoose	3 Aug. 2016
Lepomis gibbosus	Pumpkinseed	15 Aug. 2019
Lithobates catesbeianus	American bullfrog	3 Aug. 2016
Muntiacus reevesi	Muntjac deer	3 Aug. 2016
Myocastor coypus	Coypu	3 Aug. 2016
Nasua nasua	Coati	3 Aug. 2016
Nyctereutes procyonoides	Raccoon dog	2 Feb. 2019
Ondatra zibethicus	Muskrat	2 Aug. 2017
Orconectes limosus	Spiny-cheek crayfish	3 Aug. 2016
Orconectes virilis	Virile crayfish	3 Aug. 2016
Oxyura jamaicensis	Ruddy duck	3 Aug. 2016
Pacifastacus leniusculus	Signal crayfish	3 Aug. 2016
Percottus glenii	Amur sleeper	3 Aug. 2016
Plotosus lineatus	Striped eel catfish	15 Aug. 2019
Procambarus clarkii	Red swamp crayfish	3 Aug. 2016
Procambarus fallax f. *virginalis*	Marbled crayfish	3 Aug. 2016
Procyon lotor	Raccoon	3 Aug. 2016
Pseudorasbora parva	Stone moroko	3 Aug. 2016
Sciurus carolinensis	Grey squirrel	3 Aug. 2016
Sciurus niger	Fox squirrel	3 Aug. 2016
Tamias sibiricus	Siberian chipmunk	3 Aug. 2016
Threskiornis aethiopicus	Sacred ibis	3 Aug. 2016
Trachemys scripta	Cumberland sliders	3 Aug. 2016
Vespa velutina nigrithorax	Asian hornet	3 Aug. 2016

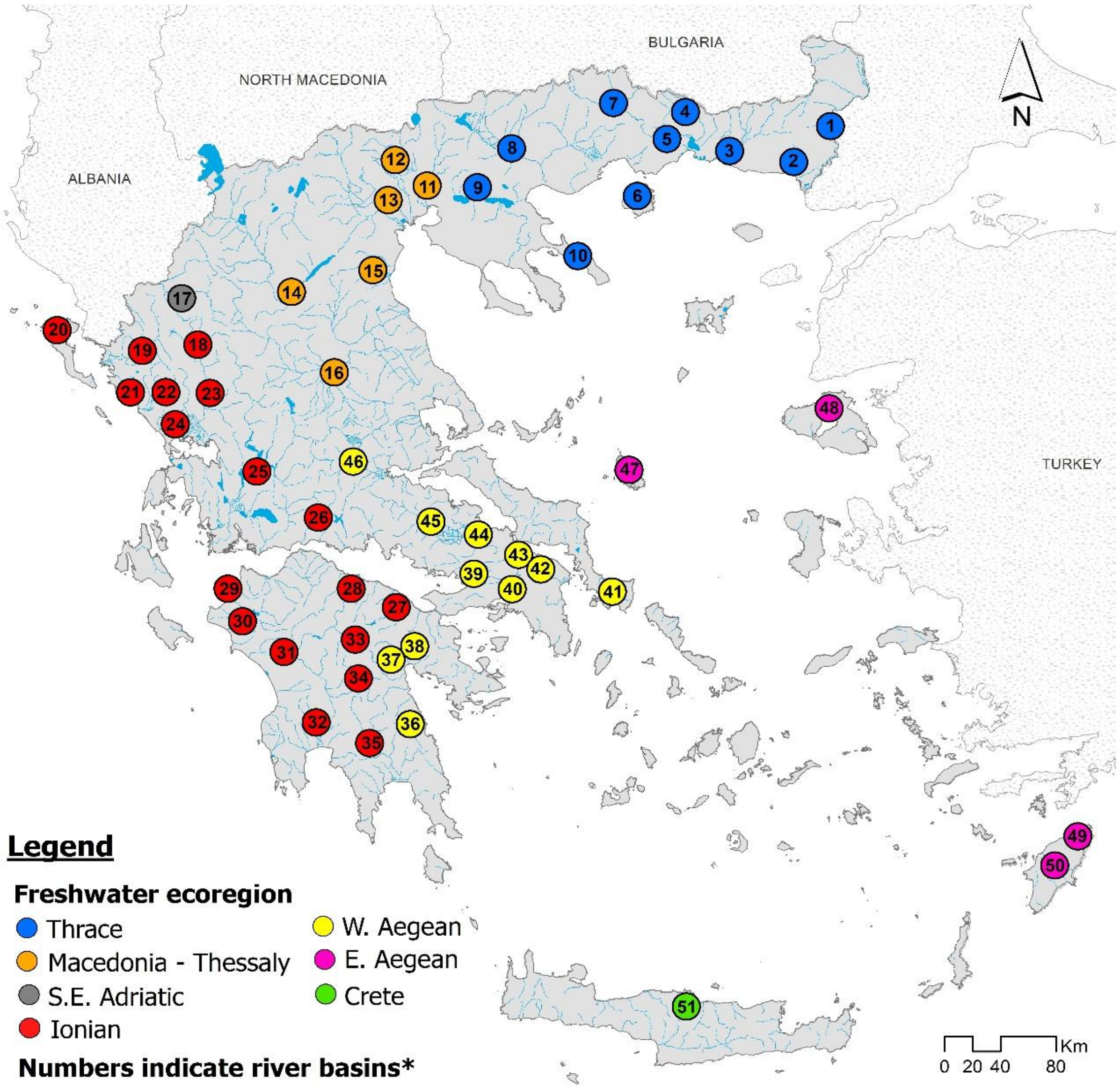

Figure A.3.1. River network of Greece and the 51 river basins per freshwater ecoregion used in the analysis.

***Thrace:** 1. Evros/Meric, 2. Apokrimno, 3. Bospos, 4. Kompsatos, 5. Kossynthos, 6. Thasos, 7. Nestos/Mesta, 8. Strymonas/Struma, 9. Volvi, 10. Agion Oros; **Macedonia-Thessaly:** 11. Gallikos, 12. Axios/Vardar, 13. Loudias, 14. Aliakmonas, 15. Mavroneri, 16. Pinios Thes.; **S.E. Adriatic:** 17. Aoos/Vjose; **Ionian:** 18. Pamvotida, 19. Kalamas, 20. Fonissa, 21. Kalodiki, 22. Acheron, 23. Arachthos, 24. Louros, 25. Acheloos, 26. Mornos, 27. Asopos Pel., 28. Krathis, 29. Vergas, 30. Pinios Pel., 31. Alfeios, 32. Pamisos, 33. Kandila, 34. Taka, 35. Evrotas; **W. Aegean:** 36. Dafnonas, 37. Lerni, 38. Inachos, 39. Psatha, 40. Kifisos Att., 41. Rigia, 42. Charadros, 43. Asopos Viot., 44. Yliki, 45. Kifisos Viot., 46. Spercheios; **E. Aegean:** 47. Skyros, 48. Lesvos, 49. Gadouras, 50. Kremastinos; **Crete:** 51. Almyros Irakliou.

Table A.3.1. Average dissimilarity (%) between network's modularity classes of non-indigenous fish assemblages according to Simper analysis.

Modularity class	0	1	2	3	4	5	6	7	8	9	10	11
0		77.2	83.3	100	100	100	84.1	100	100	100	100	54.8
1			76.7	100	100	100	81.6	100	100	100	100	87.7
2				100	100	100	86.7	100	100	100	100	89.7
3					100	100	100	100	100	100	100	100
4						100	99.4	100	100	100	100	100
5							100	100	100	100	100	100
6								100	100	100	100	89.2
7									100	100	100	100
8										100	100	100
9											100	100
10												100
11												

Table A.3.2. The Biotic and environmental factors excluded from the ordination analysis of the study according to Spearman correlation coefficient (rho>0.75).

Excluded Factors	Jan Temp	Jul Temp	Dist. source	Native Rich	Pearson (D)	Fine Sub	Fast flow	Forest
Mean Temp*	0,89	0,85						
UpArea*			0,80					
Shannon (H')*				0,78	-0,92			
Coarse Sub*						-0,88		
Slow flow*							-0,78	
Agricultural								-0,89

**: mean annual air temperature; upstream catchment area; Shannon Index; agricultural land cover; coarse substrate and slow-flow habitats)*

Table A.4.1 Non-indigenous occurrences of sailfin molly (*Poecilia latipinna*) by Continent/Country.

Continent/Country	Occurrences	ISO 3166 Codes	References*
Americas			
Bahamas	2	BS	[5, 39]
Brazil	2	BR	[4, 23, 24]
Canada	1	CA	[14]
Colombia	1	CO	[35, 41]
Dominican Republic	1	DO	[17]
Mexico	6	MX	[31, 36]
Puerto Rico	4	PR	[6, 30]
United States of America	41	US	[30]
Venezuela	2	VU	[32]
Asia			
Bahrain	1	BH	[13]
China	1	CN	[28, 42]
Guam	1	GU	[10]
India	2	IN	[38]
Indonesia	1	ID	[9, 19]
Iran	3	IR	[18, 12]
Iraq	1	IQ	[15]
Northern Mariana Islands (US)	1	MP$_{(US)}$	[16, 30]
Oman	2	OM	[34]
Philippines	5	PH	[13, 25]
Saudi Arabia	2	SA	[1, 2]
Singapore	1	SG	[7, 29]
Taiwan	1	TW	[43]
Thailand	1	TH	[40]
Europe			
Greece	1	GR	[20]
Oceania			
Australia	5	AU	[3, 8, 21]
Fiji	1	FJ	[37]
Hawai (US)	9	HI$_{(US)}$	[11, 22, 30, 33]
New Zealand	1	NZ	[26, 27]

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Table A.4.2. Site details of the 100 non-indigenous occurrences of sailfin molly (*Poecilia latipinna*). In few occasions, no specific coordinates were given in the available literature, hence, in those cases, we provide coordinates marking the middle of each country (denoted by asterisk). Geographic coordinates are expressed in decimal degrees.

Country	Region	Locality	Latitude	Longtitude
Australia	Brisbane & Harvey Bay	Streams and rivers around Hervey Bay	25.27° S	152.58° E
Australia	Northern Territory	waters in the vicinity of Darwin	12.84° S	131.00° E
Australia	Queensland	Burrum River near Buxton town	25.29° S	152.87° E
Australia	Queensland	Ross river	19.32° S	146.74° E
Australia	Sangate	Dowse Lagoon	27.31° S	153.06° E
Bahamas	New Providence	Lake Cunningham	25.07° N	77.42° W
Bahamas	New Providence	Lake Killarney	25.05° N	77.45° W
Bahrain	Sitra Island	Sitra Island	26.1° N	50.55° E
Brazil	Sobradinho region	São Francisco River	22.3° S	47.38° W
Brazil	Vieiras municipality	Santo Antonio Creek	20.56° S	42.17° W
Canada	Alberta	Thermal spring in Banff National Park	51.1° N	115.17° W
China	*no specific region record*	*no specific locality record*	35.93° N	103.93° E
Colombia	Northwest Colombia	Magdalena watershed	2.41° N	75.43° W
Dominican Republic*	*no specific region record*	*no specific locality record*	19.04° N	70.51° W
Fiji*	*no specific region record*	*no specific locality record*	17.81° S	177.95° E
Greece	Attica, Athens	geothermal Lake Vouliagmneni	37.81° N	23.79° E
Guam	Santa Rita town	Fena Reservoir	13.36° N	144.7° E
India	Tamil Nadu, Chennai	Kolathur	13.12° N	80.21° E
India	Tamil Nadu, Chennai	Perugalathur	12.91° N	80.09° E
Indonesia	Java Island	Sundaland hotspot	8.64° S	120.87° E
Iran	Isfahan Province	Zayandehrud Basin, Jarghoyeh qanat	32,36° N	52,76° E
Iran	Isfahan Province	Zayandehrud Basin, Malvajerd	32,07° N	52,59° E
Iran	Khuzestan Province, SE Abadan	Tigris River Basin, Arvand River, Choibdeh canal	30,21° N	48,56° E
Iraq	Basrah Province	east Al-Hammar Marsh	30.71° N	47.59° E
Mexico	Baja California	irrigation channel between Ejido Nayarit and Ejido Sonora	32.29° N	115.26° W
Mexico	Baja California	Laguna Salada at Paraíso fishery camp	32.63° N	115.65° W

Country	Region	Locality	Latitude	Longtitude
Mexico	Baja California	Río Colorado at Ejido Yucumuri	32.08° N	115.20° W
Mexico	Baja California	Río El Mayor at Campo Sonora	32.00° N	115.30° W
Mexico	Baja California	Río Hardy at Campo Mosqueda	32.08° N	115.20° W
Mexico	California gulf	California gulf	31.69° N	113.75° W
New Zealand	North Island	Geothermal wetlands, southern end of Lake Taupo	38.95° S	175.75° E
Oman	Arabian Gulf	Arabian Gulf	26.02° N	56.18° E
Oman	Gulf of Oman	Gulf of Oman	23.58° N	57.6° E
Philippines	Manila Bay	salt ponds of Cavite	14.46° N	120.9° E
Philippines	Manila Bay	saltwater fishponds of Bulacam	14.79° N	120.86° E
Philippines	Manila Bay	saltwater fishponds of Malabon	14.66° N	120.96° E
Philippines	Manila Bay	saltwater fishponds of Malolo	14.83° N	120.8° E
Philippines	Manila Bay	saltwater fishponds of Obando	14.72° N	120.92° E
Puerto Rico	Aguas Buenas	*no specific locality record*	18.24° N	66.11° W
Puerto Rico	Hato Puerco Ward	Canovanillas River	18.38° N	65.91° W
Puerto Rico	Platas Ward	Lajas Irrigation Canal	18.02° N	66.97° W
Puerto Rico	San Lorenzo city	a creek tributary of the Loiza River	18.18° N	65.95° W
Saudi Arabia	Eastern Province	Al-Qatif to Al-Hofuf region (Persian gulf)	26.67° N	50.05° E
Saudi Arabia	Riyadh Province	Wadi Haneefah stream	24.47° N	46.42° E
Singapore	NW coast of the main Singapore island	Sungei Buloh Wetland Reserve	1.45° N	103.73° E
Taiwan	Linyuan District	at the lower part (mouth) of Ai River, near Kaohsiung	22.49° N	120.38° E
Thailand*	*no specific region record*	*no specific locality record*	14.06° N	100.06° E
US Commonwealth	Northern Mariana Islands	Saipan, Garapan Drainage	15.15° N	145.71° E
USA	Arizona	Colorado River area below Imperial Dam	32.88° N	114.46° W
USA	Arizona	Gila River at Painted Rock Dam west of Gila Bend	33.07° N	112.99° W
USA	Arizona	Gila River from Colorado River to Phoenix	32.81° N	113.71° W
USA	Arizona	Gila River	33.09° N	113.71° W
USA	Arizona	lower Gila basin in canals and wastewater ponds	32.68° N	114.29° W
USA	Arizona	lower Salt River	33.42° N	112.08° W

Country	Region	Locality	Latitude	Longtitude
USA	Arizona	Phoenix metropolitan area	33.45° N	112.08° W
USA	Arizona	Roosevelt Lake on Salt River	33.67° N	111.14° W
USA	Arizona	Salt River between Stewart Mtn. Dam and Granite Reef Diversion Dam	33.56° N	111.53° W
USA	Arizona	Salt River in the vicinity of Phoenix	33.52° N	112.07° W
USA	Arizona	Verde River and downstream in canal systems	33.55° N	111.66° W
USA	California	canals around Salton Sea	33.27° N	115.91° W
USA	California	ponds and canals in Southern CA	33.19° N	116.08° W
USA	California	Salton Sea	33.33° N	115.83° W
USA	California	southern tribs to the Salton Sea	33.09° N	115.72° W
USA	California	springs of Death Valley	36.54° N	116.96° W
USA	California	Sweetwater Marsh adjacent to San Diego Bay	32.64° N	117.11° W
USA	Colorado	canals, ditches and ponds below the Weisbart farm	37.33° N	105.81° W
USA	Colorado	San Luis Valley, Rio Grande headwaters drainage	37.7° N	105.89° W
USA	Hawai	Hawaii	19.59° N	155.58° W
USA	Hawai	Kauai	21.98° N	159.59° W
USA	Hawai	Maui	20.79° N	156.27° W
USA	Hawai	Molokai	21.13° N	156.99° W
USA	Hawai	Molokai	21.09° N	157.06° W
USA	Hawai	Oahu, in Bellows	21.36° N	157.71° W
USA	Hawai	Oahu	21.37° N	158.02° W
USA	Hawai	Oahu, in Ordy's pond at Barbers Point	21.31° N	159.05° W
USA	Hawai	Oahu, in Nu'uanu reservoir	21.4° N	157.86° W
USA	Montana	Beaverhead Ditch	45.38° N	112.76° W
USA	Montana	Trudau Pond	45.23° N	112.13° W
USA	Montana	Upper Yellowstone River	47.69° N	104.11° W
USA	Montana	warm spring at Beaverhead Rock	45.38° N	112.46° W
USA	New Mexico	lower Rio Grande	32.28° N	106.83° W
USA	Nevada	Alamo, ash Springs	37.46° N	115.19° W

Country	Region	Locality	Latitude	Longtitude
USA	Nevada	Blue Point Spring near Lake Mead	36.39° N	114.43° W
USA	Nevada	Bradford Spring in Ash Meadows	36.4° N	116.3° W
USA	Nevada	Colorado drainage	34.16° N	114.44° W
USA	Nevada	Death Valley, springs	36.48° N	117.1° W
USA	Nevada	Indian Springs	36.56° N	115.67° W
USA	Nevada	Jack Rabbit Spring in Ash Meadows	36.39° N	116.28° W
USA	Nevada	lower Virgin River	36.5° N	114.34° W
USA	Nevada	Meadow Valley Wash drainage, Lincoln County	37.83° N	114.35° W
USA	Nevada	Moapa (Muddy) River drainage	36.61° N	114.74° W
USA	Nevada	Pahranagat Valley	37.25° N	115.12° W
USA	Nevada	Point of Rocks Spring in Ash Meadows	36.4° N	116.27° W
USA	Nevada	Rogers Spring (Overton Arm of Lake Mead)	36.36° N	114.43° W
USA	Nevada	several springs in Ash Meadows National Wildlife Refuge	36.43° N	116.35° W
USA	Nevada	warm springs flowing into Lake Mead in southern NV	36.13° N	114.14° W
USA	Texas	San Antonio River, spring-influenced central Texas headwaters	32.38° N	99.78° W
USA	Utah	Spring ponds North of the Great Salt Lake	41.69° N	112.83° W
Venezuela*	Nueva Esparta	*no specific locality record*	10.57° N	64.00° W
Venezuela	Carupano	Small ditches 3km west of Carupano	10.67° N	63.38° W

Table A.5.1. Abbreviation and spatial occurrence within river drainages of all species included in the RDA analysis.

Species	Abbreviation	Acheloos	Alfeios	Aliakmon	Aoos	Arachthos	Asopos Pel.	Dafnonas	Evinos	Evrotas	Kalamas	Krathis	Louros	Nestos	Pamisos	Prespa	Spercheios
Alburnoides economoui	*Aeco*																●
Alburnoides prespensis	*Apre*				●												
Alburnoides thessalicus	*Athe*			●													
Alburnus scoranza	*Asco*				●												
Anguilla anguilla	*Aang*				●				●	●	●		●		●		
Barbus balcanicus	*Bbal*			●													
Barbus macedonicus	*Bmac*			●													
Barbus peloponnesius	*Bpel*	●	●			●			●		●				●		
Barbus prespensis	*Bpre*				●												
Barbus sperchiensis	*Bspe*																●
Barbus strumicae	*Bstr*													●			
Caspiomyzon graecus	*Cgra*												●				
Chondrostoma vardarense	*Cvard*			●	●												
Cobitis hellenica	*Chel*												●				
Cobitis ohridana	*Cohr*				●												
Economidichthys pygmaeus	*Epygm*												●				
Gasterosteus gymnurus	*Ggym*												●		●		
Gobio bulgaricus	*Gbul*			●													
Gobio skadarensis	*Gska*				●												
Luciobarbus albanicus	*Lalb*	●				●			●		●		●				

Species	**Abbreviation**	**Acheloos**	**Alfeios**	**Aliakmon**	**Aoos**	**Arachthos**	**Asopos Pel.**	**Dafnonas**	**Evinos**	**Evrotas**	**Kalamas**	**Krathis**	**Louros**	**Nestos**	**Pamisos**	**Prespa**	**Spercheios**
Luciobarbus graecus	*Lgre*																●
Oncorhynchus kisutch	*Okis*		●														
Oncorhynchus mykiss	*Rainbow*		●	●	●	●	●	●		●		●	●		●		●
Oxynoemacheilus pindus	*Opind*				●												
Pachychilon pictum	*Ppict*				●												
Pelasgus laconicus	*Plac*									●							
Pelasgus marathonicus	*Pmar*																●
Pelasgus sp.	*Pelasgus sp*						●										
Pelasgus stymphalicus	*Pstym*		●						●								
Pelasgus thesproticus	*Pthe*												●				
Pseudorasbora parva	*Pparv*	●															
Romanogobio elimeius	*Reli*			●													
Rutilus panosi	*Rpan*	●															
Salaria fluviatilis	*Sflu*	●															
Salmo farioides	*Native*	●	●		●	●			●		●						●
Salmo lourosensis	*Native*												●				
Salmo macedonicus	*Native*													●			
Salmo pelagonicus	*Native*			●													
Salmo peristericus	*Native*															●	
Salmo trutta	*Stru*		●														
Squalius keadicus	*Skea*									●							
Squalius orpheus	*Sorph*													●			

Species	Abbreviation	Acheloos	Alfeios	Aliakmon	Aoos	Arachthos	Asopos Pel.	Dafnonas	Evinos	Evrotas	Kalamas	Krathis	Louros	Nestos	Pamisos	Prespa	Spercheios
Squalius pamvoticus	*Spam*					●							●				
Squalius peloponensis	*Spel*	●	●				●	●	●			●			●		
Squalius sp. Aoos	*SspAoos*				●												
Squalius vardarensis	*Svard*			●													●
Telestes pleurobipunctatus	*Tpleu*	●	●			●			●		●		●				
Tropidophoxinellus spartiaticus	*Tspar*									●					●		

Table A.5.2. The co-occurrence of rainbow and native trout.

Site	Species	Abundance	Density			Total density (ind*ha^{-1})
			>20 cm	10<X<20 cm	<10 cm	
ARAP_UP	*O. mykiss*	51	144	337	10	490
	Native trout	41	77	317	0	394
ARAP_UP	*O. mykiss*	17	0	360	320	680
	Native trout	35	40	520	840	1400
ARAP_UP	*O. mykiss*	44	143	556	0	698
	Native trout	22	127	222	0	349
GORGO_DW	*O. mykiss*	2	0	0	40	40
	Native trout	28	40	440	80	560
GORGO_DW	*O. mykiss*	1	0	0	25	25
	Native trout	101	75	725	1725	2525
GORGO_CONFL	*O. mykiss*	12	15	59	103	176
	Native trout	9	0	44	88	132
GOURA	*O. mykiss*	1	0	50	0	50
	Native trout	1	50	0	0	50
MG	*O. mykiss*	5	0	100	67	167
	Native trout	18	67	333	233	633
REDSTONE	*O. mykiss*	2	63	0	0	63
	Native trout	21	219	188	250	656
VOID_AOOS	*O. mykiss*	3	23	0	0	23
	Native trout	56	92	31	308	431
SM	*O. mykiss*	2	36	36	0	71
	Native trout	39	393	929	71	1393
TIMIOS	*O. mykiss*	3	38	0	0	38
	Native trout	1	13	0	0	13

Table A.6.1. Studies on microplastics contamination in freshwater fish populations.

Family	Species	Authority	Freshwater system	Country	Reference*
Ailiidae					
	Laides longibarbis	(Fowler, 1934)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
Anabantidae					
	Anabas testudineus	(Bloch, 1792)	River	Malaysia	Sarijan et al., 2019
Bagridae					
	Bagrus bayad	(Forsskål, 1775)	River	Egypt	Khan et al., 2020
	Hemibagrus spilopterus	Ng & Rainboth, 1999	River	Thailand	Kasamesiri & Thaimuangphol, 2020
Callichthyidae					
	Hoplosternum littorale	Hancock, 1828	River	Brazil	Silva-Cavalcanti et al., 2017
Catostomidae					
	Carpoides cyprinus	(Lesueur, 1817)	River	USA	McNeish et al., 2018
	Catostomus commersoni	(Lacepède, 1803)	River	Canada, USA	Campbell et al., 2017; McNeish et al., 2018
Centrarchidae					
	Lepomis auritus	Linnaeus, 1758	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Lepomis cyanellus	Rafinesque, 1819	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Lepomis humilis	Girard, 1858	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Lepomis macrochirus	Rafinesque, 1819	Estuary, River, Lake	USA, South Korea	Phillips & Bonner, 2015; Peters & Bratton, 2016, Park et al., 2020
	Lepomis megalotis	Rafinesque, 1820	Estuary, River, Lake	USA	Phillips & Bonner, 2015; Peters & Bratton, 2016
	Lepomis microlophus	Günther, 1859	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Micropterus salmoides	(Lacepède, 1802)	Estuary, River, Lake	USA, South Korea	Phillips & Bonner, 2015; Hurt et al., 2020; Park et al., 2020
	Micropterus sp.	-	River	USA	McNeish et al., 2018
Channidae					

Family	Species	Authority	Freshwater system	Country	Reference*
	Channa argus	Cantor, 1842)	River	South Korea	Park et al., 2020
	Parachanna obscura	(Günther, 1861)	Lake	Nigeria	Adeogun et al., 2020
Characidae					
	Astyanax mexicanus	De Filippi, 1853	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Astyanax rutilus	(Jenyns, 1842)	Estuary	Argentina	Pazos et al., 2017
	Oligosarcus oligolepis	(Steindachner, 1867)	Estuary	Argentina	Pazos et al., 2017
Cichlidae					
	Coptodon zillii	(Gervais, 1848)	Lake	Nigeria	Adeogun et al., 2020
	Hemichromis fasciatus	Peters, 1857	Lake	Nigeria	Adeogun et al., 2020
	Herichthys cyanoguttatus	Baird & Girard, 1854	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Oreochromis aureus	Steindachner, 1864	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Oreochromis mossambicus	(Peters, 1852)	Estuary	Malaysia, South Africa	Naidoo et al., 2020; Sarijan et al., 2019
	Oreochromis niloticus	(Linnaeus, 1758)	River, Lake	Egypt, Tanzania	Biginagwa et al., 2016; Khan et al., 2020
	Sarotherodon melanotheron	Rüppell, 1852		Nigeria	Adeogun et al., 2020
Clariidae					
	Clarias gariepinus	(Burchell, 1822)	River	Malaysia	Sarijan et al., 2019
Claroteidae					
	Chrysicthys nigrodigitatus	(Lacépède, 1803)	Lake	Nigeria	Adeogun et al., 2020
Clupeidae					
	Dorosoma cepedianum	(Lesueur, 1818)	Estuary, River, Lake	USA	Phillips & Bonner, 2015; McNeish et al., 2018; Hurt et al., 2020
	Dorosoma petenense	Günther, 1867	Estuary, River, Lake	USA	Phillips & Bonner, 2015
Curimatidae					
	Cyphocharax voga	Hensel, 1836	Estuary	Argentina	Pazos et al., 2017

Family	Species	Authority	Freshwater system	Country	Reference*
Cyprinidae					
	Campostoma anomalum	Rafinesque, 1820	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Carassius auratus	Linnaeus, 1758	Lake	China	Jabeen et al., 2017; Yuan et al., 2019
	Carassius cuvieri	Temminck & Schlegel, 1846	River	South Korea	Park et al., 2020
	Cyclochelichthys apogon			Malaysia	Sarijan et al., 2019
	Cyclochelichthys repasson	(Bleeker, 1853)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Cyprinella lutrensis	Baird & Girard, 1853	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Cyprinella spiloptera	(Cope, 1865)	River	USA	McNeish et al., 2018
	Cyprinella venusta	Girard, 1856	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Cyprinus carpio	Linnaeus, 1758	Estuary, Lake	Argentina, China, South Korea	Jabeen et al., 2017; Pazos et al., 2017; Park et al., 2020
	Gobio gobio	Linnaeus, 1758	River	Belgium, France, Poland	Sanches et al., 2014; Kuśmierek & Popiołek, 2020, Slootmaekers et al., 2020
	Henicorhynchus siamensis	(Sauvage, 1881)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Hemiculter bleekeri	Warpachowski, 1888	Lake	China	Jabeen et al., 2017
	Hypophthalmichthys molitrix	Valenciennes, 1844	Lake	China	Jabeen et al., 2017
	Labeo chrysophekadion	(Bleeker, 1849)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Labiobarbus siamensis	(Sauvage, 1881)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Megalobrama amblycephala	Yih, 1955	Lake	China	Jabeen et al., 2017
	Mystus bocourti	(Bleeker, 1864)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Notropis amabilis	(Girard, 1856)	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Notropis atherinoides	Rafinesque, 1820	River	Canada, USA	Campbell et al., 2017; McNeish et al., 2018
	Notropis hudsonius	(Clinton, 1824)	River	USA	McNeish et al., 2018

Family	Species	Authority	Freshwater system	Country	Reference*
	Notropis sabinae	Jordan & Gilbert, 1886	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Notropis stramineus	(Cope, 1865)	Estuary, River, Lake	USA	Phillips & Bonner, 2015; McNeish et al., 2018
	Notropis volucellus	(Cope, 1865)	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Pimephales vigilax	Baird & Girard, 1853	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Pimephales promelas	Rafinesque, 1820	River	Canada, USA	Campbell et al., 2017; McNeish et al., 2018
	Puntioplites proctozyson	(Bleeker, 1865)	River	Thailand	Kasamesiri & Thaimuangphol, 2020
	Pseudorasbora parva	Temminck and Schlegel, 1846	Lake	China	Jabeen et al., 2017
	Rutilus rutilus	(Linnaeus, 1758)	River	UK, Poland	Hotron et al., 2018; Kuśmierek & Popiołek, 2020
	Squalius cephalus	(Linnaeus, 1758)	River	France	Collard et al., 2018
	Squalius vardarensis	Karaman, 1928	River	Greece	Present study
<u>Esocidae</u>					
	Esox lucius	Linnaeus, 1758	River	Canada	Campbell et al., 2017
<u>Fundulidae</u>					
	Fundulus diaphanus	(Lesueur, 1817)	River	USA	McNeish et al., 2018
	Fundulus notatus	Rafinesque, 1820	Estuary, River, Lake	USA	Phillips & Bonner, 2015
<u>Gasterosteidae</u>					
	Culaea inconstans	(Kirtland, 1840)	River	Canada	Campbell et al., 2017
<u>Gobiidae</u>					
	Neogobius melanostomus	(Pallas, 1814)	River	USA	McNeish et al., 2018
<u>Eleotridae</u>					
	Oxyeleotris marmorata	(Bleeker, 1852)	River	Malaysia	Sarijan et al., 2019
<u>Hepsetidae</u>					
	Hepsetus odoe	(Bloch, 1794)	Lake	Nigeria	Adeogun et al., 2020
<u>Ictaluridae</u>					
	Ameiurus natalis	Lesueur, 1819	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Ictalurus punctatus	Rafinesque, 1818	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Noturus gyrinus	Mitchill, 1817	Estuary, River, Lake	USA	Phillips & Bonner, 2015

Family	Species	Authority	Freshwater system	Country	Reference*
Latidae					
	Lates niloticus	(Linnaeus, 1758)	Lake	Tanzania, Nigeria	Biginagwa et al., 2016; Adeogun et al., 2020
Loricariidae					
	Hypostomus commersoni	Valenciennes, 1836	Estuary	Argentina	Pazos et al., 2017
Pangasiidae					
	Pangasius hypophthalmus	(Sauvage, 1878)	River	Malaysia	Sarijan et al., 2019
Percidae					
	Etheostoma artesiae	Hay, 1881	Estuary, River, Lake	USA	Phillips & Bonner, 2015
Pimelodidae					
	Luciopimelodus pati	(Valenciennes, 1835)	Estuary	Argentina	Pazos et al., 2017
	Parapimelodus valenciennis	(Lütken, 1874)	Estuary	Argentina	Pazos et al., 2017
	Pimelodus maculatus	Lacepéde, 1803	Estuary	Argentina	Pazos et al., 2017
	Pseudoplatystoma corruscans	(Spix & Agassiz, 1829)	Estuary	Argentina	Pazos et al., 2017
Poeciliidae					
	Gambusia affinis	Baird & Girard, 1853	Estuary, River, Lake	USA	Phillips & Bonner, 2015
	Gambusia holbrooki	Girard, 1859	Wetlands	Australia	Su et al., 2019
Prochilodontidae					
	Prochilodus lineatus	(Valenciennes, 1836)	Estuary	Argentina	Pazos et al., 2017
Siluridae					
-	*Silurus asotus*	Linnaeus, 1758	River	South Korea	Park et al., 2020

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Appendix B

List of scientific publications broadly associated with PhD subject (e.g.: “non-indigenous fish species”, “freshwater introductions”, “aquatic invasions”, “invasion risk assessments” etc.) produced either through the thesis *per se* or by further collaboration with several experts within the field of invasion biology, during the period 2016-2020. The five papers (four published and one submitted) based on or directly related to the PhD work are marked with an asterisk (*).

1. Perdikaris C., **Koutsikos N.**, Vardakas L., Kommatas D., Simonović P., Paschos I., Detsis V., Vilizzi L. and Copp G.H. **2016**. Risk screening of non native, translocated and traded aquarium freshwater fish in Greece using FISK. *Fisheries Management and Ecology,* 23: 32-43.

2. Piria M., Copp G.H., Dick J.T., Duplić A., Groom Q., Jelić, D., Groom Q., Jelić D., Lucy F.E., Roy H.E., Sarat E., Simonović P., Tomljanović T., Tricarico E., Weinlander M., Adámek Z., Bedolfe S., Coughlan N.E., Davis E., Dobrzycka-Krahel A., Grgić Z., Kırankaya Ş.G., Ekmekçi G.F., Lajtner J., Lukas J.A.Y., **Koutsikos N.,** Mennen G.J., Mitić B., Pastorino P., Ruokonen T.J., Skóra M.E., Smith E.R.C., Šprem N., Tarkan A.S., Treer T., Vardakas L., Vehanen T., Vilizzi L., Zanella D. and Caffrey J.M. **2017**. Tackling invasive alien species in Europe II: threats and opportunities until 2020. *Management of Biological Invasions,* 8(3): 273-286.

3. **Koutsikos N.,** Economou A.N., Vardakas L., Kommatas D. and Zogaris S. **2017**. First confirmed record of an established population of sailfin molly, Poecilia latipinna (Actinopterygii: Cyprinodontiformes: Poeciliidae) in Europe. *Acta Ichthyologica et Piscatoria,* 43(3): 311-315.

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5. *** Koutsikos N.**, Vardakas L., Kalogianni E. and Economou A.N. **2018.** Global distribution and climatic match of a highly traded ornamental freshwater fish, the sailfin molly *Poecilia latipinna* (Lesueur**,** 1821). *Knowledge and management* of *aquatic ecosystems,* 419, 23.

6. *** Koutsikos N.**, Vardakas L., Zogaris S., Perdikaris C. Kalantzi O.I. and Economou A.N. **2019.** Does rainbow trout justify its high rank among alien invasive species? Insights from a nationwide survey in Greece. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 29(3): 409-423.

7. *** Koutsikos N.,** Zogaris S., Vardakas L., Kalantzi O.I., Dimitriou E. and Economou A.N. **2019.** Tracking non-indigenous fishes in lotic ecosystems: Invasive patterns at different spatial scales in Greece. *Science of the Total Environment,* 659: 384-400.

8. Vilizzi L., Copp G.H., Adamovich B., Almeida D., Chan J., Davison P.I., Dembski S., Ekmekçi F.G., Ferincz Ă., Forneck S.C., Hill J.E., Kim J.E., **Koutsikos N.,** Leuven R.S.E.W., Luna S.A., Magalhães F., Marr S.M., Mendoza R., Mourão C.F., Neal J.W., Onikura N., Perdikaris C., Piria M., Poulet N., Puntila R., Range I.L., Simonović P., Ribeiro F., Tarkan A.S., Troca D.F.A., Vardakas L., Verreycken H., Vintsek L., Weyl O.L.F., Yeo D.C.J. and Zeng Y. **2019.** A global review and meta-analysis of applications of the freshwater Fish Invasiveness Screening Kit. *Reviews in Fish Biology and Fisheries,* 1-40.

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10. * Koutsikos N., Vavalidis Th., Kalogianni, E., Vardakas L., Kalantzi O.I., Dimitriou E. and Zogaris S. **(submitted)**. *Defining non-indigenous fish assemblages types in Mediterranean rivers: Network analysis and management implications.*

11. Tricarico E., Tarkan A.S., Vilizzi L., Bilge G., Ekmekçi F.G., Filiz H., Giannetto D., İlhan A., Killi N., Kırankaya Ş.G., **Koutsikos N.,** Kozic S., Kurtul I., Lazzaro L., Marchini A., Occhipinti-Ambrogi A., Perdikaris C., Piria M., Pompei L., Sarı H., Smeti E., Stasolla G., Top N., Tsiamis K., Vardakas L., Yapıcı S., Yoğurtçuoğlu B. and Copp G.H. **(submitted)**. *Risk of invasiveness of non-native aquatic species in the eastern Mediterranean under current and projected climate conditions.*

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