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**Development of a fish-based index to assess the ecological quality of transitional waters:
the case of French estuaries.**

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Abstract

The Water Framework Directive requires the assessment of the ecological status of transitional waters considering the fish component. An original methodology, based on a pressure-impact approach, was established to develop a multimetric fish-based index to characterize the ecological quality of French estuaries. An index of contamination, based on the chemical pollution affecting aquatic systems, was used as a proxy of anthropogenic pressure. The fish metric selection was based on their response to disturbances tested via statistical models (generalized linear models) taking into account sampling strategy and estuarine features. Four metrics, for which discriminating responses to level of pressure were demonstrated, were retained to constitute the estuarine multimetric fish index. This new tool appeared particularly relevant to detect the contamination effects on fish communities in estuaries. It could help managers to take decisions in order to maintain or reach the good status required by the Water Framework Directive for 2015.

Keywords: multimetric fish index, anthropogenic disturbances, ecological quality status, estuaries, generalized linear models

1. Introduction

Transitional waters¹ are highly variable environments. They are influenced by fluctuating marine and freshwater flows, which lead to large and quick variations of their physico-chemical features. By providing many species with basic requirements for their life cycle, these particular areas support a wide range of fauna, from resident to freshwater and marine species. Estuaries are especially essential to fish due to their ecological functions related to refuge area, habitats for reproduction, permanent habitat, nursery grounds and migration routes (Elliott et al., 2007). However, they are impacted by multiple anthropogenic disturbances such as fishing, dredging, pumping and pollution (Blaber et al., 2000; Cloern, 2001; Dauvin, 2008).

In this context, the assessment of the ecological status of transitional waters is required, especially in the European Union (EU Water Framework Directive; WFD - Directive 2000/60/EC; Anonymous, 2000). Fish communities appear to be a relevant biological element to evaluate aquatic ecosystems' health (Karr, 1981; Karr et al., 1986; Elliott et al., 1988; Fausch et al., 1990; USEPA, 2000; Whitfield and Elliott, 2002; Harrison and Whitfield, 2004). Several types of indices exist: some are based on only one criterion as the Community Degradation Index (CDI; Ramm, 1988) and the Biological Health Index (BHI; Cooper et al., 1994), while others consist of a combination of several metrics, *i.e.* the Indices of Biotic Integrity (IBI; Karr, 1981; Roset et al., 2007). A metric is defined as “a measurable factor that represents various aspect of biological assemblage, structure, function, or other community component” (USEPA, 2000; Coates et al., 2007). Thus, multimetric indices are expected to provide information about various aspects of fish assemblages and lead to a more holistic, integrative and functional approach (Roset et al., 2007).

¹ surface waters in the vicinity of river mouths, partly saline in character as a result of their proximity to coastal waters but substantially influenced by freshwater flows (WFD - Directive 2000/60/EC)

Based on fish data for 13 estuaries in France, the aim was here to develop a multimetric fish-based index to assess the ecological status of French estuaries, as required by the WFD. Fish metrics were constructed using ecological guilds (Elliott and Dewailly, 1995). Their response to anthropogenic pressures was analysed, taking into account the effects of the sampling design (Mouillot et al., 2006). Then a selection was realised based on the ability of these metrics to discriminate between different levels of pressure. Last, selected metrics were combined to develop a multimetric index of estuarine ecological status (Roset et al., 2007).

2. Materials and methods

2.1. Fish data

Thirteen estuaries along the French Atlantic and English Channel coasts were sampled in 2005 and 2006 (Fig. 1) following a standardised sampling protocol (Lepage and Girardin, 2006). The fishing events occurred in Spring and Autumn. Two types of beam trawls were used according to the size of estuaries. One had an opening of 3 x 0.5 m and a 20 mm stretched mesh in the codend and was used in large estuaries only; the other had an opening of 1.5 x 0.5 m and 16 mm stretched mesh in the codend and was preferred in small estuaries. Trawling was performed during daytime, against the current, for about 15 min at a speed ranging from 1.5 to 3 knots. The number of trawl hauls ranged from 12 to 78 per estuary and per season (Table 1). Hauls were distributed, as far as possible, all along the salinity gradient inside the estuaries but obvious sewage points were avoided when known. All fishes caught were identified, measured and counted. After each fishing event, salinity was recorded. This dataset included 734 trawl hauls.

2.2. General methodology

The lack of pristine sites and historical data to establish reference conditions as required by the WFD, led us to adopt a methodology based on statistical modelling (Pont et al., 2006): thresholds and reference points were defined using pressure/impact models (DPSIR approach - Borja et al., 2006). A first approach based on such models showed the negative impact of proxies of anthropogenic disturbances on the nursery function of estuaries for marine fish species (Courrat et al., 2009). This work highlighted the need to take into account the variability in fish metrics due to the sampling protocol and some estuarine features, as they represented a great part of the variability in fish data (Nicolas et al., In press). This method appeared particularly appropriate in a context of « Estuarine Quality paradox », *i.e.* when the effects of natural and anthropogenic stress on estuarine biota are difficult to discriminate (Elliott and Quintino, 2007; Dauvin and Ruellet, 2009). Hence, in order to build a multimetric fish based index for French transitional waters, we adopted the following steps (Fig. 2): (1) Indices based on human pollutions and activities were elaborated to describe the anthropogenic disturbances that potentially affect fishes. (2) Candidate fish metrics were selected with regard to literature and previous knowledge. (3) The impact of overall contamination on candidate metrics was tested *via* statistical modelling. The models were realized at the trawl haul sampling scale and took into account the variability due to sampling protocol and environmental features. (4) Metrics whose response to pressure was significant were selected. (5) A methodology was developed to calculate thresholds distinguishing between three levels of contamination. (6) Redundant metrics and metrics with non-discriminant thresholds were removed. (7) Scores (1-3-5) were attributed by comparing metric values, calculated from the survey data, to the above-mentioned thresholds according

to spatio-temporal conditions of sampling and estuarine features. (8) These scores were combined to provide a general assessment of the ecological status of each estuary. (9) A comparison between levels of pressure and multimetric scores was realized.

2.3. Candidate metrics

Candidate fish metrics were selected to characterize both fish diversity and the various ecological functions associated with transitional waters. The global diversity, described by the taxonomic richness, *i.e.* the number of species caught, and the total density were considered. A review of existing studies (Claridge et al., 1986; Elliott et al., 1990; Pomfret et al., 1991; Elliott and Dewailly, 1995; Deegan et al., 1997; Marshall and Elliott, 1998; Cabral et al., 2001; Thiel and Potter, 2001; Lobry et al., 2003; Thiel et al., 2003; Breine et al., 2004; Coates et al., 2004; Harrison and Whitfield, 2004; Maes et al., 2005; Breine et al., 2007; Elliott and Quintino, 2007) led us to focus on a guild approach to characterize the functional aspects of transitional waters for fish. Root (1967) defined a guild as a group of species that exploit the same class of environmental resources in a similar way. We used an extended definition to include a description of the way species use transitional waters, as well as their feeding mode and their vertical distribution in the water column (Elliott and Dewailly, 1995; Lobry et al., 2003; Franco et al., 2006; Elliott et al., 2007; Table 2). All fish species present in the dataset were classified for each type of guild. Number of species and density were selected as potential metrics and calculated for each guild. In the aim of assessing estuaries' ecological status, the retained metrics were supposed to have significant trends with increasing degradation (Breine et al., 2004; Breine et al., 2007). Thus, for each metric, the expected response with increasing degradations was defined according to literature and expert's

judgements and only the metrics known to negatively respond to increasing anthropogenic pressures were retained (Table 3).

2.4. Modelling for the fish metrics

All metrics were modelled at the trawl haul scale (one trawl haul = one line in the dataset) to take into account the metrics' variability due to sampling protocol (Courrat et al., 2009). Each fish metric was modelled with Generalized Linear Models (GLMs) using R software (R Development Core Team, 2005).

Models options depended on data distribution for the different metrics. For species richness (SR), global and by guilds, the GLMs were based on a Poisson law:

$$\text{Log}(\text{SR}) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

where variable[1,2,..,n] represents the introduced descriptors that can be either continuous covariates or class factors.

The metrics expressed in density were composed of 0 inflated data, which made inappropriate the use of linear models. Thus, a delta type model that consisted in a combination of two models was used (Stefansson, 1996). The first was a binomial model on the presence-absence data ($D_{0/1}$).

$$\text{Logit}(D_{0/1}) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

The second model tested the positive densities (D_+) which were log-transformed using a Gaussian law (Le Pape et al., 2003; Nicolas et al., 2007; Courrat et al., 2009):

$$\text{Log}(D_+) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

2.5. Variables included in the models for the description of the sampling protocol and some estuarine features

Courrat et al. (2009) highlighted the importance of descriptors of the sampling protocol and estuarine features to explain the variability of the number of species and the abundances of marine juvenile migrants in French estuaries. The present work is based on an extrapolation of these results to other metrics. The models estimated species richness and fish densities in different guilds at the sampling scale; while taking into account some factors describing protocol variability and environmental/hydromorphologic features, they analysed the effects of anthropogenic pressures on these metrics.

2.5.1. Variables for the description of the effect of the sampling protocol on fish metrics

Fish assemblages in transitional waters change according to seasons (Elliott et al., 1990; Araùjo et al., 1998; Thiel et al., 2003; Koutrakis et al., 2005; Franco et al., 2006; Lobry et al., 2006), hence the effect of sampling season (spring or autumn) was tested as a class factor. Transitional waters are characterized by a strong salinity gradient, known to have a significant effect on fish assemblages (Thiel et al., 1995; Marshall and Elliott, 1998; Lobry et al., 2006; Franco et al., 2008b). Salinity was tested within models as a class factor. Salinity class boundaries were adapted from the Venice system (1958): oligohaline class ([0-5]), mesohaline class ([5-18]) and polyhaline class (>18).

2.5.2. Variable for the description of the effect of environmental and hydromorphologic features at a larger scale on fish metrics

As French coasts of Atlantic and English Channel sheltered different fish assemblages (Coates et al., 2004; Franco et al., 2008a), an ecoregion factor was also taken into account in the models.

Estuarine size has been pointed out as a significant predictor of taxonomic richness (Monaco et al., 1992; Elliott and Dewailly, 1995; Roy et al., 2001; Nicolas et al., In press). Thus, the estuarine size was included in the models, as a class factor distinguishing the large (>100 km²) from the small estuaries. As, the large estuaries were sampled with the large beam trawl and the small estuaries with the small one (except for the Loire estuary which was sampled with the small gear in its oligohaline area), estuarine size and type of beam trawl were strongly correlated, which induced redundancy in the models. One of these two variables has to be removed. Hence, the variable “size of estuary”, replaced the variable “type of beam trawl” used by Courrat et al. (2009) as, in the context of the WFD requirements, it appears more consistent to define thresholds varying according to water bodies’ physical characteristics.

2.6. *Indices of anthropogenic pressures*

An index of contamination was used as a proxy for human disturbances impacting estuaries. We used data on the chemical contamination collected by the French monitoring network of the marine environment (RNO; www.ifremer.fr/envlit). All 13 estuarine areas considered in this study correspond to sites investigated by this network. Concentrations of five heavy metals (Cd, Zn, Cu, Hg and Pb) and two organic pollutants (polychlorobiphenyle and polycyclic aromatic hydrocarbons) in two mollusc species (mussels *Mytilus edulis* and oysters *Crassostrea gigas*) were standardized (Beliaeff et al., 1998; Gilliers et al., 2006). For each site, median values were computed over 6 years: from 2000 to 2005. Medians were preferred to means because they are more robust with regard to outliers (Beliaeff et al., 1998). A normed Principal Component Analysis (PCA) was computed to synthesize the number of variables (contaminants, in column) used to describe the overall chemical contamination of

the sites (individuals, in row). This PCA allowed to compute two distinct indices of contamination (Courrat et al., 2009): the first axis was strongly influenced by the metals whereas the second axis was related to the organic pollutants. Hence, an index of heavy metal pollution and an index of organic pollution were derived from the factorial scores of the estuaries along each axis. Finally, the index of overall contamination (used in models as a proxy of the cumulative effects of human disturbances in estuaries) was obtained by summing the scaled values (factorial scores) of the first two indices (Fig. 3).

2.7. Statistical analyses

The models, including effects of protocol (salinity class, season), estuarine features (ecoregion, estuarine size) and anthropogenic pressure proxy, can be written as follows:

$$\text{Metric} \approx \text{factor}(\text{season}) + \text{factor}(\text{salinity}) + \text{factor}(\text{size}) + \text{factor}(\text{ecoregion}) + \text{covariate}(\text{contamination index})$$

“Correlating the results of a metric to the stressor gradient is a central part of the procedure” (Hering et al., 2006). We calculated the statistical significance of each descriptor and pressure index effect at the level of 5% in GLMs (Chi-squared test). The non-significant descriptors were removed from the models. The nature (positive or negative) of the impact of each variable and of the pressure index on the fish metrics was determined from the sign of the corresponding coefficient(s). A graphical analysis of the residuals was carried out for each GLM to verify underlying hypotheses.

2.8. Metric selection

The checkout of initial hypotheses concerning the expected trends of the metrics with increasing degradation is an essential step to build multimetric indices (Roset et al., 2007). A metric (density or species richness, global or by guild) was retained only when the initial hypothesis of its trend with increasing anthropogenic disturbances was confirmed by models. A second selection consisted in testing the correlations (Pearson correlation test) between each pair of metrics. When two metrics were strongly correlated, only one was kept to avoid redundancies.

2.9. Test of discriminance in response to pressure and thresholds calculation

Thresholds were established using fitted values from the GLMs and corresponding confidence intervals. For each selected metric, a model was constructed with a combination of the significant variables among season, salinity class, estuarine size and ecoregion. Then fitted values of these models were calculated for 3 levels of anthropogenic pressure. In the studied estuaries, the highest value of the overall contamination index was found in the Seine estuary and the lowest in the Mont Saint Michel Bay (Fig. 3); these values were used to respectively simulate high and low pressure. Then the mean of these two extreme values was used to characterize an intermediate level of pressure.

To define confidence intervals, we simulated 5000 virtual observations for the different models (glm Poisson) and submodels (glm Binomial and glm Gaussian), with means equal to the corresponding predicted values and standard deviation equal to corresponding predicted standard errors. For metrics of densities, the 5000 simulated probabilities of presence and the 5000 simulated log-densities were then multiplied to produce 5000 simulated densities. Finally, for each metric, the 10% and 90% quantiles of the 5000 simulated densities or number of species were used as confidence intervals.

Thresholds were defined by considering the gap between the confidence intervals around the predicted value obtained for the 3 simulated levels of pressure. The thresholds were expressed as numbers of species for the metrics related to species richness and as log-densities for the metrics of total density or density per guild. Hence, for each metric discriminating between the three levels of pressure, two thresholds distinguishing three quality classes were calculated according to the protocol and environmental conditions (Fig. 4a). However, when the confidence intervals overlapped for most of the combinations of natural variables, we considered that the risk of uncertainty was too high and we decided not to maintain the metric into the multimetric index (Fig. 4c); when confidence intervals only overlapped under certain conditions, the metric was kept (Fig. 4b).

2.10. Scoring

For each metric, the value calculated from the dataset was compared to the thresholds defined previously to estimate scores (Roset et al., 2007). A score was attributed only if the number of hauls realised for the corresponding season and salinity class was considered as sufficient to provide a reliable representation of the fish metric. Some preliminary analyses (unpublished data) based on bootstrapping on a pool of virtual trawl hauls generated by the models were made in order to determine what is this “sufficient” number of trawl hauls. Though these analyses have still to be improved, results tend to show that with 6 trawl hauls, it is possible to get an assessment of fish densities with an acceptable error, which means that this error does not lead to a misclassification of the salinity class ecological status. Here it was thus assumed that 6 trawl hauls per salinity class and per season may allow to assess accurately the ecological status of a salinity class.

A score equal to (Fig. 4):

- 1 was attributed if the metric value was situated below the prediction realized for the maximum of pressure *i.e.* the worst class of quality,
- 3 was attributed if the metric value was situated between the predictions for the 2 levels of pressure *i.e.* the class of moderate quality,
- 5 was attributed if the metric value was situated above the prediction for the minimum of pressure *i.e.* the best class of quality.
When the 10% and 90% quantiles of two consecutive levels of pressure overlapped, a score of 2 or 4 was attributed for the metric values situated into the uncertainty area (Fig. 4b).
The scores obtained for each season and each salinity class were added and divided by the maximal potential score (5 x number of summed scores) to get one value for each metric and each estuary. These values per metric were then averaged to get the final value of the multimetric index for each estuary.
Finally, a linear regression was performed to test the relevance of the final multimetric fish index regarding the increasing index of contamination.

3. Results

3.1. Metric selection

Freshwater and fish feeder species were absent in more than 90% of the trawl hauls, so modelling for them was irrelevant. The models testing the effect of the pollution index are described in Table 4. The descriptors of the protocol and the environmental features were significant for most of the metrics and they partly explained their variability. 11 metrics showed significant trends with increasing pressure, 10 being negative (Table 4) thus identical

to the expected effect of pressure (Table 3). Hence, these 10 metrics were retained in this first step of the analysis to potentially be included in the final multimetric fish index.

The study of the correlations between metrics revealed that benthic species and benthic feeder species were strongly correlated. The Pearson correlation coefficient reached 94% between the number of species of these two guilds and 100% for the densities. Hence, to avoid redundancy in the final fish index, only metrics concerning benthic species, easier to identify, were selected to compose the final index and benthic feeder metrics were not retained.

3.2. Discriminance of metrics with regard to different levels of anthropogenic disturbances

The 10% and 90% quantiles of two consecutive levels of pressure overlapped for all the metrics expressed in number of species (Table 4). Thus, these metrics were considered as irrelevant for assessing the ecological status of French estuaries with the present approach. The quantiles also overlapped for the densities of diadromous and marine juveniles in the small Atlantic estuaries. Nevertheless, these metrics were maintained into the multimetric index, as the uncertainty area was limited to specific conditions.

Finally, four metrics were retained: total density (TD), density of diadromous migrant species (DDIA), density of marine juvenile migrants (DMJ) and density of benthic species (DB) (Table 4). For each of them, thresholds between different levels of contamination were calculated for the different combination of factors (season, salinity class, estuarine size, ecoregion).

3.3. Multimetric index values

According to comparisons between in situ data and thresholds (Fig. 4), scores were attributed for each fish metric in each combination of factor describing sampling protocol and estuarine features; then these scores were combined in a multimetric index value for each estuary. For the 13 Atlantic and English Channel estuaries, the multimetric fish index values ranged from 0.26 in the Gironde estuary to 0.95 in the Mont St Michel Bay (Fig. 5). A linear regression showed a significant negative relationship ($r^2=0.35$, $p.value<0.05$) between this estuarine multimetric fish index and the index of contamination (Fig. 6).

4. Discussion

This paper presents the development of a fish-based index for defining the ecological status of transitional waters using a pressure/impact approach. The methodology is in agreement with the processes recommended by Hering et al. (2006) and Stoddard et al. (2008) for the creation of multimetric indices. Despite the ‘estuarine quality paradox’, *i.e.* the fact that features of anthropogenic stress coincide with those of natural stress in estuaries (Elliott and Quintino, 2007), we showed significant relationships between anthropogenic disturbances and various aspects of fish assemblages. Thresholds were identified for metrics presenting significant trends with increasing pressure and a system of scoring was elaborated to qualify estuaries’ quality by combining the selected fish metrics.

4.1. Pressure index

The anthropogenic pressure index used - *i.e.* index of contamination - focuses on only one type of anthropogenic pressures affecting estuaries. It was difficult to obtain homogenous data on the different types of anthropogenic pressures that can be found for each of the 13 studied

estuaries. The data used for this work were the most precise and homogenous data that corresponded to the present fish sampling period. It would be fruitful to improve this pressure index especially considering hydromorphological modifications like polderisation and loss of intertidal mudflat leading to loss of habitat. In the present study, the index of contamination was used as proxy for the overall anthropogenic disturbances impacting these estuaries (Courrat et al., 2009). Aubry and Elliott (2006) qualified these indicators of disturbance in their contribution to the development of the Environmental Integrative Indicators, as “potential”, because (i) the process of the biological response to an increase of this measure of pressure was not demonstrated here, even if they are known from previous approaches (Courrat et al., 2009) (ii) the measure of pressure did not take into account the whole anthropogenic disturbances.

4.2. A general method to test the effect of anthropogenic pressure with regard to other sources of variability

The degree of spatial and temporal variability in transitional waters is high (McLusky, 1981) and previous studies emphasised the need to take into account the effects of sampling protocol and estuarine features on fish metrics (Whitfield and Elliott, 2002; Mouillot et al., 2006; Roset et al., 2007; Courrat et al., 2009). The present study confirmed that fish metrics highly depend on sampling and that it is necessary to consider these metrics at the sampling site scale in estuaries’ status assessment to take into account the patterns of natural variations. On the contrary, designing fish metrics at the scale of the estuary would restrict the analysis to the number of sampled systems (here: 13) and would lead to use very simple models, few synthetic descriptors and spurious approach to take into account differences in sampling protocol. In conclusion, the effects of the sampling design cannot be summarized at the scale

of estuary, making the use of fish metrics at this scale for testing anthropogenic disturbance effects irrelevant (Courrat et al., 2009). This conclusion is essential to assess ecological quality, as a non-accurate description of the natural variability can hamper the sensitivity of fish indices to human disturbance (Roset et al., 2007). Furthermore, working at the sampling scale will allow for testing the effects of other descriptors linked to intra-estuarine variability. Moreover, in the aim of monitoring estuarine quality, this approach could allow to estimate different ecological status in different part of a system, at least along an upstream - downstream salinity gradient.

A large part of the metric variability was not explained by the models used in this study (from 65 to 82%). The addition of more environmental variables likely to influence estuarine fish assemblages can improve the interpretation of the metric variability. Especially, further descriptors of habitat richness and diversity, in addition to the single description of estuarine size, could improve models accuracy. However, this would complicate the calculation of thresholds and the scoring process by increasing the number of combinations of factors describing estuarine features to account for in the statistical predictions. Furthermore, the WFD requires a limited number of thresholds. An exhaustive list of all the environmental factors affecting fishes can not be included in models. The present approach was a balance between available exhaustive descriptors, models parsimony and limited complexity in threshold calculation. In spite of a large residual variability, this approach provides a simple and robust method to estimate fish metric levels with regard to estuaries' quality.

4.3. Selected metrics, ecosystem functioning and ecological status

Among the 16 candidate metrics, 10 were found to respond significantly to anthropogenic pressure in the expected direction. Species richness and fish densities are usually considered as good indicators for ecological status of estuaries (Hughes et al., 1982). Most of the selected metrics describe the structure of fish assemblages using the concept of guilds. The guild approach has been recognized to be a good scheme to describe and explain transitional waters' community structure and estuarine functionalities (Deegan et al., 1997; 2004; Coates et al., 2004; Harrison and Whitfield, 2004; Breine et al., 2007; Elliott et al., 2007; Franco et al., 2008a; Franco et al., 2008b). Moreover, in such inter-site studies, it allows to overcome the differences in fish assemblages through geographical gradients and to obtain robust indices (Mouillot et al., 2006).

Metrics of species richness

Species richness usually decreases with a decrease of the habitat quality (Hughes et al., 1982; Gibson, 1994). Here, fish species richness appeared to significantly decrease when anthropogenic pressure is enhanced. This has been verified on the global species richness and also per guild. Thus, anthropogenic disturbances appeared to have significant negative effects on almost all metrics of species richness.

Metrics of fish density

The metric of total density was selected for the multimetric fish index. The total density appeared to be a common surrogate for system productivity and a good element to evaluate ecosystem health (Karr, 1981; Deegan et al., 1997). Moreover, metrics of global abundances satisfy the WFD (Anonymous, 2000) which asks to monitor composition and abundance for fish in transitional waters.

Several metrics related to density per guilds were also retained:

- density of diadromous migrant species. Diadromous migrants use transitional waters as migration routes (Deegan et al., 1997; Roth et al., 1998; Oberdorff et al., 2002; Levrel, 2007) and are highly sensitive to many sources of pollution and degradation, particularly to migration barriers, which make this metric an important indicator of connectivity (McDowall and Taylor, 2000; Elliott and Hemingway, 2002).

- density of marine juvenile migrants. Many studies have demonstrated the negative effect of anthropogenic degradations on marine juveniles and the nursery function of the estuaries (Gibson, 1994; Deegan et al., 1997; Gilliers et al., 2006; Breine et al., 2007; Le Pape et al., 2007) and Courrat et al. (2009) focused on this guild with the same data to develop the present approach.

- density of benthic and benthivorous fish. Benthic species are particularly sensitive to siltation and oxygen deficiency (Oberdorff and Porcher, 1994; Kestemont et al., 2000) and are considered as early-warning indicators of anthropogenic disturbance (Hughes et al., 1998). This metric appeared particularly interesting to assess the degree of perturbation of benthic habitats and has already been used in other multimetric index (Borja et al., 2004; Breine et al., 2004; Coates et al., 2004; Harrison and Whitfield, 2004; Uriarte and Borja, 2009). Benthic feeders were shown to respond negatively to anthropogenic pressure but their redundancy with benthic species led to the removal of this metric from the final index. Even if trophic guild metrics should be interesting tools to characterize ecological function of estuaries (Elliott and Dewailly, 1995) as they provide an insight into the community structure (Hughes et al., 1982), none was retained in the final index.

The aim of the present approach was to analyse the response of fish metrics, considered as relevant to estimate the ecological status of estuaries, to potential indicators of disturbance (Aubry and Elliott, 2006) based on chemical contamination (Courrat et al., 2009). It

highlighted the negative impact of a proxy of anthropogenic pressures on different metrics representing ecological and functional integrity of estuaries. Even if, for some metrics, the effect was not significant (and even opposite to expected for positive densities of marine seasonal species), the methodology developed by Courrat et al. (2009) for the marine juveniles appears efficient to test the effects of anthropogenic pressures on other fish metrics and can be extrapolated to other components of the fish structure.

4.4. The multimetric fish index

4.4.1. Thresholds

Two thresholds, separating 3 classes, have been calculated from 3 levels of anthropogenic disturbance. The lower and the upper levels are associated with the best and the worst status observed among the sampled sites. This could correspond to good and poor status referring to the WFD terminology. No site corresponds to pristine site (high status) or to heavily disturbed site (bad status). Extrapolations from model's predictions below or up to the observed level of contamination could allow to assess metric values in reference situation, as it has already been realized with the method of quintiles or the Maximum Value Lines (Oberdorff and Hughes, 1992; Oberdorff et al., 2001; Harrison and Whitfield, 2004; Breine et al., 2007; Coates et al., 2007).

Among the 10 metrics presenting a significant response in the expected direction, four were finally selected for the estuarine multimetric index. All these metrics are expressed in density. Although metrics expressed in number of species decreased significantly with anthropogenic pressure, developing thresholds was not realistic because of overlapping quality class intervals. This is the consequence of the low number of species caught per sample: it is difficult to discriminate between quality statuses if the expected number of species per sample

in each guild is low. This is called by Hering et al. (2006) “the numerically unsuitable metrics”. Further approaches would have to be tested in the future to include species richness in the indicators (Nicolas et al., In press).

Nevertheless, our approach reached reliable estimates and was able to distinguish different levels of disturbances (Quataert et al., 2007). This method implies a large enough sampling survey, well distributed on the whole transitional area. Such standardization is essential to provide consistency in the assessment based on fish indices (Roset et al., 2007).

4.4.2. Combining metrics in a composite index

The multimetric fish index provided relatively accurate classification of the estuaries. Indeed, the Gironde and Seine estuaries obtained the lowest values concerning fish aspect and were also the most perturbed in agreement with our proxy based on chemical contamination but also with expert knowledge. Conversely, the Mont Saint Michel Bay and the Charente estuary, where the perturbation index is low, presented high scores for the multimetric fish index. A significant negative correlation was found between the level of contamination and the fish index. However, the explained percentage of variability was low and the regression was strongly influenced the 2 large polluted estuaries, Gironde and Seine. This low correlation was not surprising for several reasons:

- The residual variability of the metrics not explained by the models was important, thus the related multimetric estimate of ecological status is associated with uncertainty.

- Contamination data for the sampled sites were chosen as descriptors of the anthropogenic pressures as they were available everywhere, directly correlated to the level of human pressure and appeared as good proxies for it. However, other human disturbances (habitat loss, anoxia, etc.; Peterson, 2003) are not taken into account with such proxy and the single effect of contamination did not quantify the whole anthropogenic pressure. To assess the

502 impact of anthropogenic disturbances on ecological status of estuaries, one of the main
503 challenges is to determine relevant descriptors of these disturbances. While such a measure
504 does not exist, the strength and accuracy of the link between index of ecological status and
505 anthropogenic pressure can not be estimated and discrepancies in scoring pressure relations
506 are difficult to analyse. Nevertheless, the proposed methodology allowed to build a fish index
507 able to detect the impacts of the human activities on fishes and to evaluate the quality status
508 of the fish communities in transitional waters.

509
510 To our knowledge, it is the first time that different scores are attributed according to the
511 season and the salinity class in an estuarine fish-based index. These scores were added and the
512 method employed to combine the metrics consisted in simple averaging. Systems to weight
513 metrics exist (*e.g.* Kestemont et al., 2000; Aubry and Elliott, 2006) but the choice of non-
514 weighing the metrics is the most commonly adopted and appears quite legitimate as the
515 diagnostic supplied by the different metrics appeared of the same interest and not redundant.
516 The present selection of non-redundant metrics reduces their number in the final index and
517 validate this scoring approach. Another method consists in organizing the fish metrics
518 according to the best balance between type I (falsely declaring the status of a site as disturbed
519 while it is not) and type II (falsely declaring a disturbed site as undisturbed) error and
520 stopping the addition of supplementary metrics in the index when the AUC criterion (Area
521 Under the error Curve) is the lowest (Breine et al., 2007). Such method appeared very
522 interesting to limit misclassification errors but the low number of selected metrics in this work
523 prevents from using such complex selection approach.

524 Indeed, the number of selected metrics in this study seemed low compared with other
525 multimetric fish indices and future works to consider additional metrics will be helpful to
526 improve the assessment of ecological status in estuaries. Further alternatives can be used to

provide complementary metrics. Metric calculation at the salinity class scale instead of for each sample should provide interesting prospects. This approach could be fruitful to integrate new metrics, and especially those based on species richness. First it will allow to reach higher level of estimation for this family of metrics (global or by guilds), as the number of species has been demonstrated to increase log-linearly with the number of samples (Krebs, 1999; Nicolas et al., In press). Indeed, the species richness is higher when samples are pooled than for a single one. This will probably allow to obtain significant thresholds as difference between class (in number of species) will increase with the estimate average. Nevertheless, it would be based on pooled data thus on fewer degrees of freedom and that would increase problems to include natural contrasts and sampling strategy in the models. Hence, further thorough work has to be done in this direction (Nicolas et al., In press) to improve the present index.

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Figure captions

Fig. 1. Location of the 13 French estuaries considered in the present study.

Fig. 2. General methodology for testing the impacts of anthropogenic disturbances on transitional water functions from fish metrics and building a multimetric fish index (adapted from Courrat et al. (2009)).

Fig. 3. Indices of heavy metals, organic and overall contaminations for the 13 studied estuaries classified according to an ascending level of overall contamination. Arrows indicate the levels of pressure used for prediction from fish metrics' models.

Fig. 4. Test of discriminance of the effect of anthropogenic pressures on predicted values of fish metrics, with associated thresholds (horizontal lines) and scores. Example for a metric expressed in log-density. a: Illustration of non overlapping quantiles. b: Methodology adopted for the scoring when quantiles slightly overlapped. c: strong overlapping leading to reject the metric. Circles represent the metric values calculated for high (black), intermediate (grey) and low (white) values of the anthropogenic pressure index (fitted values from the models). Triangles correspond to 10 and 90% quantiles.

Fig. 5. Values of the multimetric fish index calculated for each of the 13 Atlantic and English Channel estuaries.

Fig. 6. Relationship between the multimetric fish index and the index of contamination for the 13 studied estuaries.

Table 1.

Number of trawl hauls performed in each estuary according to seasons and salinity gradient.

	Spring				Autumn			
	oligohaline	mesohaline	polyhaline	Σ	oligohaline	mesohaline	polyhaline	Σ
Adour	10	6	3	19	16	5	8	29
Authie	3		12	15	4	1	10	15
Bidassoa		3	9	12		1	21	22
Canche	3	3	9	15	3	1	13	17
Charente	4	5	8	17	11		11	22
Gironde	14	41	23	78	2	37	34	73
Loire	7	10	11	28	22	10	13	45
Mont Saint Michel Bay	6		22	28	8		25	33
Orne	7	2	8	17	7	1	10	18
Seine	11	2	24	37	10	6	27	43
Seudre			17	17			23	23
Somme	2	6	18	26	1	4	22	27
Veys Bay	2	2	25	29		3	26	29

Table 2.

List of the ecological, trophic and vertical distribution guilds (adapted from Elliott and Dewailly (1995)).

Criteria	Guilds	Definition
Ecology	DIA: Diadromous migrant species	Species that use transitional waters to pass between salt and fresh waters for spawning and feeding
	ER: Estuarine resident species	Species that spend their entire life in the transitional waters
	FW: Freshwater species	Freshwater species that occasionally come into transitional waters but not dependant of these systems
	MA: Marine adventitious visitors	Species that appear irregularly in the transitional waters but have no apparent transitional water requirements
	MJ: Marine juvenile migrant species	Species that use the transitional waters primarily as a nursery ground
	MS: Marine seasonal migrants species	Species that have regular seasonal visits to the transitional waters, usually as adults
Trophic	V: Herbivorous	Species that feed by grazing on macroalgae or other macrophytes
	Z: Zooplankton predators	Species that feed exclusively or mainly on zooplankton
	IB: Benthic invertebrate predators	Species that feed exclusively or mainly on benthic invertebrate
	IS: Suprabenthic invertebrate	Species that feed exclusively or mainly on suprabenthic invertebrate
	F: Fish feeders	Species that feed exclusively or mainly on fish
	O: Omnivorous	Species that feed on all the available resources that can be consumed
Vertical distribution	P: Pelagic species	Species that live in the main water column
	D: Demersal fishes	Species that live in the water layer just above the bottom
	B: Benthic species	Species that live on or in the substratum

Table 3.

Candidate metrics and their expected response with increasing degradations defined according to expert judgment. (-) Decreasing.

Candidate metrics	Abbreviations	Expected response face to increasing degradation
<i>Diversity and abundance descriptors</i>		
1. Species richness	SR	(-)
2. Total density	TD	(-)
<i>Functionality descriptors</i>		
<u>Ecological guilds</u>		
3. Number of diadromous migrant species	NSDIA	(-)
4. Density of diadromous migrant species	DDIA	(-)
5. Number of freshwater species	NSFW	(-)
6. Density of freshwater species	DFW	(-)
7. Number of marine juvenile migrant species	NSMJ	(-)
8. Density of marine juvenile migrant species	DMJ	(-)
9. Number of marine seasonal migrant	NSMS	(-)
10. Density of marine seasonal migrant	DMS	(-)
<u>Trophic guilds</u>		
11. Number of benthic invertebrate feeder species	NSIB	(-)
12. Density of benthic invertebrate feeder species	DIB	(-)
13. Number of fish feeder species	NSF	(-)
14. Density of fish feeder species	DF	(-)
<u>Vertical distribution guilds</u>		
15. Number of benthic species	NSB	(-)
16. Density of benthic species	DB	(-)

Table 4.

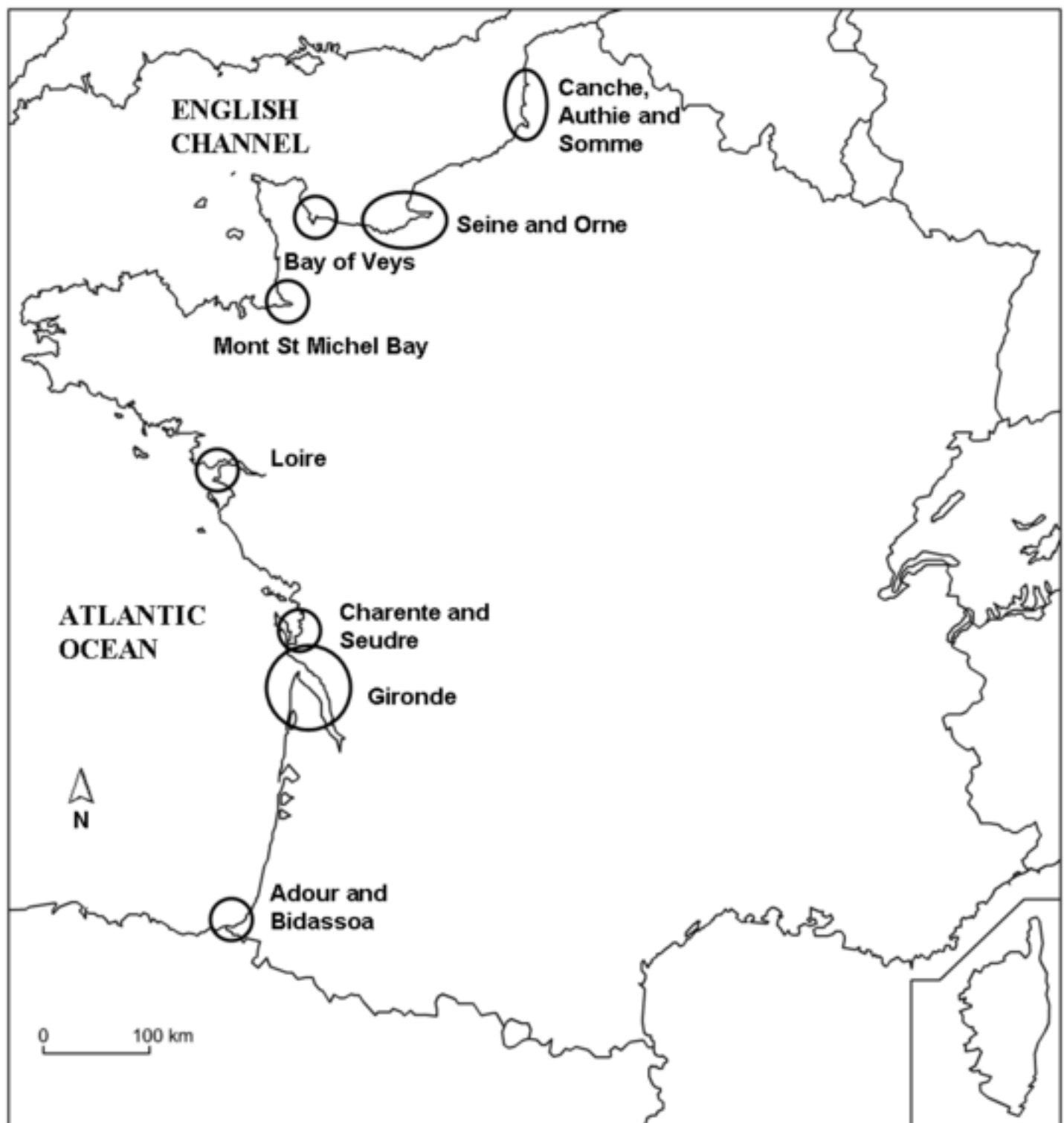
Summary of the models used for each candidate metrics. *, **, *** the factors or pressure effects are significant at respectively the 0.05, 0.01 and 0.001 levels. The metric significantly decreased (-), increased (+) or showed no significant trend (NS) with increasing pressure. (4.6): the percentage of the variability explained. Resid. df: Residual degree of freedom. Comp.: Comparison between expert judgements and models: =: the pressure effect is the same; ≠: is different. Corr.: Pairs of metrics (a1-a2) strongly correlated. Disc. thres.: the calculated thresholds were discriminant (Y) or not (N).

Candidate metrics	Model	Protocol and environmental descriptors				Pressure effect	Resid. df	Comp.	Corr.	Disc. thres.	Selected metrics
		Season	Salinity	Surface	Ecoregion						
<i>Diversity and abundance descriptors</i>											
SR	Poisson		*** (4.6)	*** (12.5)	*** (5.6)	-*** (2.5)	728	=		N	
TD	log-normal			*** (9.0)	*** (9.3)	-*** (5.6)	688	=			
	Binomial			*** (3.9)	*** (4.9)	NS	730			Y	X
<i>Functionality descriptors</i>											
<u>Ecological guilds</u>											
NSDIA	Poisson	** (1.1)	*** (7.7)	*** (11.3)	*** (8.3)	-*** (2.7)	727	=		N	
DDIA	log-normal		*** (6.2)	*** (6.5)	*** (12.0)	-*** (9.8)	375	=			
	Binomial	*** (1.5)	*** (6.4)	*** (7.1)	*** (8.9)	NS	727			Y	X
NSMJ	Poisson		*** (13.9)	*** (5.0)	*** (8.2)	-*** (3.9)	728	=		N	
DMJ	log-normal	* (0.8)		*** (6.3)	*** (4.2)	-*** (7.1)	436	=			
	Binomial		*** (9.6)	*** (1.5)	*** (5.3)	-*** (3.7)	728			Y	X
NSMS	Poisson		*** (10.2)	*** (5.6)	*** (2.7)	NS	728				
DMS	log-normal	*** (2.4)	* (1.2)	*** (36.1)		+*** (1.5)	311	≠			
	Binomial	** (1)	*** (9.3)	*** (6.2)	* (0.5)	NS	727				
<u>Trophic guilds</u>											
NSIB	Poisson		* (0.9)	** (1.0)	*** (6.7)	-*** (2.1)	728	=	a1		
DIB	log-normal		*** (2.0)	*** (12.4)	*** (6.7)	-*** (6.1)	625	=	b1		
	Binomial	* (0.7)			* (0.8)	NS	730				
<u>Vertical distribution guilds</u>											
NSB	Poisson		*** (5.3)	*** (2.4)	*** (4.3)	-*** (1.6)	728	=	a2	N	
DB	log-normal		*** (2.2)	*** (12.1)	*** (6.7)	-*** (6.1)	621	=	b2	Y	
	Binomial		* (1.3)		* (1.2)	NS	729			X	

Figure1

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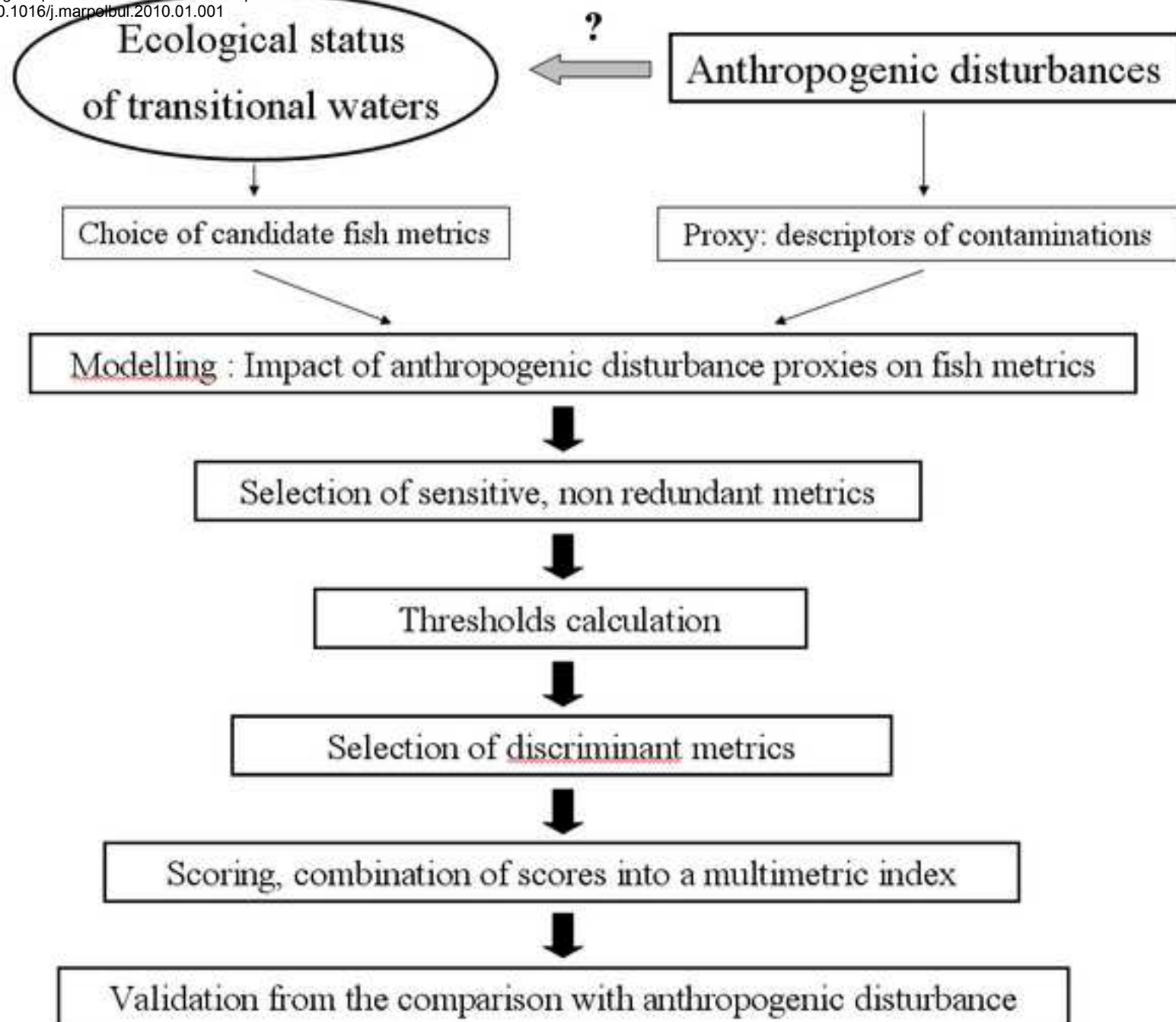


Figure3
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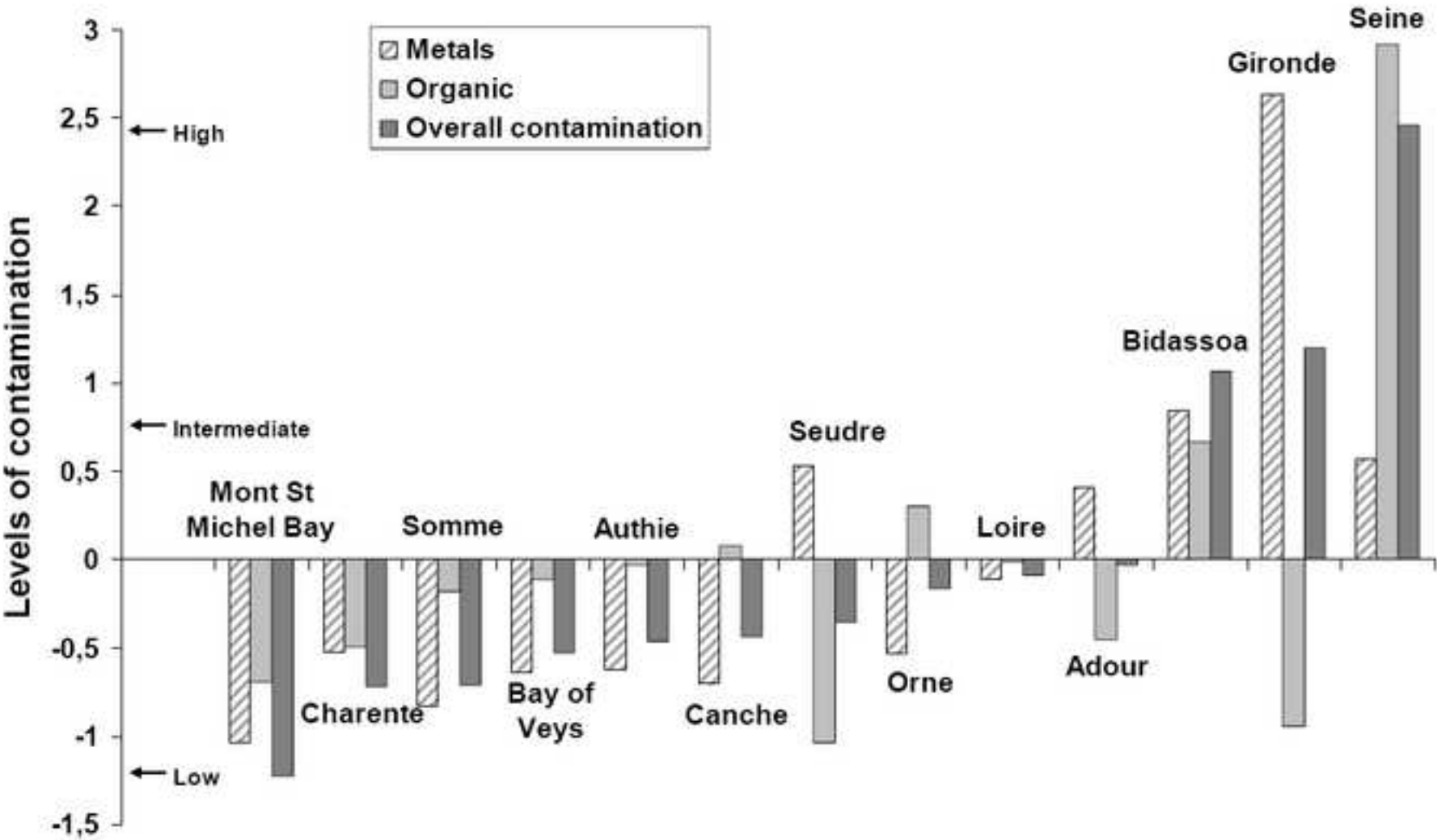


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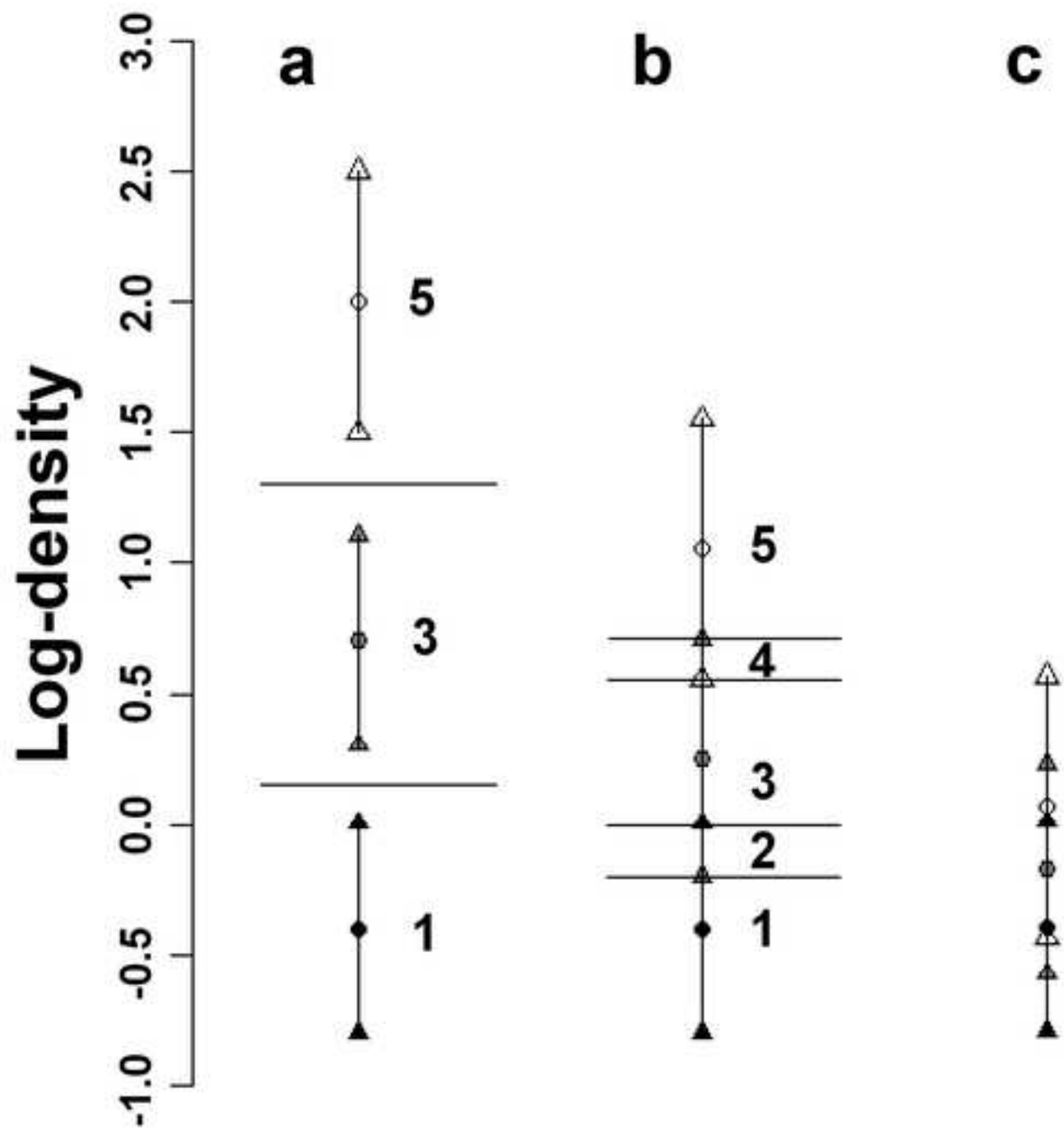


Figure5
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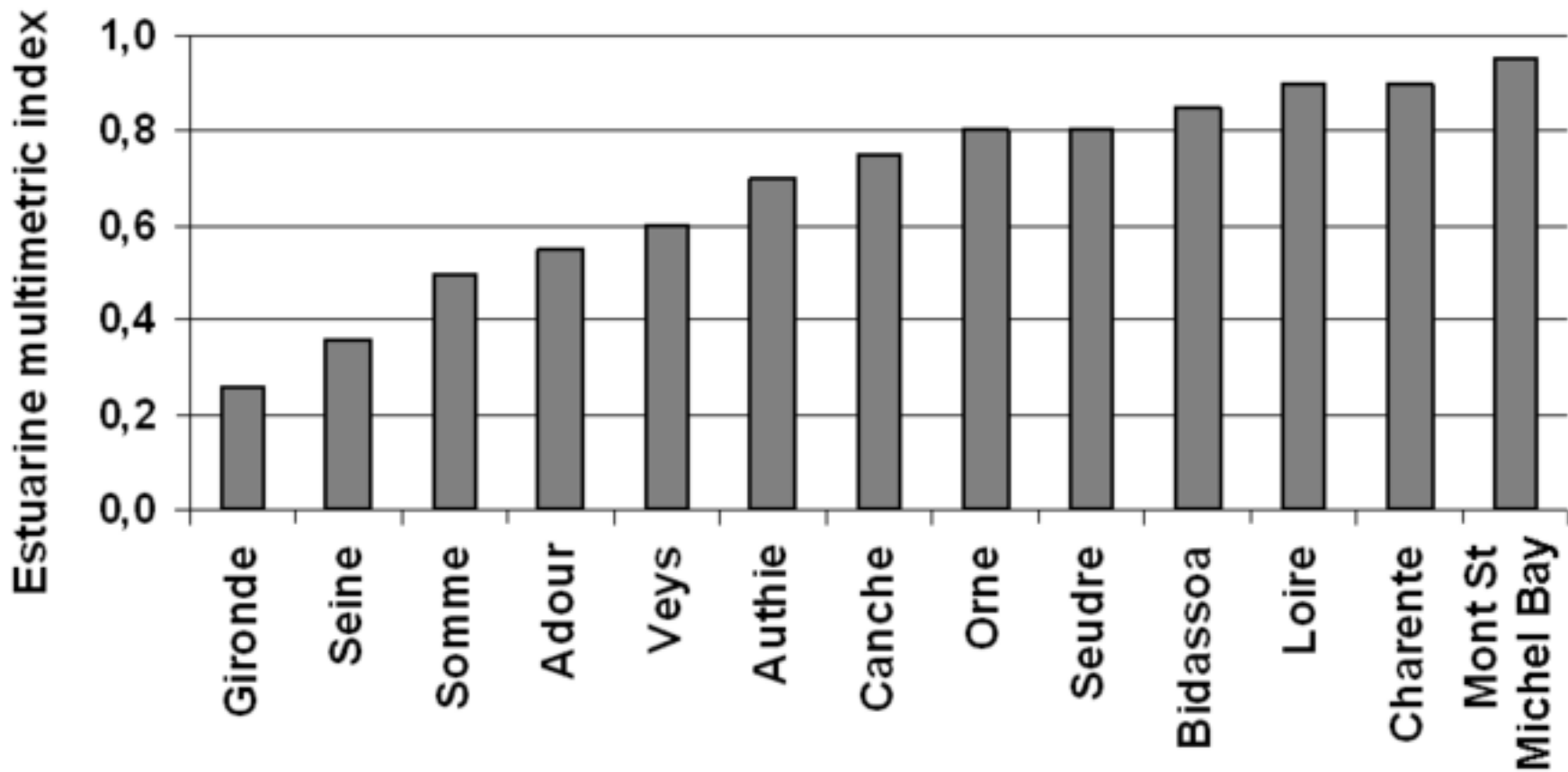


Figure6
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