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## Developing policy-relevant river fish monitoring in Greece: Insights from a nationwide survey

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### Abstract

A wide-ranging river fish survey was executed in the summer of 2009 as part of the preparatory actions for the establishment of a monitoring programme for the EU Water Framework Directive (WFD). This was the first extensive electrofishing campaign for WFD standardised bioassessment in Greece and the experience and insights gained are used here to provide a review of fish-based assessment conditions and requirements in this country. The survey sampled 85 sites on 25 rivers throughout mainland Greece, collecting 70 species of freshwater fish. Quantitative site-based assemblage data is used for taxonomic and ordination analyses revealing a strong biogeographic regionalisation in the distribution of the ichthyofauna. The structural and spatial organisation of the fish fauna using species-level and community-level data analyses is explored in three ecoregions where data was deemed sufficient. Transitions in community taxonomic composition among ecoregions were abrupt and concordant with geographical barriers, reflecting the influence of historical biogeographic processes. Community-based analysis revealed a substantial degree of variation in quantitative attributes of the fish assemblages among ecoregions. Key conclusions of this work are: (a) the fish-based bioassessment system must be regionalised to reflect biogeographic variation; (b) high faunal heterogeneity among ecoregions (taxonomic, structural), and to a lower degree among basins, constrains the transferability of bioassessment metrics and indices created for explicit regions to other regional frameworks; (c) faunal depauperation in most of the study areas reduces the utility of functional bioassessment metrics and also limits the utilisation of rare species and the applicability of the classical form of the “Index of Biotic Integrity” concept. Recommendations to cope with these problems are discussed.

**Keywords:** Bioassessment, freshwater fishes, Water Framework Directive, Mediterranean rivers, biogeographical regionalisation.

### Introduction

The EU Water Framework Directive (WFD) requires member states to establish biological monitoring programmes for the purpose of systematically assessing the ecological status of surface waters and determining the level of human impact on ecosystems. The WFD Article 8 specified that monitoring programmes should be launched by the end of the year 2006 and summary reports providing an overview of the monitoring network and procedures should be submitted to the Commission by March 2007. Greece failed to follow these deadlines, and in June 2008 the European Commission initiated an infringement procedure for non-compliance (case A2007/2490). To satisfy information needs for the reporting requirements, a research project was carried out in 2009 as part of preparatory actions for the implementation of bioassessment monitoring in Greek waters. The project was implemented by a consortium of research groups, each focusing on a specific category of waters, and aimed to achieve two objectives: to formulate the sampling techniques and protocols, and to identify appropriate bioassessment methods for the aquatic ecosystems of Greece. The realisation of a field sampling survey for

standardisation purposes and preliminary ecological status assessments was included in the project mandate. The Inland Waters section of the Hellenic Centre for Marine Research assumed responsibility for the category “rivers”. The fieldwork schedule included sampling of three biological elements (fish, macroinvertebrates and macrophytes) and measurements of physicochemical, chemical and habitat parameters. The ichthyological component of the river survey work is the focus of the present article.

The biological monitoring system demanded by the WFD is neither a simple data collection process, nor an autonomous activity with an end in itself. Rather, it is an enormous and technically complicated operation that involves various tasks (network design and field protocol development, routine and standardised sampling, data processing and interpretation, quality controls, reporting and advice), and is itself part of a larger coordinated effort aiming to improve the manner of water resource exploitation and management (EU, 2003a). Should the monitoring system fail to perform as intended, the WFD environmental goals will be compromised, irrespective of how well other aspects of the Directive are implemented. Building a WFD-compliant monitoring system is a challenging undertaking. Technical complexities and

uncertainties associated with nearly all steps of a monitoring development process have the capacity to weaken the reliability of the monitoring results (Hatton-Ellis, 2008; Nöges *et al.*, 2009; Hering *et al.*, 2010; Marzin *et al.*, 2014) and even jeopardise the legal basis and societal acceptance of the WFD environmental objectives (Kallis & Butler, 2001; Ollivier, 2004; Raadgever *et al.*, 2010; van der Keur *et al.*, 2010; Josefsson, 2012; Bouleau & Pont, 2015). However, the WFD is flexible enough to deal with incomplete information and low-performance bioassessment systems, encouraging regular updates of the monitoring procedures in light of new information and scientific progress. Feedback control is therefore an essential element of this “adaptive approach” to monitoring design and implementation. The whole monitoring system can be viewed as an evolving dynamic process subject to amendments and revisions based on informational feedback from previous rounds of sampling.

In this general context, it is reasonable to ask which data collection strategy and data analysis methodology can best contribute to fish monitoring capacity-building in Greece. When the project described in this article was launched, there was no previous monitoring experience, nor a sound informational basis, to assist in the formulation of the survey plan and procedures. Moreover, the sampling framework targeted different taxonomic groups concurrently, and did not specifically address the demands of fish monitoring. Given this paucity of information, the strategy for reaching the project’s objective consisted in making the best use of what already existed (workforce, budgetary sources, equipment, datasets) and directing the data analysis towards developing an in-depth understanding of the mechanisms that shape fish community diversity at different spatial scales and across biogeographic gradients.

In this article we describe the sampling plan and procedures of the 2009 survey project and we provide an account of the survey results, together with considerations on their limitations, potential utilities and methodological implications for bioassessment monitoring. We discuss ways to optimise the network design and sampling protocols, and we seek to identify information needs and tasks to be addressed in a future revision of the monitoring programme. Within this conceptual framework, we address three specific issues relevant to monitoring and bioassessment tool development:

### ***Sampling procedures and analysis of spatial heterogeneities***

Fieldwork planning (typically addressing network design, instrumentation needs, sampling protocol development and standardisation issues) plays a critical role in the success of a monitoring programme; field sampling produces the data upon which all subsequent analyses and interpretations are based (Strobl & Robillard, 2008). A key requirement is that the sampling locations and

field procedures produce a reasonable representation of the regional species pools and ecological communities (Angermeier & Karr, 1986; Roset *et al.*, 2007). This is a challenging requirement, especially when the area being surveyed is highly heterogeneous in assemblage structure and taxonomic composition, or the spatial distribution of species is very patchy, or assemblages contain a high proportion of rare species. It is therefore of practical importance to examine how the spatial resolution and intensity of sampling may affect the monitoring results and their use in bioassessment. Specific issues to be addressed in the present article include: sampling effort required to produce reasonably complete regional inventories of stream fishes; and whether a sampling strategy yielding larger samples or more samples is appropriate for bioassessment analysis.

### ***Biogeographical regionalisation***

Biogeographic regionalisation is an important consideration for fish-based bioassessment, especially because freshwater fish can only disperse via freshwater corridors, and their distributions strongly reflect historical patterns of vicariance and drainage connections (Reyjol *et al.*, 2007). For the implementation of bioassessment programmes, the WFD proposes the use of Illies (1978) biogeographical classification of Europe. Zogaris *et al.* (2009a) asserted that this system does not partition taxonomic variability of fish communities in Greek freshwaters sufficiently well, and that an ichthyogeographic regionalisation is more appropriate for fish bioassessments. A variety of ichthyogeographic classifications have been produced for the Balkans through the use of different datasets and methods of data analysis (reviewed by Skoulikidis *et al.*, 2009; Barbieri *et al.*, 2015). However, they have all been derived through the analysis of qualitative (presence/absence) data aggregated at basin or broader spatial scales. In the present article an ichthyogeographic classification of Greece based on site-specific quantitative (abundance) is presented. We examine how this regionalisation compares with previous regionalisations and whether it satisfies the WFD’s principles and requirements for bioassessment monitoring.

### ***Bioassessment options***

The first phase of WFD implementation saw an explosive development of bioassessment indices which have been based on different principles and conceptions, and many have not been empirically substantiated and sufficiently standardised (Hatton-Ellis, 2008; Nöges *et al.*, 2009; Hering *et al.*, 2010; Birk *et al.*, 2012). The prevailing approach to fish bioassessment is based on the concept of “biotic integrity”, upon which the “Index of Biotic Integrity” (IBI) was formulated (Karr, 1981). The IBI approach to bioassessment is founded on the premise that an integration of structural and functional attributes

of fish communities can provide a reliable assessment of ecosystem health and effects of human impacts. Despite various criticisms, the IBI remains the most widely accepted method of bioassessment using fish assemblages (Ruaro & Gubiani, 2013). A number of authors have questioned the applicability of the classical “biotic integrity” approach in species-poor fish faunas, on the grounds that these faunas are often dominated by tolerant species with low functional specialisation (e.g. Benejam *et al.*, 2015). It is relevant in this context to examine patterns of species diversity and degree of rarity across spatial scales in order to evaluate the strength and weaknesses of alternative bioassessment strategies, also taking into account cost-effective considerations and transferability issues.

## Materials and Methods

### *Sampling plan and procedures*

The survey took place between June 17 and July 30, 2009. Sampling site designation was based on the available biological and ecological data at the time and was influenced by accessibility. Undisturbed and minimally disturbed sites were included in the network for the determination of reference conditions. At each site, three biological quality elements (fish, macroinvertebrates and macrophytes) were sampled. Habitat features were recorded and physicochemical and chemical parameters were measured at each site. Environmental and habitat elements are not reported in this study. Fish sampling was carried out with electrofishing in wadeable reaches (less than upper-thigh deep) at 85 lotic stretches (Fig. 1); sites 73 and 82 were removed from the analysis due to poor sampling conditions.

At each site, a representative river reach containing typical fish habitats (i.e. riffle, run, pool, glide) was electrofished, and key environmental and habitat parameters were recorded. Fish sampling and environmental/habitat data collection and recording followed standardised procedures developed during the EU-funded research project FAME (Schmutz *et al.*, 2007) with some necessary modifications (see IMBRIW, 2013). Briefly, a stream section about 100 m (ranging from 80 to 150 m) was sampled, a single electrofishing pass was conducted and no stop nets were used. In small rivers the entire river channel was surveyed. When the river channel exceeded 30 m width, or when the water column exceeded waist-depth, sampling was conducted partially from one riverbank. A common practice involved the operator thrusting the anode at a distance ahead to surprise the fish and limit fish escape. The same field crew participated in the survey in order to ensure that methods were consistent among sites. Throughout the surveys, two 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 V), 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, 600 V), which was used in deeper streams and rivers.

Fish were identified to species level following Kotelat & Freyoff (2007) as the main taxonomic reference. All fish were measured to Total Length (TL), grouped in 5 cm length class intervals, and returned alive to the river at the site of capture. In cases of dubious fish identifications (usually juveniles), samples were preserved in formalin for laboratory identification. Fish abundance data at each site (numbers per single run fishery) were converted to a real densities (dividing numbers by the wetted surface area sampled). Fish densities could not be adequately estimated in 32 sites with deep pools or fished partially from one bank due to high fish escape rates. Due to unreliable density data from these sites, only abundance data (sample size, typically representing the number of fish caught in an approximately 100 m sampled channel length) were used as a Catch Per Unit Effort measure.

### Data analysis

Species abundances per site were square root transformed and analysed by hierarchical clustering (group average linkage method) to classify sites into homogeneous groups with respect to fish species associations and abundances. The analysis was performed using Ward’s hierarchical procedure for joining the clusters and the Euclidean distance as a similarity measure. Non-parametric multi-dimensional scaling (NMDS) ordination was used as an independent technique to verify results from hierarchical cluster analysis, as recommended by Kwak & Peterson (2007). The grouping in the NMDS ordination was based on the calculation of the Bray–Curtis similarity index (Clarke & Warwick, 1994). Non-native species were excluded from both analyses. Areas with relatively homogeneous fish faunas (fish ecoregions) were identified through these analyses and served as the geographic frame for organising ichthyological information at regional scales.

Sampling sufficiency and the extent to which the samples collected were representative of the regional fish communities were tested through generating sample-based species accumulation curves (Gotelli & Colwell, 2010). The procedure for adding samples to the curves was statistically randomised 50 times to obtain a smoothed curve using the EstimateS software package, version 8.2.0 (Colwell, 2006). Ecoregions for which this analysis failed to reveal data adequacy were excluded from some of the subsequent analyses.

For analyses of the distribution, diversity and assemblage characteristics of the Greek ichthyofauna we

employed two approaches: R-mode (species-level) analysis, which compares species for details of their distribution, and Q-mode (assemblage-level) analysis, which compares areas according to the characteristics of their assemblages (see Simberloff & Connor, 1979; Bell, 2003). R-mode analysis was performed through the use of ranked-occupancy and ranked abundance diagrams, following methodological principles outlined by Colwell (2009) and Jenkins (2011). These diagrams describe patterns of species distributions and abundances in relative terms, i.e. how widespread or abundant a species is relative to others in a defined spatial scale. The dynamics between species distribution and abundance within ecoregions was explored through the use of occupancy-local abundance diagrams (Gaston & Blackburn, 2000). Native species were only considered for this analysis. Q-mode analysis was designed to describe the compositional and structural characteristics of fish assemblages in each ecoregion and to explore relationships between ecoregions, initially with the help of boxplots for 'J' Pielou's evenness, H' Shannon-Wiener's diversity index, and log-transformed values of the number of species and the total abundance per sample. An ANOVA test was performed on the same indices in order to test for significant differences. A post-hoc Tukey test was later applied in order to check for pairwise significant differences among the ecoregions.

The outcomes of the Q-mode and the R-mode analyses were used in combination with information from the species accumulation curves to explore patterns of species diversity and distribution among and within ecoregions and the degree to which the sampling design and the samples collected were sufficient and representative of the areas and fish communities investigated.

## Results

### Overview of survey results

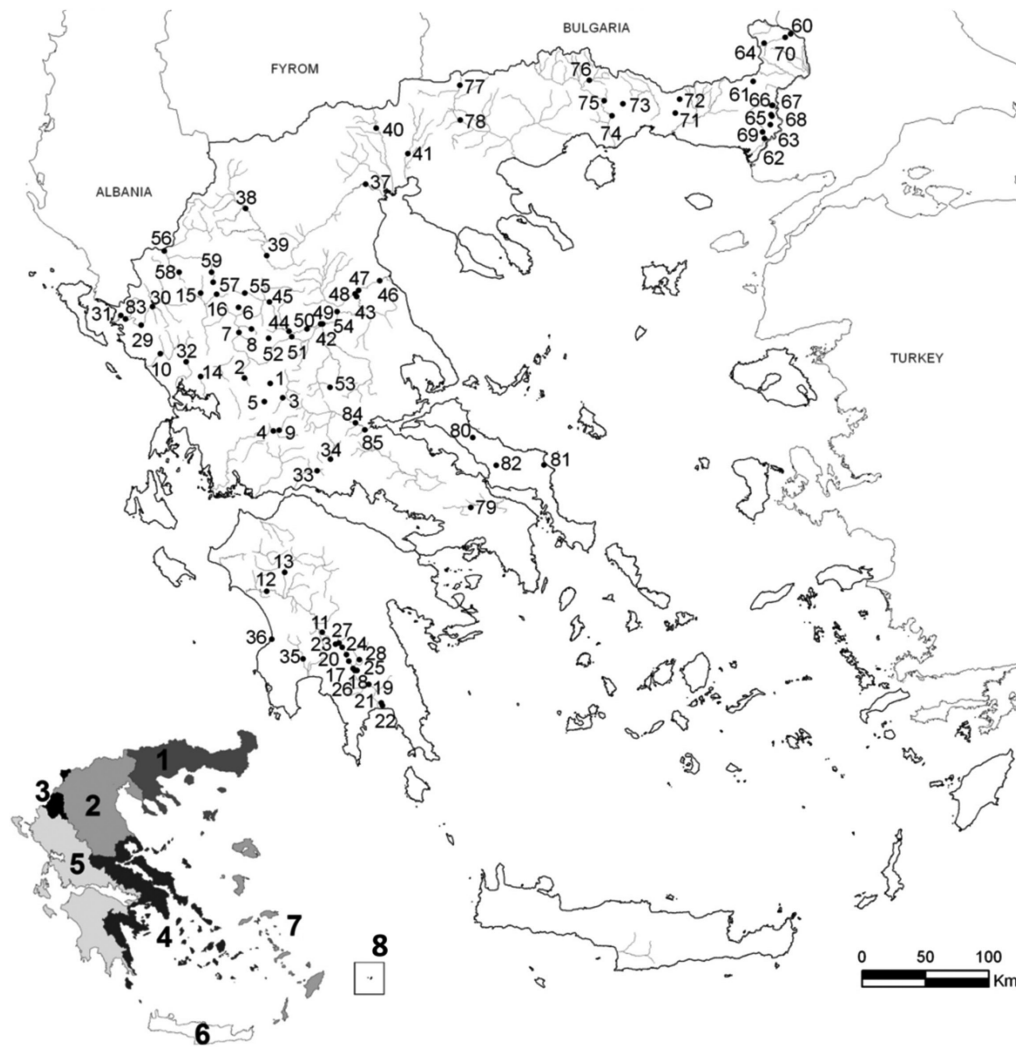
Detailed information on species occurrences and abundances in the survey area is presented in Table A1 (in Appendix). Table 1 provides summary data on fish community composition for each ecoregion and for the whole survey area. Altogether, 25,489 individuals belonging to 73 taxa (Appendix Table A1) in 14 families of freshwater fish were recorded. Three taxa could not be assigned to species level and were excluded from the analyses. The Cyprinidae family was strongly dominant with 49 species, accounting for 87.8 % of the total number of individuals sampled. Members of this family occurred in all ecoregions. Only two genera, *Barbus* and *Squalius*, were found in all ecoregions. The cyprinids *A. bipunctatus* and *S. vardarensis*, recorded from 24 and 22 sites respectively, were the most widespread of the native species collected. Among non-indigenous species, *G. holbrooki* and *L. gibbosus* were the most widespread, with 14 and 6 site occurrences respectively. *C. carpio* and *T. tinca*, native to northeastern Greece, were recorded as translocated to non-indigenous locations. Out of the 70 species sampled, 64 are native to Greece and six are introduced. Species endemic to Greece and/or to the southern Balkans comprised 61.4% of the total number of species collected.

### Ichthyogeographic regionalisation of the study area

Hierarchical clustering revealed a clear grouping pattern of assemblages (Fig. 2). All major groups showed remarkable geographical coherence, i.e. sites of a certain geographical region were classified closely within the dendrogram. To maintain consistency with previous work, all sampling sites were assigned to one of the five freshwater ecoregions defined by Zogaris *et al.* (2009b)

**Table 1.** Summary of fish community characteristics in the five ecoregions.

	Thraki	Makedonia Thessaly	Ionian	Western Aegean	Southeastern Adriatic	Total
Number of sampling sites	19	19	36	7	4	85
Number of families	9	7	8	3	4	14
Number of genera	27	22	17	9	8	41
Number of species	31	26	22	11	8	73
Number of individuals	5517	7901	9441	1481	1149	25489
Mean number of species/site	4.6	7.03	6.8	2	1.7	7
Mean number of individuals/site	290.4	415.8	262.3	211.6	287.3	300
Endemics species to Balkans	9	11	1	1	6	21
Endemics species to Greece	2	3	12	5	0	22
Total endemics species	11	14	13	6	6	43
% endemics species	35.5	53.8	59.1	54.5	75	58.9
Total endemic individuals	2417	5689	8601	819	693	18219
% endemic individuals	43.8	72	91.1	55.3	60.3	71.48
Alien species (incl. translocated)	4	4	5	2	0	8
% alien species	12.9	15.3	22.7	18.2	0	8.5
Alien individuals (incl. translocated)	1082	412	72	2	0	1568
% alien individuals	19.6	5.2	0.7	0.1	0	6.15

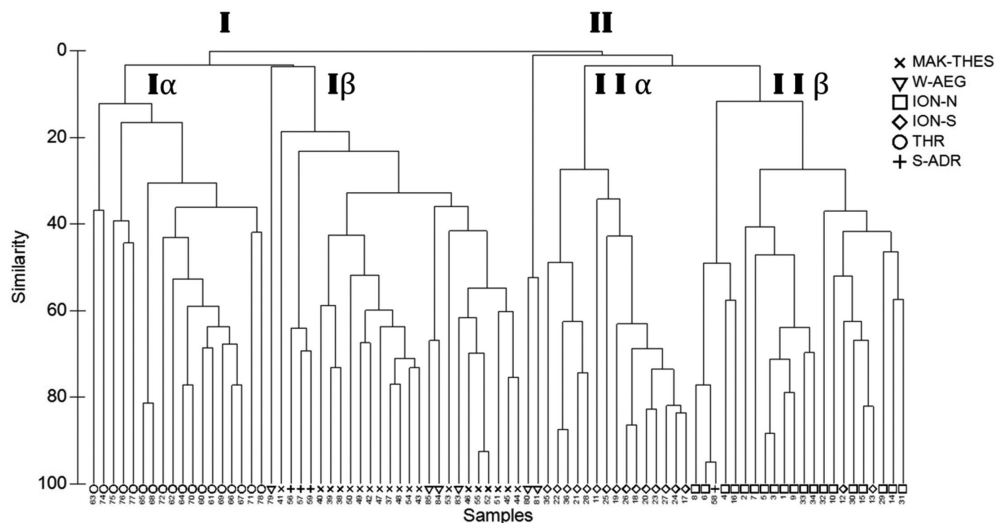


**Fig. 1:** Sampled site names: IONIAN: 1. Anargyri, 2. Argyri, 3. G. Papadias, 4. Kleidi, 5. Kryoneri, 6. PER12, 7. PER9.1, 8. PER6.1, 9. Trikfara, 10. Glyki, 11. Anthochori, 12. Arxaia Olympia, 13. Foloï, 14. Arta, 15. Vardas, 16. Votonosi, 17. Bioxios, 18. Ds Viologikos, 19. Gefchiliomodou, 20. Gefkollinon, 21. GefSkalas, 22. GefiraEkbolis, 23. Giakoumeika, 24. Pardali, 25. Sparti Ds ger, 26. Us GefSpartis, 27. Us kolliniotiko, 28. Us Skoura, 29. Ds Neraida, 30. Gribovo, 31. Ragio, 32. Agios Georgios, 33. Mornos Md, 34. Mornos Up, 35. Valyra, 36. Kalonero. MAKEDONIA-THESSALY: 37. Niseli, 38. Platania, 39. Sim Ben, 40. Axioupoli, 41. Gallikos, 42. Enipeas, 43. Gyrtoni, 44. Litheos, 45. P410, 46. P027, 47. P073, 48. P088, 49. P266 (Farkadona), 50. P297, 51. Pamisos, 52. Pyli, 53. Sophaditiko, 54. Tourkogefira, 55. Malakasiotis. SOUTHEAST ADRIATIC: 56. Melisopetra, 57. Moura, 58. Void up, 59. Vrazit Vovoussa. THRAKI: 60. Ardas, 61. Erythrotamos, 62. Feres, 63. Fleva, 64. Kiprinos, 65. Lira, 66. Magazi, 67. Magazi 2, 68. Mavrorema, 69. Mega rema, 70. Rizia, 71. Mesochori, 72. Symvolo, 73. Kossinthos, 74. Paradisos, 75. Stavropouli, 76. Agiabarbara, 77. Promaxon, 78. Strimonikos. WESTERN AEGEAN: 79. Dafnoula; 80. Kirefs, 81. Manikiotiko DW, 82. Psaxna, 83. Kastri, 84. Lamia, 85. Sperchios. Inset map: Freshwater ecoregions of Greece and their abbreviations: 1. Thraki, 2. Makedonia-Thessaly, 3. Southeast Adriatic, 4. Western Aegean, 5. Ionian, 6. Kriti, 7. Eastern Aegean, 8. Southern Anatolian.

in continental Greece, termed “ecoregions” hereafter: Thraki (THR), Makedonia-Thessaly (MAK-THES), Ionian (ION), southeastern Adriatic (S-ADR), and Western Aegean (W-AEG) (Fig. 1).

Two major clusters (I and II), each split into two sub-clusters, and a third species-poor cluster can be distinguished. Cluster I includes sites located in rivers draining to the northern Aegean Sea and is subdivided into two distinct branches that contain dissimilar and geographically well delineated assemblages: an “eastern” branch, which corresponds to sites from THR (I $\alpha$ ); and a “western” branch, which corresponds to all sites of

MAK-THES, with six more interspersed sites from other regions (I $\beta$ ). Three of the interspersed sites (56, 57, 59 in Fig.1) belong to S-ADR. The remaining three sites (83, 84, 85 in Fig. 1) are located in the Sperchios R. (W-AEG), and are aggregated with the sites of the adjacent Pinios basin of MAK-THES. Cluster II includes all sites located in rivers draining to the Ionian Sea (ION) and is subdivided into two geographically segregated groups, one north (II $\alpha$ ) and one south of the Patraikos Gulf (II $\beta$ ). Thus, the splitting of Zogaris’ (2009b) ION ecoregion into two provisional subregions (ION-NORTH and ION-SOUTH) was deemed necessary to account for diversity



**Fig. 2:** Dendrogram of a hierarchical clustering of the sampled river sites using group average clustering from Bray-Curtis similarities on square root transformed fish species abundance data. Two major biotic groups each comprising two subgroups are identified (see text). Sites are enumerated as in Figure 1.

patterns identified. NMDS ordination (stress coefficient 5%) applied on the same set of transformed data (Fig. 3) broadly revealed the same groupings: two “Ionian” groups and two “North Aegean” groups.

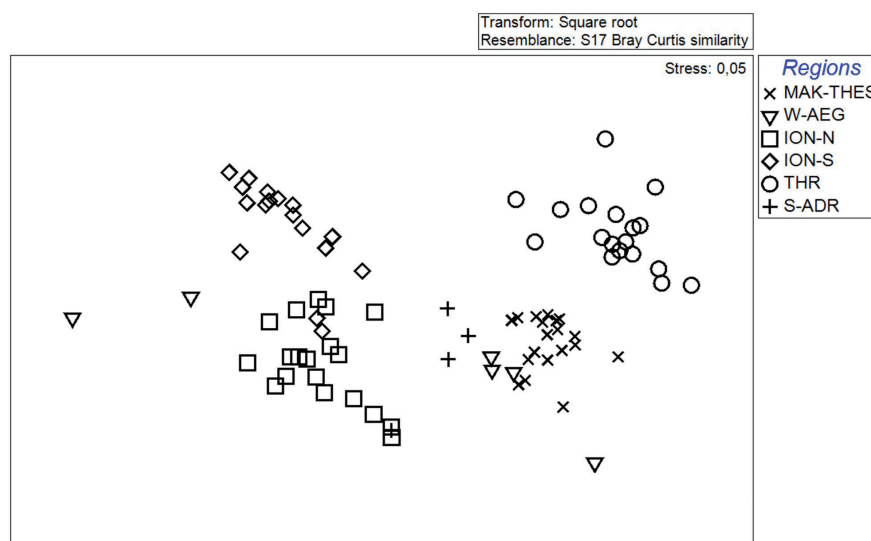
**Sampling sufficiency and inventory completeness**

Sample-based species accumulation curves for S-ADR and W-AEG were far from reaching asymptotes (Fig. 4), suggesting that the samples were too few to be representative of the regional taxonomic diversity. ION, THR and MAK-THES were more comprehensively sampled; although the data do not allow acceptable estimates of “true” species richness by extrapolations to asymptotes, they do provide reasonable approximations of saturation trends and community characteristics in a comparative

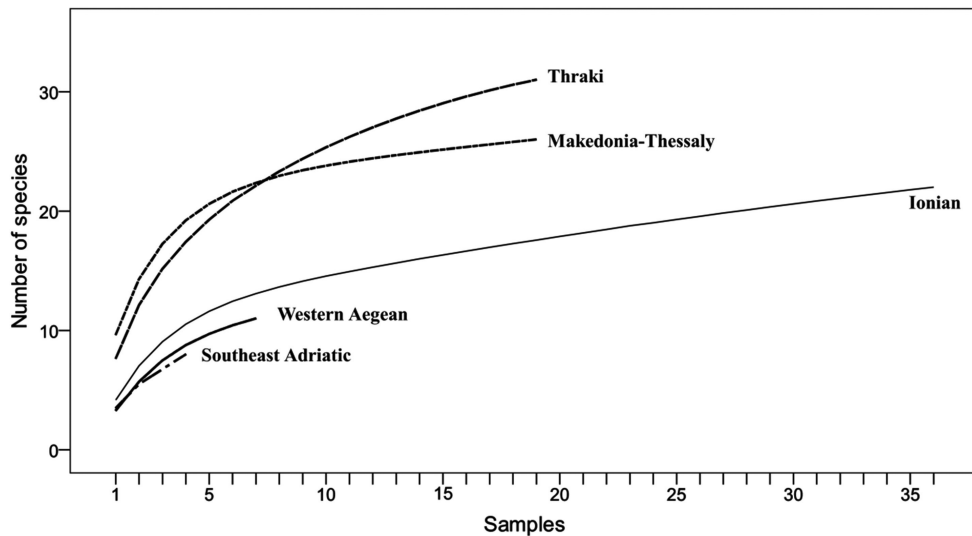
sense. However, only the curve for MAK-THES showed a levelling off, indicating adequate representation of the regional species pool. The curve for THR rose throughout the sampling sequence without showing signs of flattening. Finally, the curve for ION exhibited an early curvature but it did not level off sufficiently for sampling effort to be considered adequate. Thus, more sampling in the latter two ecoregions may reveal more species.

**Spatial patterns of occupancy and abundance**

Species were assigned to three spatial scales - sites, basins and ecoregions - and ranked in descending order of occupancies (occurrences) at each scale. The diagrams for ecoregion and basin occupancies (Fig. 5) have a negative exponential trend and are approximated by



**Fig. 3:** Two-dimensional NMDS plot with sites designated by groups from the results of the hierarchical clustering. Biotic groups correspond with the similarity clusters in Figure 2. Region code symbols are reported in Figure 2.

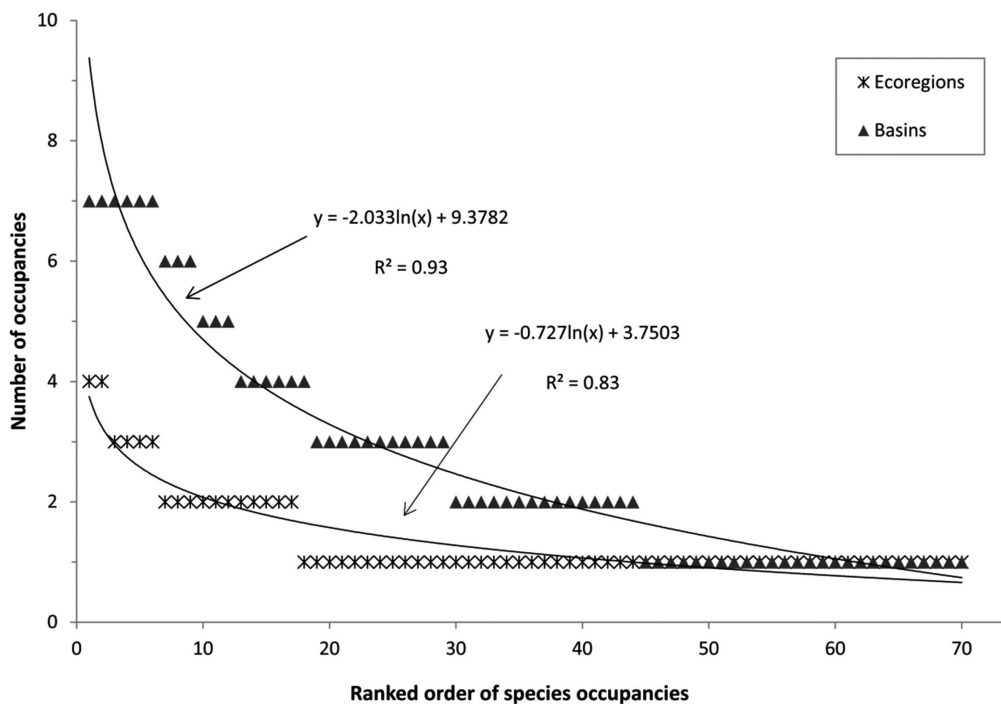


**Fig. 4:** Randomised species accumulation curves for the five ecoregions illustrating the cumulative site species richness versus number of sites sampled.

logarithmic regressions. The initially steep slope of the curve for ecoregion occupancy indicated an exceptionally high turnover rate, suggesting that the species composition of each ecoregion is unique. Indeed, 53 of the total 70 species sampled were found to occupy only one ecoregion, and 11 species were observed in two ecoregions. Four species and two species appeared in three and four ecoregions respectively. No species was found in all five ecoregions. The curve pattern for basin occupancy indicated a substantial degree of faunal differentiation

between basins: 40 of the 70 species sampled were recorded from only one or two basins.

We explored the degree of taxonomic affinity between ecoregions by calculating the Jaccard similarity coefficient between pairs of regional communities, separately for all ( $J_{total}$ ) and for native ( $J_{native}$ ) species (Table 2). Table 3 shows their basin and site occupancies by ecoregion and provides information on their provenance (native or non-native status) and ecological groups (primary freshwater or diadromous). Overall, 17 species occurring in two or more ecore-



**Fig. 5:** Ranked occupancy diagrams plotting the species ranked in descending order of occupancies (occurrences) at ecoregional and basin scales (horizontal axis) against the corresponding occupancy value at each scale (vertical axis). Each species is indicated by a point.



**Table 2.** Matrix of Jaccard similarity (J) showing the fish faunal affinity between ecoregions based on shared species. Upper-right: all species ( $J_{total}$ ). Lower-left: native species ( $J_{native}$ ) only.

	MAK-THES	THR	S-ADR	IONIAN	W. AEG	
MAK-THES		21,3	6,3	6,7	15,6	$J_{total}$
THR	14,0		2,6	8,2	5,0	
S-ADR	7,1	2,9		7,1	11,8	
IONIAN	0,0	2,3	8,7		6,5	
W. AEG	14,8	2,9	13,3	4,0		
			$J_{native}$			

**Table 3.** Species shared between ecoregions and regional occupancy characteristics (basin and site occurrences). Diadromous (including potentially diadromous) are lightly shaded; non-native species are indicated by darker shaded highlighting.

	IONIAN		S-ADR		W. AEG		MAK-THES		THR	
	Basins	Sites	Basins	Sites	Basins	Sites	Basins	Sites	Basins	Sites
<i>Alburnoides bipunctatus</i>			1	3	1	3	3	17	1	1
<i>Chondrostoma vardarensis</i>			1	1			3	15	2	4
<i>Sabanejewia balcanica</i>							2	5	1	2
<i>Gobio bulgaricus</i>							3	3	4	10
<i>Rutilus rutilus</i>							2	8	2	8
<i>Squalius vardarensis</i>					1	3	4	19		
<i>Vimba melanops</i>							3	8	1	5
<i>Barbus sperchiensis</i>					1	1	1	12		
<i>Pelagus marathonicus</i>					2	4	1	1		
<i>Anguilla anguilla</i>	7	15	1	1	1	1				
<i>Gasterosteus gymnourus</i>	1	1							1	1
<i>Salmo farioides</i>	3	11	1	3						
<i>Cyprinus carpio</i>	1	1			1	1	2	4	2	2
<i>Pseudorasbora parva</i>	1	1					1	1	1	2
<i>Gambusia holbrooki</i>	2	2					3	7	2	5
<i>Lepomis gibbosus</i>							1	4	1	2
<i>Carassius gibelio</i>							3	7	2	6

gions were identified: nine native, five non-native to the systems in which they were recorded, and three potentially and/or presently diadromous. MAK-THES and THR presented the highest faunal similarity to each other with ten species in common (six native and four introduced). Calculations from the samples showed that shared species accounted for 29.6 % of the total number of individuals sampled in the latter two ecoregions. If the introduced species are excluded from the analysis, the percentage drops to 18.5 %. S-ADR and W-AEG had distinct fish faunas, sharing relatively few species with other ecoregions. ION presented the most distinctive native fish fauna, having no primary freshwater species in common with other ecoregions.

Non-native species tended to be disproportionately shared among ecoregions (five shared out of eight species in total) relative to native species (nine out of 62 species). Of the three species designated as “diadromous”, only eel (*A. anguilla*) has a marine stage for dispersal in present-day environments. The two other species (*S. farioides* and *G. gymnurus*) are no longer diadromous in the Mediterra-

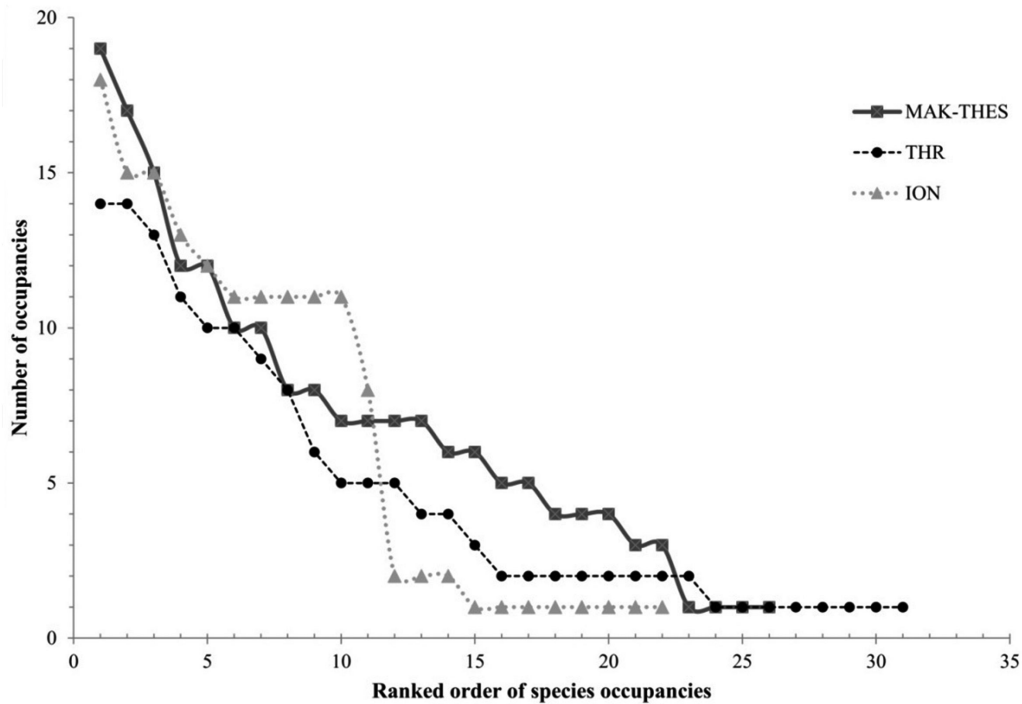
nean (see Bianco & Nordlie, 2008), but they have diadromous origins and presumably have had marine dispersal opportunities during former glacial periods.

For three ecoregions where the species inventories were more complete (MAK-THES, THR and ION), ranked species occupancy diagrams at site scale were generated (Fig. 6). In ION, a sharp decline in site occupancy values at about the middle of the respective species rank sequences reflects the existence of many narrowly distributed species. Indeed, about half of the species sampled in this ecoregion were obtained from only one or two sites. In MAK-THES, the progression of occupancy values with ranking position is more continuous, which implies a higher uniformity in species distributions. Only 15.4% of the species sampled in this ecoregion were collected from one site, and none from two sites. Ranked species abundance diagrams for the same three ecoregions (Fig. 7) show that few species are numerically abundant, while most species are numerically scarce. The three dominant species made up more

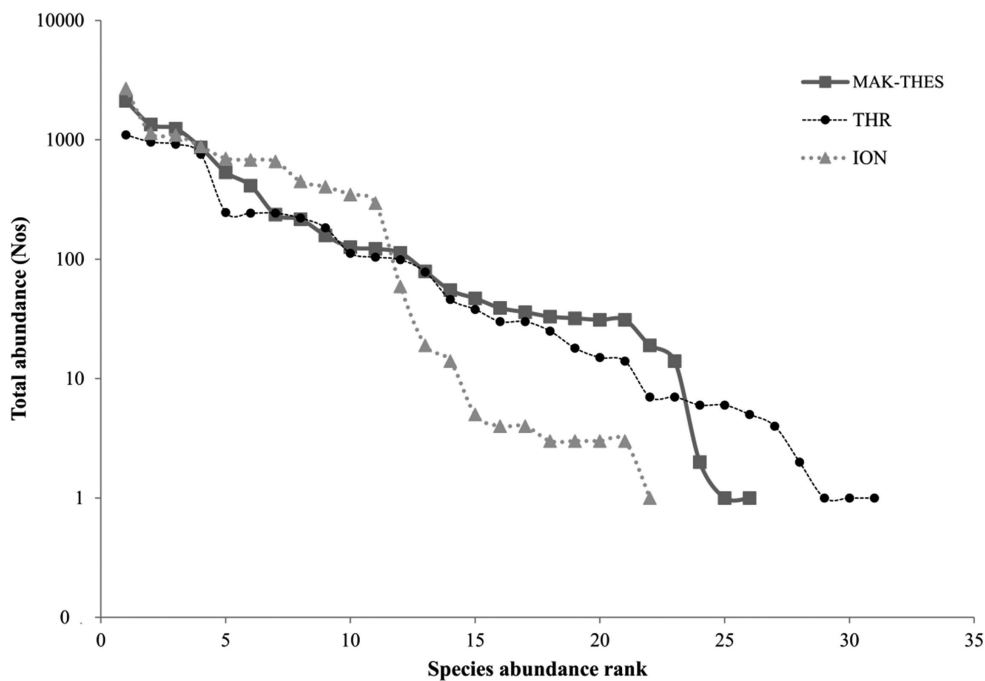
than 50% of the total catch in all three ecoregions. The ten least abundant species, together, comprised 0.73% (THR), 2.08% (MAK-THES) and 0.62% (ION) of all individuals collected during the survey.

Scatterplots of site occupancies versus local abundances for the ecoregions MAK-THES, THR and ION in-

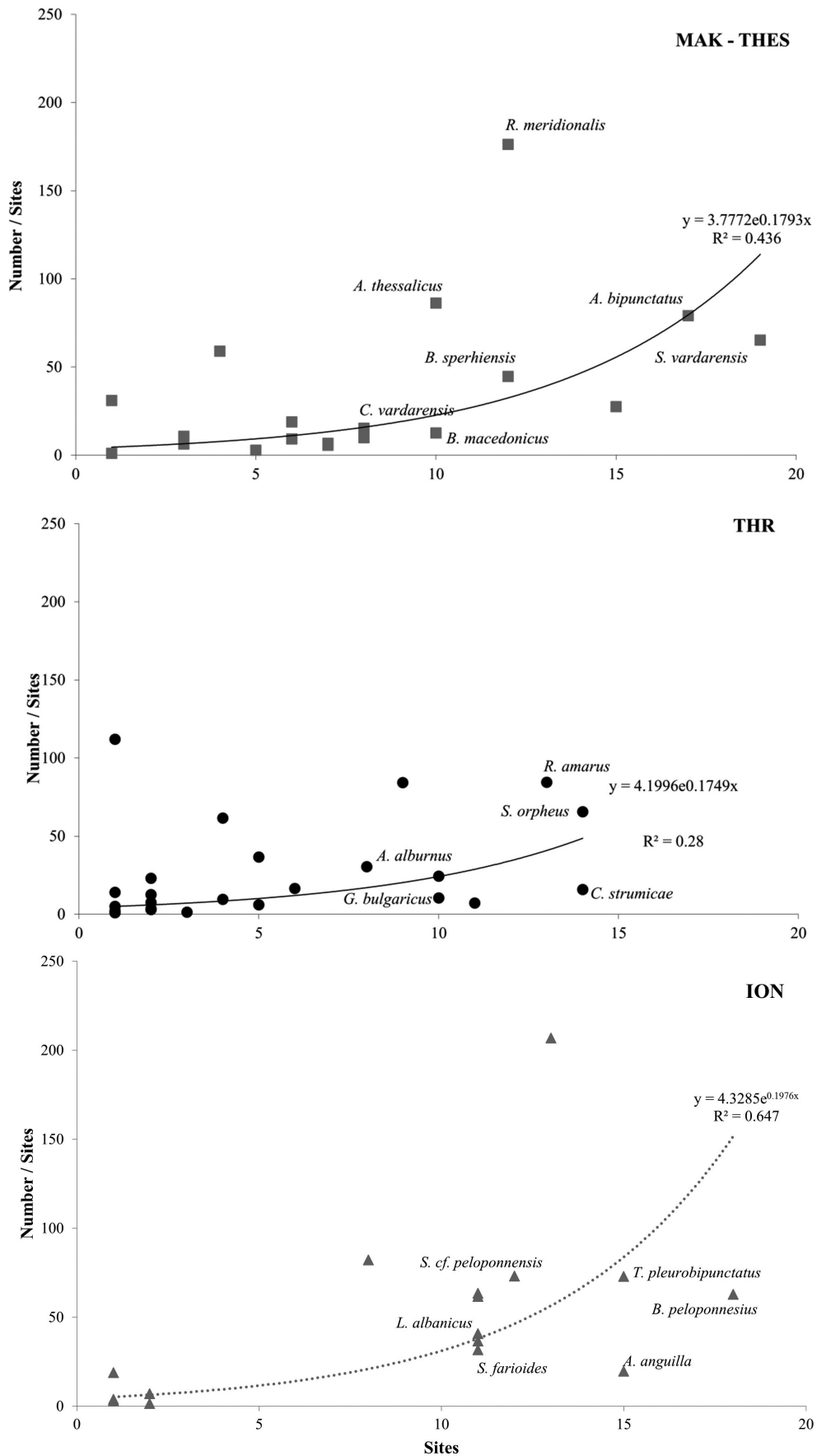
dicated a positive association between these two variables (Figure 8), suggesting that most species are either spatially restricted and locally rare, or widespread and locally abundant. Widespread and locally abundant species play an important role in food web dynamics and ecosystem function and are potentially useful indicators of ecosystem integrity.



**Fig. 6:** Ranked species occupancy at the site scale for three ecoregions. Species are arranged according to a decreasing order of site occupancies against the corresponding occupancy values. Each species is indicated by a point.



**Fig. 7:** Rank abundance for three ecoregions plotting the species abundances (measured on a log scale in the y-axis) against species abundance rank (species sorted in descending order of total abundance in the x-axis). Each species is indicated by a point.



**Fig. 8:** Species site occupancies plotted against mean species local abundances (mean number of individuals per site in the sites of the species' occurrence) for three ecoregions. Each dot represents a species and full names are given to the most widespread "guiding species". Only native species are depicted. Region code symbols are reported in Figure 3.

**Table 4.** List of “guiding species” within samples from three ecoregions.

Makedonia – Thessaly	Ionian	Thraki
<i>A. bipunctatus</i>	<i>A. anguilla</i>	<i>B. cyclolepis</i>
<i>A. thessalicus</i>	<i>B. peloponnesius</i>	<i>R. amarus</i>
<i>B. macedonicus</i>	<i>L. albanicus</i>	<i>S. orpheus</i>
<i>C. vardarensis</i>	<i>S. farioides</i>	<i>A. alburnus</i>
<i>S. vardarensis</i>	<i>S. cf. peloponensis</i>	<i>G. bulgaricus</i>
<i>R. meridionalis</i>	<i>T. pleurobipunctatus</i>	<i>C. strumicae</i>
<i>B. sperchiensis</i>		

These species were characterised as “guiding species” for ecological status evaluations. The guiding species identified through this analysis (Table 4) comprise 50.3%, 77.2% and 75.9% of the individuals collected in THR, MAK-THES and ION respectively.

### Structural organisation of regional fish faunas

According to the boxplots of the four community indices (abundance, species richness, Pielou evenness and Shannon-Wiener diversity) ION presented the lowest means, followed by THR, and MAK-THES presented the highest ones (Figure 9). The ANOVA test indicated significant difference ( $P < 0.01$ ) only for Shannon-Wiener diversity and species richness. The Tukey test further showed that there is a significant difference between the ION ecoregion and the other two (MAK-THES, THR), the ION having significantly lower diversity ( $P < 0.05$ ) and species richness ( $P < 0.01$ ) values.

### Discussion

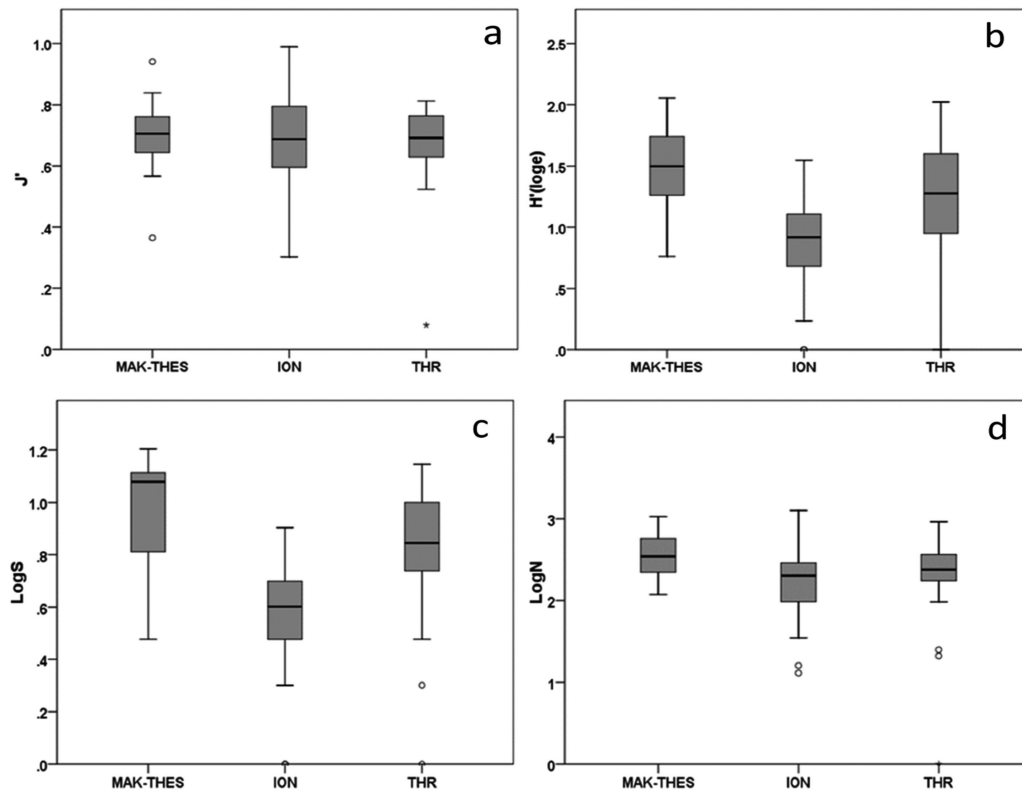
Our survey was the first to collect fish data from the running waters of Greece at a nationwide scale with a consistent and standardised sampling method. We recorded 70 species, less than half of the about 160 fish species known from the freshwater Greece (Barbieri *et al.*, 2015). However, our survey targeted the smaller fluvial systems and was not specifically structured to include lakes and other outstanding habitat types for fish biodiversity (i.e. deep river reaches, wetlands and other lentic waters). Records of species found in new localities have been reported by Koutsikos *et al.* (2012).

The survey results have contributed to tracking the spread of non-native species. Six alien species were collected and, together with translocated species, represented 8.5 % of the total number of species sampled, and 6.15 % of the total number of individuals recorded in non-native localities. Four aliens (*G. holbrooki*, *L. gibbosus*, *P. parva* and *C. gibelio*) were found at relatively high frequencies and abundances. Two aliens (*G. holbrooki* and *L. gibbosus*) were recorded from new localities, indicating an invasive spreading tendency. These results show that

non-native species make a relatively small contribution to the number of species and individuals occurring in the rivers, contrary to the picture emerging from literature-based compilations of alien species occurrences or introductions in Greek freshwaters (Economou *et al.*, 2007). Apparently, alien species invasion has not yet become such a serious problem in Greek rivers compared to other European Mediterranean countries, such as Italy (Gherardi *et al.*, 2008) and the Iberian Peninsula (Clavero & Garcia-Berthou, 2006). Bianco (1990b) showed that Greece had one of the lowest incidences of alien fish species distribution in Europe. Low invasion rates could be due to the lack of dams and associated reservoirs in the smaller Greek river basins. Dams have been linked to the persistence and spread of aliens in other Mediterranean countries (Clavero & Garcia-Berthou, 2006; Zogaris *et al.*, 2012). However, it can also be attributed to the poor development of sport angling, which is an important driver for alien species introduction. Ornamental fish trade represents a little-explored but potentially important introduction pathway (Papavaslopoulou *et al.*, 2013). From the perspective of evaluating and abating the threats imposed by alien species on the native biota, it is imperative to closely examine the habitat and anthropogenic pressure data in the locations of alien species occurrences in order to research conditions favouring establishment and population growth.

### An ichthyogeographic regionalisation of Greece based on quantitative data

Ichthyogeographic regionalisations of the Balkans have been pursued since the beginning of the 20<sup>th</sup> century (Petit, 1930; Stephanidis, 1939; Berg, 1948; Bianco, 1990a; Economidis & Bănărescu, 1991; Bănărescu, 2004; Abell *et al.*, 2008; Zogaris *et al.*, 2009b; Bobori, 2012; Oikonomou *et al.*, 2014). Most of these research efforts have taken a historical biogeographic perspective, focussing on clarifying the species’ geographic origins and major dispersal and vicariance events that have shaped their current distributions and associations. Data were obtained from scientific and grey literature, and were highly heterogeneous in quality and content, representing different collection times, geographical scales and sampling methods. Almost invariably, the data used in biogeographic studies were qualitative in character (species presence/absence) and were aggregated at coarse spatial scales (river drainage or broader). Qualitative data contain important taxonomic and distributional information, including information on extinct and rare species, and can greatly contribute to the field of historical biogeography (Leveque *et al.*, 2008; Filipe *et al.*, 2009; Levy *et al.*, 2009). However, qualitative data fail to address the evenness (relative abundance) component, which is important for clarifying ecological dynamics (Chiarucci *et al.*, 2011). Studies from a range of plant and animal communities have underlined that biogeographic classifications derived from presence-absence data may not be appro-



**Fig. 9:** Variation among ecoregions in structural properties of fish assemblages: (a) Pielou's evenness index; (b) Shannon–Wiener diversity index; (c) species richness (log transformed); and, (d) fish abundance (log transformed). Box plots depict the range of site values, the 25th and the 75th percentiles, and the mean as the central tendency; symbols o and \* indicate outliers and extreme values, respectively.

appropriate for research pursuing ecological objectives (Haila *et al.*, 1987; Ranta & Jarvinen, 1987; Blanchette *et al.*, 2008; Howard *et al.*, 2014; Veloz *et al.*, 2015). Bioassessment under the WFD has an ecological focus, addressing the evaluation of human impacts on aquatic ecosystems through comparisons of the communities in the assessed localities with reference communities. Species abundances and proportional relationships are explicitly required by the WFD for ecological status assessments in freshwaters (EU, 2003b) and are invariably included in currently used bioassessment indices (Pont *et al.*, 2006; Schmutz *et al.*, 2007; Murphy *et al.*, 2013; Benejam *et al.*, 2015). We therefore allow ourselves the speculation that an ichthyogeographic regionalisation based on quantitative data is more appropriate for the scope of WFD bioassessments. What really matters is that the regionalisation system consistently identifies spatial gradients and breaks in the fish community organisation pattern, whether the taxonomic make-up of the community has changed greatly or not.

The picture emerging from our analysis points out a hierarchical biogeographic structure of the Greek ichthyofauna, with the degree of faunal similarity increasing from broader to lower spatial scale. At a broad scale, continental Greece falls under two ichthyogeographic divisions, an eastern (North Aegean) and a western (Ionian). The two divisions are separated by the Pindus mountain range and

contain taxonomically dissimilar fish faunas. In fact, not a single primary freshwater species was found on both sides of Pindus, and only secondary freshwater species were shared. The Ionian fauna appeared to be poorer in terms of regional species richness (22 species), but endemism levels were higher, with dominance of range-restricted endemics. The North Aegean division was found to support richer fish faunas (31 and 26 species in THR and MAK-THES respectively) and contained fewer endemics, most of which have a distribution spreading to neighbouring Balkan countries. These two ecoregions share a number of species, but also have their own distinctive endemics.

The North Aegean and Ionian faunal groups also exhibited remarkable structural differences. The Ionian fish assemblages had lower richness, diversity and species abundance values, and they exhibited higher evenness variation than those the North Aegean assemblages. These assemblage characteristics indicate a species-poor and spatially variable Ionian fish fauna, and support the contention that this fauna comprises a regionally well-defined pool of species. Two non-competing explanations can account for the unique taxonomic composition and species depauperation of the Ionian assemblages: a historical explanation (long isolation of the Ionian region due to the existence of mountain and marine barriers since the Miocene), and an ecological one (hydrographically frag-

mented network composed of many small autonomous river basins) (Economou *et al.*, 2007; Skoulikidis *et al.*, 2009). In contrast, the two North Aegean ecoregions are crossed by large rivers that originate from neighbouring countries and flow through extensive lowland areas to the Aegean Sea. Moreover, their fish faunas had relatively recent (Pliocenic) contacts with Danubian and Black Sea fish faunas (Economidis & Banareescu, 1991).

An interesting pattern to observe is that the THR, MAC-THES and ION ecoregions are roughly defined by the river-mouth location (marine area) of the rivers flowing through them. THR and MAC-THES encompass rivers having estuaries in the Thracian Sea and the Thermaikos Gulf, respectively. A notable exception to the pattern is that sites on the more southerly located Sperchios R. showed a tight clustering with the sites of MAC-THES. ION contains a “northern” subregion and a “southern” subregion, which include rivers flowing to coasts north and south of the Patraikos Gulf, respectively. The consistency of the pattern is compelling and implies that a regional dispersal process has acted to homogenise the species pools of neighbouring rivers. Dispersal enabled by lowland connections of rivers sharing the same coast during the more than 100 m marine regressions of the Pleistocene could account for the observed pattern (Bianco, 1986, 1990a). While such a dispersal process can possibly explain faunal exchanges among adjacent rivers that have outlets in the Thermaikos Gulf and in the Thracian Sea, the relatively steep bathymetric gradients in the Ionian Sea would have prevented widespread river connections during the Pleistocene lowstands. Therefore, it is tempting to speculate that older regression events, possibly during the dramatic sea level drop in the arid phases of the Messinian Salinity crisis, when the sea level dropped by several hundred meters, were more influential in shaping the distribution of freshwater fish species in the Ionian region.

Previous biogeographic studies have recognised the distinctiveness of the North Aegean and Ionian fish faunas and have proposed uninterrupted vicariance across the Pindus barrier as the dominant diversification mechanism (Economou *et al.*, 2007; Zogaris *et al.*, 2009a; Drakou *et al.*, 2009; Oikonomou *et al.*, 2014). However, opinions diverge over finer divisions and their boundaries. For example, there is no consensus over whether Makedonia-Thessaly and Thraki should be considered as a single or as separate biogeographic entities (see Bianco, 1990a; Economidis & Banareescu, 1991; Maurakis *et al.*, 2001; Zogaris *et al.*, 2009a; 2009b; Oikonomou *et al.*, 2014). The present ichthyogeographic classification shows a good agreement with the classification proposed by Zogaris *et al.* (2009b). This is probably because, despite substantial differences in data types and methodological approaches (contemporary sample data versus historical species occurrence records, site versus drainage scales of data aggregation, quantitative versus qualitative information), similar clustering techniques and the same taxonomy were used. An interest-

ing case of disagreement concerns the geographic placement of the Sperchios R. In our classification, Sperchios falls in the MAK-THES ecoregion, whereas in all previous classifications it is considered to be part of the W-AEG (also called Attiko-Beotia) ecoregion. Sperchios shares only three species with MAK-THES. However, these species were found to be widespread and locally abundant in both ecoregions, and therefore weighted strongly in our similarity ratings. In contrast, previous classifications were based on analyses of presence/absence data and highlighted taxonomic variability. Our analysis also supports a clear-cut biogeographic distinction between MAK-THES and THR. This surprisingly robust biogeographic separation between the Thracian and the Macedonian-Thessalian fish faunas may also reflect the quantitative nature of our dataset, namely the influence of few dominant species unique to either ecoregion in the similarity scores.

The biogeographic status of W-AEG and S-ADR cannot be explicitly addressed by our study. Both regions appear to be species-poor with a high proportion of endemics. However, data were retrieved from a limited number of sites and do not adequately describe the species composition and diversity characteristics of the regional fish faunas. Note that S-ADR is represented in our samples only by the upper part of the Aaos R., which flows through Albania to the southern part of the Adriatic Sea. Surprisingly, three sites on the Aaos R. showed stronger affinity to the MAK-THES ecoregion, which lies across Pindus, rather than to the adjacent ION ecoregion. This affinity is driven by the occurrence in the Aaos R. of two cyprinid species (*A. bipunctatus* and *C. vardarensis*) which are widespread and abundant in MAK-THES. However, a recent study (Geiger *et al.*, 2014) identified the Adriatic and Aegean populations of these two species as genetically distinct. There is no doubt that these populations will be raised to species status when they are studied in more detail (e.g. for *A. bipunctatus*, see Stierandová *et al.*, 2016).

### **Patterns of species distribution and abundance**

Quantifying large-scale variation in species abundances and distributions allows the detection of scale-dependent processes and patterns that may not be revealed by analysis confined at small scales (Magurran *et al.*, 2011). A dominant pattern emerging from our analysis is the positive relationship between the geographic range of species (occupancy) and their local abundance. Occupancy and local abundance are not directly related variables, and their positive association implies an underlying mechanism that controls both range expansion and local-scale population size. This is one of the most consistent patterns in large-scale community ecology. It has been widely documented for a diverse array of animal and plant species (Blackburn *et al.*, 2006), including freshwater fishes (Pyron, 1999; Tales *et al.*, 2004; Granado-Lorencio *et al.*, 2005; Taylor

*et al.*, 2006; Faulks *et al.*, 2015). This pattern has received considerable theoretical and empirical research attention due to its practical applications in many disciplines such as conservation biology, invasive ecology and large-scale monitoring (Blackburn *et al.*, 2006; Gaston, 1999; Gaston & Blackburn, 2000; Holt *et al.*, 2002; Taylor *et al.*, 2006; Buckley & Freckleton, 2010).

In the context of bioassessment monitoring, occupancy-abundance relationships could be used to highlight species that are potential indicators of ecosystem condition. Species that are spatially rare in a specific region may also be numerically rare in this region, and these species are likely to have specialised habitat and narrow ecological requirements (Brown, 1984). While this evidence labels rare species as intolerant to environmental stressors and human impairment, the relative utility of rare and common species in bioassessment has been a much-debated issue (Cao *et al.*, 1998; Marchant, 1999; Nijboer & Schmidt-Kloiber, 2004; Arscott *et al.*, 2006; Gaston, 2008; Karr & Chu, 1999; Hawkins *et al.*, 2000; Heino & Soininen, 2010). For example, Cao *et al.* (1998, 2001) proposed that more emphasis should be placed on rare species for the derivation of bioassessment metrics due to their high responsiveness to environmental degradation. By contrast, Gaston (2008, 2011) assigned greater diagnostic value to the common species for the important influence they have on ecosystem structure, function and energy turnover by virtue of their abundance and widespread occurrence. Fish-based bioassessment methods reflect this controversy, and there have been arguments for and against the inclusion of rare taxa in ecological status evaluations (Kennard *et al.*, 2006; Wan *et al.*, 2010; Poos *et al.*, 2012).

We acknowledge that rare species provide very useful functional metrics that can be highly informative of the ecological status. Also, that a satisfactory representation of rare species in the dataset is desirable for better defining the regional species pools and is potentially important for biodiversity conservation. However, the benefits of targeting rare species should be evaluated against relevant costs, possible drawbacks and other potential options. An effective bioassessment tool must operate not only with sensitivity, but also with precision (Wan *et al.*, 2010). In our study, the majority of species were collected in small numbers at few localities. Increasing sampling effort at the level required to obtain statistically adequate data on populations of rare species would increase the cost, workforce and time required for sampling/analysis far beyond the available budgetary resources and institutional possibilities. It can reasonably be inferred that rare species cannot consistently and reliably be used to indicate human pressures. Clearly, a key issue to be addressed in future monitoring is balancing sampling effort and cost with statistical confidence and reliability, given limited resources and time for field work. This line of reasoning points to basing bioassessment monitoring primarily on common species, for which most metrics will be calculated. Common species can be caught in statistically

sufficient numbers for metric computations and their use in bioassessment confers two major advantages; they are likely to provide metrics that can be applied consistently across large areas, and they typically occur in several river types and habitats within each drainage network.

Placing the emphasis on common species generates two methodological implications for the design of the national monitoring scheme. First, bioassessment should be strongly weighted towards compositional (relative abundance) metrics; these are determined primarily by abundant species and assign little weight to rare species (see Wan *et al.*, 2010, for a relevant discussion). Second, the field data collection plan should be tailored to provide the data required for this bioassessment approach. If the focus is on common species, there is no need to use expensive field time for collecting samples larger than needed for confident evaluations. The “guiding” species identified in this research were present across wide areas and comprised the largest fraction (between 50.0% and 76.0%) of the captures. We construe this as an indication that site-specific sampling effort was sufficient for obtaining representative estimates of the proportional abundance and variation of common species. It should be added that some guiding species have a distribution spanning two or more ecoregions or have ecologically similar relatives in other ecoregions. This may be advantageous from a scale perspective in allowing development of transferable metrics among regions. These considerations lead us to conclude that sampling should be targeted towards more samples, rather than larger samples, in order to include more water bodies in the bioassessment process. Nonetheless, our analysis was based on limited datasets, and the sampling protocol was not designed for biodiversity research. Sampling intensity can have a strong effect on species detection probabilities and observed abundances, thereby influencing the results of bioassessment surveys (Pritt & Frimpong, 2014). Methodological issues of sampling should be given due considerations in future monitoring efforts.

### ***Monitoring design and bioassessment strategy***

Aquatic ecosystem monitoring and ecological research constitute tightly interconnected fields and should be integrated to achieve a cohesive strategy; scientific knowledge and expertise obtained from ecological research may enhance monitoring design and procedures, while the monitoring results may reveal patterns and phenomena that can contribute to ecological research (Franklin *et al.*, 1999; Lindenmayer & Likens, 2009). Our study has provided basic information about the structure and spatial organisation of fish communities that can contribute to monitoring capacity building from methodological and empirical perspectives, also revealing knowledge gaps, research needs and challenges with the work. Two main conclusions can be drawn from our data and analysis. First, a biogeographic regionalisation deems necessary

to delineate appropriate spatial frameworks for bioassessment. The regionalisation used in the present study needs to be refined with additional data, especially from central-eastern Greece. Another pending issue for future research is to examine whether a sub-regionalisation is required for the Ionian ecoregion in order to control for considerable faunal heterogeneity within this ecoregion. And second, inclusion of rare species may skew bioassessment for purely probabilistic reasons. Common species are less affected by sampling variability and can be monitored with reasonable sampling effort per site. Future research should provide a better understanding of the ecology, life history and population dynamics of common species in order to identify regionally appropriate metrics.

These considerations provide a context for evaluating alternative bioassessment options with respect to their appropriateness for the lotic freshwaters of Greece. Policies and approaches for assessing the ecological status of aquatic ecosystems have been developed in different parts of the world, and a variety of bioassessment techniques are now available (see reviews by Simon and Lyons, 1995; Murphy *et al.*, 2002; Roset *et al.*, 2007; Reyjol *et al.*, 2014). The index of biotic integrity (IBI) is the most widely accepted method for fish-based assessments. Originally developed by Karr (1981) for fish in North American rivers, the IBI was subsequently adapted to other regions by modification, addition or deletion of metrics (Miller *et al.*, 1988; Simon & Lyons, 1995), and some versions have been developed for European rivers (e.g. Oberdorff *et al.*, 2002; Pont *et al.*, 2007; Roset *et al.*, 2007). Perhaps it is more appropriate to consider the IBI not as an “index”, but as a flexible conceptual framework that can be adapted on a regional scale (Scardi *et al.*, 2006).

Fish IBIs typically employ a combination of taxonomic, structural and functional metrics that measure human-induced degradation of aquatic systems as a combined function of population and community attributes, indicator taxa (stress intolerant) and ecological guild. Despite the numerous modifications, IBIs retain the original holistic concept of biotic integrity, and explicitly incorporate functional attributes of the fish community into the assessment procedure. Generalisability and transferability are important issues to consider when creating IBIs. Early versions were developed for single watersheds; subsequent versions were adapted for use at broader (e.g. ecoregion) scales (Simon & Lyons, 1995). A persistent problem with attempts to generalise has been that different river drainage systems often harbour different fish communities, due to which they may not be comparable in terms of bioassessment protocols. As a solution to the problem, it has been proposed to base bioassessment mainly or only on functional metrics. Functional metrics cluster species in generalised guild or trait categories (trait-based approach); they can be highly indicative for the ecological quality, and their geographical distribution is less variable than that of taxonomic metrics (Pont *et*

*al.*, 2006). Functional traits are being increasingly considered in bioassessment studies, not only for reducing the problems associated with geographic differences in the taxonomic composition of communities, but also for enhancing the sensitivity of the bioassessment tool to environmental degradation (e.g. Noble *et al.*, 2007; Dolédec & Statzner, 2010; Logez & Pont, 2011; Van den Brink *et al.*, 2011; Bouleau & Pont, 2015).

Application of the IBI to Mediterranean-climate regions has been challenging. Fish faunas in Mediterranean rivers have experienced relatively long periods of isolation and environmental harshness, and are characterised by low species richness, high endemism ratios and relatively broad environmental tolerances (Ferreira *et al.*, 2007). These conditions limit the applicability of the classical IBI bioassessment approach, which has been designed for use in relatively species-rich communities. It is especially a problem to include functional metrics in the IBI, given that most Mediterranean rivers are dominated by tolerant species with low ecological specialisation. Under these circumstances, the deployment of specialised functional metrics may introduce bias and undesirable noise, thereby creating similar problems as discussed above for rarity. Based on such considerations, several authors have ascertained that functional (guild) metrics have limited utility in the species-depauperate and highly diversified Mediterranean fish faunas, and that compositional and population age/size structure metrics are more appropriate (Moyle & Randall, 1998; Lyons, 2006; Ferreira *et al.*, 2007; Magalhaes *et al.*, 2008; Hermoso *et al.*, 2010; Monaghan & Soares, 2010; Aparicio *et al.*, 2011; Ayllón *et al.*, 2012; Benejam *et al.*, 2005, 2015). We subscribe to this view, and we consider that the national bioassessment system should have a reduced number of functional metrics. We recognise, however, that certain “common” functional traits, including feeding habitat and degree of rheophily, have their place in a Greek bioassessment system.

Within this general framework, two bioassessment options need to be considered and tested for best performance and applicability: developing local (basin-scale) indices that have the capacity to incorporate local community attributes, and creating regional indices that are more broadly applicable (Roset *et al.*, 2007; Ferreira *et al.*, 2007; Schmutz *et al.*, 2007; Hermoso *et al.*, 2010; Benejam *et al.*, 2015). The “local” approach to index development copes well with the strong biogeographic structure and spatial heterogeneity of the Greek ichthyofauna, and is a logical option for some basins with high endemism levels. The “regional” approach has to be based largely on “guiding” species and is preferable from cost and practicability perspectives. However, as our study has shown, ecoregions contain substantial biological heterogeneity within them, and caution is needed when extrapolating approaches developed for one area to other areas. These considerations underscore the criti-



cal need for fundamental research focused on community dynamics and ecological processes. Research should also explore the possibility of using models for the derivation of reference conditions. Model-based indices have the capacity to simulate ecological processes over large spatial extents, which is advantageous when variation in local communities changes considerably with regional scale (Scardi *et al.*, 2006; Hawkins *et al.*, 2010).

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## Appendix A

**Table A1.** Species sampled, abundances (N, numbers of individuals collected), occupancies (S, number of sites; B, number of basins; E, number of ecoregions), provenance status (N=Native, A=Alien, T=Translocated) and endemism level (GR=Endemic to Greece and BL=Endemic to Balkans).

Species	Authority	N	S	B	E	Provenance status	Endemism level
<b>Anguillidae</b>							
<i>Anguilla anguilla</i>	(Linnaeus, 1758)	299	15	7	3	N	
<b>Blenniidae</b>							
<i>Salaria fluviatilis</i>	(Asso, 1801)	448	11	5	1	N	
<b>Centrarchidae</b>							
<i>Lepomis gibbosus</i>	(Linnaeus, 1758)	43	6	2	2	A	
<b>Clupeidae</b>							
<i>Alosa fallax</i>	(La Cepede, 1803)	1	1	1	1	N	
<b>Cobitidae</b>							
<i>Cobitis arachthosensis</i>	Economidis & Nalbant 1997	4	1	1	1	N	GR
<i>Cobitis punctulata</i>	Erk'akan, Atalay-Ekmekci & Nalbant 1998	5	1	1	1	N	
<i>Cobitis strumicae</i>	Karaman 1955	221	14	4	1	N	BL
<i>Cobitis vardarensis</i>	Karaman 1928	47	7	3	1	N	BL
<i>Sabanejewia balcanica</i>	(Karaman, 1922)	60	7	3	2	N	BL
<b>Cyprinidae</b>							
<i>Alburnoides bipunctatus</i>	(Bloch, 1782)	2231	24	6	4	N	
<i>Alburnus alburnus</i>	(Linnaeus, 1758)	243	10	1	1	N	
<i>Alburnus</i> sp.		30	1	1	1	N	
<i>Alburnus thessalicus</i>	Stephanidis 1950	862	10	3	1	N	BL
<i>Alburnus vistonicus</i>	Freyhof & Kottelat 2007	1	1	1	1	N	GR
<i>Aspius aspius</i>	(Linnaeus, 1758)	1	1	1	1	N	
<i>Barbus</i> sp.		31	5	3	1	N	
<i>Barbus balcanicus</i>	Kotliik, Tsigenopoulos, Rad & Berrebi 2002	236	4	3	1	N	
<i>Barbus cyclolepis</i>	Heckel 1837	758	9	2	1	N	BL
<i>Barbus euboicus</i>	Stephanidis 1950	181	1	1	1	N	GR
<i>Barbus macedonicus</i>	Karaman 1928	126	10	3	1	N	BL
<i>Barbus peloponnesius</i>	Valenciennes 1842	1132	18	7	1	N	GR
<i>Barbus rebeli</i>	Koller 1926	525	3	1	1	N	
<i>Barbus sperchiensis</i>	Stephanidis 1950	765	13	2	2	N	GR
<i>Barbus strumicae</i>	Karaman 1955	183	5	2	1	N	
<i>Carassius gibelio</i>	(Bloch, 1782)	257	13	5	2	A	
<i>Chondrostoma vardarensis</i>	Karaman 1928	469	20	6	3	N	
<i>Cyprinus carpio</i>	Linnaeus 1758	62	8	6	4	N,T	
<i>Gobio bulgaricus</i>	Drensky 1926	136	13	7	2	N	BL
<i>Gobio feraeensis</i>	Stephanidis 1973	113	6	1	1	N	GR
<i>Leucaspis delineatus</i>	(Heckel, 1843)	6	2	2	1	N	
<i>Luciobarbus albanicus</i>	(Steindachner, 1870)	348	11	4	1	N	GR
<i>Luciobarbus graecus</i>	(Steindachner, 1896)	66	3	1	1	N	GR
<i>Pachychilon macedonicum</i>	(Steindachner, 1892)	39	7	2	1	N	BL
<i>Pachychilon pictum</i>	(Hechel & Kner, 1858)	3	1	1	1	N	BL
<i>Pelagus laonicus</i>	(Kottelat & Barbieri, 2004)	878	12	2	1	N	GR
<i>Pelagus marathonicus</i>	(Vinciguerra, 1921)	150	5	3	2	N	GR
<i>Pelagus stymphalicus</i>	(Valenciennes, 1844)	3	2	2	1	N	GR

(continued)

Table 1 (continued)

<i>Pelagus thesproticus</i>	(Stephanidis, 1939)	14	2	2	1	N	BL
<i>Petroleuciscus borysthenicus</i>	(Kessler, 1859)	246	4	3	1	N	
<i>Phoxinus strymonicus</i>	Kottelat 2007	112	1	1	1	N	(GR)
<i>Pseudorasbora parva</i>	(Temminck & Schlegel, 1846)	24	4	3	3	A	
<i>Rhodeus amarus</i>	(Bloch, 1782)	1098	13	3	1	N	
<i>Rhodeus meridionalis</i>	Karaman 1924	2115	12	3	1	N	BL
<i>Romanogobio elimeius</i>	(Kattoulas, Stephanidis & Economidis, 1973)	19	3	2	1	N	BL
<i>Rutilus rutilus</i>	(Linnaeus, 1758)	365	16	4	2	N	
<i>Rutilus sp. Sperchios</i>	In Kottelat & Freyoff 2007	70	2	1	1	N	GR
<i>Scardinius acarnanicus</i>	Economidis 1991	3	1	1	1	N	GR
<i>Squalius cf. peloponensis</i>	(Valenciennes, 1844)	678	11	7	1	N	GR
<i>Squalius keadicus</i>	(Stephanidis, 1971)	698	11	1	1	N	GR
<i>Squalius orpheus</i>	Kottelat & Economidis 2006	918	14	4	1	N	BL
<i>Squalius sp. Aeos</i>	In Kottelat & Freyoff 2007	2	1	1	1	N	BL
<i>Squalius sp. Evia</i>	In Kottelat & Freyoff 2007	337	2	2	1	N	GR
<i>Squalius vardarensis</i>	Karamam 1928	1286	22	5	2	N	BL
<i>Squalius sp. Evinos</i>	In Kottelat & Freyoff 2007	658	8	2	1	N	GR
<i>Telestes pleurobipunctatus</i>	(Stephanidis, 1939)	1094	15	7	1	N	GR
<i>Tinca tinca</i>	(Linnaeus, 1758)	5	1	1	1	T	
<i>Tropidophoxinellus spartiaticus</i>	(Schmidt-Ries, 1943)	2690	13	3	1	N	GR
<i>Vimba melanops</i>	(Heckel, 1837)	109	13	4	2	N	BL
<b>Esocidae</b>							
<i>Esox lucius</i>	Linnaeus 1758	4	3	1	1	N	
<b>Gasterosteidae</b>							
<i>Gasterosteus gymnaurus</i>	Cuvier 1829	21	2	2	2	N	
<b>Gobiidae</b>							
<i>Knipowitschia caucasica</i>	(Berg, 1916)	6	2	2	1	N	
<i>Knipowitschia sp.</i>		3	1	1	1	N	
<i>Knipowitschia thessala</i>	(Vinciguerra, 1921)	55	6	1	1	N	GR
<i>Proterorhinus semillunaris</i>	(Heckel, 1837)	78	11	1	1	N	
<b>Nemacheilidae</b>							
<i>Oxynoemacheilus bureschi</i>	(Drensky, 1928)	7	2	2	1	N	BL
<i>Oxynoemacheilus pindus</i>	(Economidis, 2005)	6	1	1	1	N	BL
<b>Percidae</b>							
<i>Sander lucioperca</i>	(Linnaeus, 1758)	1	1	1	1	N	
<i>Perca fluviatilis</i>	Linnaeus 1758	15	2	1	1	N	
<b>Poeciliidae</b>							
<i>Gambusia holbrooki</i>	Girard 1859	1233	14	7	3	A	
<b>Salmonidae</b>							
<i>Oncorhynchus mykiss</i>	(Walbaum, 1792)	1	1	1	1	A	
<i>Salmo farioides</i>	Karaman 1938	553	14	4	2	N	BL
<i>Salmo cf. trutta</i>	Linnaeus 1758	1	1	1	1	A	
<b>Siluridae</b>							
<i>Silurus glanis</i>	Linnaeus 1758	1	1	1	1	N	