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

C. Delpech, A. Courrat, Stéphanie Pasquaud, Jérémy Lobry, Olivier Le Pape, D. Nicolas, Philippe Boët, Michel Girardin, Mario Lepage

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17 **Abstract**

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

30

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

33 **1. Introduction**

34

35 Transitional waters¹ are highly variable environments. They are influenced by fluctuating
36 marine and freshwater flows, which lead to large and quick variations of their physico-
37 chemical features. By providing many species with basic requirements for their life cycle,
38 these particular areas support a wide range of fauna, from resident to freshwater and marine
39 species. Estuaries are especially essential to fish due to their ecological functions related to
40 refuge area, habitats for reproduction, permanent habitat, nursery grounds and migration
41 routes (Elliott et al., 2007). However, they are impacted by multiple anthropogenic
42 disturbances such as fishing, dredging, pumping and pollution (Blaber et al., 2000; Cloern,
43 2001; Dauvin, 2008).

44 In this context, the assessment of the ecological status of transitional waters is required,
45 especially in the European Union (EU Water Framework Directive; WFD - Directive
46 2000/60/EC; Anonymous, 2000). Fish communities appear to be a relevant biological element
47 to evaluate aquatic ecosystems' health (Karr, 1981; Karr et al., 1986; Elliott et al., 1988;
48 Fausch et al., 1990; USEPA, 2000; Whitfield and Elliott, 2002; Harrison and Whitfield,
49 2004). Several types of indices exist: some are based on only one criterion as the Community
50 Degradation Index (CDI; Ramm, 1988) and the Biological Health Index (BHI; Cooper et al.,
51 1994), while others consist of a combination of several metrics, *i.e.* the Indices of Biotic
52 Integrity (IBI; Karr, 1981; Roset et al., 2007). A metric is defined as “a measurable factor that
53 represents various aspect of biological assemblage, structure, function, or other community
54 component” (USEPA, 2000; Coates et al., 2007). Thus, multimetric indices are expected to
55 provide information about various aspects of fish assemblages and lead to a more holistic,
56 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)

57

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

65

66 **2. Materials and methods**

67

68 *2.1. Fish data*

69

70 Thirteen estuaries along the French Atlantic and English Channel coasts were sampled in
71 2005 and 2006 (Fig. 1) following a standardised sampling protocol (Lepage and Girardin,
72 2006). The fishing events occurred in Spring and Autumn. Two types of beam trawls were
73 used according to the size of estuaries. One had an opening of 3 x 0.5 m and a 20 mm
74 stretched mesh in the codend and was used in large estuaries only; the other had an opening of
75 1.5 x 0.5 m and 16 mm stretched mesh in the codend and was preferred in small estuaries.
76 Trawling was performed during daytime, against the current, for about 15 min at a speed
77 ranging from 1.5 to 3 knots. The number of trawl hauls ranged from 12 to 78 per estuary and
78 per season (Table 1). Hauls were distributed, as far as possible, all along the salinity gradient
79 inside the estuaries but obvious sewage points were avoided when known. All fishes caught
80 were identified, measured and counted. After each fishing event, salinity was recorded. This
81 dataset included 734 trawl hauls.

82

83 2.2. General methodology

84

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

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

110

111 2.3. *Candidate metrics*

112

113 Candidate fish metrics were selected to characterize both fish diversity and the various
114 ecological functions associated with transitional waters. The global diversity, described by the
115 taxonomic richness, *i.e.* the number of species caught, and the total density were considered.
116 A review of existing studies (Claridge et al., 1986; Elliott et al., 1990; Pomfret et al., 1991;
117 Elliott and Dewailly, 1995; Deegan et al., 1997; Marshall and Elliott, 1998; Cabral et al.,
118 2001; Thiel and Potter, 2001; Lobry et al., 2003; Thiel et al., 2003; Breine et al., 2004; Coates
119 et al., 2004; Harrison and Whitfield, 2004; Maes et al., 2005; Breine et al., 2007; Elliott and
120 Quintino, 2007) led us to focus on a guild approach to characterize the functional aspects of
121 transitional waters for fish. Root (1967) defined a guild as a group of species that exploit the
122 same class of environmental resources in a similar way. We used an extended definition to
123 include a description of the way species use transitional waters, as well as their feeding mode
124 and their vertical distribution in the water column (Elliott and Dewailly, 1995; Lobry et al.,
125 2003; Franco et al., 2006; Elliott et al., 2007; Table 2). All fish species present in the dataset
126 were classified for each type of guild. Number of species and density were selected as
127 potential metrics and calculated for each guild. In the aim of assessing estuaries' ecological
128 status, the retained metrics were supposed to have significant trends with increasing
129 degradation (Breine et al., 2004; Breine et al., 2007). Thus, for each metric, the expected
130 response with increasing degradations was defined according to literature and expert's

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

133

134 *2.4. Modelling for the fish metrics*

135

136 All metrics were modelled at the trawl haul scale (one trawl haul = one line in the dataset) to
137 take into account the metrics' variability due to sampling protocol (Courrat et al., 2009).

138 Each fish metric was modelled with Generalized Linear Models (GLMs) using R software (R
139 Development Core Team, 2005).

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

$$142 \text{Log}(\text{SR}) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

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

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

$$149 \text{Logit}(D_{0/1}) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

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

$$152 \text{Log}(D_+) \approx \text{constant} + \text{variable}_1 + \text{variable}_2 + \dots + \text{variable}_n$$

153

154 *2.5. Variables included in the models for the description of the sampling protocol and some*
155 *estuarine features*

156

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

164

165 *2.5.1. Variables for the description of the effect of the sampling protocol on fish metrics*

166 Fish assemblages in transitional waters change according to seasons (Elliott et al., 1990;
167 Araùjo et al., 1998; Thiel et al., 2003; Koutrakis et al., 2005; Franco et al., 2006; Lobry et al.,
168 2006), hence the effect of sampling season (spring or autumn) was tested as a class factor.
169 Transitional waters are characterized by a strong salinity gradient, known to have a significant
170 effect on fish assemblages (Thiel et al., 1995; Marshall and Elliott, 1998; Lobry et al., 2006;
171 Franco et al., 2008b). Salinity was tested within models as a class factor. Salinity class
172 boundaries were adapted from the Venice system (1958): oligohaline class ([0-5]),
173 mesohaline class ([5-18]) and polyhaline class (>18).

174

175 *2.5.2. Variable for the description of the effect of environmental and hydromorphologic*
176 *features at a larger scale on fish metrics*

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

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

191

192 2.6. *Indices of anthropogenic pressures*

193

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

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

212

213 *2.7. Statistical analyses*

214

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

217 $Metric \approx factor(season) + factor(salinity) + factor(size) + factor(ecoregion) +$
218 $covariate(contamination\ index)$

219

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

227

228 *2.8. Metric selection*

229

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

237

238 *2.9. Test of discriminance in response to pressure and thresholds calculation*

239

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

248 To define confidence intervals, we simulated 5000 virtual observations for the different
249 models (glm Poisson) and submodels (glm Binomial and glm Gaussian), with means equal to
250 the corresponding predicted values and standard deviation equal to corresponding predicted
251 standard errors. For metrics of densities, the 5000 simulated probabilities of presence and the
252 5000 simulated log-densities were then multiplied to produce 5000 simulated densities.
253 Finally, for each metric, the 10% and 90% quantiles of the 5000 simulated densities or
254 number of species were used as confidence intervals.

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

265

266 *2.10. Scoring*

267

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

279 A score equal to (Fig. 4):

280 - 1 was attributed if the metric value was situated below the prediction realized for the
281 maximum of pressure *i.e.* the worst class of quality,
282 - 3 was attributed if the metric value was situated between the predictions for the 2 levels of
283 pressure *i.e.* the class of moderate quality,
284 - 5 was attributed if the metric value was situated above the prediction for the minimum of
285 pressure *i.e.* the best class of quality.
286 When the 10% and 90% quantiles of two consecutive levels of pressure overlapped, a score of
287 2 or 4 was attributed for the metric values situated into the uncertainty area (Fig. 4b).
288 The scores obtained for each season and each salinity class were added and divided by the
289 maximal potential score (5 x number of summed scores) to get one value for each metric and
290 each estuary. These values per metric were then averaged to get the final value of the
291 multimetric index for each estuary.
292 Finally, a linear regression was performed to test the relevance of the final multimetric fish
293 index regarding the increasing index of contamination.

294

295 **3. Results**

296

297 *3.1. Metric selection*

298

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

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

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

311

312 *3.2. Discriminance of metrics with regard to different levels of anthropogenic disturbances*

313

314 The 10% and 90% quantiles of two consecutive levels of pressure overlapped for all the
315 metrics expressed in number of species (Table 4). Thus, these metrics were considered as
316 irrelevant for assessing the ecological status of French estuaries with the present approach.

317 The quantiles also overlapped for the densities of diadromous and marine juveniles in the
318 small Atlantic estuaries. Nevertheless, these metrics were maintained into the multimetric
319 index, as the uncertainty area was limited to specific conditions.

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

325

326 *3.3. Multimetric index values*

327

328 According to comparisons between in situ data and thresholds (Fig. 4), scores were attributed
329 for each fish metric in each combination of factor describing sampling protocol and estuarine
330 features; then these scores were combined in a multimetric index value for each estuary. For
331 the 13 Atlantic and English Channel estuaries, the multimetric fish index values ranged from
332 0.26 in the Gironde estuary to 0.95 in the Mont St Michel Bay (Fig. 5). A linear regression
333 showed a significant negative relationship ($r^2=0.35$, $p.value<0.05$) between this estuarine
334 multimetric fish index and the index of contamination (Fig. 6).

335

336 **4. Discussion**

337

338 This paper presents the development of a fish-based index for defining the ecological status of
339 transitional waters using a pressure/impact approach. The methodology is in agreement with
340 the processes recommended by Hering et al. (2006) and Stoddard et al. (2008) for the creation
341 of multimetric indices. Despite the ‘estuarine quality paradox’, *i.e.* the fact that features of
342 anthropogenic stress coincide with those of natural stress in estuaries (Elliott and Quintino,
343 2007), we showed significant relationships between anthropogenic disturbances and various
344 aspects of fish assemblages. Thresholds were identified for metrics presenting significant
345 trends with increasing pressure and a system of scoring was elaborated to qualify estuaries’
346 quality by combining the selected fish metrics.

347

348 *4.1. Pressure index*

349

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

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

364

365 *4.2. A general method to test the effect of anthropogenic pressure with regard to other*
366 *sources of variability*

367

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

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

386

387 A large part of the metric variability was not explained by the models used in this study (from
388 65 to 82%). The addition of more environmental variables likely to influence estuarine fish
389 assemblages can improve the interpretation of the metric variability. Especially, further
390 descriptors of habitat richness and diversity, in addition to the single description of estuarine
391 size, could improve models accuracy. However, this would complicate the calculation of
392 thresholds and the scoring process by increasing the number of combinations of factors
393 describing estuarine features to account for in the statistical predictions. Furthermore, the
394 WFD requires a limited number of thresholds. An exhaustive list of all the environmental
395 factors affecting fishes can not be included in models. The present approach was a balance
396 between available exhaustive descriptors, models parsimony and limited complexity in
397 threshold calculation. In spite of a large residual variability, this approach provides a simple
398 and robust method to estimate fish metric levels with regard to estuaries' quality.

399

400 *4.3. Selected metrics, ecosystem functioning and ecological status*

401

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

412

413 *Metrics of species richness*

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

419

420 *Metrics of fish density*

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

426 Several metrics related to density per guilds were also retained:

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

432 - density of marine juvenile migrants. Many studies have demonstrated the negative effect of
433 anthropogenic degradations on marine juveniles and the nursery function of the estuaries
434 (Gibson, 1994; Deegan et al., 1997; Gilliers et al., 2006; Breine et al., 2007; Le Pape et al.,
435 2007) and Courrat et al. (2009) focused on this guild with the same data to develop the
436 present approach.

437 - density of benthic and benthivorous fish. Benthic species are particularly sensitive to
438 siltation and oxygen deficiency (Oberdorff and Porcher, 1994; Kestemont et al., 2000) and
439 are considered as early-warning indicators of anthropogenic disturbance (Hughes et al., 1998).
440 This metric appeared particularly interesting to assess the degree of perturbation of benthic
441 habitats and has already been used in other multimetric index (Borja et al., 2004; Breine et al.,
442 2004; Coates et al., 2004; Harrison and Whitfield, 2004; Uriarte and Borja, 2009). Benthic
443 feeders were shown to respond negatively to anthropogenic pressure but their redundancy
444 with benthic species led to the removal of this metric from the final index. Even if trophic
445 guild metrics should be interesting tools to characterize ecological function of estuaries
446 (Elliott and Dewailly, 1995) as they provide an insight into the community structure (Hughes
447 et al., 1982), none was retained in the final index.

448

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

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

458

459 *4.4. The multimetric fish index*

460

461 *4.4.1. Thresholds*

462 Two thresholds, separating 3 classes, have been calculated from 3 levels of anthropogenic
463 disturbance. The lower and the upper levels are associated with the best and the worst status
464 observed among the sampled sites. This could correspond to good and poor status referring to
465 the WFD terminology. No site corresponds to pristine site (high status) or to heavily disturbed
466 site (bad status). Extrapolations from model's predictions below or up to the observed level of
467 contamination could allow to assess metric values in reference situation, as it has already been
468 realized with the method of quintiles or the Maximum Value Lines (Oberdorff and Hughes,
469 1992; Oberdorff et al., 2001; Harrison and Whitfield, 2004; Breine et al., 2007; Coates et al.,
470 2007).

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

477 in each guild is low. This is called by Hering et al. (2006) “the numerically unsuitable
478 metrics”. Further approaches would have to be tested in the future to include species richness
479 in the indicators (Nicolas et al., In press).
480 Nevertheless, our approach reached reliable estimates and was able to distinguish different
481 levels of disturbances (Quataert et al., 2007). This method implies a large enough sampling
482 survey, well distributed on the whole transitional area. Such standardization is essential to
483 provide consistency in the assessment based on fish indices (Roset et al., 2007).

484

485 *4.4.2. Combining metrics in a composite index*

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

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

497 - Contamination data for the sampled sites were chosen as descriptors of the anthropogenic
498 pressures as they were available everywhere, directly correlated to the level of human
499 pressure and appeared as good proxies for it. However, other human disturbances (habitat
500 loss, anoxia, etc.; Peterson, 2003) are not taken into account with such proxy and the single
501 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

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

539

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547

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757 **Figure captions**

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

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

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

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

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

774 Fig. 6. Relationship between the multimetric fish index and the index of contamination for the
775 13 studied estuaries.

776

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	=		Y	X
	Binomial			*** (3.9)	*** (4.9)	NS	730				
<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	=		Y	X
	Binomial	*** (1.5)	*** (6.4)	*** (7.1)	*** (8.9)	NS	727				
NSMJ	Poisson		*** (13.9)	*** (5.0)	*** (8.2)	-*** (3.9)	728	=		N	
DMJ	log-normal	* (0.8)		*** (6.3)	*** (4.2)	-*** (7.1)	436	=		Y	X
	Binomial		*** (9.6)	*** (1.5)	*** (5.3)	-*** (3.7)	728				
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	X
	Binomial		* (1.3)		* (1.2)	NS	729				

Figure1

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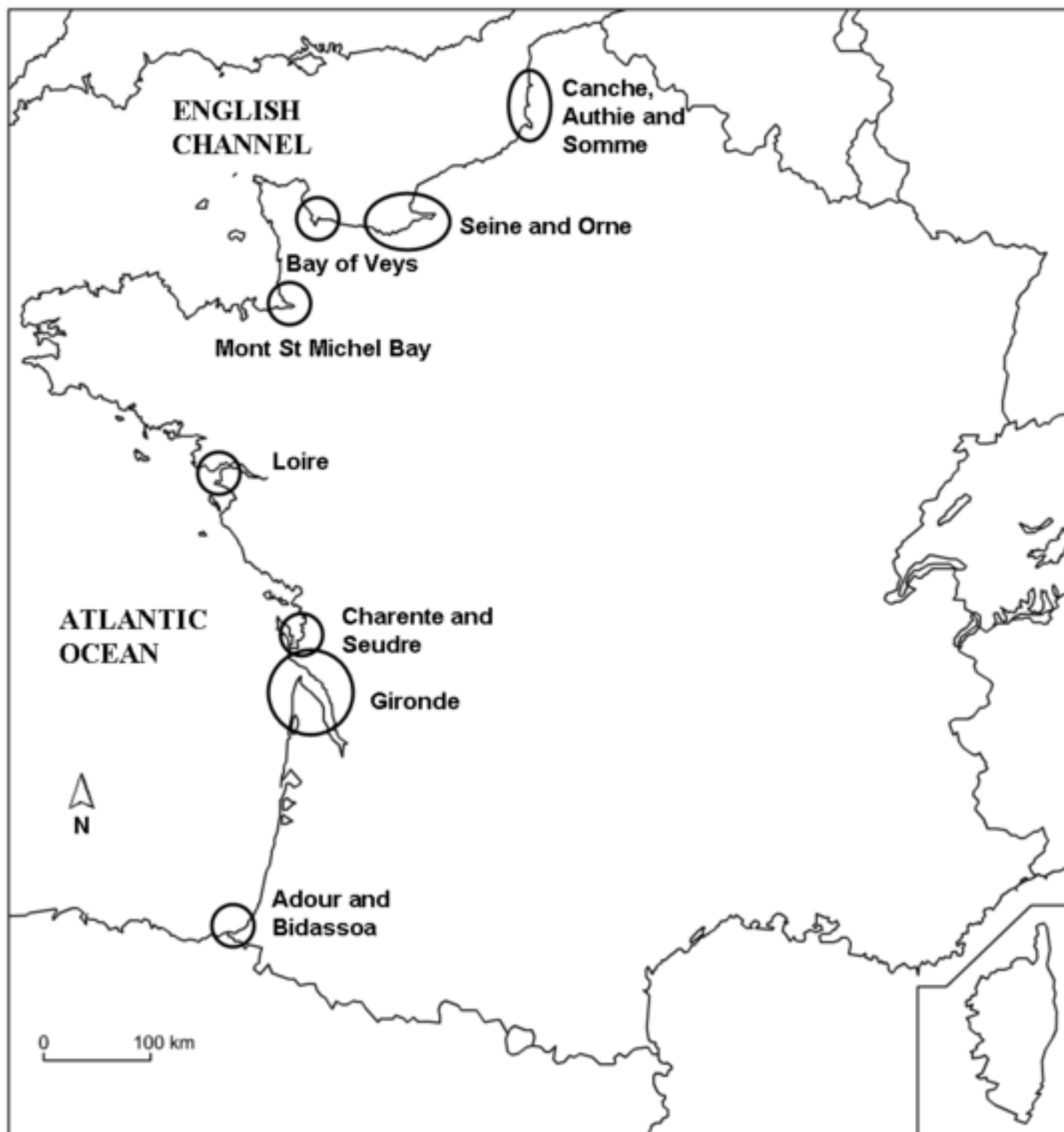


Figure2

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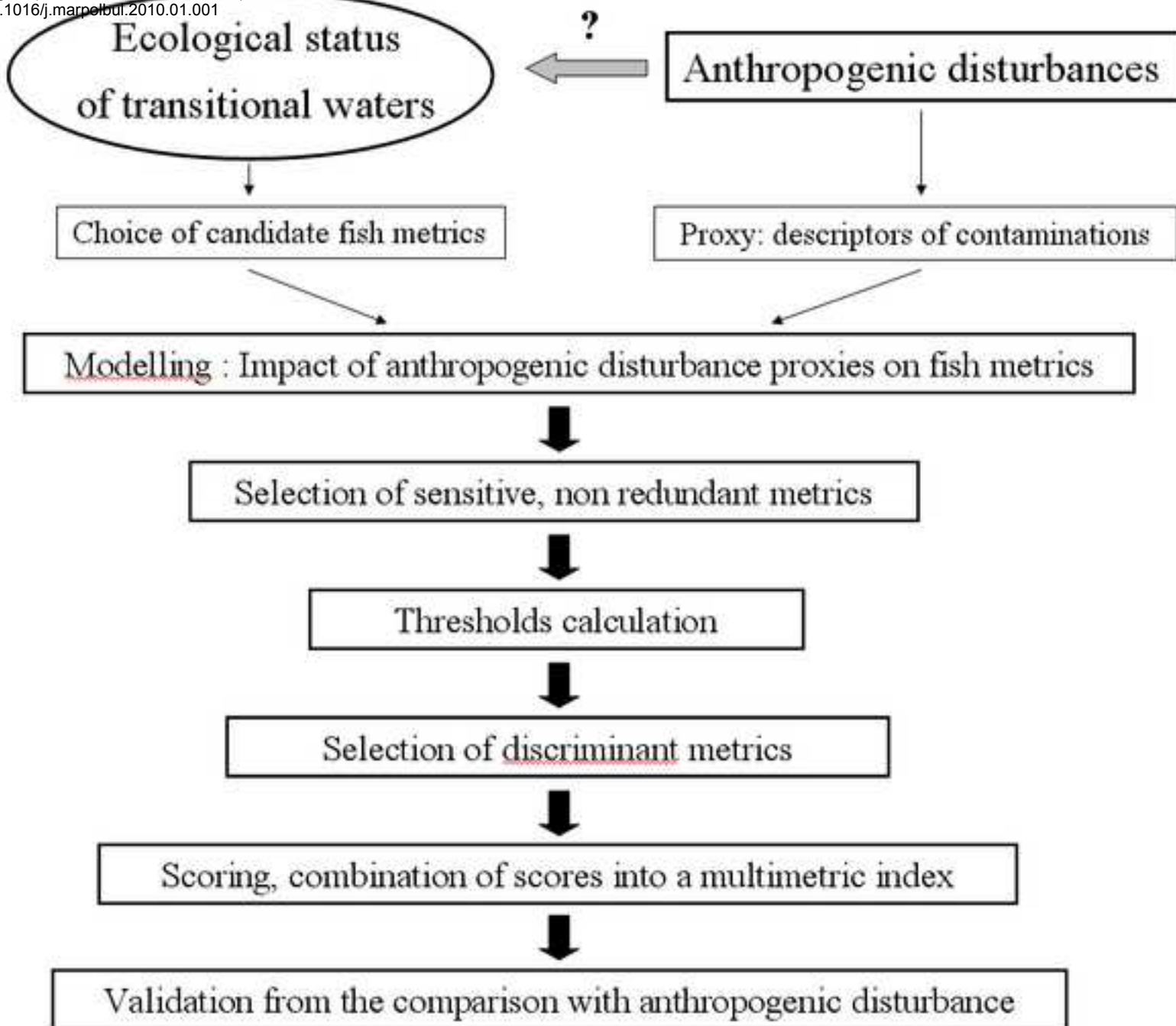


Figure3

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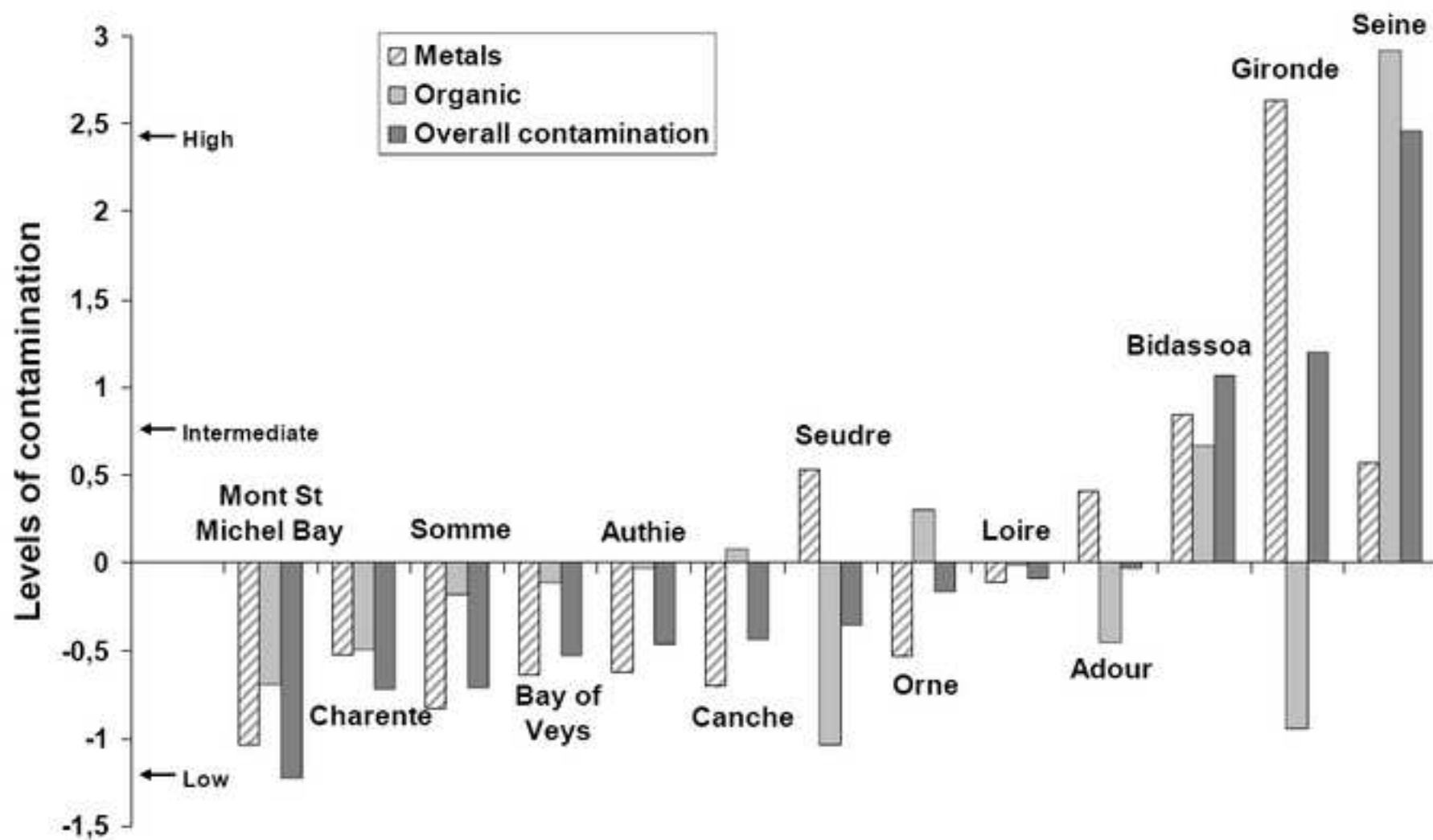


Figure4

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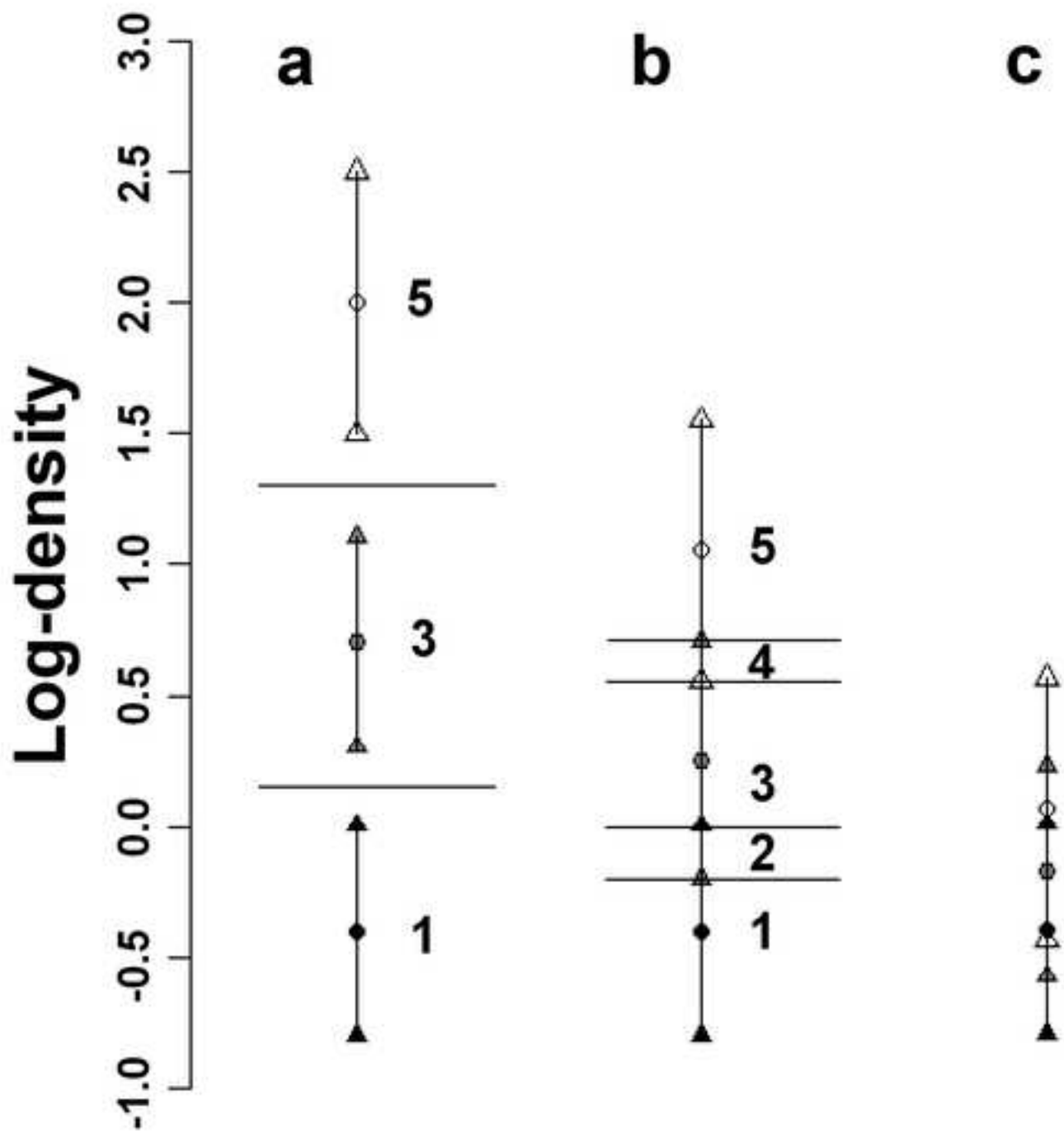


Figure5

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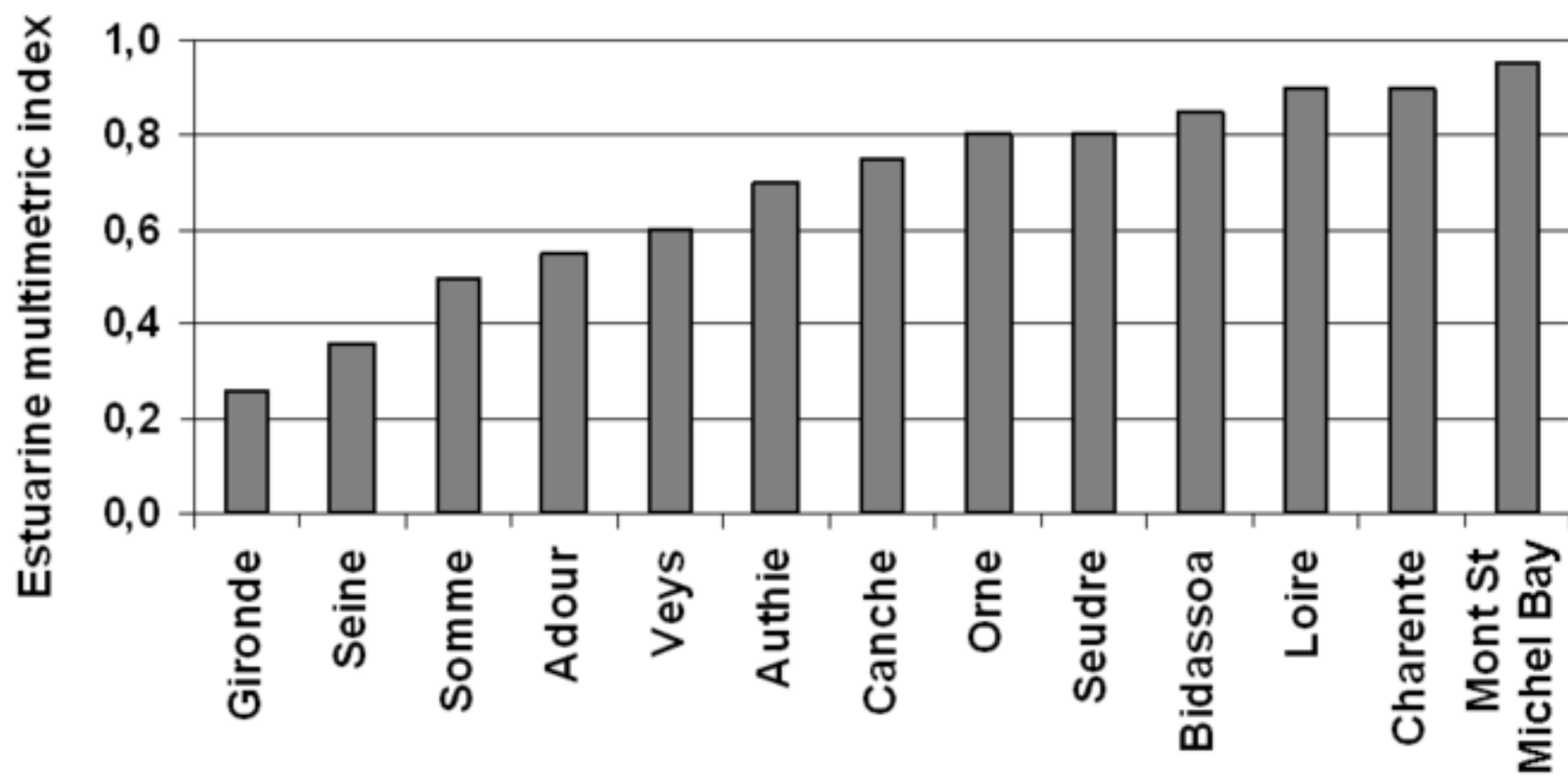


Figure6

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