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Assessing fish quality status in transitional waters, within the European Water Framework Directive: Setting boundary classes and responding to anthropogenic pressures

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ABSTRACT

Validation of the AZTI's Fish Index (AFI), proposed for the Basque Country (northern Spain), in assessing fish quality within the Water Framework Directive (WFD), is undertaken. The response to anthropogenic pressure is investigated, in setting the boundaries between the different quality status classes. Hence, 12 estuaries were sampled, at different frequencies, between 1989 and 2007, by means of a beam trawl. Significant ($p < 0.0001$) correlations were found between the AFI and oxygen saturation and ammonia. Oxygen quality standards are used to set boundaries between quality classes. Then, the AFIs obtained are compared with different anthropogenic pressures, including urban and industrial discharges, engineering works and dredging. The effects of the removal of some of these pressures are also studied. The total number of pressures within an estuary shows significant ($p < 0.009$) negative correlation with AFI, explaining between 51 and 62% of the variability in fish quality. The impact of pressures upon fish and demersal assemblages is detected as required by the WFD. Nonetheless, further investigation and intercalibration of the methods used, are necessary.

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1. Introduction

The European Water Framework Directive (WFD; Directive 2000/60/EC) states the need to achieve 'a good ecological status', by 2015, for all European water bodies, including transitional (estuaries) and coastal waters (for details, see Borja et al., 2004; Borja, 2005). Biological elements are especially important, in assessing such a status, e.g. phytoplankton, macroalgae, angiosperms, benthos and fish. A similar approach has been adopted by the new European Marine Strategy Directive (MSD; Directive 2008/56/EC), in assessing the environmental status within offshore waters (Borja, 2006), together with other legislation world-wide (Borja et al., 2008).

In the particular case of fish, the WFD specifies that they must be assessed in freshwaters and transitional waters (and not in coastal waters), taking into account species composition, abundance and the proportion of disturbance-sensitive species. In fact, the trends in one or more of the community attributes (such as composition, trophic structure, diversity, abundance or biomass) can be used to monitor the ecological functioning, and health, of an estuarine ecosystem (Moore et al., 1995; Whitfield and Elliott, 2002).

As stated by Coates et al. (2007), most of the methods used to assess the ecological status, based upon fish, are derived from the metric-scoring system used in assessing the 'biotic integrity' of North American fish communities (Karr, 1981), i.e. the 'index of biotic integrity' (IBI). Derivations from this method have been used as a classification tool for fish quality assessment, world-wide (Deegan et al., 1997; Harrison et al., 2000; Gibson et al., 2000; Hughes et al., 2002; Whitfield and Elliott, 2002; Harrison and Whitfield, 2004, 2006); in recent times, it has served as basis for several methodologies applied under the WFD (Borja et al., 2004, 2009a; Breine et al., 2004, 2007; Coates et al., 2007), being some of them compared in Martinho et al. (2008). Recently, some of these methods have been applied to coastal waters under the MSD (Henriques et al., 2008).

According to the WFD, biological element methodologies used to assess ecological status should respond to anthropogenic pressures, rather than to natural variability (Solimini et al., 2006). However, very few studies have focused upon the response of these fish assessment methods to human pressures (Harrison et al., 2000; Cabral et al., 2001; Breine et al., 2007; Vasconcelos et al., 2007). Hence, there is a need to validate the proposed fish methodologies, against transitional water pressures, as has been undertaken for benthos (Borja et al., 2009b).

The WFD states that any sign of distortion, from type-specific conditions in the species composition and abundance of fish,

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together with the abundance of the disturbance-sensitive species, must be attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements. This distortion is calculated by means of the Ecological Quality Ratio (EQR), which represents the differences between monitored data and reference conditions (see Borja et al., 2004). The EQR, which ranges between 0 and 1, is divided into five quality classes (i.e. bad, near to 0; poor; moderate; good; and high, near to 1, status), according to the normative definitions within the WFD. The validation of the methodologies used in the assessment requires also the determination of boundaries between such quality classes.

Hence, the aim of this contribution is to validate the methodology proposed by Borja et al. (2004), in assessing fish quality within the WFD (named AZTI's Fish Index (AFI)), by studying the response to anthropogenic pressures; likewise, setting boundaries between the different quality status classes.

2. Methods

2.1. Sampling

A network of monitoring trawl lines along the 12 main Basque estuaries, from the inner, middle and outer reaches (three to five trawl lines, per estuary), was established by the Basque Government; this network provides water, sediment and biological quality information from a total of 39 sampling locations (Fig. 1). The demersal assemblage sampling was carried out every September–October, at high tide, between 2002 and 2007, once every 3 years at each of the estuaries. Moreover, some parts of the estuaries were sampled on the basis of long-term annual time-series: Barbadún (three trawl lines) and Nervión (five trawl lines), since 1989–1990; and Butroe (three trawl lines), since 1997; whilst others were sampled, discontinuously, since 1995 (Table 1). Locations were determined by the suitability of the sea-bed for trawling, as well as by the requirement to incorporate the whole of the salinity range within each of the estuaries.

At each of these trawl lines, three hauls (replicates) were collected, using a 1.5 m wide beam trawl with a tickler chain; the first part of the net has 10 mm mesh size and 8 mm mesh size cod end; and towed for 10 min at ~1.5 knots (sometimes the trawl period might differ, when rocks or other obstacles made the trawling difficult). Finally, fish and crustacean density were calculated taking into account then fishing effort calculated from the beam width, the time of trawling and the boat speed. Similar methodologies have been used by other authors, such as Elliott and Hemingway (2002), Johnson et al. (2008) and Selleslagh and Amara (2008). Samples were identified and counted on-board immediately. Species which could not be identified were fixed in a solution of 4% formalin, then examined in the laboratory.

The Basque estuaries can be divided into 14 water bodies (although, for this contribution, only 13 were considered; this was because the Oka estuary was considered as a single water body, instead of two bodies). These water bodies are distributed among three transitional types (see 'delimitation criteria', in Borja et al., 2004): (1) Type I – small river-dominated estuaries; (2) Type II – estuaries with extensive intertidal flats; and (3) Type III – estuaries with extensive subtidal areas (Table 1).

2.2. Pressures and environmental data

The estuaries and coasts of the Basque Country were investigated, to identify relevant and significant human pressures (Borja et al., 2006b). This information, together with the new information obtained after that study, is summarised in Tables 2 and 3. An overall pressure index was calculated for each estuary (see Table 2), using data from significant pressures listed within Table 8 in Borja et al. (2006b). Hence, a relative rating (3, 2, 1, and 0, respectively) has been allocated to each of the pressure levels described there (high, moderate, low, and without pressure, respectively). Subsequently, a 'mean overall pressure index' was calculated, for each of the estuaries. The lowest pressure indices were those of the Lea and Barbadún estuaries; the highest were those of the Nervión and Oiartzun (Table 2).

The main significant pressures identified for the Basque Country include urban and industrial discharges (affecting organic matter increase and oxygen consumption), and hydromorphological pressures (dykes and port construction, dredging, and land reclamation). Conversely, positive actions include the removal of discharges and water treatment programmes (at catchment and estuary levels, including wastewater treatment plants) (Table 3).

Hypoxia and ammonia are considered as being harmful for estuarine fishes (Eby et al., 2005; Eddy, 2005). Hence, oxygen saturation and ammonia have been used as environmental variables, to determine their effects on fish quality assessment. Data used in this investigation are those obtained from the Nervión estuary, which has an extensive dataset since 1989; this includes low tide bottom oxygen saturation and ammonia (on the basis of eight to 12 annual surveys). Oxygen was measured using membrane polarographic probes, whilst ammonia concentrations were determined by segmented-flow analysis, with Technicon AAIII systems, following Hansen and Grashoff, 1983. Mean oxygen and ammonia values have been derived on the basis of a 12 month sampling period (up to 12 data, from October of 1 year, to September of the next year); these were used to establish the

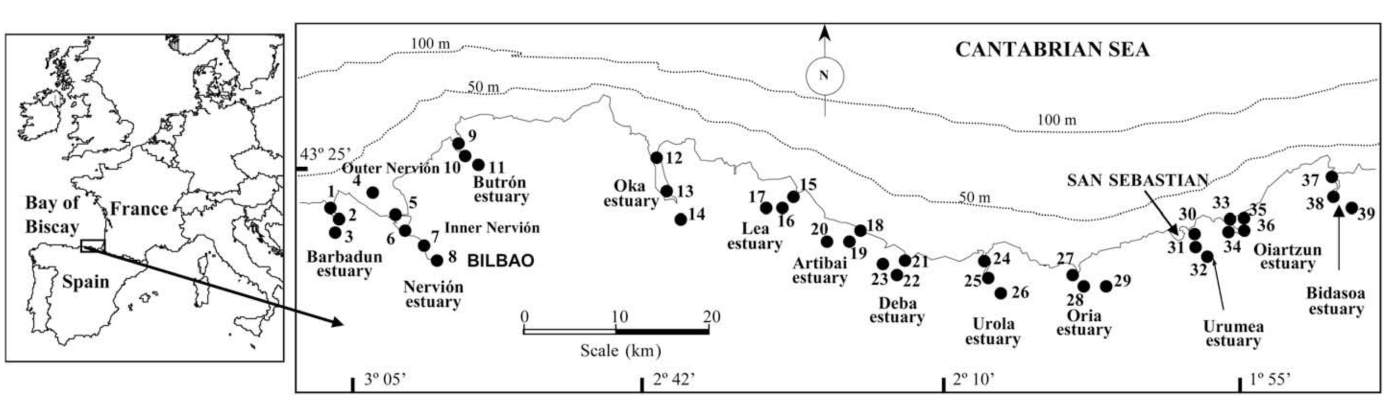

Fig. 1. Sampling locations (trawl lines) and water bodies within the estuaries of the Basque Country.

Table 1
Characteristics of each water body (typology and area), together with the names of the stations sampled, the percentage of the water body assigned to each station (in the same order), total number of samples available and the sampling period. Notes: (a) for station locations, see Fig. 1; and (b) the area of each water body has been obtained from Borja et al. (2006b).

Water body	Typology	Area (km^2)	Stations	Station area (%)	Samples (number of hauls)	Sampling period (years)
Barbadún	II	0.75	1, 2, 3	54, 40, 6	135	1990–2006
Outer Nervión	III	19.10	4, 5	80, 20	114	1989–2007
Inner Nervión	III	10.14	6, 7, 8	31, 31, 38	138	1989–2007
Butroe	II	1.60	9, 10, 11	68, 16, 16	66	1997–2005
Oka	II	10.28	12, 13, 14	33, 22, 45	18	2002, 2005
Lea	II	0.50	15, 16, 17	50, 40, 10	18	2002, 2005
Artibai	II	0.46	18, 19, 20	60, 25, 15	18	2002, 2005
Deba	I	0.74	21, 22, 23	20, 30, 50	27	1996, 2003, 2006
Urola	II	0.83	24, 25, 26	66, 22, 12	27	1996, 2004, 2007
Oria	II	2.36	27, 28, 29	37, 40, 23	27	1996, 2003, 2006
Urumea	I	1.40	30, 31, 32	55, 30, 15	27	1995, 2004, 2007
Oiartzun	III	1.00	33, 34, 35, 36	30, 20, 35, 15	45	1995, 2001, 2004, 2007
Bidasoa	III	6.83	37, 38, 39	45, 22, 33	54	1995, 2001, 2004, 2005, 2007

influence of oxygen on demersal assemblages, as sampled in October.

The fate and behaviour of dissolved oxygen is of critical importance to marine organisms, in determining the severity of adverse impacts; this is the reason for its importance as one of the physico-chemical elements supporting biological elements, within the WFD (Bald et al., 2005; Best et al., 2007). Taking into account the effects of dissolved oxygen on fish assemblages and health, in transitional waters (Maes et al., 2007), if a significant correlation is found between EQR and oxygen, then some oxygen saturation standards can be applied as the basis for the calculation of the EQR class boundaries. Hence, we propose here to use only generally accepted standards, as outlined below.

- 100% of oxygen saturation might be used as the threshold between high and good status.
- 80%, which corresponds to the quality standards for some uses of marine waters, such as shellfishing and aquaculture (79/923 Shellfish Waters Directive), might be used as the threshold, between good and moderate status. This boundary is the most important within the WFD, because if 'good status' is not achieved, some actions to remove the pressures are necessary.
- 60%, which corresponds to the minimum value to be reached at any time and anywhere in the Nervión estuary, as an objective of the management authority (Borja et al., 2006a; García-Barcina et al., 2006), might be used as the threshold, between moderate and poor status. In addition, values >60% are preferred for salmon migration, after Priede et al. (1988).
- 40%, which corresponds to a value below which the area could present some hypoxia, even anoxia, events might be used as the threshold, between poor and bad status. Hence, fish (i.e. salmon) movement is inhibited below this boundary and fish mortality is probable, after Priede et al. (1988).

2.3. Biological data

The method used in this contribution (AFI), developed by Borja et al. (2004) and slightly modified subsequently (Borja et al., 2009a), is based upon two different models: that of the U.K. (Whitfield and Elliott, 2002) and that of Belgium (Breine et al., 2004, 2007). One of the problems in adapting these models here is the small size of the Basque estuaries (Table 1), which contain only a small number of 'estuarine resident' fish species. Hence, Borja et al. (2004) proposed the incorporation of the crustaceans, as a characteristic demersal component of the estuaries, when assessing Types I and II; then using fishes alone, for Type III (the Nervión, Oiartzun and Bidasoa estuaries). The AFI, as described in Borja et al. (2004, 2009a) incorporates: (1) the richness (number of species); (2) indicator and introduced species (percentage of individuals); (3) fish health (percentage affected); (4) trophic composition (percentage of omnivorous and piscivorous); and (5) resident estuarine species (number and percentage of individuals) (see Table 4). Some of these metrics are being used elsewhere in determining fish assemblages (Elliott and Dewailly, 1995; Elliott et al., 2007; Franco et al., 2008).

Table 2
Main driving forces acting in the Basque Country, for each estuary, together with the total number of pressures, a global pressure index, and some significant pressures. Data updated from Borja et al. (2006b), for explanation on units, see that contribution.

	Driving forces				Pressures									
Estuary	Population	Industry	Ports	Agriculture	Total pressures	Pressure index	Nutrient discharge	Water pollution	Sediment pollution	Dredged sediments	Shoreline reinforcement		Intertidal losses	Berths
	(n° km^{-2})	(n°)	(n°)	(n°)	(n°)		(kg N d^{-1} km^{-2})	(%)	(%)	(10^4 $m^3 \cdot y^{-1}$)	Ports (%)	Other (%)	(%)	(n°)
Barbadún	320.7	407	0	396	52	0.9	2005	10	0.0	0	0.0	46.4	81	3
Nervión	3623.5	65,337	5	2264	499	2.8	904	27	82.8	32	90.7	2.1	30	1555
Butroe	207.5	728	1	890	78	1.1	1342	13	0.0	11.8	7.1	22.3	37	407
Oka	85.3	421	1	1000	137	1.2	210	12	0.0	3	1.9	51.4	30	356
Lea	175.2	577	1	444	45	0.8	2016	4	0.0	0	11.3	58.4	15	178
Artibai	305.8	1054	1	435	83	1.3	2788	8	34.1	10.5	19.1	32.2	40	202
Deba	111.4	931	1	507	198	1.8	9445	33	60.8	0.2	3.8	56.9	45	128
Urola	211.7	844	1	349	144	1.8	5427	23	52.9	6	10.0	36.4	57	638
Oria	89.8	1082	1	562	149	1.4	5331	29	17.6	4.5	12.3	40.7	59	168
Urumea	2957.7	1329	0	328	145	1.2	3075	28	46.1	0	0.0	43.8	88	5
Oiartzun	2151.3	24,164	3	606	144	2.9	1629	39	70.0	20.1	66.8	24.6	55	200
Bidasoa	1020.5	7013	5	707	270	1.9	1233	19	5.5	1.1	13.2	62.4	60	1682

Table 3
Main significant pressures (producing negative effect on fishes) and actions taken (positive effects on fishes) detected at each transitional water body, within the Basque Country. The year(s) of the pressure or action are shown in brackets. Data updated from Borja et al. (2006b). Note: for estuary locations, see Fig. 1.

Water Body	Pressures	Actions
Barbadún	Oil refinery, urban discharge	Oil refinery effluent deviation (1999)
Inner Nervión	Changes in morphology, pollutants	Water Treatment Plant (1990–2001)
Outer Nervión	Dredging (2001), port construction (1993–1997)	Water Treatment Plant (1990–2001)
Butroe	Small urban discharges, dredging (2001)	Water Treatment Plant (1997)
Oka	Urban discharge, Shipyard, dredging (1995, 1998, 1999, 2003)	Urban discharge deviation
Lea		Water Treatment Plant (1995, 2005)
Artibai	Urban & industrial discharges	Basin water treatment
Deba	Urban & industrial discharges	Basin pollutants removal
Urola	Dredging (2000–2005), port construction (1997–1998)	Basin and estuarine water treatment (2007)
Oria	Land-claim (2001), port construction (2005)	Basin water treatment
Urumea	Urban & industrial discharges	Water Treatment Plant
Oiartzun	Morphology, pollutants, dredging (decreasing since 1995)	Water Treatment Plant (1996, 2001)
Bidasoa	Urban discharges (1995), port construction (1998–2000)	Water Treatment Plant (2000, 2003)

Each of the nine indicators used in Table 4 has an associated score (1, 3 or 5). The addition of all the scores provides the final quality classification for this element, as follows: high quality: 39–45 scores; good quality: 31–38; moderate quality: 24–30; poor quality: 17–23; and bad quality: 9–16. These values have been converted into an EQR, lying between 0 (9 scores) and 1 (45 scores). An Excel file template, which automatically calculates the EQRs (=AFI) from trawl line abundance data, is available upon request to the authors.

The AFI was calculated for each trawl line (after pooling all replicates); the results were integrated then at the water body level, following the methodology proposed by Borja et al. (2008, 2009a). In essence, each trawl line is representative of a certain surface area, within the water body, measured from maps and *Geographical Information Systems* (see percentages, associated with each location, in Table 1). Hence, having derived the AFI for each trawl line, the total AFI for the water body can be calculated directly by, weighting by that area. As an example, the integration of data for 1992, within the Barbadún estuary, is presented in Table 5. This approach was proposed because the WFD requires the classification at the water body level. Likewise, Coates et al. (2007) have highlighted that fish populations do not aggregate spatially within estuaries; they are highly variable throughout the year. As such, it is recommended that pooling the data be undertaken on an annual survey basis, for all of the reaches.

Table 4
Estuarine demersal indicators in the Basque Country, with the assigned scores (adapted from Borja et al. (2004, 2009a)), permitting the AZTI's Fish Index (AFI) calculation. Key: F – fishes; and C – crustaceans. The addition of scores provides the status: high (39–45), good (31–38), moderate (24–30), poor (17–23), bad (9–16). Types I and II utilise fish and crustacean data; Type III only fish data.

Indicator	Scores		
	1	3	5
1. – Richness (F and C) (n° sp.)	<3	4–9	>9
2. – Pollution indicator species (F and C) (% individuals)	>80	30–80	<30
3. – Introduced species (F and C) (% individuals)	>80	30–80	<30
4. – Fish health (damage, diseases …) (% affection)	>50	5–49	<5
5. – Flat fish presence (% individuals)	<5	5–10 or >60	10–60
6. – Trophic composition (% omnivorous)	<1 or >80	1–2.5 or 20–80	2.5–20
7. – Trophic composition (% piscivorous)	<5 or >80	5–10 or 50–80	10–50
8. – Estuarine resident (F and C) (n° sp.)	<2	2–5	>5
9. – Resident species (F and C) (% individuals)	<5 or >50	5–10 or 40–50	10–40

2.4. Statistical treatment of the data

In order to study the effect of pressures and actions taken to remove them, two analyses were performed: (1) when assessing the impacts associated to one-off pressures or actions taken within a limited space of time, descriptive measures such as mean and standard deviations and Student's t test for comparison of the groups data were undertaken; and (2) to assess how well the demersal assemblages were correlated with abiotic variables and pressures, a pair-wise Pearson's correlation between variables was carried out. As pressures were measured in 2004, only the AFI derived on the basis of fish and demersal data from 2004 to 2006, were used as the dependent variable.

Statgraphics Plus 5.0 was used to undertake the statistical analyses performed.

3. Results

Since 1989, the number of fish and crustacean species identified in the Basque estuaries were 52 and 33, respectively (Table 6). In total, 65 taxa were identified in the Nervión estuary, between 20 and 26 taxa were identified in the Barbadún, Butroe, Oiartzun and Bidasoa estuaries, and between 10 and 20 taxa in the remainder. It can be seen that the number of species is higher in Type III estuaries and those with longer monitoring programmes. Indeed, there is a significant correlation between the total number of hauls available (see Table 1) and the number of fish species identified (r: 0.92; $p < 0.0001$), the number of crustaceans species (r: 0.76; p: 0.004), and the total number of species found within each estuary (r: 0.90; p: 0.0001). However, several species are common to all of the estuaries, such as *Gobius niger, Platichthys flesus* (except in Oiartzun), *Pomatoschistus* sp., *Solea solea*, and *Syngnathus* spp. (except in Urola) within the fishes, and *Carcinus maenas, Crangon crangon*, and *Palaemon* spp., within the crustaceans (Table 6). Other common fish are *Anguilla anguilla* and *Diplodus sargus*. In general, the highest densities are recorded in the Nervión estuary (Table 6).

When the AFI was calculated for the dataset of the Nervión estuary, using fishes alone or together with crustaceans, a significant positive correlation ($p < 0.0001$) was found when comparing AFI with mean

Table 5
Example of integrating the ecological status of several locations, into a unique value, for the whole Barbadún water body (for 1992) using AZTI's Fish Index (AFI) values. The final status result corresponds to that show in Fig. 3b. Notes: for boundaries, in assessing the final status, see text (data adapted from Borja et al., 2008, 2009a). Sampling location positions are as shown in Fig. 1. Surface and rate (R) are obtained from Table 1 ('area' and 'station area' columns, respectively).

Sampling location:	1	2	3	Total
Ecological status	Good	Moderate	Poor	
AFI (E)	0.61	0.44	0.33	
Surface (km^2)	0.41	0.30	0.04	0.75
Rate (per one) (R)	0.54	0.40	0.06	1.0
Total AFI (E × R)	0.33	0.18	0.02	0.53
Global status				Moderate

Table 6
List of species identified within the Basque estuaries, including the mean density (n°. haul^{-1}) and total number of fish and crustacean species, together with the total number of taxa per estuary.

	Barbadun	Nervión	Butroe	Oka	Lea	Artibai	Deba	Urola	Oria	Urumea	Oiartzun	Bidasoa
Fishes												
Anguilla anguilla	4.1	2.8	1.0		1.0	3.7	3.0		1.3			
Aphia minuta		6.0										
Arnoglossus imperialis		1.7										
Arnoglossus laterna		19.4									6.0	
Arnoglossus sp.		17.0										
Arnoglossus thori		1.6										3.0
Aspitrigla cuculus		1.0										
Atherina presbyter	1.0	4.3										
Buglossidium luteum		17.5										
Callionymus lyra		5.4	1.0					1.0	1.2		2.3	
Callionymus maculatus		28.0										
Chelon labrosus	2.3	1.8										
Crystallogobius sp.		2.0										
Ctnolabrus rupestris		1.0			2.0						1.0	
Dicentrarchus labrax	3.0	1.0	2.0									
Dicentrarchus punctatus		1.0										
Dicologlossa cuneata		29.6										
Diplodus annularis								4.7				
Diplodus cervinus		1.0										
Diplodus puntazzo		2.0										
Diplodus sargus	6.6	11.8	8.0	1.0	3.0	2.7	2.0	3.5				1.1
Diplodus sp.			1.0									
Echiichthys vipera				5.0	2.0				1.0			
Engraulis encrasicolus	1.0	3.4	1.0									
Eutrigla gurnardus		1.0										
Gobius niger	5.8	11.6	2.3	1.0	4.0	2.0	1.7	2.0	4.0	2.0	2.0	2.7
Hippocampus hippocampus			1.3	1.3					3.0	1.0		2.0
Lesueurigobius friesii		1.5	2.0									
Lithognathus mormyrus		3.3	1.0									
Mullus surmuletus	1.0	6.3	5.0	1.0								1.0
Pagellus acarne		1.0										
Pagellus bogaraveo				1.0								
Pagellus sp.				1.0								
Parablennius sp.												1.0
Platichthys flesus	3.8	1.0	1.4	3.0	1.0	1.5	1.0	1.6	1.6	5.7		2.0
Pomatoschistus sp.	62.2	126.2	111.4	275.8	52.3	34.3	53.0	43.3	105.7	25.5	18.9	42.8
Scophthalmus maximus			1.0									
Scorpaena scrofa		2.0									1.0	1.0
Scorpaena sp.		1.0										
Serranus cabrilla		2.5										
Solea senegalensis								3.0				1.3
Solea solea	2.4	21.3	5.4	4.3	3.5	1.6	2.0	6.8	3.4	1.0	2.6	2.0
Symphodus melops									1.0			
Syngnathus abaster			2.0									
Syngnathus acus	1.0	1.3		4.8	2.5	1.0			2.0	2.0	1.0	3.0
Syngnathus rostellatus							5.0					
Syngnathus sp.			1.0									
Trachurus trachurus		2.4										
Trigla lyra		1.0										
Trigla lucerna		1.0										
Trisopterus luscus		7.4										
Umbrina canariensis												1.0
Total fish species	12	38	17	11	9	7	7	8	10	6	8	13
Crustaceans												
Alpheus glaber		3.3										
Carcinus maenas	85.4	109.6	52.0	40.0	64.9	9.4	14.7	11.0	45.8	34.4	3.7	24.1
Clibanarius erythropus												70.0
Crangon crangon	14.2	174.4	40.1	216.5	64.8	2.0	58.4	13.2	63.1	21.2	2.0	13.6
Diogenes pugilator		31.1		3.3							1.0	43.0
Eriphia verrucosa		1.0									1.0	
Galathea squamifera		1.0									1.0	
Goneplax rhomboides		14.0										
Grapsidae sp.	2.0											
Inachus dorsettensis		1.0										
Liocarcinus depurator		16.2		1.0				2.0			9.0	2.0
Liocarcinus holsatus		7.6										
Macropodia rostrata	1.8	5.4		2.7	1.0		6.0	1.5	1.0		1.6	4.3
Maja squinado		1.4						1.0			1.0	2.0
Munida intermedia		6.0										
Munida rugosa		1.0										
Necora puber		2.8									1.0	

Table 6 (*continued*)

	Barbadun	Nervión	Butroe	Oka	Lea	Artibai	Deba	Urola	Oria	Urumea	Oiartzun	Bidasoa
Pachygrapsus marmoratus	4.0	3.3	6.6	13.5	29.0		8.9	5.1	17.3	3.3	1.0	14.0
Pachygrapsus sp.			4.0		10.0				2.0			3.0
Pagurus prideauxi		4.9									1.0	
Pagurus sculptimanus		2.0										
Pagurus sp.		1.0										
Palaemon serratus		49.9										
Palaemon sp.	34.4		18.2	12.0	11.4	7.5	15.6	14.0	9.4	29.5	5.3	12.7
Pasiphaea sivado		2.5										
Pilumnus hirtellus		1.5	1.2					1.0	1.0		2.0	2.5
Pisa tetraodon												1.0
Pisidia longicornis	1.0	4.6					1.0	2.0	1.0		1.0	3.3
Polybius henslowii		1.0										
Portunus latipes		1.0										
Processa parva		2.0										
Upogebia pusilla	2.4	3.3	3.0		52.0					1.0		
Xantho pilipes											1.0	
Total crustacean species	8	27	7	7	7	3	6	9	8	5	15	13
Total taxa	20	65	24	18	16	10	13	17	18	11	23	26

annual bottom oxygen saturation (Figs. 2a,b). The explained variability of AFI is 73% for fishes and 80% for fishes and crustaceans (Figs. 2a,b). Conversely, a significant negative correlation ($p < 0.0001$) was found when comparing AFI with mean bottom ammonia concentration, explaining between 69 and 78% of the variability (Figs. 2c,d).

As the Nervión estuary is a Type III water body (with only fishes being used in the quality assessment), the equation presented in Fig. 2a was used in setting the boundary classes. Hence, taking into account the oxygen saturation objectives and the quality standards (explained in Section 2), the boundaries between the different quality status classes were determined as: 0.17 for bad/poor boundary; 0.34 for poor/moderate; 0.56 for moderate/good; and 0.82 for good/high.

The use of these boundaries has permitted assessment of the status and investigation of its evolution, over time, in some of the areas with long-term demersal trawling monitoring (Fig. 3). Within the Nervión estuary, the inner part, which presented low levels of oxygen until recent times, showed bad to moderate quality, until 2001 (Fig. 3a). The completion of the Water Treatment Plant, together with the biological treatment (Table 3), produced an increase in the dissolved oxygen and physico-chemical quality. Further, demersal fishes have recolonised this area, increasing its quality (Fig. 3a). Such discharges removal produces a significant ($p < 0.005$) improvement in fish quality, with mean AFI values of 0.22 before the total discharge removal to 0.56 after that (see Table 7).

Conversely, the outer water body, with less physico-chemical alterations, showed better quality (Fig. 3a). However, the worsening in conditions between 1993 and 1996, and after 2001, over this area, coincides with the construction of a large commercial port and a very large dredging programme undertaken there, for sand extraction, respectively (Table 3). Probably, these pressures have led to damage in the reproduction or feeding areas, reducing the quality to a poor status (in the first case) and to moderate status (in the second). The port construction produces a significant ($p < 0.05$) drop in fish quality, with mean AFI values of 0.73 before the

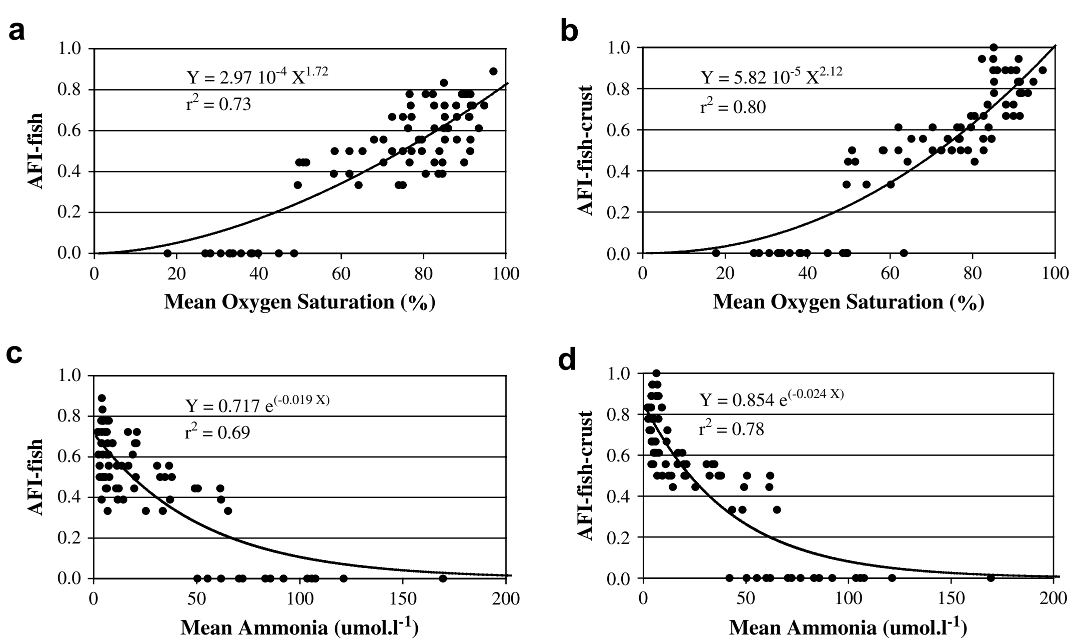

Fig. 2. Power and exponential regressions, calculated using the Nervión dataset (1989–2007), between: (a) mean annual bottom oxygen saturation and AZTI's Fish Index (AFI), calculated using fishes; (b) mean annual bottom oxygen saturation and AFI, calculated using fishes and crustaceans; (c) mean annual bottom ammonia concentration and AFI, calculated using fishes; and (d) mean annual bottom ammonia concentration and AFI, calculated using fishes and crustaceans.

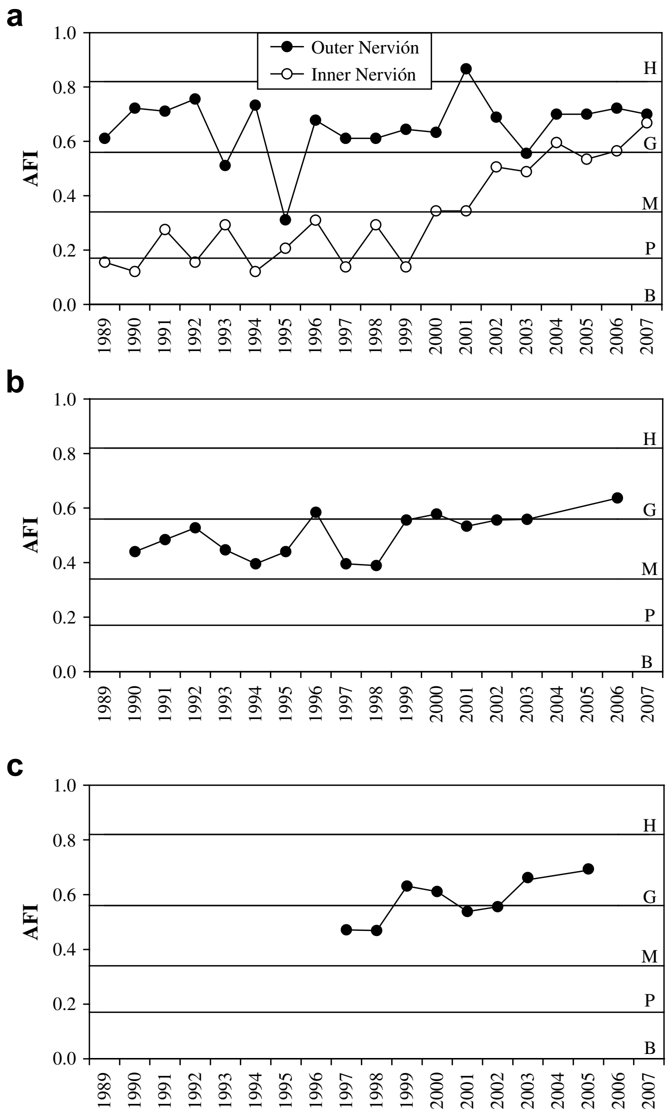

Fig. 3. Evolution of AZTI's Fish Index (AFI), calculated integrating data at the water body level: (a) inner and outer Nervión, using fishes; (b) Barbadún, using fishes and crustaceans; and (c) Butroe, using fishes and crustaceans. Key: H – high status; G – good status; M – moderate status; P – poor status; and B – bad status.

construction to 0.41 after that (see Table 7). In turn, dredging produces a decrease in quality (mean AFI values from 0.71 to 0.62), but the change is not significant (Table 7).

In 1999, the discharge of oil refinery effluent into Barbadún water body was terminated (Table 3). Before this date, demersal quality within the estuary was mostly moderate, improving the quality to good status, following the pressure removal (Fig. 3b). Such discharges removal produces a significant ($p < 0.005$) improvement in fish quality, with mean AFI values of 0.44 before the total discharge removal to 0.57 after that (see Table 7). However, the AFI values which lie near the limit between moderate and good status, might be associated with the malfunctioning of the Water Treatment Plant.

The water treatment programme on the Butroe water body was completed by 1997 (Table 3); at that time, the demersal quality within the water body was moderate (Fig. 3c). After 2 years, the demersal quality started to improve, from moderate to good, with a clear positive trend (Fig. 3c). The discharges removal produces a significant ($p < 0.005$) improvement in fish quality, with mean AFI values of 0.47 before the total discharge removal to 0.62 after that (see Table 7). In 2001–2002, the quality dropped to moderate status (Fig. 3c); this was affected probably by the channel dredging undertaken in 2001 (Table 3). The quality then improved until good status was achieved, after 2002. This quality decrease is significant ($p < 0.05$), with mean AFI values of 0.62, before dredging, dropping to 0.55 after that.

The remainder of the estuaries has been sampled, discontinuously, only between 2 and 5 years (Fig. 4). In general, although these estuaries are relatively small (between 0.4 and 10.3 km^2, see Borja et al. (2006b) for details), a small gradient in the quality status, from the inner part (more degraded), to the outer part (in a better status), can be observed in most of them (Fig. 4). The Oka, Lea, Artibai, and Deba estuaries were classified around the limit between good and moderate status (Figs. 4a–d). In some cases, i.e. Artibai and Deba estuaries, a slight improvement can be detected; this is due, probably, to the water treatment programmes within the catchment (Table 3). Other estuaries have been classified as in good status (e.g. Urola, Oria) even if in some of the internal parts, the quality is moderate (Figs. 4e,f). The slight improvement of these estuaries in recent times could be related also with water treatment, both in the catchment and within the estuary (Table 3). The Urumea estuary experienced degradation in 2007, from previous good status to moderate (Fig. 4g); this was related, probably, to periodical wastewater discharges to the estuary that year.

The Oiartzun and Bidasoa estuaries have shown a progressive improvement, from moderate (even poor) status to good–moderate, in recent years (Figs. 4h,i). This pattern is more evident in the external and middle parts of the estuaries, than in the inner part; it is due to presence of the water treatment plants within both water bodies (Table 3). Such discharges removal produces a significant ($p < 0.05$) improvement in fish quality in Bidasoa, but not in Oiartzun (Table 7), with mean AFI values of 0.38 and 0.46, respectively, before the total discharge removal to 0.50 and 0.53 and after that (see Table 7).

When the correlation between driving forces and pressures with AFI for the whole water body, for the period 2004–2006, were established, all of the drivers showed negative correlations; however, only industry (pollution) and the number of ports

Table 7
Comparison, using a Student's t-test, of mean AZTI's Fish Index (AFI) values before and after pressures or actions taken to remove them, at limited space of time. The years used within the analysis are shown for each water body. Key: SD – standard deviation; NS – correlation not significant ($p > 0.05$).

Water body	Pressure/action	Before pressure or action		After pressure or action		Student's t-test	Significance
		Years	Mean AFI ± SD	Years	Mean AFI ± SD		
Inner Nervión	Discharge removal	1989–2001	0.22 ± 0.09	2002–2007	0.56 ± 0.07	−9.23	$p < 0.005$
Oiartzun	Discharge removal	1995, 2001	0.46 ± 0.12	2004, 2007	0.53 ± 0.11	−0.86	NS
Bidasoa	Discharge removal	2001, 2004	0.38 ± 0.05	2005, 2007	0.50 ± 0.09	−2.86	$p < 0.05$
Barbadún	Discharge removal	1993–1998	0.44 ± 0.07	1999–2006	0.57 ± 0.04	−3.81	$p < 0.005$
Butroe	Discharge removal	1997–1998	0.47 ± 0.00	1999–2000	0.62 ± 0.01	−14.80	$p < 0.005$
Butroe	Dredging	1999–2000	0.62 ± 0.01	2001–2002	0.55 ± 0.01	5.48	$p < 0.05$
Outer Nervión	Dredging	1999–2001	0.71 ± 0.13	2002–2003	0.62 ± 0.09	0.92	NS
Outer Nervión	Port construction	1990–1992	0.73 ± 0.02	1993, 1995	0.41 ± 0.14	3.16	$p < 0.05$

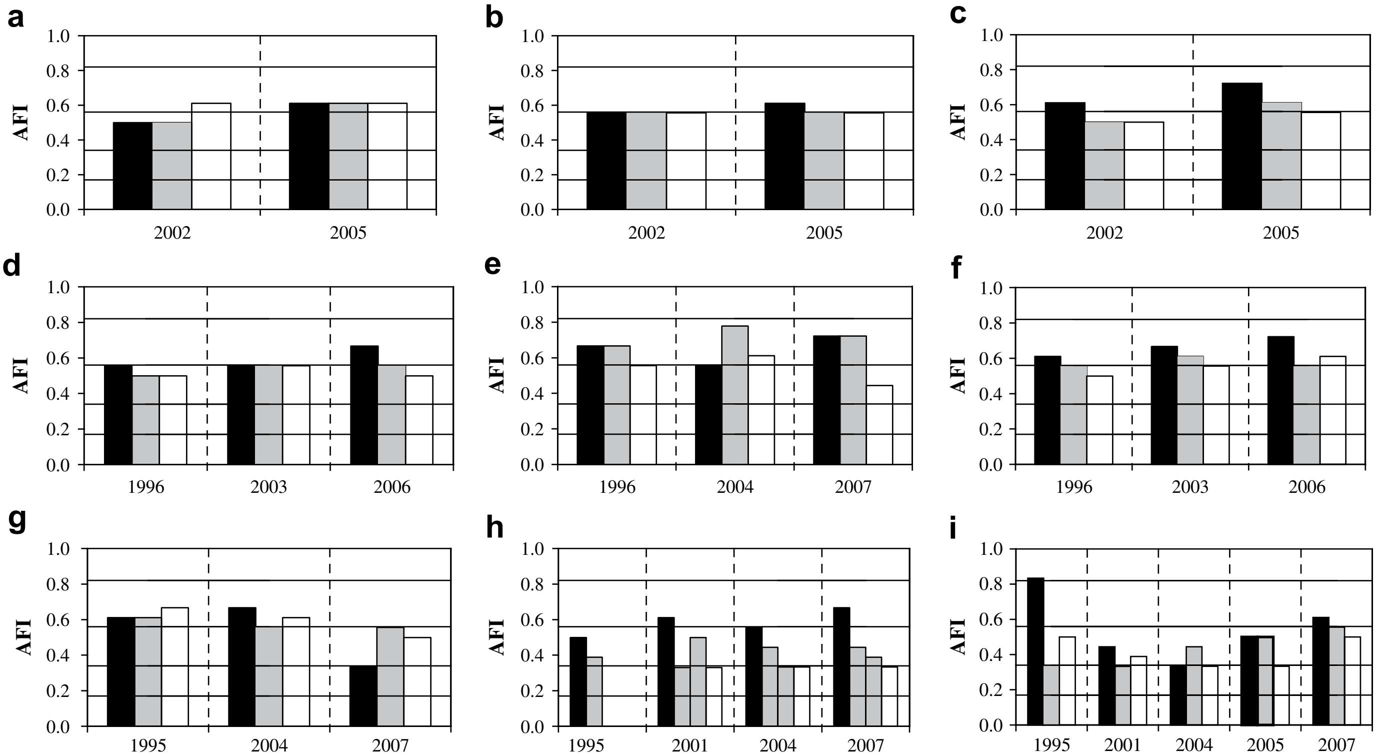

Fig. 4. Evolution of AZTI's Fish Index (AFI), calculated at each of the trawl line within the water body: (a) Oka; (b) Lea; (c) Artibai; (d) Deba; (e) Urola; (f) Oria; (g) Urumea, using fishes and crustaceans; (h) Oiartzun; and (i) Bidasoa, using fishes. Notes: black bars represent data from outer reaches; grey bars from middle reaches; and white bars from inner reaches. Horizontal lines represent, from top to bottom, the boundaries of high, good, moderate, poor, and bad status.

(hydromorphological changes) presented a significant correlation ($p < 0.02$) (Table 8). From the study of pressures, it is interesting to note that the total number of pressures within the estuaries and the pressure index, shown highly significant ($p < 0.009$) negative correlation (Table 8). Other significant ($p < 0.05$) and negative correlations were detected for shoreline reinforcement and the number of ship berths (Table 8).

The increasing number of human pressures and the pressure index, within the estuaries, explains between 51 and 62% of the variability in fish quality, as measured by the methodology explained here (Fig. 5). It would appear that only very few pressures (or small magnitude of them) can provide a high quality in fish or demersal assemblages, as measured in this investigation.

4. Discussion

4.1. Class boundaries definition

To our knowledge, all fish assessment methods used previously adopted fixed ratings between classes, in assessing the final status, normally by dividing the scale into portions of 20–25% of the total (see Harrison and Whitfield, 2004). In the present investigation, a class boundary setting, linked to the response of the fish AFI to environmental variables (oxygen saturation), has been preferred. Hence, this approach provides an 'independent' way to define the quality class boundaries associated to human pressures, as required by the WFD.

Moreover, aquatic systems are impacted upon by multiple, rather than individual, pressures; this makes it more difficult to study the response of fish assemblages, to these pressures. Hence, in the regression equations presented in Fig. 5, the absence of pressure produces AFI values of between 0.66 and 0.75, classifying the status as good, instead of high. Probably, this outcome means that the response of fishes and demersal assemblages, to the first steps of an increasing pressure (especially multiple) is not linear; this, in turn, produces a rapid degradation in the quality, which further can be linear, as shown in Fig. 5. Such rapid degradation, with increasing pressure, can be observed with ammonia; for this, increases >10 μmol l^{-1} produce a rapid decrease in quality (Fig. 2d). In this way, an assumption of many of the multimetric approaches is that changes in fish and demersal assemblages are related linearly to degradation (Harrison and Whitfield, 2004). As shown with the response of fish AFI to oxygen and ammonia and, probably on the basis of the results shown in Fig. 5, this assumption is no longer valid; as such, boundaries between classes cannot be established linearly. However, the definitive boundaries should be determined after intercalibration with other methodologies, as has been undertaken with benthic communities (Borja et al., 2007).

Basque estuaries, as with many other estuaries located within industrialised regions/countries (see Harrison and Whitfield,

Table 8
Correlations between main driving forces and pressures (obtained from Table 2) and AZTI's Fish Index (AFI) calculated for each of the 12 estuaries, within the period 2004–2006.

Drivers	*r*	*p*	Pressures	*r*	*p*
Population	−0.54	0.067	Total pressures	−0.72	0.009
Industry	−0.67	0.017	Pressure index	−0.79	0.002
Ports	−0.93	0.000	Nutrient discharges	0.22	0.485
Agriculture	−0.47	0.121	Water pollution	−0.38	0.218
			Sediment pollution	−0.46	0.136
			Dredging	−0.49	0.104
			Shoreline reinforcement (ports)	−0.70	0.011
			Shoreline reinforcement (other)	0.16	0.628
			Intertidal loss	0.11	0.727
			Berths	−0.74	0.006

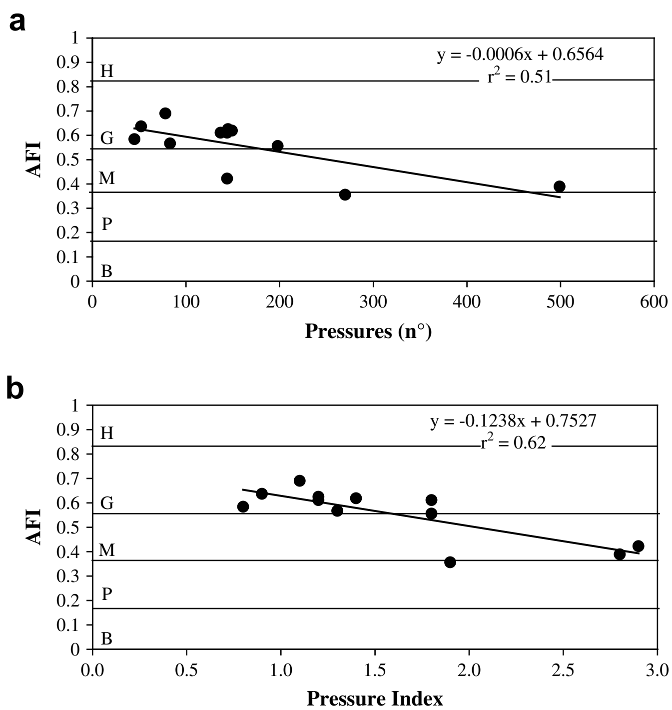

Fig. 5. Regressions between the AZTI's Fish Index (AFI) and total number of pressures (a); and overall pressure index (b), for the period 2004–2006. Note: H – high status; G – good status; M – moderate status; P – poor status; and B – bad status.

2006), are subject to a variety of effects; these range from industrial, agricultural and domestic effluent discharges, physical disturbance, alterations in floodplain land use, canalisation, dredging, etc. A particular advantage, in using fish and demersal assemblages to assess estuarine quality, is that these biotic indicators integrate the effects of a range of environmental effects (such as water quality and habitat destruction) as demonstrated in this particular investigation. In the case of the Basque estuaries, due to their generally small size, most are very susceptible to anthropogenic disturbances, cf. Harrison and Whitfield (2006), for South African estuaries. Moreover, sampling effort, together with different environmental factors (such as salinity, turbidity, etc.) might mask some of the fish and crustacean assemblage responses, to human pressures (Johnson et al., 2008; Selleslagh and Amara, 2008).

Although fish are effective at integrating environmental conditions, over large spatial and temporal scales (Fausch et al., 1990), the above comments related to the size of the systems, the different environmental conditions, the number and nature of the pressures, etc., makes it urgent to define European transitional typologies, in order to compare and intercalibrate methods within the same water body type (Borja et al., 2007). Hence, some previous approaches in establishing transitional water typologies can be used, in such an investigation (Harrison et al., 2000; Elliott and McLusky, 2002; McLusky and Elliott, 2007).

4.2. Response of fish and demersal assemblages, to pressures

The estuaries of the Basque Country received a high load of pollutants, until the end of the 1990's (Cearreta et al., 2004; García-Barcina et al., 2006; Borja et al., 2009b). As a consequence, depending upon the load, the morphological structure of the water bodies, residence times, etc., some of them (i.e. the innermost parts of the Nervión and Oiartzun estuaries) were azoic, for many years. The methodology proposed by Borja et al. (2004, 2009a), for the assessment of fish and demersal assemblages, classifies these systems into bad to moderate status, detecting clearly the impacts produced by such pressures (Figs. 3 and 4). A similar response of fish communities, to wastewater discharges, has been described by Hall et al. (1997) and Jones (2006). Most of this response is due to the low oxygen concentrations (even anoxia) in the water column, together with the high amount of dissolved nutrients, which produces damage and mortality in fishes (Araújo et al., 2000; Jones, 2006).

However, the methodology used here has detected also changes in quality due to hydromorphological pressures, such as dredging, channelling, land-reclamation, marina construction, or the presence of ports. These pressures result in a reduction of two or three levels in quality (between 12 and 44% in AFI values); when these are temporal, the recovery to a previous quality classification takes 2 or 3 years after the impact (see examples, in Figs. 3a,c, Table 7). This pattern of impact and recovery has been detected also, at the same locations, for benthic communities (Borja et al., 2009b).

Impacts on fishes produced by dredging have been detected also in other studies (Pérez-Ruzafa et al., 2006; Breine et al., 2007; Vasconcelos et al., 2007). It is known also that channel morphology and habitat niche requirements influence fish and demersal assemblages (Elliott and Hemingway, 2002). Hence, these hydromorphological pressures show an impact upon fish assemblages, through habitat losses and disturbances on the food webs (Madon, 2008). Dyke and breakwater construction produce impacts on fish and demersal assemblages, by changing their composition and density (Pérez-Ruzafa et al., 2006).

The decreases in fish and demersal ecological status, due to the increased number of pressures, indicate a general degradation in the health of the ecosystem; therefore, in the diversity and abundance of the fish assemblages, as detected also by Coates et al. (2007) and Vasconcelos et al. (2007). This change in quality is likely to reflect the impact of the urbanised and morphologically-modified Basque estuaries, providing few habitats for juvenile and adult fish; however, the spatial changes within the estuaries, from the inner to the outer parts, more than the pressure gradients, reflect also the greater freshwater influence of the inner parts; these support less species diversity, than the lower reaches (Coates et al., 2007). Finally, the small size (<5 km^2) of most of the Basque estuaries can be linked with the limited number of resident fish species; this, in turn, can probably influence the methodology (see above). Hence, Types I and II estuaries have a low number (normally <13) of fish taxa (Table 6). With this limitation, Borja et al. (2004) proposed the incorporation of crustaceans into the assessment, for Types I and II.

Regarding the hydromorphology, two of the Basque estuaries (the Nervión and Oiartzun) have been classified as 'heavily modified water body' (HMWB), under the WFD; as such, they have to meet the requirements of 'good ecological potential' (Borja and Elliott, 2007). The criteria for ecological potential require that a water body should not deteriorate and will most probably be compared to the same reference conditions as those that are not heavily modified; however, the boundary criteria may be different (Coates et al., 2007). Nonetheless, in this contribution, both estuaries have been studied using the same criteria as those in the remaining estuaries.

The study undertaken here has been done using multiple pressures and stressors within the water bodies, as shown in Tables 2 and 3 (see also Borja et al., 2006b). It could have been interesting to have a separate analysis for each hydromorphological pressure, in order to demonstrate the effect of a given pressure, and/or to demonstrate the effect of cumulated hydromorphological pressure. This exercise has been made as far as possible (see Table 7);

however, it is difficult to separate the effects of each pressure when they are multiple and, as demonstrated by Crain et al. (2008), cumulative effects of multiple stressors will often be worse than expected based on single stressor impacts.

4.3. Response of fish and demersal assemblages, to the removal of pressures

Point-source pollution generally originates from wastewater discharged from industrial facilities and municipal sewage. In recent years, most of these pressures have been removed from the Basque aquatic systems, producing a positive evolution in estuarine quality (Borja et al., 2009a). The removal of an oil refinery effluent from a transitional water body (Fig. 3b, Table 7) produced an increase in demersal quality, from moderate to good (increase in AFI values of 22.5%), in 2–3 years. Previous studies have shown that oil refinery discharges produce lower fish abundance, richness and biodiversity in small estuaries (Vallières et al., 2007). The time it takes for an area to recover, following the cessation of oil refinery effluent, varies and depends upon the area and the type of organisms involved. However, Wake (2005) mentions also 2–3 years, for several locations, in relation to benthic communities recovery.

Water treatment in several of the Basque river catchments and estuarine systems commenced in the late 1980s, e.g. in the Nervión estuary (García-Barcina et al., 2006). Most of the engineering works within this particular estuary finished in 2001, when the biological water treatment started; this coincided with a distinct recovery in the physico-chemical and benthic elements (Borja et al., 2009a,b).

The positive trends observed in the fish status are due mainly to the reduction, in recent years, in nutrient discharges and an increase in dissolved oxygen, in some cases, from anoxic or hypoxic situations, to well-oxygenated bottom layers e.g. as observed in the Oiartzun and Nervión estuaries (García Barcina et al., 2006; Borja et al., 2006a). In previously less affected areas, recovery took around 3 years; however, in the most impacted water bodies, recovery took 8–10 years, to achieve a good fish status (see Fig. 3a). Within the systems with significant response the increase in AFI values after pressure removal represents between 24 and 60% (Table 7).

This paradigmatic biological response, illustrating the fish recovery pattern, is similar to other Basque aquatic systems, with water treatment plans undertaken over several years i.e. Oiartzun and some parts of the Bidasoa; but also in other estuaries, elsewhere (Whitfield and Elliott, 2002; Jones, 2006). Probably, some of this recovery depends upon the previous recovery of benthic communities, on which the fishes feed (Borja et al., 2009a,b).

5. Conclusions

On the basis of various examples of urban and industrial discharges into Basque Country water bodies, the absence of oxygen, or the recovery of enough oxygen concentration (following water treatment) to support life in previous azoic areas, has been demonstrated as being an important pressure, driving fish and demersal quality status.

Moreover, the methodology adopted here (AFI) has identified impacts produced by different hydromorphological pressures, including dredging, channelling, or harbour construction, on fish and demersal assemblages; also, in quality recovery following the cessation of such pressures.

The use of oxygen quality standards, in the determination of quality class boundaries for fish assessment, appears to be useful in the implementation of the WFD; they permit the investigation of the evolution and responses of fish assemblages, to changes in human pressures. However, as other factors not affecting the level of oxygen (i.e. heavy metal loads) can lead also to a bad ecological status, the final boundaries must be intercalibrated with other methodologies and countries.

Although the present analysis has provided a valuable insight into assessing the response of fish and demersal assemblages, to different pressures, as required by the WFD, some future investigations are required: (1) refinement and intercalibration, with investigations from other countries, of the methodology developed here; (2) the proposed boundaries need to be tested with other typologies with, probably, some adaptation needed; and (3) the HMWBs need to be taken into account, in the definition of the fish ecological potential.

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References

Araújo, F.G., Williams, W.P., Bailey, W.P., 2000. Fish assemblages as indicators of water quality in the Middle Thames Estuary, England (1980–1989). Estuaries 23, 305–317.
Bald, J., Borja, A., Muxika, I., Franco, J., Valencia, V., 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: a case-study from the Basque Country (Northern Spain). Marine Pollution Bulletin 50, 1508–1522.
Best, M.A., Wither, A.W., Coates, S., 2007. Dissolved oxygen as a physico-chemical supporting element in the Water Framework Directive. Marine Pollution Bulletin 55, 53–64.
Borja, A., 2005. The European Water Framework Directive: a challenge for nearshore, coastal and continental shelf research. Continental Shelf Research 25 (14), 1768–1783.
Borja, A., 2006. The new European Marine Strategy Directive: difficulties, opportunities, and challenges. Marine Pollution Bulletin 52, 239–242.
Borja, A., Elliott, M., 2007. What does 'Good Ecological Potential' mean, within the European Water Framework Directive? Marine Pollution Bulletin 54, 1559–1564.
Borja, A., Franco, J., Valencia, V., Bald, J., Muxika, I., Belzunce, M.J., Solaun, O., 2004. Implementation of the European Water Framework Directive from the Basque Country (northern Spain): a methodological approach. Marine Pollution Bulletin 48, 209–218.
Borja, A., Muxika, I., Franco, J., 2006a. Long-term recovery of soft-bottom benthos following urban and industrial sewage treatment in the Nervión estuary (southern Bay of Biscay). Marine Ecology Progress Series 313, 43–55.
Borja, A., Galparsoro, I., Solaun, O., Muxika, I., Tello, E.M., Uriarte, A., Valencia, V., 2006b. The European Water Framework Directive and the DPSIR, a methodological approach to assess the risk of failing to achieve good ecological status. Estuarine, Coastal and Shelf Science 66, 84–96.
Borja, A., Josefson, A.B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., Rodríguez, J.G., Rygg, B., 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin 55, 42–52.
Borja, A., Bricker, S.B., Dauer, D.M., Demetriades, N.T., Ferreira, J.G., Forbes, A.T., Hutchings, P., Kenchington, R., Marques, J.C., Zhu, C., 2008. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine Pollution Bulletin 56, 1519–1537.
Borja, A., Bald, J., Franco, J., Larreta, J., Muxika, I., Revilla, M., Rodríguez, J.G., Solaun, O., Uriarte, A., Valencia, V., 2009a. Using multiple ecosystem components in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. Marine Pollution Bulletin. doi:10.1016/j.marpolbul.2008.11.010.
Borja, A., Muxika, I., Rodríguez, J.G., 2009b. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. Marine Ecology. doi:10.1111/j.1439-0485.2008.00272.x.

Breine, J., Simoens, I., Goethals, P., Quataert, P., Ercken, D., van Liefferinghe, C., Belpaire, C., 2004. A fish-based index of biotic integrity for upstream brooks in Flanders (Belgium). Hydrobiologia 522, 133–148.

Breine, J.J., Maes, J., Quataert, P., van den Berg, E., Simoens, I., van Thuyne, G., Belpaire, C., 2007. A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). Hydrobiologia 575, 141–159.

Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect environmental changes? Climate Research 18, 119–126.

Cearreta, A., Irabien, M.J., Pascual, A., 2004. Human activities along the Basque coast during the last two centuries: geological perspective of recent anthropogenic impact on the coast and its environmental consequences. In: Borja, A., Collins, M. (Eds.), Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series 70, pp. 27–50.

Coates, S., Waugh, A., Anwar, A., Robson, M., 2007. Efficacy of a multimetric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin 55, 225–240.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11, 1304–1315.

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., Buonaccorsi, J., 1997. Development and validation of an estuarine biotic integrity index. Estuaries 20 (3), 601–617.

Eby, L.A., Crowder, L.B., McClellan, C.M., Peterson, C.H., Powers, M.J., 2005. Habitat degradation from intermittent hypoxia: impacts on demersal fishes. Marine Ecology Progress Series 291, 249–261.

Eddy, F.B., 2005. Ammonia in estuaries and effects on fish. Journal of Fish Biology 67 (6), 1495–1513.

Elliott, M., Dewailly, F., 1995. The structure and components of European estuarine fish assemblages. The Netherlands Journal of Aquatic Ecology 29, 397–417.

Elliott, M., Hemingway, K.L. (Eds.), 2002. Fishes in Estuaries. Blackwell Publishing Ltd., Oxford, 658 pp.

Elliott, M., McLusky, D.S., 2002. The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science 55, 815–827.

Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish and Fisheries 8, 241–268.

Fausch, K.D., Lyons, J., Karr, J.R., Angermeier, P.L., 1990. Fish communities as indicators of environmental degradation. American Fisheries Society Symposium 8, 123–144.

Franco, A., Franzoi, P., Torricelli, P., 2008. Structure and functioning of Mediterranean lagoon fish assemblages: a key for the identification of water body types. Estuarine, Coastal and Shelf Science 79, 549–558.

García-Barcina, J.M., González-Oreja, J.A., De la Sota, A., 2006. Assessing the improvement of the Bilbao estuary water quality in response to pollution abatement measures. Water Research 40, 951–960.

Gibson, G.R., Bowman, M.L., Gerritsen, J., Snyder, B.D., 2000. Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance. EPA 822-B-00-024. US Environmental Protection Agency, Office of Water, Washington, DC.

Hall, J.A., Frid, C.L.J., Gill, M.E., 1997. The response of estuarine fish and benthos to an increasing discharge of sewage effluent. Marine Pollution Bulletin 34, 527–535.

Hansen, H.P., Grashoff, K., 1983. Automated chemical analysis. In: Grashoff, K., Erhardt, M., Kremling, K. (Eds.), Methods of Seawater Analysis. Verlag Chemie, Weinheim, pp. 347–395.

Harrison, T.D., Whitfield, A.K., 2004. A multi-metric fish index to assess the environmental condition of estuaries. Journal of Fish Biology 65, 683–710.

Harrison, T.D., Whitfield, A.K., 2006. Application of a multimetric fish index to assess the environmental condition of South African estuaries. Estuaries and Coasts 29, 1108–1120.

Harrison, T.D., Cooper, J.A.G., Ramm, A.E.L., 2000. Geomorphology, Ichthyofauna, Water Quality and Aesthetics of South African Estuaries. Department of Environmental Affairs & Tourism, Pretoria, South Africa, p. 228.

Henriques, S., Pais, M.P., Costa, M.J., Cabral, H., 2008. Efficacy of adapted estuarine fish-based multimetric indices as tools for evaluating ecological status of the marine environment. Marine Pollution Bulletin 56, 1696–1713.

Hughes, J.E., Deegan, L.A., Weaver, M.J., Costa, J.E., 2002. Regional application of an index of estuarine biotic integrity based on fish communities. Estuaries 25, 250–263.

Johnson, D.D., Rotherham, D., Gray, C.A., 2008. Sampling estuarine fish and invertebrates using demersal otter trawls: effects of net height, tow duration and diel period. Fisheries Research 93, 315–323.

Jones, P.D., 2006. Water quality and fisheries in the Mersey estuary, England: a historical perspective. Marine Pollution Bulletin 53, 144–154.

Karr, J.R., 1981. Assessment of biotic integrity using fish communities. Fisheries 6, 21–27.

Madon, S.P., 2008. Fish community responses to ecosystem stressors in coastal estuarine wetlands: a functional basis for wetlands management and restoration. Wetlands Ecology and Management 16 (3), 219–236.

Maes, J., Stevens, M., Breine, J., 2007. Modelling the migration opportunities of diadromous fish species along a gradient of dissolved oxygen concentration in a European tidal watershed. Estuarine, Coastal and Shelf Science 75, 151–162.

Martinho, F., Viegas, I., Dolbeth, M., Leitão, R., Cabral, H.N., Pardal, M.A., 2008. Assessing estuarine environmental quality using fish-based indices: performance evaluation under climatic instability. Marine Pollution Bulletin 56, 1834–1843.

McLusky, D.S., Elliott, M., 2007. Transitional waters: a new approach, semantics or just muddying the waters? Estuarine, Coastal and Shelf Science 71, 359–363.

Moore, J.A., Lew, A.A., Cunningham, J.A., Kachuba, M., 1995. Fish communities as indicators of environmental quality in the West River watershed. Yale Forestry and Environmental Studies Bulletin 100, 178–196.

Pérez-Ruzafa, A., García-Charton, J.A., Barcala, E., Marcos, C., 2006. Changes in benthic fish assemblages as a consequence of coastal works in a coastal lagoon: the Mar Menor (Spain, Western Mediterranean). Marine Pollution Bulletin 53, 107–120.

Priede, I.G., Solbé, J.F.d.L.G., Nott, J.E., O'Grady, K.T., Cragg-Hine, D., 1988. Behaviour of adult Atlantic salmon, *Salmo salar* L., in the estuary of the River Ribble in relation to variations in dissolved oxygen and tidal flow. Journal of Fish Biology 33 (Suppl. 1), 133–139.

Selleslagh, J., Amara, R., 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). Estuarine, Coastal and Shelf Science 79, 507–517.

Solimini, A.G., Cardoso, A.C., Heiskanen, A.S., 2006. Indicators and Methods for the Ecological Status Assessment Under the Water Framework Directive. Linkages Between Chemical and Biological Quality of Surface Waters. Institute for Environment and Sustainability, Joint Research Center, European Communities, 262 pp.

Vallières, G., Munkittrick, K.R., MacLatchy, D.L., 2007. Assessing fish community structure in relation to water quality in a small estuarine stream receiving oil refinery effluent. Water Quality Research Journal of Canada 42 (2), 72–81.

Vasconcelos, R.P., Reis-Santos, P., Fonseca, V., Maia, A., Ruano, M., Franca, S., Vinagre, C., Costa, M.J., Cabral, H., 2007. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: a multi-metric index and conceptual approach. Science of The Total Environment 374, 199–215.

Wake, H., 2005. Oil refineries: a review of their ecological impacts on the aquatic environment. Estuarine, Coastal and Shelf Science 62, 131–140.

Whitfield, A.K., Elliott, M., 2002. Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. Journal of Fish Biology 61, 229–250.