Template for reporting on Intercalibration of new or revised ecological assessment methods according to finalised Intercalibration results (Gap 2)

DEVELOPMENT OF A NATIONAL CLASSIFICATION METHOD FOR THE ECOLOGICAL STATUS OF RIVERS IN GREECE USING THE BIOLOGICAL QUALITY ELEMENT "FISH" AND FITTING THE METHOD TO THE RESULTS OF THE COMPLETED INTERCALIBRATION OF THE MED GIG

UPDATED VERSION 3

(Dec 24th 2016; Annex in separate volume)

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This report should be cited as:

Tachos V., Zogaris S., Oikonomou E. & Economou A.N. Dec 2016. Development of a national classification method for the ecological status of rivers in Greece using the Biological Quality Element "Fish" and fitting the method to the results of the completed intercalibration of the Med GIG. Unpublished Report submitted to WG ECOSTAT through the Special Secretariat for Water, Hellenic Ministry of Environment and Energy. Institute of Marine Biological Resources and Inland Waters- HCMR, Athens, 23 pp. (not including Annex report).

Athens, December 2016

Development of a national classification method for the ecological status of rivers in Greece using the biological quality element "fish" and fitting the method to the results of the completed Intercalibration of the MED GIG

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1. INTRODUCTION

- Hellenic Republic
- BQE Fish (rivers)
- IC types R-M1, R-M2, R-M4 and R-M5

The Hellenic Republic is now using a state-wide assessment method for the BQE fish in rivers, the Hellenic Fish Index, HeFI (Tachos *et al*. 2016; Zogaris *et al*. in prep.). In Greece, since 2002, six fish-based assessment methods have been developed by scientists involved in fish-based bioassessment research and working within relevant EU projects (e.g. FAME, STAR). The earlier indices (both spatially-based and model-based – see Annex) were important preparatory steps and extremely valuable for developing knowledge and understanding of the nation's river ecosystems. However, a precautionary approach was taken in the development of a state-wide interregional index due to remarkable spatial and temporal variability in fish assemblages and diverse lotic conditions, within the territory of Greece. Greece, thus, lagged behind completion of a state-wide index (Pont *et al*. 2011). The Hellenic Fish Index (HeFI) was developed and tested during the last two years, within an international cooperation project involving Greek and Austrian scientists; a complete description of this new index is given in the Annex section, accompanying this report.

The aim of this report is to test if the Hellenic Fish Index (HeFI) is compliant with the completed MED-GIG intercalibration exercise and if that the instructions of the CIS Guidance Document no 30 (EU 2015) have been followed. In particular, we examined whether all relevant parameters indicative of the BQE "fish" are covered, class boundaries are set in line with the WFD's normative definitions, the method is applicable for nearly all major river systems in mainland Greece, including the IC types R-M1, R-M2, R-M4 and R-M5, and that HeFI performs well in discriminating human pressures.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHOD

The Hellenic Fish Index (HeFI) is an interregional model-based fish index, geared to be applied in a wide variety of river types in the southern Balkans. References are built based on standard environmental parameters at the river site level (five environmental parameters were retained in the model), through a modeling approach. The index was developed by first defining least disturbed river sites forming a "calibrated reference site dataset" and secondly quantifying and analyzing differences of fish metrics between reference and impaired sites. Out of many potential metrics tested, four fish metrics showed the best ability to explain natural variance of fish communities or to explain the distance between reference and impaired sites. 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).

2.1. METHODS AND REQUIRED BQE PARAMETERS

HeFI was developed using fish data and relevant environmental parameters collected through standardised procedures, during the national monitoring programme for rivers (and through projects which applied the same sampling method in previous years (2002 to 2014). Methods for the designation of reference conditions, the identification of appropriate metrics and the setting of class boundaries fulfill the WFD normative requirements. Fish collected during the sampling operations are identified at the species level (or similar taxonomic level) and are assigned to ecological guilds. The use of guild-based metrics allows us to overcome issues associated with the high taxonomic diversity in Greek freshwaters and the biogeoraphic difference among different ecoregions. Four metrics showed high capacity to discriminate between impacted and unimpacted sites (Table 1).

Table 1. Overview of the metrics included in the Hellenic Fish Index (HeFI)

MS	Taxonomic composition	Abundance	Age-Class
GR	YES (species level and functional guild categories define attributes)	YES (relative abundance is used since the population sampled is semi-quantitative data; catch-per-unit-effort relates in the areal density of specimens collected) • Density of Potamodromous fishes (all species present in sampled assemblage) <dens.POTAD.p.all> • Density of benthic fishes smaller than 15 cm (TL) <dens.BENTH.p.150small> • Density of omnivorous fishes smaller than 10 cm (TL) <dens.OMNI.p.100small> • Density of insectivorous fishes larger than 10 cm (TL) <dens.INSV.p.100large>	YES (size class boundaries are used as a proxy in three metrics of the "Abundance" category)

Combination rule used in the method

The final EQR is calculated as an arithmetic average of the single EQRs of the four metrics.

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)

All indicative parameters for fish in rivers (composition, abundance, age-classes) are included in the index procedure. The index performed well in discriminating human pressure classes, giving a significant negative linear response to a gradient of degradation. This approach worked in both small and large rivers and among 6 different ecoregions.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

For the development of the initial index data were acquired through site-specific sampling using electrofishing devices. A total of 640 samples were considered for the initial analyses; these are fairly evenly distributed across the mainland of Greece and include two major islands as well, but not the island of Crete (which is considered a separate freshwater ecoregion). Samples were selected from representative sites in a variety of river types and human-induced impairment conditions. Only samples in which at least 15 specimens were captured were considered in the analyses.

Sampling time and frequency

Sampling was targeted during the low-flow or near low flow period, usually during the summer, ranging from April to October, depending on altitude and river type. In small xerothermic Mediterranean conditions "summer" sampling may begin in April through June, while in large rivers and canals the summer season is also extended to cover September and most of October if flows remain low (i.e. before heavy autumn rains). Operational monitoring included two samples per year (spring and summer). Surveillance monitoring included two samples (spring and summer) in a single year, once in the river basin management cycle. For the purposes of index development and intercalibration only one sample per site (the most representative and recent) was utilised in the dataset.

Sampling method

Standardised sampling was undertaken using an electrofishing method that follows the FAME project guidelines (Schmutz *et al.* 2007a; 2007b) and roughly abides by CEN standards. Due to limitations in personnel and resources (and eventually in crew number and field-time allocation), some practices followed a rapid assessment approach and do not fully conform to CEN. One anode is utilised in all cases irrespective of the lotic bodies wetted width, a single pass was conducted, and no stop-nets were used. Fish lengths were recorded during sampling, using a meter ruler attached to the anode pole, and grouped in 5-cm intervals. In boat and bank-based electrofishing surveying the anode is usually thrown to catch larger fish or surprise fish shoals; it is not thrown during the backpack electrofishing sampling. The method has been outlined in a detailed manual (IMBRIW-HCMR 2012) and has also been utilised in other countries in the eastern Mediterranean (Zogaris et al. 2012; 2015). Special attention was given to the length of river section and area sampled. As described in detail in the Annex of the present report, the ten to twenty-fold criteria (i.e. wetted width per length) is followed except for a small number of sites with highly homogeneous fish fauna, e.g. mono-species or low-species sites such as in small "mountain barbel type" streams or species-poor shallow mountain streams. Although this method is usually defined as a semi-quantitative one, special assessment of sampling effort and many other fish-habitat and sampling environment conditions are recorded in the protocol.

Three electrofishing sampling approaches are utilised in the standard procedure in different lotic water conditions:

- Backpack electrofishing (usually employing a battery-powered unit) DC pulsed, 1,5 KW output power, max. 850 v used in streams (1-10 m wide)
- Bank-based electrofishing DC unpulsed, 7,0 KW output power, 600 V used in wider rivers (>10 m wide and with deeper waters up to 1m depth).
- Boat-based electrofishing DC unpulsed, 8,0 KW output power, 600 V used in deeper streams and rivers.

The fish sampled are identified to species level (or defined sub-specific unit for some non-valid taxa), measured (in five-centimeter class intervals, TL) and then returned alive to the river at the site of capture.

A variety of habitat parameters are collected at the sampled river site. In each sampling site the wetted surface area sampled is estimated and used to convert fish numbers into areal densities; this provides a measure of catch-per-unit-effort (CPUE).

Site characteristics, landscape features and key environmental and habitat parameters are recorded in a field protocol at all sampled locations. This protocol (IMBRIW-HCMR, 2012) is a version of the FAME (2005) protocol, as modified for Greece, and accommodates fields for sampling details, hydrological characteristics, habitat variables, substrate composition, physicochemical parameters and important anthropogenic pressures affecting the site or the river segment. Habitats are divided in five types (pools, glides, runs, riffles and rapids). The physicochemical parameters are recorded using multiparametric instruments measuring water temperature, conductivity, turbidity, dissolved oxygen and pH. Additional parameters are recorded or measured from samples brought to the laboratory (e.g. BOD). Also, hydromorphological analysis is performed in many stations with the use of the River Habitat Survey (RHS) method and riparian vegetation (QBR). Photographs and video of the sampled localities and specimens caught are taken for documentation and follow up evaluations. New archival systems, relational databases and online web-based archives have been established to inventory this data and associated materials.

Anthropogenic pressure assessment is applied for each sample in each site. Knowledge of pressures at the site, reach and basin scale was important. Twelve anthropogenic pressures that impact fishes at the site, reach and basin scale were assessed (usually during a desk study; although this has recently been incorporated in the field form procedure as well). These pressures are scored on a 1 to 5 scale based on knowledge and judgment by the principle researcher of the sampling work (see Table 2).

Data processing

Data are stored in a site-specific database. Statistical analyses were implemented using R (version 2.13.1; R Development Core Team).

Identification level

Fishes are identified to species level or as-yet-undescribed species-unit level (i.e. former subspecies or non-valid operational taxonomic units) as promoted in the national checklist (Barbieri *et al.* 2015). During sampling fishes are retuned alive to the river; in cases of doubt in identification (usually juveniles) samples are preserved in formalin solution/ and or ethanol for laboratory identification.

2.3. NATIONAL REFERENCE CONDITIONS

Detailed description of setting of national reference conditions

Following procedures that have been established in Europe (Schmutz *et al.* 2007a), two index-building approaches have been thus far developed in Greece: a type-based (or spatially-based), and a site-based approach (See Annex). The traditional type-based approach requires a detailed and biologically relevant typology of river ecosystems. Type-specific reference conditions development has been slow and difficult in areas with high levels of environmental and biological heterogeneity, and this is a problem especially in Mediterranean countries (e.g. Ferreira *et al.* 2007; Magalhães *et al.* 2008). In Greece in particular, additional difficulties for developing type-specific reference conditions are high biogeographic variation (eight ecoregional entities have been defined, see Barbieri *et al.* 2015) and marked differences in assemblage structure and taxonomic composition among ecoregions and basins (Economou et al. 2007; 2016). The site-specific approach does not use a river classification through an overriding typology and instead utilises modelling to predict the reference values of the fish metrics, from the environmental features of the sites. In the EU an inter-regional approach for a pan-European site-based approach has been followed since the FAME project (FAME 2005) and this has resulted in efforts to broaden the spatial scale of the indices, resulting in model-based EFI and EFI+ (see Roset *et al.* 2007; Pont *et al.* 2007; EFI+Consortium 2009). In terms of reporting and assisting intercalibration approaches this general screening-level index has been important as it is the only way to surpass biogeographic variability at the continental scale. However, the application of EFI/EFI+ in some species-poor areas, such as in Ireland, southern Balkans and the Mediterranean has been problematic (e.g. Jepsen and Pont 2007).

In HeFI reference conditions are modeled in this work from a “calibrated dataset” of sites that were pre-assessed as not or minimally impaired by human-induced pressures. Calibrated (low impacted) sites were identified using fish-relevant indicators of anthropogenic impact, following procedures similar to those described by Degerman *et al.* (2007). A five-class classification scheme was established through this approach. The calibrated dataset included sites having a pre-classified low impact score (1 or 2 in every pressure).

For the development of the index, a total of nine environmental descriptors, known to influence fish assemblages (Pont *et al.* 2005), were chosen in a first step for analyses: altitude, slope, annual variation of air temperature, mean August air temperature, mean January air temperature, altitude of river source, distance to source, catchment area, and theoretical flow. These attributes were either measured in the field or derived from flow statistics, satellite data or/and or geographical information systems data. These variables were assumed to be not (or only slightly) modified by local anthropogenic activities. Correlations among descriptors were analyzed using Principal Component Analyses. As a first step, the two PCA axis were plotted to identify correlating descriptors. Afterwards, PCA was redone and plotted without redundant descriptors. The first two axes of the final PCA explained 37% and 30% of the inherent variance (see annex for details). Finally, five environmental descriptors were retained for reference model: altitude, slope, mean January air temperature, altitude of source and catchment area. There is evidence from other research efforts that these natural parameters are important in influencing the longitudinal distribution and biotic classification of streams and rivers in Greece (Economou *et al.* 2003; Zogaris *et al.* 2004; Economou *et al.* 2007; Zogaris 2009; Vardakas *et al.* 2015).

Additionally, biogeographical characterization of Greece's territory was imposed by combining "southern" (AEGEAN, IONIAN) and "northern" (ADRIATIC, MAC-THES, THRACE) freshwater ecoregions, since especially the latter show biogeographical affinities (Zogaris *et al.* 2009). This division is based on the fact that there is an overriding boundary among south/southwest and northern part of the southern Balkan Peninsula (Economou *et al.* 2016). The distinctive species-rich faunas of Danubian origin in the "northern" super-region, is an important first biogeographical break for this regionalization application and suites the reference model development.

Interpreting reference model results

Functional guild definitions were created 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 (Noble *et al.* 2007; see annex). Each species was assigned to one of the different categories of a trait (24 categories; see annex). We assigned species to categories based on published accounts (e.g., Holcik *et al.* 1989; 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 (Barbieri *et al.* 2015).

Direction of response (positive or negative) of particular ecological traits was predefined according to ecological expectations (Table 2). 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).

Classification and regression trees (CRT) were used as a recursive partitioning method, to model fish metrics as a function of environmental characteristics (analyzed in R-project CRAN, version 3.2.4). 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. The tree's 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.

The four selected metrics are interpreted in relation to dominating abiotic environmental parameters. Under reference conditions the spatial pattern of the metric proportion of large insectivorous fish (>=100 mm) 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 the "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 reference models in Annex report).

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

The index was constructed by averaging the 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).

2.5. PRESSURES ADDRESSED

Please describe the pressures addressed by the method and provide pressure-response relationship (graph, equation)

Anthropogenic alterations of sampled rivers were assessed according to standardised classifications. Based on information of pressure variables a cumulative pressure index was calculated by adding pressure scores for each sample (Table 2). The selected metric responded well to the cumulative pressure index (Fig. 2, Fig. 3) and these responses are interpretable from basin ecological understanding of human-induced pressures on fish assemblages.

Table 2: Scoring of pressures into 5 classes and definition of reference and impacted dataset. Note that for the Intercalibration exercise a similar set of pressures was used (see below).

	Class				
Pressures	**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)**	

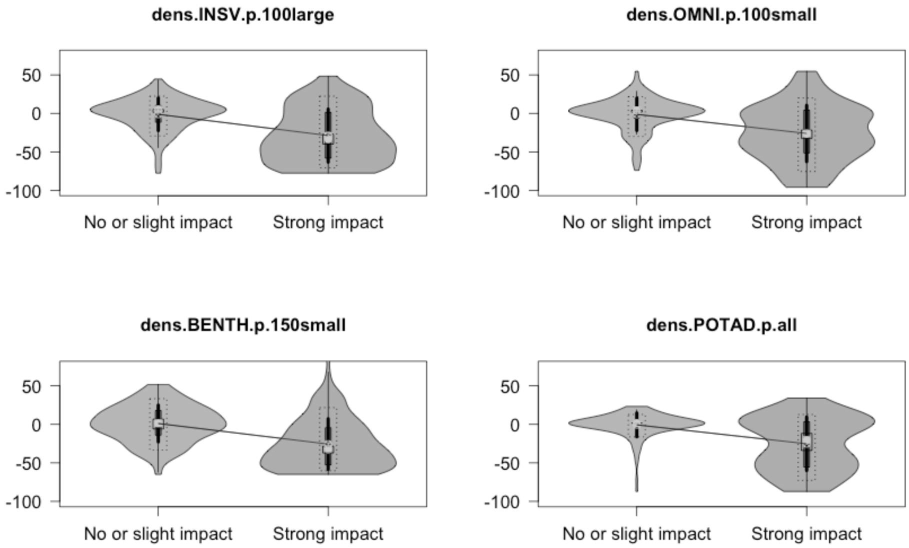


Figure 1. Response of individual metrics to pressures.

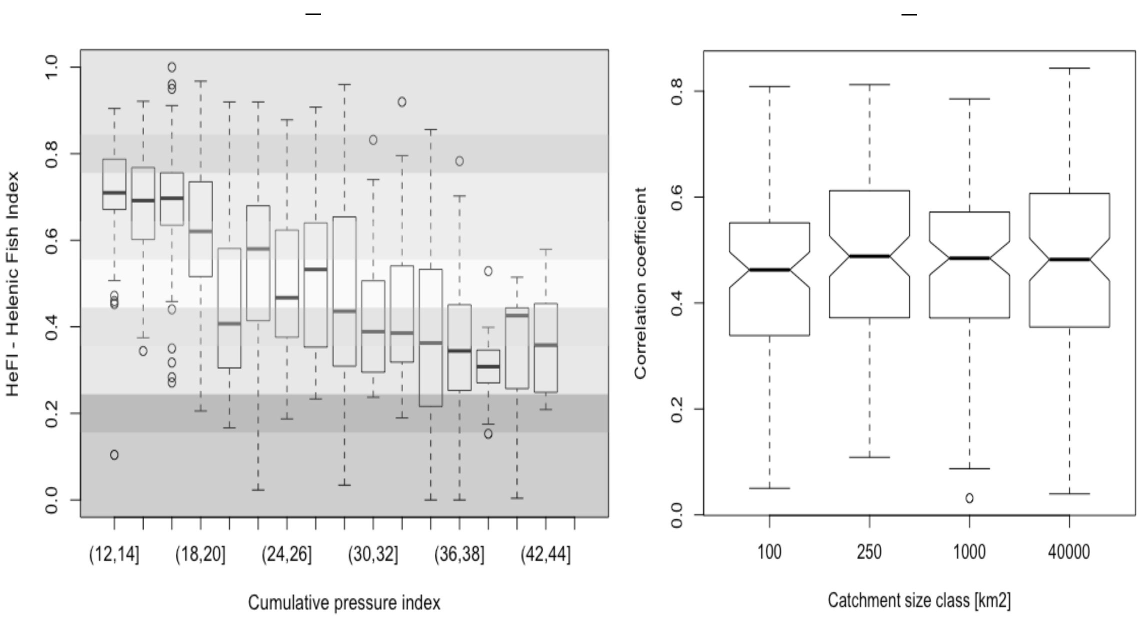


Figure 2. A) HeFi index and its responsiveness with the cumulative pressure index of samples and B) index response for different catchment size (Spearman rank correlations).

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 3. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES; **the thresholds of the five ecological status classes were defined by splitting the index range in five equally spaced classes with class boundaries at 0.8, 0.6, 0.4 and 0.2.**
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES; **HeFI is an average of four metrics which cover all relevant parameters.**
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES; **many of the assessed sites were assigned to IC common types R-M1, R-M2, R-M4 and R-M5.**
The water body is assessed against **type-specific near-natural reference conditions**	YES; **however the index is based on a model for site-specific reference development. Sites pre-assessed as being in natural and near-natural condition (calibrated reference data-set) were used for the establishment of reference conditions.**
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") clearly has to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

HeFI is a model-based index for the derivation of reference conditions and provides a site-based typological development similar to the EFI+ methodology. Specific physical attributes of the investigated river site are utilised to model the site's reference conditions (at each sampled site). The five environmental descriptors that were selected after the statistical tests for reference models are: altitude, slope, mean January air temperature, altitude of source and catchment area. It is important to note that these attributes do help to produce a complete interpretation of lotic water fish communities and there is much evidence for this in earlier fish-environment studies in Greece. Altitude is an important surrogate for water temperature and there has been shown to be a distinct cold water/warm water community break-point in Greek fish assemblages (Economou *et al.* 2007; Zogaris 2009). Slope is significant since it surrogates flow velocity and separates the strictly lotic from lentic natural flow/hydromorphological conditions in running waters. January temperatures signal the areas in Greece that are influenced by a colder continental climate zones (in contrast to the warmer/drier Mediterranean climate zones) and this is not always overlapping geographically with altitude. "Altitude of source" is known to be important since it indicates the general pattern of the longitudinal gradient and catchment area (measured from upstream of the sampled site); this gives a good measure of the longitudinal position of the site in the River Continuum Concept perspective.

The HeFI can be used in most rivers in mainland Greece and addresses the four IC types defined for the Mediterranean (R-M1, R-M2, R-M4 and R-M5). Additionally it applies to other river types (including R-M3 and "large rivers"), which are not included in the common IC types. Table 4 gives the number of sampling sites in the four IC types for the MED-GIG.

Table 4. Sampling sites in the IC types for the MED GIG.

Common IC Type[1]	River characterization	Catchment (km^2)	Geology	Flow regime	Number of sites
R-M1	Small Mediterranean streams	10-100	Mixed (except silicious)	Highly seasonal	**20**
R-M2	Medium Mediterranean streams	100-1000	Mixed (except silicious)	Highly seasonal	**52**
R-M4	Mediterranean mountain streams		Non-silicious	Highly seasonal	**42**
R-M5	Temporary streams			Temporary	**N/A for Greece**

[1] Another 28 samples fall into the river type R-M3 (large, lowland, catchment area 1000-10000 Km2, geology mixed, highly seasonal) and 15 samples fall in the river type "very large rivers" (large, lowland, catchment area >10000 Km2, geology mixed, highly seasonal), which are not included in the common IC types.

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

HeFI index responds to various pressures that can impact fish communities, including hydro-morphological changes, water quality degradation and disruption of connectivity (see annex).

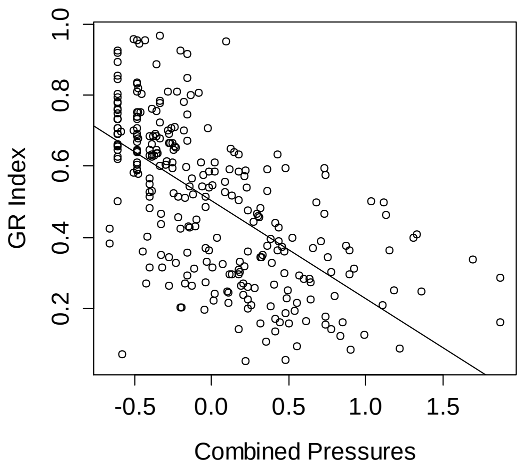
During the intercalibration process a series of common pressures (Table 5) was addressed and tested for responsiveness, across HeFI index values.

Table 5. Common pressures estimated and addressed for IC exercise.

VARIABLE	EXPLANATION	SCALE	PRESSURE INTENSITY				Nb of categ.
P_barrier	Presence of downstream artifical barriers on the catchment scale	catchment	no	low	high		3
P_barrierup	**Artificial barriers upstream from the site**	segment	no	low	medium	high	**4**
P_barrierdown	**Artificial barriers downstream from the site**	segment	no	low	medium	high	**4**
P_impoundment	**Impoundment**	site	no	low	high		**3**
P_hydropeaking	**Hydropeaking**	site	no	low	high		**3**
P_waterabsrt	**Water abstraction**	site	no	low	medium	high	**4**
P_reservoir	**Colinear connected reservoir (fish farms, fish ponds,...)**	segment	no	high			**2**
P_dam	**Upstream dams influence**	site	no	low	high		**3**
P_watertemp	**Water temperature modification (excuding dam effect)**	site	no	high			**2**
P_chan	**Channelisation / Cross section alteration (segment scale)**	segment	no	low	medium	high	**4**
P_vegrip	**Riparian vegetation**	site	no	low	medium	high	**4**
P_habalt	**Local Habitat alteration (site scale)**	site	no	low	medium	high	**4**
P_dyke	**Dykes (flood protection)**	segment	no	low	medium	high	**4**
P_tox	**Toxic Risk. Priority substances list**	segment	no	low	high		**3**
P_waterac	**Water acidification**	segment	no	low	high		**3**
P_waterqualindex	**National water quality index (segment scale)**	segment	no	low	medium	high	**4**
P_navigation	**Navigation**	segment	no	high			**2**
P_recreational	Recreational use with high intensity (angling, boating,...)	site	no	high			2
P_specimp	Impairment of indigenous species	segment	no	high			2
P_predation	Heavy predation	site	no	high			2
P_stockact	Major effect on indigenous populations by stocking activities	segment	no	high			2

*in bold/yellow, pressures considered in the definition of reference sites
*in blue, maximal pressure intensity accepted for a site to be accepted as reference site

For the IC exercise a combined index of pressure was established, based on the 13 pressures listed above and using a MCA (*Multivariate Correspondence Analysis*). The HeFI index seems to respond well to the combined index of pressures (Fig. 3). At the same time, HeFI index also responds quite well to the total number of pressures at level "High", among the 13 pressures (Fig 4). The response of HeFI index to individual common pressures is represented in Figure 5.




```
Coefficients:
                Estimate   Std. Error   t value   Pr(>|t|)
(Intercept)      0.50392    0.01015      49.66    <2e-16 ***
global_pressure -0.27535    0.01974     -13.95    <2e-16 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1701 on 279 degrees of freedom
Multiple R-squared:  0.4107,  Adjusted R-squared:  0.4086
F-statistic: 194.5 on 1 and 279 DF, p-value: < 2.2e-16

sqrt(0.4107) =  0.6408588
```

Figure 3. HeFI (GR Index) response to IC combined pressures and relative summary statistic results from (IC exercise).

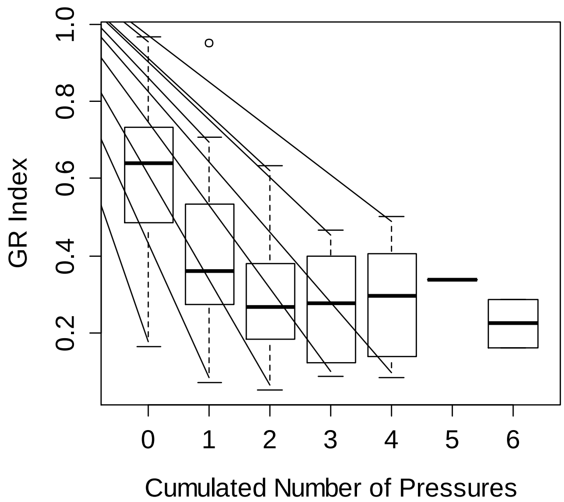


Figure 4. HeFI (GR Index) response to the total number of pressures at level “High” (IC exercise).

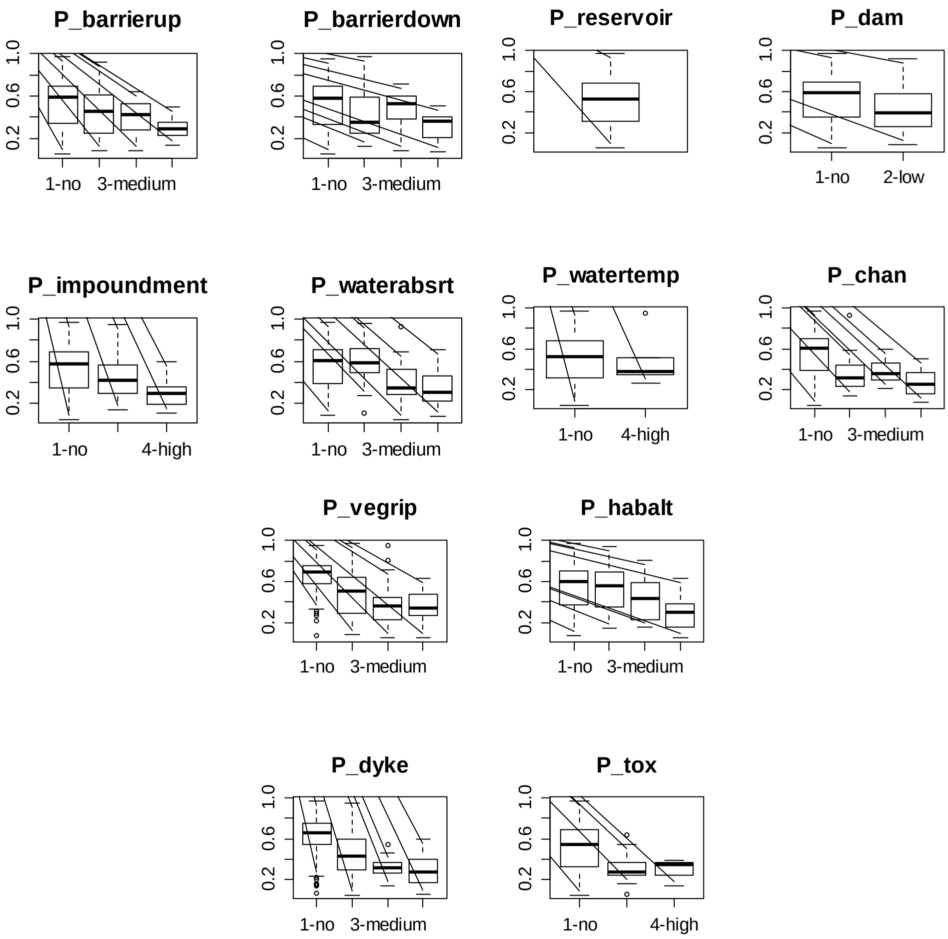


Figure 5. HeFI (GR Index) response to individual common pressures (IC exercise).

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

HeFI is a multimetric, model-based index, scoring the deviations from reference conditions at the site scale. The method assesses the ecological status based on species composition, relative abundance and size structure. Reference conditions, from a reference calibrated dataset (no or minimal disturbance), are used to construct the model-index. Species are assigned to ecological guilds. Guilds identified, and represented into metric fitted selection, are migration (potamodromy), habitat preferences and feeding habits. The classification of study sites takes into account metric values predicted for reference conditions and those arising by sampling data. The assessment concept of HeFI is in line with other European model-based indices (e.g. EFI+).

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

HeFI provides an effective interregional assessment tool which strives to develop into a general standard for assessment and reporting in the Hellenic Republic (see Annex). IC exercise successfully tested HeFI index in relation to other Mediterranean indices, from Spain and Portugal, which seem to share shame ichthyofaunal and climatic characteristics.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

Description of the IC option and benchmark standardization used in the completed IC exercise;

Selection of the correct procedure to use for intercalibrating new classification method.

Sampling sites are located in different biogeographic regions that host dissimilar fish communities and show a remarkable heterogeneity in hydrological and other environmental conditions (see annex). For the purpose of this bioassessment index development, the biogeographic classification of Greece was simplified and a regionalisation scheme comprising two freshwater ecoregions, a "northern" and a "southern", was adopted. Moreover, metric development was based on ecological and biological traits, which helps to overcome the problems associated with the taxonomic heterogeneity in Greek freshwaters, thereby conferring generalisability to the index. However, it is not yet clear if metrics are truly independent of finer-scale biogeographic and natural environmental conditions. Under these circumstances, setting class boundaries by splitting the index range in five equally spaced classes is a logical option. This issue will be re-examined in the years to come when more sample data will become available.

Although it may seem difficult to intercalibrate the Greek assessment method with other Mediterranean national fish assessment methods, which are already intercalibrated within the Med GIG, this was successfully accomplished. Barriers to comparisons among the Med regions include the following: a) The respective regions harbour taxonomically different fish faunas and possibly there are also structural and functional differences in fish assemblage composition which have not yet been explored; b) The Greek fish fauna is characterised by high endemicity: The freshwaters of Greece harbour 160 species, of which 137 are native, and about 90 of these are endemic to Greece or to the southern Balkans (Barbieri *et al.* 2015); c) Species traits among the fishes are poorly known, empirical evidence is scarce for many species; and d) The reference conditions in the lowland and intermittent flowing river reaches are especially difficult to develop due to lack of near-reference natural sites or the difficulty of assessing hydromorphological and ecological integrity in sites that have centuries of anthropogenic pressure at the landscape scale.

5.2. DESCRIPTION OF IC DATASET

A total of 281 samples collected through standardised procedures, from different stream types were considered for the IC exercise. The dataset covers most of the prevailing pressures and pressure gradients.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

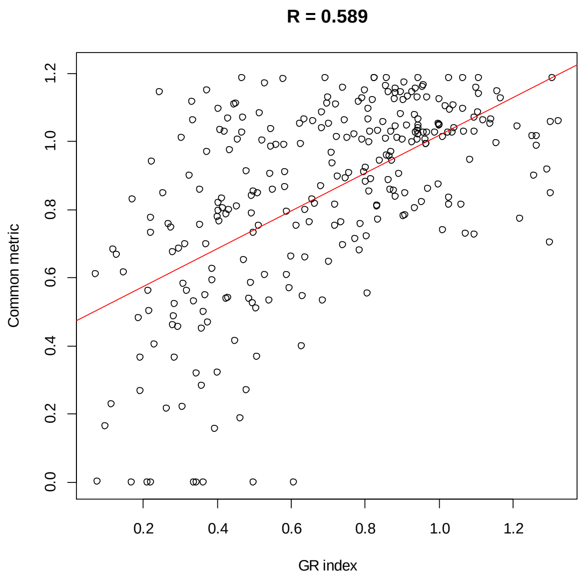
- Benchmark standardization
- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC)
- Translation of national boundaries to ICM or BRINC
- Calculating boundary bias

- Harmonizing of boundaries

Below, the results from the IC exercise between HeFI index and common metrics are presented. Tests were conducted in accordance with Mediterranean Group values (Spain & Portugal).

Correlation between HeFI (GR_index) and "common metrics"

The approach used for the Fish River IC exercise in the Mediterranean Group is the case A1 (IC Option 1 or 2 using reference/benchmark sites) without piecewise transformation. Before checking the correlation between common metrics and the national indices, values are transformed in EQR (cf IndexAll_Mean in file tab_RAWEQR_GR).



Coefficients:

	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	0. 46278	0.03431	13.49	<2e-16 ***
x	0.55538	0.04566	12.16	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2308 on 279 degrees of freedom
Multiple R-squared: 0.3465, Adjusted R-squared: 0.3442
F-statistic: 147.9 on 1 and 279 DF, p-value: < 2.2e-16

Multiple R-squared =0.3565 R = 0.58864251

Regression slope between 0.5 and 1.5 and correlation coefficient > 0.5

Figure 6. Regression plot between HeFI (GR Index) and common metrics (Mediterranean Group IC exercise)

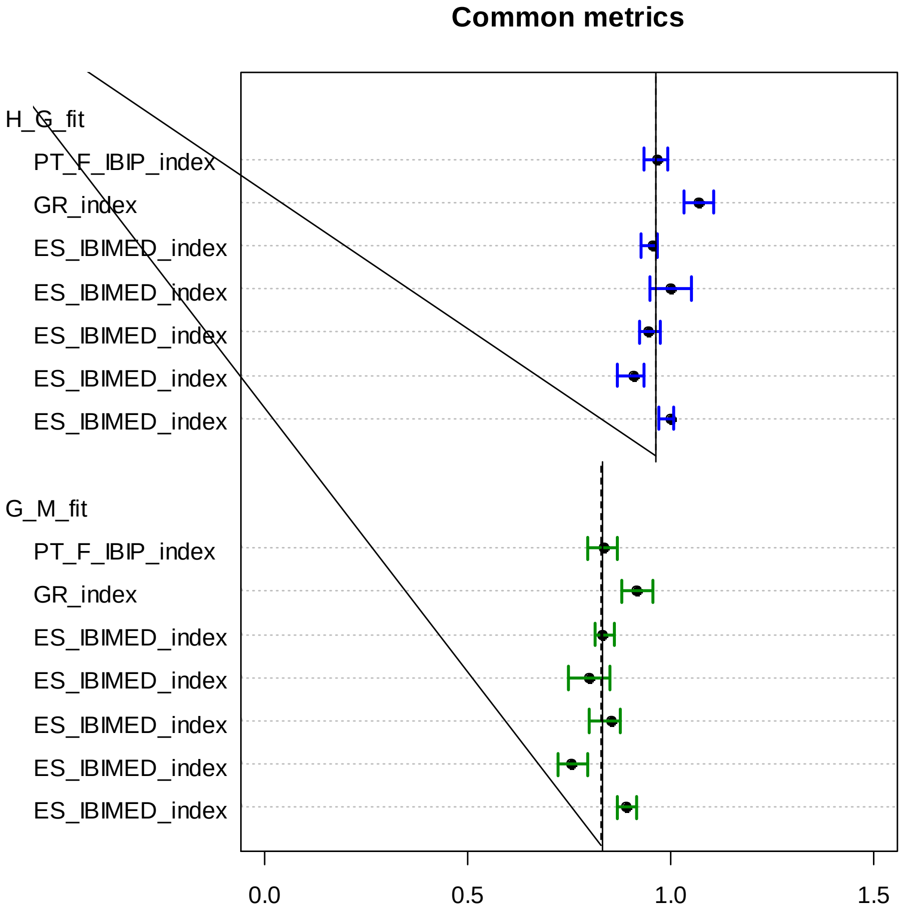


Figure 7. Intercalibration index values of HeFI (GR Index) aligned with relative values of other Mediterranean Group index values (Spain & Portugal).

The HeFI boundaries (H_G_raw and G_M_raw) lie in the range (distance with the median value < +/- a fourth of a class, e.g H_G_lwr and H_G_upr) or over the range.

Method_country	Type	H_G_raw	G_M_raw	H_G_fit	H_G_lwr	H_G_upr	G_M_fit	G_M_lwr	G_M_upr
ES_IBIMED_index	T2	10.58	9.15	0.999	0.972	1.007	0.89	0.869	0.917
ES_IBIMED_index	T3	17.37	13.69	0.908	0.87	0.934	0.756	0.722	0.794
ES_IBIMED_index	T4	11.23	9.85	0.947	0.924	0.973	0.854	0.801	0.878
ES_IBIMED_index	T5	7.47	5.61	0.999	0.949	1.052	0.799	0.749	0.849
ES_IBIMED_index	T6	11.9	9.92	0.958	0.926	0.966	0.831	0.812	0.862
HeFI_index	**no**	**0.8**	**0.6**	**1.07**	**1.032**	**1.107**	**0.918**	**0.88**	**0.956**
PT_F_IBIP_index	no	0.85	0.675	0.966	0.934	0.994	0.836	0.794	0.869
Mean	**no**	-	-	**0.963**	-	-	**0.828**	-	-
Median	**no**	-	-	**0.962**	-	-	**0.833**	-	-

*the six last columns are expressed in common EQR as defined in the IC exercise

In the case of HeFI, the method is more severe than the two other methods from the Mediterranean Group (Spain and Portugal). This is allowed in the IC exercise.

Class agreement element of comparability

Based on guidance n°30 (p. 27) it should be noted that the class agreement element of comparability cannot (easily) be calculated for joining methods, because there is no common dataset or application of the method of every country to the data of all other countries. This step, used in Option 3 of the completed intercalibration exercise, therefore has to be suspended. It is arguable that a more stringent correlation between the joining method and the common metrics should be stipulated in the case of joining methods, to compensate for the lack of a class agreement test, but this would introduce an inconsistency with the completed exercise. In reality, the goal for any joining method is clear and it should be simpler to achieve the necessary feasibility in these annex exercises than it was in earlier rounds of intercalibration, where Member States developed their methods largely in isolation. Therefore, we conclude that the class agreement is no more obligatory, in particular in the case A1: IC Option 1 or 2 using reference/benchmark sites.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

According to the normative definitions for the fish fauna provided by the WFD, high, good and moderate ecological status shall be defined by species (taxonomic) composition, abundance, age structure and type-specific disturbance sensitive species. The guild-based modeling approach used for the development of HeFI does not allow equivalent community-level descriptions of the fish fauna at high, good and moderate status. However, four fish-based methods for the assessment of ecological status that have been developed in Greece through the spatial (type-specific) approach (see annex) fully meet the normative requirements of the WFD. These indices were designed for local and regional applications and the approaches followed for the determination of fish types, the establishment of reference conditions, selection of metrics and the placement of class boundaries are founded on principles and guidelines provided in CIS Guidance Document 10 (EC, 2003).

Generally speaking, different freshwater ecoregions of Greece host different fish faunas in terms of taxonomic composition and assemblage characteristics, but four main "fish types" are commonly encountered: trout streams, barbel-trout streams, upland cyrpinid rivers, and lowland cyprinid rivers (Zogaris et al. 2004; Zogaris 2009a; see Annex). Type-specific species (those that should always be present under undisturbed and slightly disturbed conditions) and "guiding" species (those which can have a substantial influence on the structure and function of fish communities by virtue of their abundance and distribution) were defined for all types (see Annex).

6.1 DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

At high status, the type-specific and the guiding species should be present and their relative abundance should be within the range of values expected at reference conditions. The guiding species should show a healthy age (size) structure. Absolute abundance spatial and temporal variation is too large to be utilised as a defining variable for high status. The same holds true for taxonomic composition, which shows considerable variability at spatial scales within river types. For this reason, species richness and related metrics (e.g. Shannon-Wiener diversity) cannot be reliably used to characterize high status at the river type scale. It is remarkable, though, that for the upland cyrpinid rivers and the lowland cyprinid rivers, species richness and some other community attributes (e.g. relative abundance of some species) exhibit a "Vannote-like" longitudinal trend, changing predictably (e.g. species richness increases) from upstream to downstream. Therefore, predictive models can be developed to provide estimates of expected species richness at different positions of the main river stem. Preliminary results of temporal analyses at the site scale (e.g. the same sampling station visited in successive years at the same season) indicate that the relative abundance of type-specific and guiding species exhibits a remarkable interannual stability under undisturbed conditions. The relative species composition of the entire local assemblage exhibits less stability, because rare species may absent in the catch due to stochastic reasons or sampling variability.

6.2 DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

At good status, the type-specific and the guiding species should all be present and their relative abundance should be close enough to the range of values expected at reference conditions. Some habitat specialists or species with narrow ecological requirements (e.g. local trout species) may be found outside the range of values expected at reference conditions. On the other hand, generalist species may be found at higher than expected values, indicating slight disturbance attributable to anthropogenic impacts. The age structure of guiding species may also show signs of disturbance, and some age classes may be missing. Species not typically encountered in the river type under consideration may occasionally be present. This holds especially when there is anthropogenic influence in a river position not very far away from the study location (e.g. downstream dam, stocking activity). In such cases, "atypical" species (e.g. stocked rainbow trout or limnophilic species) may be present through dispersal mechanism. Diadromous species (eel) that have been historically been recorded, may be absent if a downstream dam disrupts longitudinal connectivity.

6.3 DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

At moderate status, the composition of fish communities may differ substantially from the type-specific reference community. All guiding species should still be present, but their numerical relative contribution to the assemblage composition may be well outside the range expected at reference conditions. One or more type-specific species may be missing. Species not typically encountered in the river type in question may be present and occasionally in significant numbers. Limnophilic species, stocked species or species tolerant to environmental extremes (e.g. the non-native species *Lepomis gibbosus* and *Gambusia holbrooki*) may dominate the fish fauna. The age structure of the guiding species shows signs of major anthropogenic disturbance.

7. REFERENCES

Contributors to this report

This report reviews the process and products of several years of work in fish-based index-building in Greece. More than 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 and Annex). The authors are especially grateful to Didier Pont who was responsible for exploring intercallibration and relevant statistical analyses in this report. We should specifically acknowledge the contributions of the FAME project and the scientists that worked closely with the IMBRIW-HCMR team: S. Schmutz, U. Düssling, 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. For further assistance with data analyses we are grateful to Yorgos Chatzinikolaou, Haris Vavalidis and Nektarios Kalaitzakis. 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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