Fitting a new assessment system for rivers in Greece using fish fauna to the results of the MED GIG

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1. INTRODUCTION

This report documents the development of a new ecological classification method for rivers using the BQE Fish. The approaches followed comply with the procedures specified in CIS Guidance Document no 30 (Wilby *et al*. 2014). Greece did not intecalibrate existing fish indices in the previous rounds of the intercalibration exercise due to lack of sufficient standardised data and the absence of a state-wide fish index. Although important fish-based bioassessment projects have been worked on in Greece for more than a decade, routine fish data collection began following the establishment of a national bioassessment monitoring programme in 2012. This led to the development of the “Hellenic Fish Index” (HeFI) during 2014-2016. This index is based on the site-specific approach for defining reference conditions and offers prospects for a nation-wide application. A number of fish-based indices developed earlier through the type-specific approach could only have local application due to biogeographic variability, hydrographic idiosyncrasies and substantial biological heterogeneity in the country constraining their transferability to other areas. Methods for data collection, for setting reference conditions and class boundaries, and for selecting and calibrating metrics are described and are generally compliant with the WFD normative definitions. HeFI has undergone some preliminary validation using monitoring data. However, its performance and efficiency in some river systems remains untested.

2. BACKGROUND

Before 2012 there was no functional biological monitoring programme utilising the BQE "fish in the rivers" of Greece. However, since 2003, bioassessment was included in various projects that pursued various objectives, such as environmental protection. The fish sampling procedures have generally been following the field protocols produced by the FAME project (Schmutz *et al*. 2007a). In the summer of 2009 a single nation-wide fish survey was conducted within an initial project for river assessment following the WFD (Chatzinikolaou & Economou 2009; for results see: Economou *et al*. 2016). In 2012 a monitoring system compatible with the WFD procedural requirements was formally established. The delay in initiating monitoring affected progress in bioassessment research through limiting the opportunities for the collection of fish data upon which bioassessment tool development and testing heavily relies.

Additional difficulties arose from biogeographic variability in the country, hydrographic idiosyncrasies and biological complexities (e.g. data-gaps in endemic fish ecological knowledge, fish taxonomy revisions, etc). Greece is a hydrologically fragmented country with many medium and small sized rivers (over 120 autonomous river basins containing fish have been identified; Economou *et al*. 2007a; Koutsikos *et al*. 2012). The freshwater fish fauna is characterised by a strong biogeographic structure (eight freshwater ecoregions have been defined; Zogaris *et al*. 2009a, 2009b). Like in other Mediterranean countries (Logez *et al*. 2005; Ferreira *et al*. 2007a, 2007b; Magalhães *et al*. 2008; Hermoso *et al*. 2010; Alonso *et al*. 2011, Aparichio *et al*. 2011; Dallas 2013; Benejam *et al*. 2015), the rivers Greece are characterised by fluctuating and seasonally arid hydroclimatic conditions, and they host species-poor, highly endemic and greatly diversified fish faunas which are dominated by tolerant taxa with wide environmental tolerances and low functional specialisation (Skoulikidis *et al*. 2009; Economou *et al*. 2016). This combination of conditions limit the direct applicability of bioassessment approaches that have been developed in central and northern European countries and make make fish-based bioassessment particularly challenging (Logez *et al* 2005; Ferreira *et al*. 2007a; Pont *et al*. 2007; Magalhães *et al*. 2008; Benejam *et al*. 2015; Hermoso *et al*. 2010; Dallas 2013).

Under the circumstances described above, the strategy for advancing fish-based bioassessment consisted in making the best use of what resources were available (workforce, equipment, datasets, bioassessment tools) and covering as many aspects of river assessment as possible. Initial efforts (before monitoring was implemented) focused on the development of spatially-based (type-specific) ecological status evaluation systems. These were often the by-product of small-scale regional projects which were undertaken with other objectives. Although fish sampling was conducted with WFD-compliant methodologies, the data generated by these projects were too localised and variable in temporal time scales to serve as a reliable basis for nation-wide bioassessment analysis. Some fish-based indices were generated, which were designed for local applications. However, the substantial environmental and biological heterogeneity across the country constrained the transferability of already established indices to national or even ecoregional scales. As a result, when the intercalibration exercise was taking place, a national fish-based bioassessment system was not in place. Because of this, Greece was only an observer in the "fish BQE for rivers" intercalibration process. However, researchers maintained close ties with many team members working on bioassessment and intercalibration in Mediterranean countries, and even worked on joint projects (Pont et al. 2011).

In 2009 a state-wide biological sampling programme was established, but the programme was suspended after only one round of sampling (summer 2009) had been completed. However, this sampling exercise provided basic insights into planning and procedural aspects of survey design and field protocol development and guided subsequent research efforts towards bioassessment tool development. The results of these sampling activities have been reported by Economou *et al*. (2016).

Routine and systematic fish sampling over broad spatial and temporal scales with the use of standardised protocols began in 2012, when monitoring activities were re-initiated. Data collection through these activities enabled the development of the “Hellenic Fish Index” (HeFI), which is based on a site-specific approach for predicting reference conditions. The index offers perspectives for a nation-wide application and has undergone some preliminary validation using existing data.

3. SURVEY DESIGN, FIELD SAMPLING AND RECORDING PROCEDURES

The starting point of most bioassessment monitoring methodological tasks and analyses was work accomplished during the EU-funded research project FAME in which the HCMR participated from 2002 (Schmutz *et al*. 2007a). HCMR ichthyological sampling for bioassessment pioneered something new for Greece: the use of several field protocols concurrently at each site following a holistic potamological approach (Zogaris *et al*. 2008, Zogaris 2009).

Greece's official WFD sampling network, as defined in the Joint Ministerial Decision 140384/2011, includes 449 sampling stations (149 for operational and 300 for surveillance monitoring), in 14 major River Basin Districts. Operational stations are sampled annually with two samples per year (spring and summer). Surveillance stations are scheduled to be sampled on a 3-year rotation basis, again with two samples (spring and summer) taken in the year of sampling. An increase of the sampling stations to 490 (195 for operational and 294 for surveillance monitoring) has been decided for the next sampling cycle (beginning in early 2017).

The design of the sampling network took into account a preliminary list of river Water Bodies which was provided by the administration. The positioning of the sampling sites was determined on the basis of best available knowledge to represent the landscape and macro-habitat features and the range of prevailing human pressures. Several sites were selected to correspond with sites sampled in previous fish and macroinvertebrate surveys in order to ensure the inclusion of interannual biological variability in the datasets. Sites contained in a pre-existing hydrochemical monitoring programme (the "National Network for Monitoring the Quality of Surface Waters") were also included in the sampling scheme. The designation of sites as routine or surveillance was based on available knowledge of important pressures and assessments of impacts.

The sampling network contained undisturbed or minimally disturbed sites (reference sites) to serve for the determination of reference conditions. Reference sites were selected on the basis of pressure “exclusion criteria”, following general guidelines developed during the FAME project (Economou 2002). Data from sites with low levels of anthropogenic disturbance or affected relatively little by human pressures (e.g. low levels of pollution from agricultural activities or water abstraction) were used for the derivation of reference conditions.

Fish sampling was carried out solely with the use of electrofishing and took place at the same locations as with sampling for macroinvertebrates and other BQEs. At each sampling site measurements of chemical and physicochemical parameters were made and hydromorphological analysis was performed with the use of the River Habitat Survey (RHS) method (Raven *et al.* 1997); QBR was also used as an index of riparian condition at most sites. Photographs and video of the sampled localities and specimens caught were taken for documentation and follow up evaluations.

The official sampling site area was defined as a 500-m long river section covered by the RHS method within which sampling for the various BQEs was conducted. Within each site, a representative river reach containing typical fish habitats (i.e. riffle, run, pool, glide) was selected and electrofished. A single pass was conducted and no stop nets were used. In deep rivers the electrofishing gear was fitted on a boat. Fish sampling and environmental data recording followed standardised procedures; key environmental and habitat parameters were recorded (IMBRIW 2012).

Fish sampling procedures were adapted from the CEN standard BS EN 14011:2003 Water Quality- Guidance standard on sampling fish with electricity. With regard to the length and method of the sampled stretch, some flexibility was allowed dependant on river size and depth, flow velocity, morphological, habitat characteristics and biological conditions (e.g. number of autochthonous species, abundance of individuals). Except in fishless streams, at least 15 individual fish were set to be collected (minimum catch; if not the sampling was continued in most cases until the minimum catch as completed). Generally more sampling effort was put in sites with higher species diversity and habitat heterogeneity or when fish density was particularly low. At the end of fish sampling occasion a qualitative assessment of sampling effort and efficiency was made. Sampling effort was assigned to quality classes A, B, C, D as described in the Institute's manual (IMBRIW 2012) and was recorded in the sampling protocol (see APPENDIX Figure A3). Quality class D indicates highly insufficient and/or non-representative sampling and the results from this sampling occasion were not used for ecological status assessments. This was often the case when there were particularly strong flow conditions, great depths, or morphological obstacles preventing sampling the desired minimum sampling length. Sampling quality class D is also used for qualitative records of fish. Sampling quality C is a borderline condition, and in many cases it was also not considered in our ecological status assessment.

An important parameter is the actual length of river (and the areal cover) sampled. The 20X empirical rule (fished length at least 20 times the mean wetted stream width) was set for small wadable streams (less than 6 m wide). This rule was nearly always satisfied, and stream portion about or over 100 m was typically sampled. The rule was not met in a small number of sites (less than 10) with highly homogeneous fish fauna, e.g. mono-species or low-species sites such as in small "mountain barbel type" streams. For larger wadable streams up to 15 m wide a ten-fold rule was generally assigned. The great majority of sites (80%) satisfied the rule and between 80 and 400 m were sampled. The sampling area always exceeded $100m^2$ for small streams and $200m^2$ for larger streams. For rivers with a wetted width >15 m also a ten-fold rule was generally assigned and at least 150 m length was always sampled. Boat sampling was used in most cases and several hundred meters were usually sampled. Boat sampling always exceeded $600m^2$ sampling area, with most samples lying over $1000m^2$.

Finally, regardless of the sampling method used (wading or boat), all samples, were assigned specific sampling form categories: "one bank", "partial whole" and "whole". "One bank" category covered all habitats of the shore, "partial whole" covered all habitats of the shore plus habitats of the mid-channel and "whole" category covered all habitats of the wetted channel (when "whole" was done with significant space left un-sampled it was distinguished as "ambient"; i.e. nearly whole but more fragmented and/or dispersed sampling).

Throughout the surveys, two types of electrofishing devices were used: a) Battery-powered Backpack: Hans-Grassl GmbH (Model IG200-2, DC pulsed, 1,5 KW output power, 35-100 Hz, max. 850 V) and/or Smith-Root 24L (DC pulsed 1,5 KW, 10-100 Hz, max. 980 V) which were routinely used to sample fish in streams and small rivers; 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. When the latter gear was used, a common practice involved the operator thrusting (and throwing) the anode at a distance ahead to surprise the fish and limit fish escape. The latter gear was mounted on a small aluminum boat and used in non-wadable river sections during boat-based sampling in deeper waters.

The fish recording procedures were based on several economic and technical considerations, such as limitations in the available workforce and the restricted time frame within which the daily sampling plan and each sampling round had to be completed. Thus, the fish caught were identified to species level, measured in situ in 5 cm class intervals and returned alive to the river. Measuring at 5 cm intervals provides rapid measurement documentation and this has been effectively utilised in some EU member states such as in the FiBS application in Germany (Düssling 2009). In cases of identification problems (usually juveniles), samples were preserved in formalin solution for laboratory identification. In some localities young fry occurred in enormous densities and could not be quantitatively sampled due to large numbers and/or gear limitations. However, an index of fry abundance was assessed and recorded in the protocol. In each sampling occasion the wetted surface area sampled was estimated from geometrical characteristics (fished length and cross-sectional width). Fish densities could not be quantitatively determined because no stop nets were used (also only one anode was applied).

4. ACTIVITIES SUPPORTING BIOASSESSMENT RESEARCH

Bioassessment monitoring is an interdisciplinary operation which includes various steps and stages and strongly depends on the accomplishment of tasks that provide input to monitoring tool development (e.g. species inventories, biogeographic regionalisation and ecological trait classifications). Significant milestones accomplished include the following:

4a. State-wide distributional inventory of fish species

A basin-level ichthyological database of all major catchments of Greece has been developed, using sampling data and literature information. Initially established using distribution records for native and introduced freshwater fish species from 105 hydrographic basins (Economou *et al.* 2007), the database was subsequently expanded with the addition of new information from monitoring and other research activities (e.g. Koutsikos *et al.* 2012). The database now contains data for 127 hydrographic basins. More than 160 species are accommodated in this distributional compilation. Species lists are given for each basin area, and information on conservation status and provenance are provided. This database has supported research for ecoregional delineations, and has facilitated the creation of an inventory of fish species with documented occurrence in freshwaters (see below). It has also supported efforts for species conservation.

4b. Biogeographic classification of the southern Balkans based on fish

The WFD's typology is largely based on J. Illies' biogeographical regionalisation and important discrepancies have been identified between the WFD's proposed ecoregoinal map and current understanding of major freshwater biogeographical boundaries (Zogaris *et al.* 2009a). A freshwater ecoregional delineation in the southern Balkans based on biogeographic associations of drainage-specific fish communities was developed and refined

(Zogaris 2009, Zogaris *et al.* 2009b) (see APPENDIX Figure A1.). This work was corroborated by a classification based on site-specific assemblage data by Economou *et al.* (2016). Elaboration of the data obtained by the monitoring activities has revealed that the different freshwater ecoregions host different species and different assemblage types (e.g. APPENDIX Table A1). Fish taxonomy and especially our understanding and interpretation of systematics and phylogeography are also important in defining among-basin biogeographical affinities (Economou *et al*. 2007a).

4c. Type-specific reference conditions and fish-based biotic typology classification

The issue of reference conditions is keystone within WFD bioassesment and especially challenging with fish assemblages in Mediterranean rivers (Economou 2002, Ferreira *et al.* 2007a). Recently, several projects have researched and identified fish community patterns, such as longitudinal changes and environmental parameters affecting these assemblages (Economou *et al.* 2003, Zogaris *et al.* 2004, Economou *et al.* 2007a, Zogaris 2009, Vardakas *et al.* 2015). Specific longitudinal distribution patterns under near-natural conditions have been identified (APPENDIX Figure A2)

4d. Checklist of Greek freshwater fish species and taxonomic clarifications

There has been a serious problem in bioassessment and conservation research and applications with changing fish taxonomy (Economou *et al.* 2007a). A special project to promote national checklist management (called *fishlist.gr*) has been recently pursued by IMBRIW-HCMR and a web-based educational tool is being developed. Greece's most recent annotated checklist was published during this project (Barbieri *et al.* 2015).

4e. Sampling standardisation and field protocol development

Sampling procedures and field data collections targeting bioassessment are in accordance with the FAME (2005) project protocols and largely follow the CEN electrofishing sampling methodology. Stardardised field forms have been developed since 2003 in Greece (see APPENDIX Figure A3.) The standard method and protocols are outlined in a detailed manual (IMBRIW, 2012) and have also been utilised in other countries in the Eastern Mediterranean (Zogaris *et al*. 2012; Zogaris & Özeren 2014). Differences from CEN procedures and training event developments have been outlined in Zogaris *et al*. (2015).

4f. Species guild classifications

An important need in bioassessment tool development is to assign fish species into guild or functional trait categories for making fish metrics transferable among different basins or ecoregional units. We exploited our own natural history knowledge and published information on species ecologies, biologies and life histories, together with assessments of habitat requirements derived from our site-specific database to produce guild classifications of all species normally encountered in the riverine systems of Greece (APPENDIX Table A2.)

4g. Site-specific databases for fish and environmental parameters

Fish data, environmental attributes and human-induced pressure information relevant to ecological status assessments are accommodated in a site-specific database. These data were acquired from a number of river basins through the use of fish sampling protocols specifically designed to serve the environmental demands of the WFD. The databases were initially fed with data collected during early EU-funded research projects that were undertaken to support the WFD (FAME, STAR). Since then the databases serves as the repository of data collected during all subsequent fish surveys. Data from a pre-existing site-specific fish database (Economou *et al*. 1999) were evaluated; those assessed as being pertinent and compatible with the WFD requirements were included in the new database.

5. FISH-BASED BIOASSESSMENT INDICES

Bioassessment tools developed so far are multi-metric and are based on the reference conditions concept. Two basic approaches have been followed for reference conditions designation and tool development: the spatially-based (type-specific) approach, and the model-based (site-specific) approach.

5a. Spatially-based indices

The simplest approach, and the default in the WFD, is the spatially-based approach. This involves the establishment of a river typology that allows variability of biological conditions over a broad geographical area to be partitioned into smaller spatial units (river types), for which type-specific reference conditions are defined. Four indices have been developed through this method. All were designed for local applications and were developed in the frame of research projects which were adequately resourced to enable sampling at spatial scales sufficient for multimetric indices to be constructed.

Procedures followed the guidelines of the FAME project (Schmutz *et al*. 2007b) and involved the use of similarity analysis for identifying biologically homogeneous regions (biotic typology), discriminant analysis for assigning probabilities of class membership to sites, the characterisation of reference conditions through a combination of approaches (use of data from reference sites, historical information when available, and expert judgment), the selection of metrics representing the local taxonomic composition and community structure, metric testing (redundancy, responsiveness to pressures), and setting class boundaries to metrics. Metrics were selected to represent all relevant biological parameters defined in the WFD. To the degree possible, the instructions provided in the Guidance Document no 10 (EU, 2003; Table in page 48) were followed.

- **Upland rivers fish index**

The index was developed for use in upland areas of the following river basins: Acheloos, Aliakmon, Alpheios, Arachthos and Aoos (Economou *et al*. 2007b). This index defined three major river types and can potentially be applied to other upland rivers of Greece and the Balkans. Several other indices and a detailed analyses of pressures including riparian habitat and hydromorphological human-induced degradation were studied concurrently during this bioassessment development (Zogaris *et al*. 2009; Chatzinikolaou *et al*. 2011). Four river types were defined (see Appendix A.

- **Evrotas fish index**

Evrotas is a hydrologically impacted river basin that hosts a species-depauperate and highly endemic fish fauna. Much of the main stem of this river is considered artificially intermittent (Skoulikidis *et al*. 2012) and this presents a challenge due to significant variablility especially after droughts. An index was developed based on local conditions and non-perennial reach idiosynchrasies (Skoulikidis *et al*. 2008; Vardakas *et al*. 2009). Three river types were defined.

- **Aliakmon fish index**

This index was developed for use in upper Aliakmon where the construction of a dam expected to impact the area was scheduled (Economou *et al.* 2009; Tachos *et al.* 2013). Three river types were defined. This index can be potentially be applied to the upper portions of other rivers in the Macedonia-Thessaly ecoregion. Three main river types were defined.

- **Sperchios fish Index**

This index was recently developed during an in-depth research project (KRIPIS Project Report 2016) in a basin of the northernmost part of the Western Aegean ecoregion. The index closely follows the WFD demands for uncertainty analysis (Oikonomou *et al*. 2015) and is available in recently completed unpublished report (see Sperchios River website: http://imbriw.hcmr.gr/en/development-of-integrated-basin-management-and-associated-coastal-and-marine-zone/). Four river types were defined.

5b. Model-based indices

Effort has been invested in building interregional indices that go beyond biogeographical regions. Since the EFI+ index is not applicable to Greece, it was important to begin working on model-based indices that rely on species traits and thereby surpass biogeographically-limited basin conditions. Two indices have been developed through this approach. Both use a combination of river-landscape descriptors and environmental variables to assist in “predicting” ichthyological reference conditions at a site, with which the observed fish attributes in the samples are compared.

- ***Fish-Assessment Tool for Hellenic Rivers - FATHeR***

This model-based fish index was developed through the cooperation of Uwe Dussling (German FAME partner) using limited data from the middle and upper sections of a number of watersheds within three freshwater ecoregions (Economou *et al*. 2007b). FATHeR employs environmental variables to predict the “reference fish community” in a site and then employs sample data from the site to calculate the deviation of fish community attributes from the reference attributes.

- ***Hellenic Fish Index (HeFI)***

This model-based index was developed at HCMR between 2014 and 2016 and is largely based on procedures developed in the European Fish Index approach (Schmutz et al. 2007a). It is based on data from all relevant standardised samplings including the national monitoring programme of surface waters. The index follows the WFD requirement to utilise fish composition, abundance and age structure for assessment (although size class length is used as a proxy of age). The index construction details are provided in the next section of this report.

6. DEVELOPMENT OF A STATE-WIDE INTERREGIONAL FISH INDEX (HeFI)

Here we provide details of the development and a description of the Hellenic Fish Index (HeFI). This work was developed between 2014 and early 2016 at HCMR with the cooperation of Prof. Stefan Schmutz (BOKU, Austria) and will soon be submitted for publication (Zogaris *et al*. in prep.). This index aims to be a standard national index, applicable in a wide variety of rivers among different biogeographical regions in the southern Balkans. The index utilises fish composition, abundance, age (size) structure and disturbance-sensitive taxa for assessment, and therefore meets all the requirements of the WFD.

A total of 640 samples were considered for the analyses leading to the index; this is the largest dataset ever used for inland fish assemblage-environmental research in Greece. Sampling followed the IMBRIW protocol largely applying the CEN procedure; however only one anode was used despite the width of the river's wetted area (and this creates a semi-quantitative aspect to sampling, especially in deeper waters). The sampled sites are fairly evenly distributed across the mainland of Greece and include two major islands (Evia, Lesvos). Several islands are not included since their ichthyofaunal composition is poorly developed, and the type-specific reference conditions are especially difficult to establish as is the case throughout the Mediterranean islands and peninsulas (Zogaris *et al*. 2012, Skoulikidis *et al*. 2014). In total, the analysed dataset contains 248,178 fish of 103 species. A median number of 223 fish (25% quantile 73.8, 75% quantile 516.0) and 4 species (25% quantile 2, 75% quantile 7) were caught in each sample. Fish sampling effort was about the same in reference (median 510 m^2) and impacted samples (median 450 m^2).

Functional guild definitions were applied to developed potential bioassessment metrics. Six biological and ecological traits were considered according to previous classifications of European fish traits with regard to reproduction, trophic position, habitat preference, habitat alteration and migratory behaviour. Each species was assigned to one of the different categories of a trait (24 categories). We assigned species to categories based on published accounts (e.g., Economou *et al*. 1999; FAME 2005, Logez *et al*., 2013) and recent field observations of endemic and range-restricted species whose natural history and ecology is poorly documented. Since the number of non-native species was comparatively low they were included and not given special significance as was documented in an initial monitoring review (Economou *et al*. 2016). In total, species were classified into 13 categories out of 6 traits. Each category represents 10 to 68 species (Table 1). Figure 1 shows the proportion of fish species contributing to the four final metrics.

Direction of response of ecological traits were predefined according to ecological expectations (i.e positive or negative expected response to human-induced degradation; see Table 1). Due to the "semi-quantitative" type of sampling only relative density ("dens") and relative number of species ("rich") were considered. All metrics were additionally calculated for small (<100 mm or <150 mm total length) and large fish (>=100 mm or >=150 mm total length).

Table 1: Species traits and categories tested and selected: category, code, number of classified species, acceptable reference model established, significant response to pressures and non redundant to other metrics, and finally selected metrics used for Index. Of the metrics selected three had a positive expected response to degradation, the rest negative.

Trait	Category	Code	Number of species classified	Expected Response	Reference model	Pressure response	Non redundant metrics
Feeding habitat	benthic	BENTH	44	Negative	✓	✓	dens.BENTH.p.150small
	water column	WC	68	Positive	✓	✓	-
Habitat	eurytopic	EURY	39	Positive	-	-	-
	limnophilic	LIMNO	29	Negative	-	-	-
	rheophilic	RHEO	44	Negative	-	-	-
Reproduction	lithophilic	LITH	53	Negative	✓	-	-
	phytophilic	PHYT	47	Negative	-	-	-
Reproduction/habitat	rheolithophilic	RH_LITH	53	Negative	✓	-	-
Feeding	insectivorous	INSV	55	Negative	✓	✓	dens.INSV.p.100large
	omnivorous	OMNI	44	Positive	✓	✓	dens.OMNI.p.100small
	piscivorous	PISC	10	Negative	-	-	-
Migration	long distance	LONG	11	Negative	✓	-	-
	potamodromous	POTAD	33	Negative	✓	✓	dens.POTAD.p.all

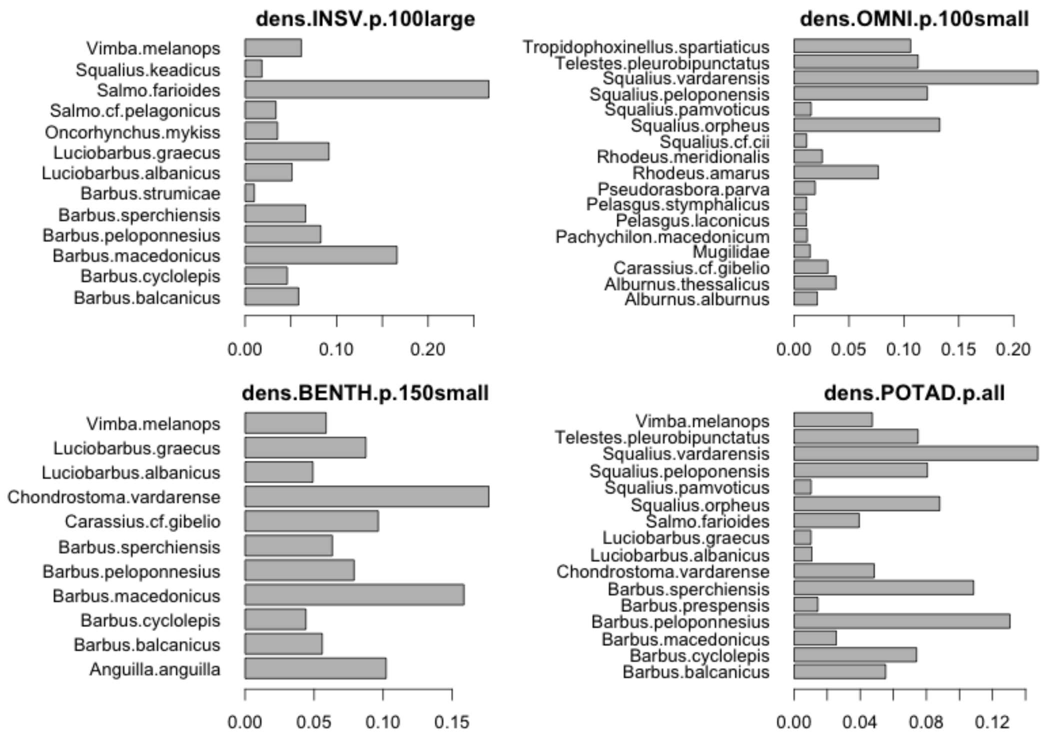

Figure 1: Proportion of fish species contributing to the four final metrics used for the Helenic Fish Index (HeFI) within the reference samples.

In order to develop the index the following pressure attributes were assessed (following Schinegger *et al* 2013) to identify near-reference and degraded sites: channel modification (channelization), Instream habitat modification, embankment, riparian vegetation modification, barrier upstream, barrier downstream, barrier basin, water abstraction, hydropeaking, hydrological modification, impoundment, pollution, urbanisation, and irrigation. Each site was assessed during a distance-based desk-study by a core team of expert field ichthyologists who understand human-induced pressures on the ichthyofauna (Figure 2). So-called "Reference sites" were defined as not or minimally impacted sites. For the selection of responsive metrics two datasets were defined: (1) “No or minimally impacted samples” (REF: class 1 and 2, n=135 sites), (2) “Strongly impacted samples” (IMPACT: class 4 and 5, n=297 sites) (see Table 2)

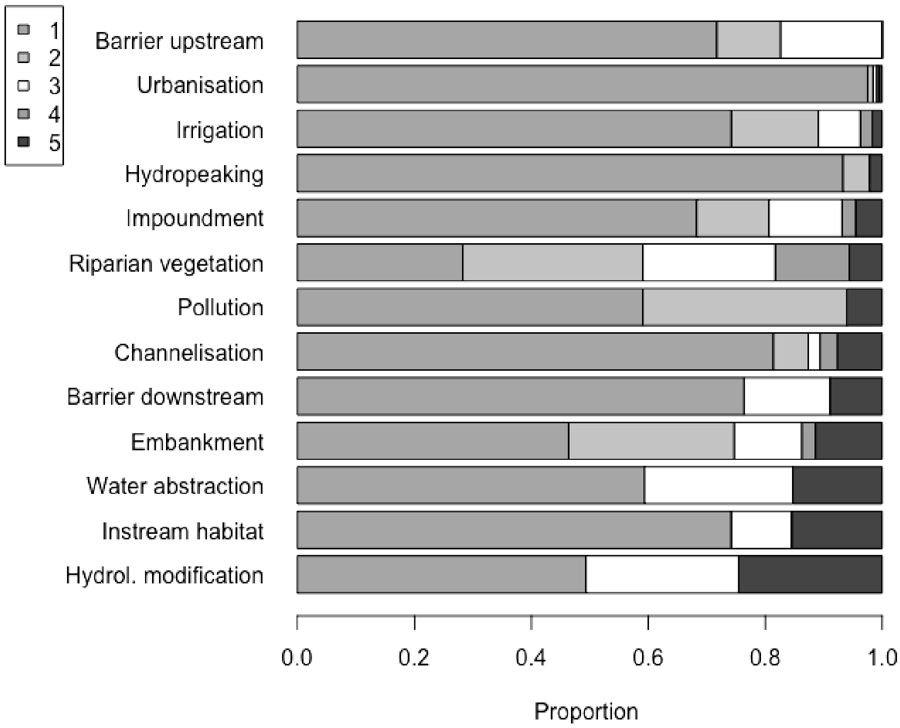

Figure 2: Classification of human-induced pressures in sites used to develop the Hellenic Fish Index (HeFI). The class-categories follow WFD practice (1=reference/high; 5=bad).

Table 2: Scoring of pressures into 5 classes and definition of reference and impacted dataset.

	Class				
Pressure information	**1**	**2**	**3**	**4**	**5**
Channel modification	1	2	3	4	5
Instream habitat modification	1	-	3	-	5
Embankment	1	2	3	4	5
Riparian vegetation modification	1	2	3	4	5
Barrier upstream	1	2	3	-	
Barrier downstream	1	-	3	-	5
Barrier basin	1	-	3	-	5
Water abstraction	1	-	3	-	5
Hydropeaking	1	2	-	-	5
Hydrological modification	1		3	-	5
Impoundment	1	2	3	4	5
Pollution	1	2			5
Urbanisation	<5%	>=5%,<10%	>=10%,<20%	>=20%,<30%	>=30%
Irrigation	<10%	>=10%,<20%	>=20%,<30%	>=30%,<40%	>=40%
N	53	82	208	36	261
Datasets	Reference data: no or minimally impacted (N=135)			Strongly impacted (N=297)	

6.a- Metric modeling and selection

Classification and regression trees (CRT), a recursive partitioning method, were used to model fish metrics as a function of environmental characteristics. Tree methods encompass several advantages: (1) nonparametric basis, (2) no implicit assumption of linearity, (3) simplicity of results for interpretation and (4) ability of predictive classification for new observations. Trees depth level was limited to 3 levels and minimum bucket size to 15 samples in order to avoid overfitting. Models performance were tested by calculating Pseudo-R2 and by 10-fold cross-validation using the intern routine of the "rpart" algorithm.

Models were then used to predict metric theoretical values in reference conditions at any site. Predictions were compared with observations and residuals (residuals = observations − predictions) were calculated. Assuming that most of the natural variability of the metrics was included in the models, the metric residuals were supposed to vary according to the intensity of human disturbances and independently of natural environmental variables (Pont *et al.*, 2006). Metrics were selected regarding model quality (Pseudo-R2 > 0.3, cross-validation results), metric sensitivity to pressure (Wilcox u-test, p<0.001, metric median deviation > 20%) and redundancy. Redundancy (Spearman rank correlations |r| >0.7) was considered by iteratively removing the metric with the highest redundancy with other metrics until redundancies among metrics were entirely eliminated.

6.b- Index computing and scoring

The index was derived by averaging selected metrics. The index derived from the untransformed metrics was rescaled to range between 0 and 1. The thresholds of the five ecological status classes (high, good, moderate, poor, or bad) were defined in agreement with European intercalibration rules by splitting the index range in five equally spaced classes with class boundaries at 0.8, 0.6, 0.4 and 0.2.

Fish index performance was tested by Spearman rank correlations comparing the fish index with the cumulative pressure index and testing the response to pressures in very small (<100 km2), small (>= 100, <250 km2), medium (>=250, <1000 km2) and large (>=1000, <40000 km2) using bootstrap method (sample size 30, 100 replicates).

6.c- Metrics selection

Reference models were derived for all but habitat traits, however, only feeding habitat, feeding, migration traits responded to pressures: (1) proportional density of insectivorous larger than 100 mm (dens.INSV.p.100large), (2) proportional density of omnivorous smaller than 100 mm (dens.OMNI.p.100small), (3) proportional density of benthic species smaller than 150 mm (dens.BENTH.p.150small) and (4) proportional density of potamodromous (dens.POTAD.p.all) (Table 1).

6.d - Reference models

Redundant environmental variables were identified by means of PCA and removed from the further analyses. Finally, altitude, slope, altitude of source, catchment and mean January air temperature were used to predict reference conditions (Figure XX).

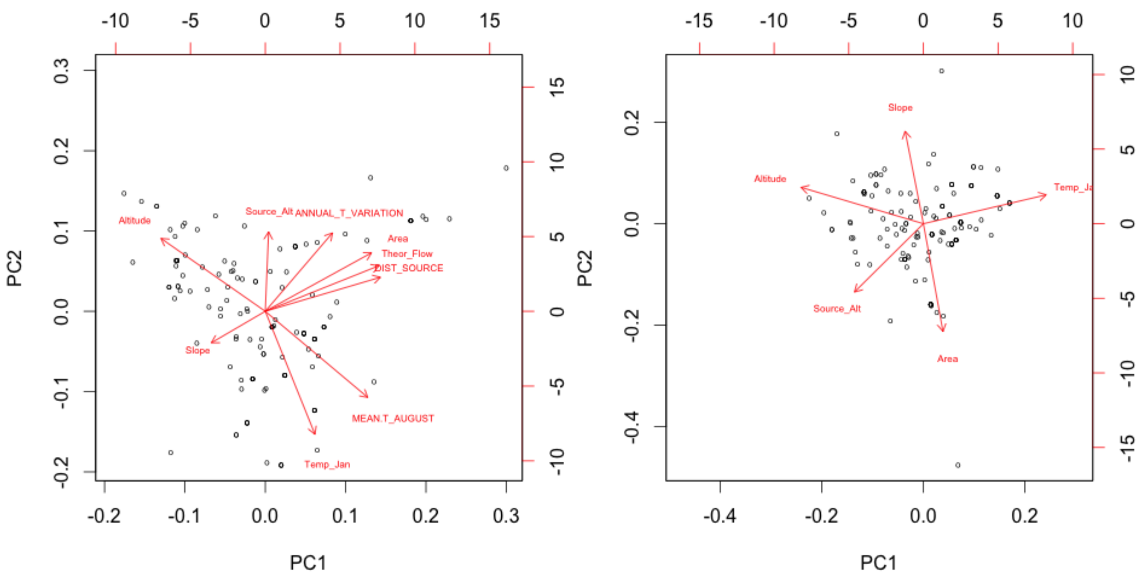

Figure 3. PCAs of environmental parameters of reference samples before (left) and after (right) removing redundant variables.

Under reference conditions the spatial pattern of the metric proportion of large (>=100 mm) insectivorous fish is mainly triggered by catchment area and altitude with high proportions in small rivers and high altitude. The proportion of small (<150 mm) benthic fish show similar patterns but with very low proportion of benthic fish at very high altitudes (>918 m). For potamodromous fish lower proportions can be expected in northern ecoregions at lower altitudes. In contrast to these three metrics, the proportion of small (< 100 mm) omnivorous fish is generally very low in all environments with the exception of southern rivers with catchment areas >208 km2 (see Figure 4).

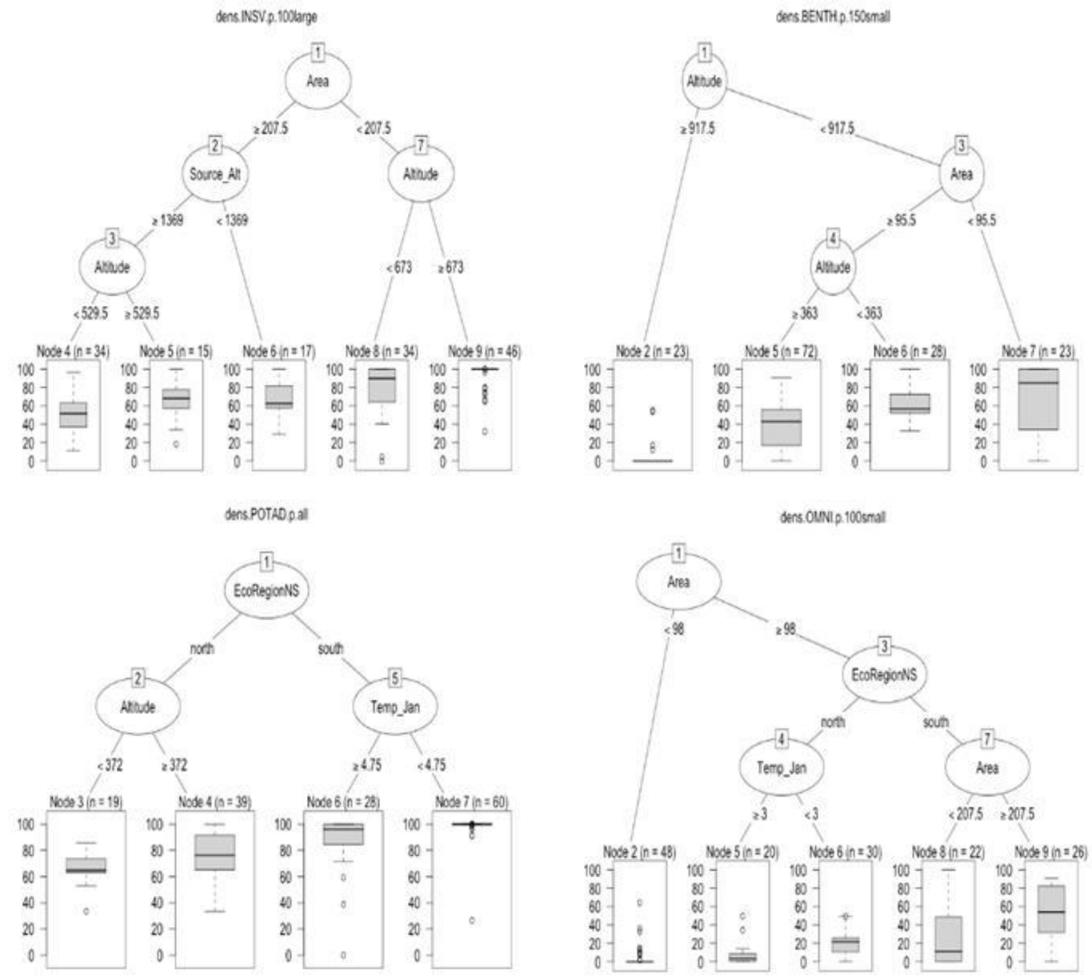

Figure 4. Reference models (decision trees) for the final metrics: (a) proportion of large (>=100 mm) insectivorous fish, (b) proportion of small (<150 mm) benthic species, (c) proportion of potamodromous species and (d) proportion of small (<100 mm) omnivorous species. Environmental parameters: Area = catchment area (km^2) upstream of sampling site, Altitude = altitude of sampling site (m), Alt_source = altitude of stream source (m), EcoRegionNS = southern or northern ecoregions, Temp_Jan = mean monthly January air temperature.

Figure 5 reflects the response of the four metrics to pressure by comparing reference samples with strongly impacted samples. The metrics show differences in the distributions of samples in the two categories: insectivorous and benthic fish respond more distinct than the other two metrics, however, potamodromous species vary less than other metrics under reference conditions. Potamodromous and omnivorous demonstrate a bimodal distribution under pressure indicating only partial response to pressures.

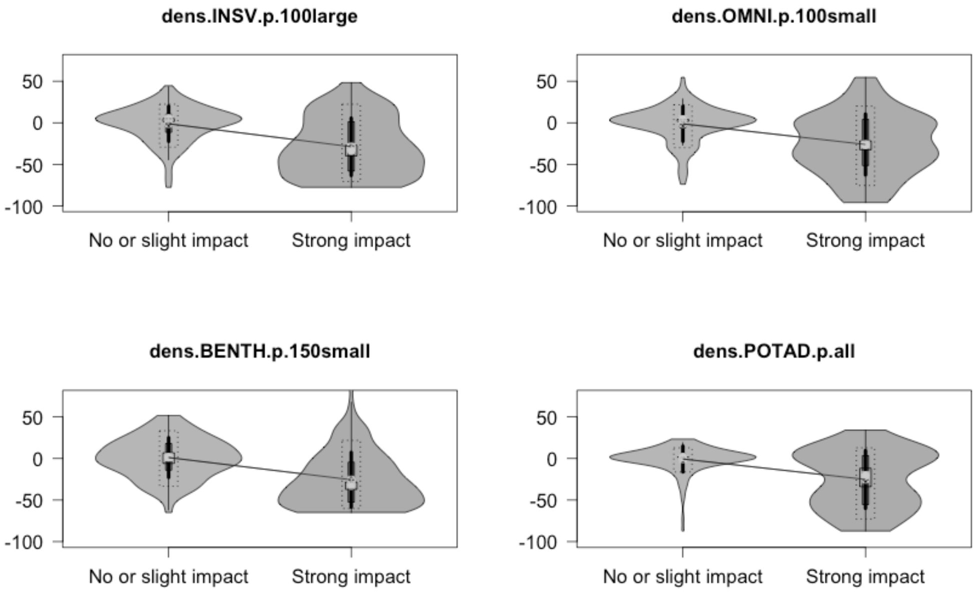

Figure 5. Response of individual metrics to site degradation (i.e.pressures).

6.e- <u>Metrics response, index scoring and index performance</u>

The performance of the index was evaluated along a gradient of human degradation (each site had been pre-classified based on a cumulative pressure index during the earlier stage). Figure 6a shows a clear but non-linear relationship between the cumulative pressure index and the fish index (Spearman rank correlation -0.537). While a slight increase in pressure is not reflected by the fish index, a pressure index >18-20 results in a significant decrease of the fish index. Variation of the fish index in response to pressures is low in case of low and high pressure but high in case of medium pressure levels. There are no influence of catchment size on index performance (Figure 6b). Therefore the index functions well both in small and large rivers.

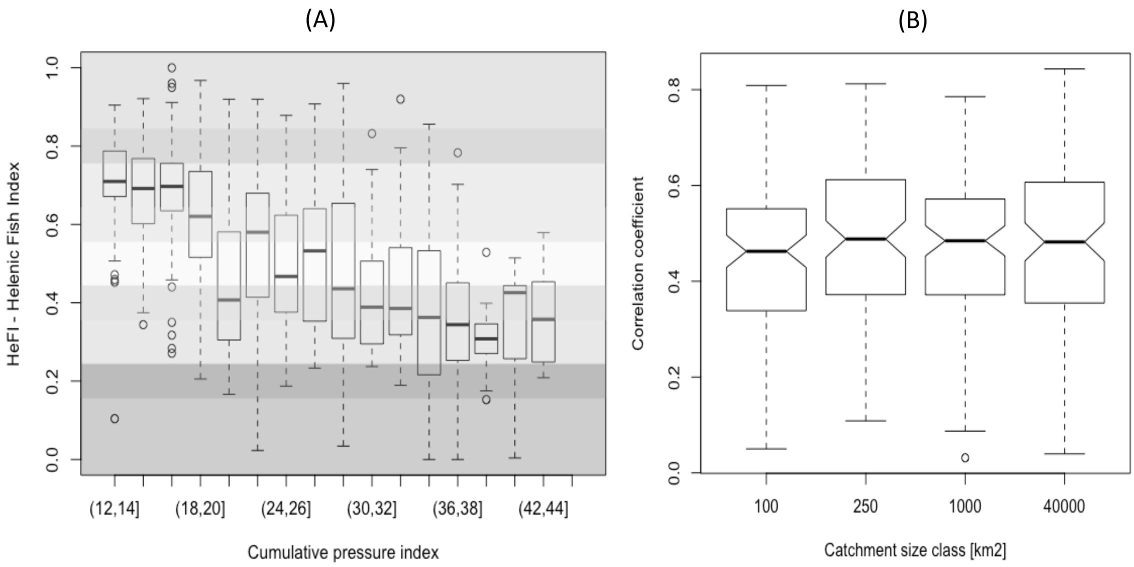

Figure 6. A) HeFi index and its responsiveness with the cumulative pressure index of samples and B) index response for different catchment size (Spearman rank correlations).

The model-based fish index performed well in discriminating human-induced degradation classes. The index scores have been preliminarily mapped using the five-scale categories indicated by the WFD (Figure 7). Based on a screening with other indices and expert knowledge of the particular sites during the sampling period, the results of the index provide evidence for a robust bioassessment tool that works across biogeographic regions in a remarkable variety of stream and river types.

Figure 7. Fish ecological status of sampled sites in Greece based on the Helenic Fish Index (HeFI).

7. DISCUSSION

The spatially-based approach is relatively straightforward and can be completed at regional scales with local datasets. It is a logical option for bioassessment studies that are applied at fine-scales, particularly in drainages with high endemicity levels and atypical landscape and hydro-morphological characteristics that do not permit the application of broad-scale approaches. A disadvantage of the spatially-based approach is that in the Greek situation, the indices created through this approach have poor generalisability and spatial transferability. No simple typology can fully capture the spatial diversity of environmental settings and biological diversity in Greek rivers. Therefore, our knowledge of the environmental factors that influence the structure and functional organisation of fish communities at regional scales must increase substantially before spatial typologies that allow to reliably predict reference conditions can be designated.

Another potential disadvantage is that river typologies cannot always control sufficiently natural variability of biological conditions, unless they are sufficiently fine-scaled to allow delineation of areas of relatively high biological homogeneity. In the main stem of some river basins we observed a longitudinal (upstream–downstream) gradient of change in metric values pertaining to species richness, assemblage composition and ecological guilds, as is predicted by the River Continuum Concept (Vannote *et al.* 1980). This longitudinal pattern has been observed by other researchers as well (e.g. Oberdorff *et al*. 2001, 2002; Grenouillet *et al*. 2004; Oliveira *et al*. 2012; Hermoso and Linke 2012) and implies that "broad" typologies cannot adequately predict reference conditions, because different reference conditions would apply to sites occupying upstream and downstream positions within a river type. Therefore, it deems essential, if the spatially-based approach is to be used for defining reference conditions, to include a model for controlling longitudinal patterns in assemblage attributes.

Model-based bioassessment indices offer a solution to the problem of poor transferability, which is associated with the spatially-based approach. This is important from a cost-effectiveness perspective. The Hellenic Fish Index (HeFI) is the most recent development and seems to be a promising tool for bioassessment in Greek rivers. However, its performance and efficiency in some river systems remains untested. The real evaluation will become possible in the years to come with more data from monitoring operations.

Currently an analysis is being conducted of parameters that are likely to influence the HeFI performance. We found it difficult to assign some fish species to rigid "guild categories" and we are now examining more closely the functional and life-history attributes of these species using data from field observations and the literature. We have also undertaken an analysis of all tasks and methodologies involved in our monitoring operations in order to identify biases and uncertainties that are likely to be associated with the survey plan and the procedures applied so far. In this context, we are examining issues of sampling scale and sampling sufficiency, spatial representativeness of the sampling network, interannual variability of catches in reference sites, and the degree to which the spatial configuration of the sampling sites ensures a fair and representative coverage of the officially designated water bodies.

8. A WAY AHEAD

Fish-based bioassessment is a very active research area in the Mediterranean (Ferreira *et al*. 2007a, Benejam et al. 2015) and our experience has shown that fish are important indicators of hydromorphological impairment, habitat degradation and connectivity disruptions. Moreover, their presence and distribution may be a good criterion for identifying "significant waters" and reforming Greece's river water body delineations (for a general current review for Greece, see Zogaris *et al*. 2016). There are also serious indications that fishes will be affected by climate change as well (Papadaki *et al*. 2015) and they are especially important as indicators for modeling environmental flow management and restoration (e.g. for an example from Greece see Muñoz-Mas *et al*. 2016).

There is important work in progress and difficult challenges ahead for WFD-relevant bioassessment using fish in rivers in Greece. The following important steps are seen as necessary to provide a way forward for fish-based bioassessment in river monitoring in this country:

1. Further development and further refinement of HeFI in order to provide a baseline interregional index for reporting and intercallibration.

2. The development of local indices at the ecoregional and basin level should also be encouraged, since these spatially-based indices may be better honed to local conditions and special environments (increasing their accuracy and precision). Furthermore, indices for small streams with depauperate ichthyofaunas should also be attempted and used in insular and peninsular small basins (e.g. see Segurado *et al*. 2014).

3. The protocols and field forms for sampling should be further refined in order to streamline field work among different sampling campaigns, and electrofishing gears. The method should be used to train all field workers sampling fish communities for bioassessment purposes in Greece. This rigorous standardization and streamlining of sampling should be promoted to assure quality control in sampling. Sampling bias is one of the most serious problems producing unwanted noise and variation since at least three methods of electrofishing are currently practiced in running waters (i.e. boat-based, bank-based and back-pack procedures).

4. Research on species assemblages, fish community dynamics, functional traits and other ecological research is important for refining existing indices.

5. A nationally agreed biotic typology scheme is an important unmet need and should be addressed urgently. Biogeographic sub-regionalisation of Greece's freshwater ecoregions is also important for regionally defined index development.

6. Research on temporal variation of samples in different sites is lacking and this is important for explaining the effects of natural variability on metrics. Work is in progress, using the available monitoring data, to study seasonal and annual biological variability in reference sites.

7. Anthropogenic pressures on fishes have been poorly studied in Greece and eastern Mediterranean river basins. Species traits and ichthyological indices should also be utilised in assessing the amelioration of ecosystem integrity after measures are applied.

APPENDIX

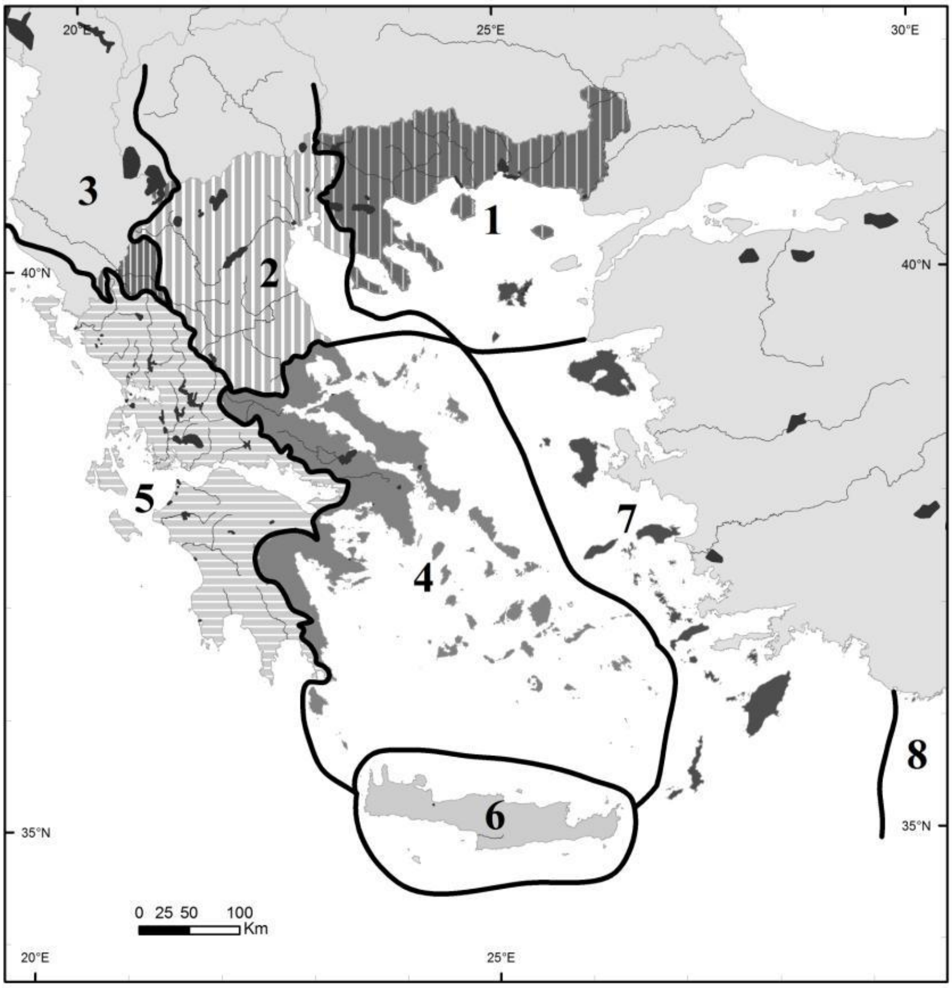

Figure A1. Freshwater Ecoregions in the Greek territory (following Zogaris 2009 with GIS cartography by Y.Chatzinikolaou and N. Koutsikos). Ecoregions by number: 1: Thrace, 2: Macedonia-Thessaly, 3: Southeastern Adriatic, 4: Western Aegean, 5: Ionian, 6: Crete, 7: Eastern Aegean, 8:Southern Anatlolia (corresponding only to the Kastellorizo Island cluster). Note that in the HeFi index the northern ecoregions (1 & 2) which have a widespread ichthyofauna of Danubian origin are grouped as "Northern" and the other ecoregions are grouped as "Southern" since they have species-depauperate assemblages dominated by range-restricted endemics.

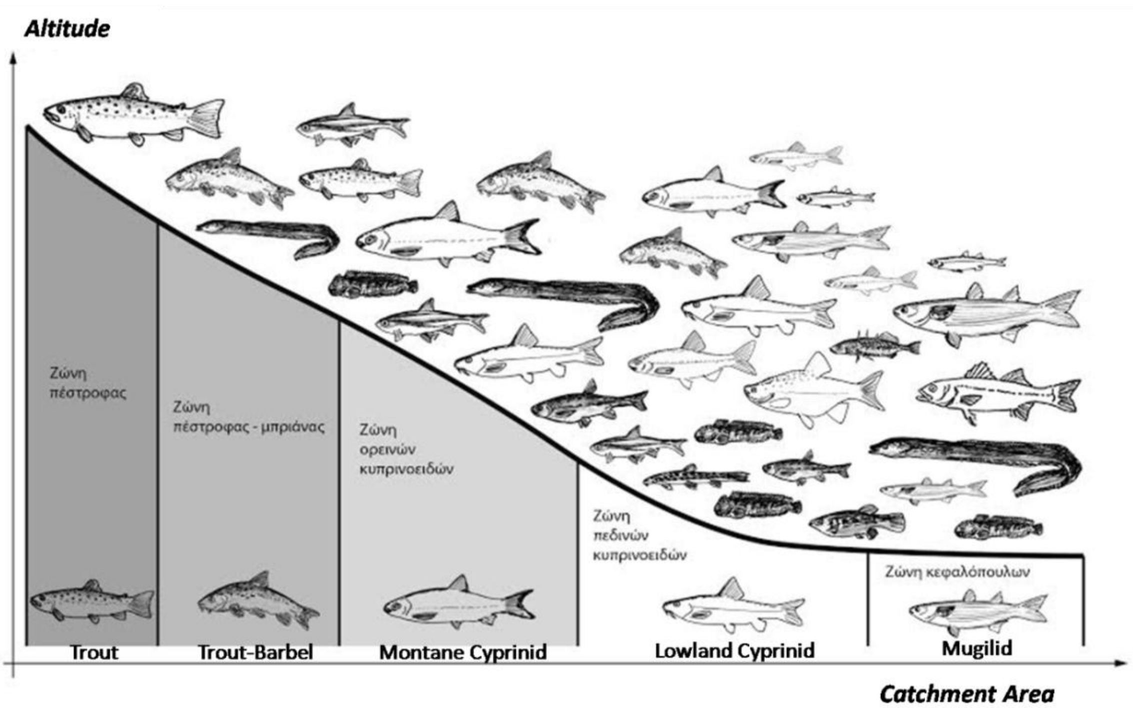

Figure A2. Reference conditions along longitudinal pattern of fish assemblages as exemplified in the Acheloos basin in the Ionian Ecoregion (adapted from Zogaris 2009). In this approach, hypothetical zones are named in order to broadly indicate the affect of combined physical attributes such as altitude, distance from river source, catchment area and slope. Of the five zones; the montane cyprinid also occurs as a mono-species "barbel zone" with small-sized *Barbus* sp (e.g. *Barbus peloponnesius* in the Acheloos river).

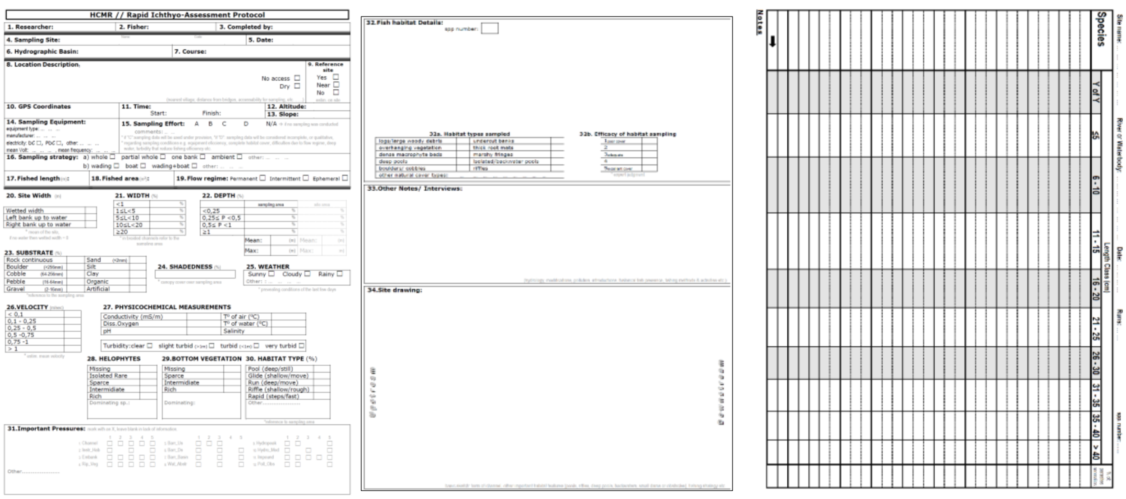

Figure A3. The standardised field forms used to record habitat and pressure data (two at L) and the fish sample data which is in size-class categories (at R). These have been published in the institute's manual (see IMBRIW 2012).

Table A1. Summary of data compiled in the HCMR fish database as of June 2016. Row titles relate to freshwater ecoregional entity (see Map on Fig. A1).

Icthyological Attributes	Thrace	Macedonia-Thessaly	Ionian	W.Aegean	E.Aegean	SE.Adriatic	Crete
Sample Number	163	271	409	178	6	20	49
Samples lacking fish	16	60	98	128	3	2	43
Total species richness	39	35	47	17	1	12	4
Sampled individuals	59673	101786	61661	11437	257	2963	218
Species per sample	7,12	7,62	3,83	2,77	1	3,44	1,33
Individuals per sample	400,5	484,7	199,5	233,4	85,7	164,6	36,3
Endemic species	15	17	26	6	1	10	0
%Endemic species	37,5	48,6	55,3	31,6	100	83,3	0
%Endemic individuals	60,4	70,5	88,0	64,2	100	57,5	0
Introduced species	5	6	9	7	0	0	2
%Introduced species	12,5	17,1	19,1	36,8	0	0	50
% Introduced individs.	6,8	7,7	3,8	12,5	0	0	78,4

Table A2. Development of HeFI: Biological traits of the fish species (following EFI+ project classification, http://efi-plus.boku.ac.at/ and adjusted to fish communities of Greece).

Species	Feeding	Migration	Repro	Hab_Repro	Hab_rheo	Hab_feed
Abramis.brama	OMNI	POTAD	PHYT	----	EURY	BENTH
Alburnoides.bipunctatus	INSV	----	LITH	RH_LITH	RHEO	WC
Alburnoides.strymonicus	INSV	----	LITH	RH_LITH	RHEO	WC
Alburnus.alburnus	OMNI	----	LITH	RH_LITH	EURY	WC
Alburnus.scoranza	OMNI	----	LITH	RH_LITH	EURY	WC
Alburnus.sp.volvi	OMNI	----	LITH	RH_LITH	LIMNO	WC
Alburnus.thessalicus	OMNI	----	LITH	RH_LITH	EURY	WC
Alburnus.vistonicus	OMNI	LONG	LITH	RH_LITH	EURY	WC
Alosa.fallax	OMNI	LONG	LITH	RH_LITH	RHEO	WC
Anguilla.anguilla	PISC	LONG	----	----	EURY	BENTH
Aphanius.fasciatus	INSV	----	PHYT	----	LIMNO	WC
Atherina.boyeri	OMNI	LONG	PHYT	----	LIMNO	WC
Barbatula.barbatula	INSV	----	LITH	RH_LITH	RHEO	BENTH
Barbus.balcanicus	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.cyclolepis	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.euboicus	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.macedonicus	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.peloponnesius	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.prespensis	INSV	POTAD	LITH	RH_LITH	EURY	BENTH
Barbus.sperchiensis	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Barbus.strumicae	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Carassius.cf.gibelio	OMNI	----	PHYT	----	LIMNO	BENTH
Caspiomyzon.graecus	----	----	LITH	RH_LITH	RHEO	BENTH
Chelon.labrosus	OMNI	LONG	----	----	EURY	WC
Chondrostoma.vardarense	----	POTAD	LITH	RH_LITH	RHEO	BENTH
Cobitis.arachthosensis	INSV	----	PHYT	----	EURY	BENTH
Cobitis.hellenica	INSV	----	PHYT	----	EURY	BENTH
Cobitis.ohridana	INSV	----	PHYT	----	LIMNO	BENTH
Cobitis.puncticulata	INSV	----	PHYT	----	LIMNO	BENTH
Cobitis.punctilineata	INSV	----	PHYT	----	EURY	BENTH
Cobitis.strumicae	INSV	----	PHYT	----	EURY	BENTH
Cobitis.trichonica	INSV	----	PHYT	----	LIMNO	BENTH
Cobitis.vardarensis	INSV	----	PHYT	----	EURY	BENTH
Ctenopharyngodon.idella	----	POTAD	----	----	EURY	WC
Cyprinus.carpio	OMNI	----	PHYT	----	EURY	BENTH
Dicentrarchus.labrax	PISC	LONG	----	----	EURY	WC
Dicentrarchus.punctatus	PISC	LONG	----	----	EURY	WC
Economidichthys.pygmaeus	OMNI	----	PHYT	----	EURY	BENTH
Economidichthys.trichonis	OMNI	----	PHYT	----	LIMNO	WC
Esox.lucius	PISC	POTAD	PHYT	----	EURY	WC
Gambusia.holbrooki	INSV	----	----	----	LIMNO	WC
Gasterosteus.gymnurus	INSV	----	PHYT	----	EURY	WC
Gobio.bulgaricus	INSV	----	LITH	RH_LITH	RHEO	BENTH

Gobio.feraeensis	INSV	----	LITH	RH_LITH	RHEO	BENTH
Gobio.skadarensis	INSV	----	LITH	RH_LITH	RHEO	BENTH
Knipowitschia.caucasica	INSV	----	PHYT	----	LIMNO	BENTH
Knipowitschia.milleri	INSV	----	PHYT	----	LIMNO	BENTH
Knipowitschia.thessala	INSV	----	PHYT	----	LIMNO	BENTH
Lepomis.gibbosus	INSV	----	PHYT	----	LIMNO	WC
Leucaspius.delineatus	OMNI	----	PHYT	----	LIMNO	WC
Leuciscus.aspius	PISC	POTAD	LITH	RH_LITH	RHEO	WC
Liza.aurata	OMNI	LONG	----	----	EURY	WC
Liza.ramada	OMNI	LONG	----	----	EURY	WC
Liza.saliens	OMNI	LONG	----	----	EURY	WC
Luciobarbus.albanicus	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Luciobarbus.graecus	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH
Mugil.cephalus	OMNI	LONG	----	----	EURY	WC
Neogobius.fluviatilis	INSV	----	LITH	RH_LITH	EURY	BENTH
Oncorhynchus.kisutch	PISC	----	LITH	RH_LITH	RHEO	WC
Oncorhynchus.mykiss	INSV	----	LITH	RH_LITH	RHEO	WC
Oxynoemacheilus.bureschi	INSV	----	LITH	RH_LITH	RHEO	BENTH
Oxynoemacheilus.pindus	INSV	----	LITH	RH_LITH	RHEO	BENTH
Pachychilon.macedonicum	OMNI	----	PHYT	----	EURY	WC
Pachychilon.pictum	OMNI	----	PHYT	----	EURY	WC
Pelasgus.laconicus	OMNI	----	PHYT	----	LIMNO	WC
Pelasgus.marathonicus	OMNI	----	PHYT	----	LIMNO	WC
Pelasgus.prespensis	OMNI	----	PHYT	----	LIMNO	WC
Pelasgus.stymphalicus	OMNI	----	PHYT	----	LIMNO	WC
Pelasgus.thesproticus	OMNI	----	PHYT	----	LIMNO	WC
Perca.fluviatilis	PISC	----	PHYT	----	EURY	WC
Petroleuciscus.borysthenicus	INSV	----	PHYT	----	LIMNO	WC
Phoxinus.strymonicus	INSV	----	LITH	RH_LITH	RHEO	WC
Proterorhinus.semilunaris	INSV	----	LITH	RH_LITH	EURY	BENTH
Pseudorasbora.parva	OMNI	----	PHYT	----	EURY	WC
Pungitius.hellenicus	INSV	----	PHYT	----	LIMNO	WC
Rhodeus.amarus	OMNI	----	----	----	EURY	WC
Rhodeus.meridionalis	OMNI	----	----	----	EURY	WC
Romanogobio.elimeius	INSV	----	LITH	RH_LITH	RHEO	BENTH
Rutilus.panosi	INSV	----	PHYT	----	EURY	WC
Rutilus.rutilus	INSV	----	PHYT	----	EURY	WC
Rutilus.sp.sperchios	INSV	----	PHYT	----	EURY	WC
Rutilus.ylikiensis	INSV	----	PHYT	----	EURY	WC
Sabanejewia.balcanica	INSV	----	PHYT	----	RHEO	BENTH
Salaria.economidisi	INSV	----	LITH	RH_LITH	LIMNO	BENTH
Salaria.fluviatilis	INSV	----	LITH	RH_LITH	RHEO	BENTH
Salmo.cf.pelagonicus	INSV	POTAD	LITH	RH_LITH	RHEO	WC
Salmo.farioides	INSV	POTAD	LITH	RH_LITH	RHEO	WC
Salmo.macedonicus	INSV	POTAD	LITH	RH_LITH	RHEO	WC
Salmo.peristericus	INSV	POTAD	LITH	RH_LITH	RHEO	WC
Sander.lucioperca	PISC	POTAD	LITH	RH_LITH	EURY	WC
Scardinius.acarnanicus	OMNI	----	PHYT	----	LIMNO	WC
Scardinius.erythrophthalmus	OMNI	----	PHYT	----	LIMNO	WC
Silurus.aristotelis	PISC	----	PHYT	----	LIMNO	BENTH
Silurus.glanis	PISC	----	PHYT	----	EURY	BENTH
Squalius.cf.cii	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.keadicus	INSV	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.moreoticus	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.orpheus	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.pamvoticus	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.peloponensis	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.sp	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.sp.aoos	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.sp.evia	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Squalius.vardarensis	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Telestes.beoticus	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Telestes.pleurobipunctatus	OMNI	POTAD	LITH	RH_LITH	RHEO	WC
Tinca.tinca	OMNI	----	PHYT	----	LIMNO	BENTH
Tropidophoxinellus.hellenicus	OMNI	----	PHYT	----	LIMNO	WC
Tropidophoxinellus.spartiaticus	OMNI	----	PHYT	----	EURY	WC
Valencia.letourneuxi	INSV	----	PHYT	----	LIMNO	WC
Valencia.robertae	INSV	----	PHYT	----	LIMNO	WC
Vimba.melanops	INSV	POTAD	LITH	RH_LITH	RHEO	BENTH

Contributors to this report

This report reviews the process and products of several years of work in fish-based index-building in Greece. Over 10 IMBRIW-HCMR scientists have been involved, the contributor's names are present in the papers and presentations that have disseminated this work (see references). We should specifically acknowledge the contributions of the FAME project and the scientists that worked closely with the IMBRIW-HCMR team: S. Schmutz, U. Dussling, M.T. Ferreira, W.R.C. Beaumont and P. Segurado. During the building of the HeFi (2014-2016) S. Schmutz was responsible for guiding the index development; his involvement has been instrumental in finalizing the proposed national fish-based index. IMBRIW-HCMR ichthyologists are also grateful to the coordination efforts of N. Skoulikidis who was responsible for the WFD monitoring project for rivers at HCMR. Finally, part of this work is based on E. Oikonomou’s doctoral dissertation “Assessing and handling uncertainty associated with WFD bioassessment and decision support tool development” at the University of Patras.

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