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Assessing estuarine environmental quality using fish-based indices: Performance evaluation under climatic instability

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ABSTRACT

The seasonal variation of five selected multimetric indices for the determination of the Ecological Quality Status (EQS) of transitional waters was evaluated, as well as the indices' responses to an extreme drought event that occurred in 2005. The database used regards the Mondego River estuary, which was sampled from June 2003 to August 2006 on a monthly basis. Among the selected indices (EBI – Deegan et al. [Deegan, L., Finn, J.T., Ayvazlan, S.G., Ryder-Kieffer, C.A., Buonaccoesi, J., 1997. Development and validation of an Estuarine Biotic Integrity Index. Estuaries 30(3), 601–617], EDI – Borja et al. [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(3–4), 209–218], EFCI – Harrison and Whitfield [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], EBI – Breine et al. [Breine, J.J., Maes, J., Quataert, P., Van den Bergh, E., Simoens, I., Van Thuyne, G., Belpaire, C., 2007. A fish-based assessment tool for the ecological quality of the brackish Scheldt estuary in Flanders (Belgium). Hydrobiologia 575, 141–159] and TFCI – Coates et al. [Coates, S., Waugh, A., Anwar, A., Robson, M., 2007. Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin 55, 225–240]), the EBI by Breine et al. (2007) was the only that evidenced clear interannual and seasonal variations. The EQS by the several indices ranged from "Low" to "High", depending on the index considered, evidencing the high level of mismatch between indices. The results are discussed in the scope of the EU Water Framework Directive, regarding monitoring strategies, application of indices and EQS assessment.

1. Introduction

The Water Framework Directive (WFD, 2000/60/EC) has set a new approach to the management and monitoring of water resources, aiming to achieve a "Good" ecological status by 2015 in all European water bodies (i.e., the values of the biological quality elements for the surface water body type should show low levels of distortion resulting from human activity, deviating only slightly from those normally associated with undisturbed conditions (Vincent et al., 2002). This directive establishes a framework for the protection of groundwater, inland surface waters, estuarine (=transitional) and coastal waters (Borja, 2005), whose main objectives are: (a) to prevent further deterioration, to protect and to enhance the status of water resources; (b) to promote sustainable water use; (c) to enhance protection and improvement of the aquatic environment, through specific measures for the progressive reduction of discharges; (d) to ensure the progressive reduction of pollution of groundwater and prevent its further pollution; and (e) to contribute to mitigating the effects of floods and droughts. Accordingly, all EU member states are required to assess the Ecological Quality Status (EQS) of water bodies, and in transitional waters (=estuaries) the measurement of biological integrity will be emphasized on phytoplankton, macroalgae, benthos and fishes.

Transitional waters are of great importance for the fish fauna, playing a vital role by providing nursery habitats, reproduction grounds, refuge from predators and migratory routes (Haedrich, 1983; Elliot and McLusky, 2002; Cabral et al., 2007; Martinho et al., 2007a,b). Nevertheless, these systems are being subjected to high environmental pressure due to anthropogenic forcing, such as eutrophication, overfishing, bank reclamation and general environmental degradation.

The use of fishes as indicators of environmental change has recently gained attention (Whitfield and Elliott, 2002), with several authors developing multimetric tools in order to assess the estuarine ecosystem status for the fish component at various latitudes

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(e.g. Deegan et al., 1997; Borja et al., 2004; Harrison and Whitfield, 2004; Breine et al., 2007; Coates et al., 2007). In addition, studies of population dynamics, food-web organization and structure of communities have been more successful than single species bioassays at predicting the effects of multiple stresses on biological systems (Schindler, 1987; Plafkin, 1989; Dolbeth et al., 2007a). The use of indicators provides the possibility to evaluate the fundamental condition of the environment without having to capture the full complexity of the system (Whitfield and Elliott, 2002), and according to the WFD guidance, the evaluation methods for the fish component should take in account both aspects of composition and abundance of fish species.

Extreme climatic events, such as floods or droughts are increasing in frequency worldwide (Mirza, 2003), and as a consequence, river discharge into many estuaries may be affected (Gleick, 2003). In the Mondego River basin, a severe drought occurred in 2005, and was classified by the Portuguese Weather Institute (http://web.meteo.pt/clima/clima.jsp) as the worst drought of the past 60 years. As a result, the decreasing precipitation and runoff induced changes in the estuary's planktonic and fish communities, with an increase in typical marine species during the drought (Marques et al., 2007; Martinho et al., 2007b). Since the implementation of the WFD by EU member states will be a continued process in time, to cope the various methodologies with climate instability is a key issue for the success of such an ambitious and promising directive. Within this framework, the objectives of the present work were to compare the results obtained by the methodologies developed by Deegan et al. (1997), Borja et al. (2004), Harrison and Whitfield (2004), Breine et al. (2007) and Coates et al. (2007) for determining the Ecological Quality Status of transitional waters using fish data, and to evaluate their responses in different climatic scenarios, namely in the presence of an extreme event (severe drought).

2. Materials and methods

2.1. Study site

The Mondego estuary is a small intertidal system, located on the Atlantic coast of Portugal (40°08′N, 8°50′W) (Fig. 1), where approximately 1072 ha correspond to wetland habitats. In its terminal part, it comprises two arms that join near the mouth, separated by an alluvium-formed island (Murraceira Island). The northern arm is deeper (average 10 m during high tide) and is the main navigation channel and the location of the commercial harbour. The southern arm is shallower (2–4 m during high tide) and water circulation is mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary system. For further detailed information on the Mondego estuary's characteristics see Teixeira et al. (2008).

2.2. Sampling procedures and data acquisition

Fish sampling was performed monthly from June 2003 to August 2006 (except in July, September, October, December 2004 and July 2006, due to technical constraints or bad weather conditions), using a 2 · 0.5 m beam trawl with one tickler chain and 5 mm mesh size in the cod end. Samples were collected during the night, at high water of spring tides and in 5 stations throughout the estuary (Fig. 1). At each station, three tows were carried out, covering at least an area of 500 m^2 each. All fish caught were identified, counted, measured (total length) and weighted (wet weight). Bottom water salinity and temperature were measured after fishing took place.

Hydrological data was obtained from INAG, Portuguese Water Institute (http://snirh.inag.pt). Monthly precipitation (from

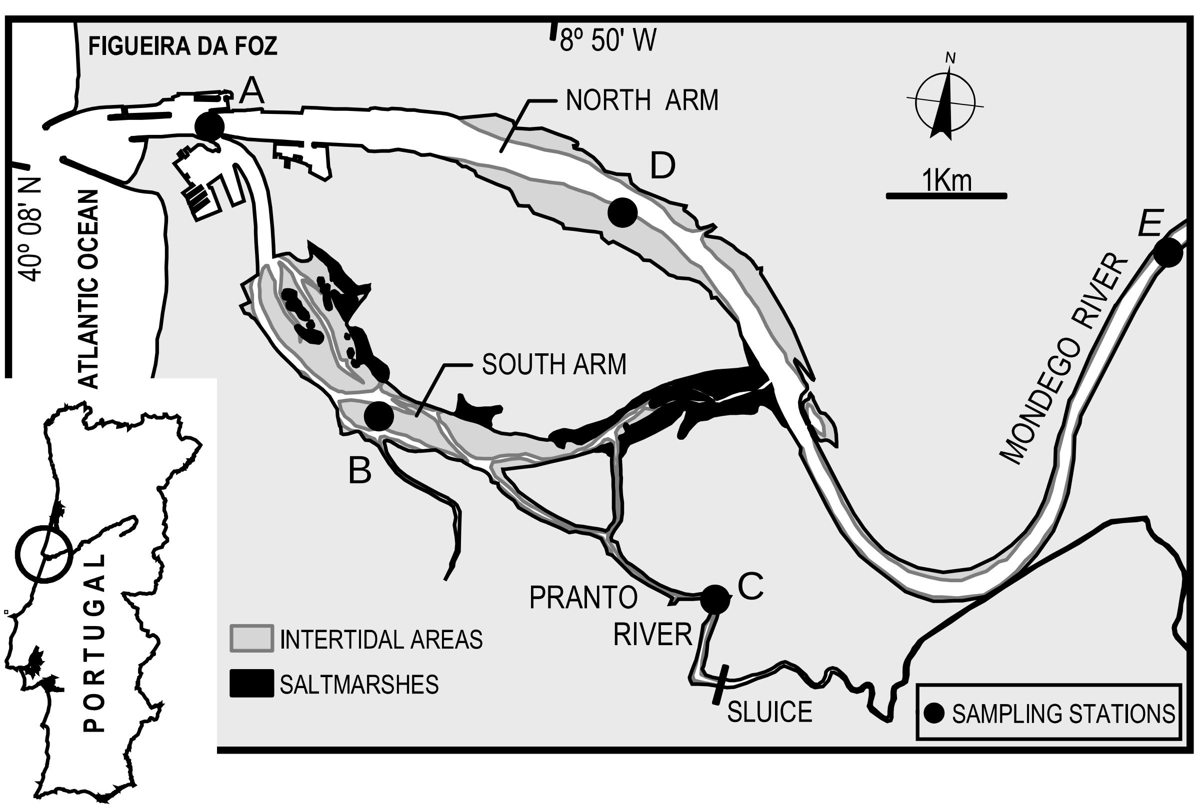

Fig. 1. Geographical location of the Mondego estuary and the five sampling stations (A–E).

January 2002 to June 2006) and long-term monthly average precipitation (from 1933 to 2006) were obtained from the Source 13F/01G station (located near the estuary). Freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra (located 40 km upstream).

2.3. Description of the multimetric indices

A variety of indices have been used to assess the Ecological Quality Status of estuarine systems. In the present study, the results of five multimetric indices and their responses to an extreme drought event were evaluated: Estuarine Biotic Integrity Index (EBI) (Deegan et al., 1997), Estuarine Demersal Indicators (EDI) (Borja et al., 2004), Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004), Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007) and the Transitional Fish Classification Index (TFCI) (Coates et al., 2007). All the methodologies and respective metrics are based on presence/absence, relative proportions and number of taxa of different species and functional groups. Unlike in larger estuaries (e.g. Breine et al., 2007; Coates et al., 2007), the Mondego estuary was not divided in sub areas, due to its relatively small size.

2.3.1. Estuarine Biotic Integrity Index (EBI) (Deegan et al., 1997)

The Estuarine Biotic Integrity Index (EBI) (Table 1) was developed using data from Waquoit Bay and validated using data from Buttermilk Bay, southern Massachusetts, USA. Each metric has an associated score of 0 or 5, and the EBI ranges from 0 to 40, being calculated as the sum of scores for each metric. Due to the inexistence of reference data, the authors only considered two Ecological Quality Status (EQS): Medium (EBI ⩾ 25); Low (EBI < 25). Although the EBI can be used with either density or biomass data, in the present case only densities were used. Sampling was carried out using a 4.8 m otter trawl (0.3 cm mesh size cod end).

2.3.2. Estuarine Demersal Indicators (EDI) (Borja et al., 2004)

The Estuarine Demersal Indicators (Table 2) were developed for the Basque Country, using fish and crustaceans data due to the small size of the Basque estuaries (in the present study, only fish were considered). Each indicator/metric has an associated score of 1, 3 or 5. The sum of all scores provides the final classification for the fish community, being then converted into the Ecological Quality Ratio (EQR), which ranges from 0 to 1 with five equal thresholds, each corresponding to an Ecological Quality Status (EQS): Bad (<0.2), Poor (0.2–0.4), Moderate (0.4–0.6), Good, (0.6–0.8) and High (0.8–1.0). The sampling method was trawling.

2.3.3. Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004)

The Estuarine Fish Community Index (EFCI) (Table 3) was developed for South African estuaries. Each metric has an associated score of 0, 3 or 5, and the EFCI is calculated as the sum of the scores. The EFCI ranges from 0 to 70 with five thresholds: Very Poor (EFCI < 20), Poor (22 ⩾ EFCI ⩽ 38), Moderate (40 ⩾ EFCI ⩽ 44), Good (46 ⩾ EFCI ⩽ 62) and Very Good (62 ⩾ EFCI ⩽ 70). Fishes were sampled using a 30 m · 1.7 m seine (15 mm bar mesh size) and a fleet of 10 · 1.7 m gillnets (45, 75 and 100 mm stretched mesh panels).

Table 1
Description of the Estuarine Biotic Integrity Index (EBI), after Deegan et al. (1997)

No.	Metric	Scores 0	Scores 5
1	Number of species (N)	<6	⩾6
2	Dominance	<3	⩾3
3	Fish abundance	<3.8	⩾3.8
4	Nursery species (N)	<3	⩾3
5	Estuarine spawners (N)	<3	⩾3
6	Resident species (N)	<4	⩾4
7	Proportion benthic fishes (%)	<0.70	⩾0.70
8	Proportion abnormal (%)	<0.01	⩾0.01

Table 2
Description of the Estuarine Demersal Indicators (EDI), after Borja et al. (2004)

No.	Metric	Scores 1	Scores 3	Scores 5
1	Number of species (N)	<3	4–9	>9
2	Pollution indicator species	Presence		Absence
3	Introduced species	Presence		Absence
4	Fish health (damage) (% affection)	>50	5–49	<5
5	Flatfish presence (%)	<5	5–10 or <60	10–60
6	Abundance of omnivorous species (%)	<1 or >80	1–2.5 or 20–80	2.5–20
7	Abundance of piscivorous species (%)	<5 or >80	5–10 or 50–80	10–50
8	Estuarine resident species (N)	<2	2–5	>5
9	Abundance of resident species (%)	<5 or >50	5–10 or 40–50	10–40

Table 3
Description of the Estuarine Fish Community Index (EFCI), after Harrison and Whitfield (2004)

No.	Metric	Scores 1	Scores 3	Scores 5
1	Number of species (N)	⩾22	<22 and ⩾12	<12
2	Rare or threatened species	Presence	Absence	
3	Exotic or introduced species		Absence	Presence
4	Species composition (% similarity)	⩾80	<80 and ⩾50	<50
5	Species relative abundance (% similarity)	⩾60	<60 and ⩾40	<40
6	Species that make up 90% of the abundance (N)	⩾8	<8 and ⩾4	<4
7	Estuarine resident species (N)	⩾5	<5 and ⩾3	<3
8	Estuarine-dependent marine species (N)	⩾14	<14 and ⩾8	<8
9	Abundance of estuarine resident species (%)	25–75	⩾10 and < 25 or >75 and ⩽90	<10 or >90
10	Abundance of estuarine-dependent marine species (%)	25–75	⩾10 and <25 or >75 and ⩽90	<0 or >90
11	Benthic invertebrate feeding species (N)	⩾7	<7 and ⩾4	<4
12	Piscivorous species (N)	⩾3	<3 and ⩾2	<2
13	Abundance of benthic invertebrate feeding species (%)	⩾10	<10 and ⩾5	<5
14	Abundance of piscivorous species (%)	⩾1	<1 and ⩾0.5	<0.5

2.3.4. Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007)

The Fish-based Estuarine Biotic Index (EBI) (Table 4) was developed for the brackish section of the Scheldt River estuary. Each metric has an associated score of 0, 0.25, 0.5, 0.75 and 1, and the EBI is calculated as the scores' average value. Due to the absence of reference data, the authors only defined the thresholds until Moderate status: Bad (EBI ⩽ 0.15), Poor (0.15 > EBI ⩽ 0.30), Moderate (EBI > 0.30). However, and since the EBI ranges from 0 to 1, it was decided to define the remaining thresholds, in order to better compare the results with the other methodologies, as follows: Moderate (0.30 > EBI ⩽ 0.55), Good (0.55 > EBI ⩽ 0.80), High (EBI > 0.80). Sampling was performed using a pair of two fyke-nets (type 120/80), deployed at low tide and emptied in the following day.

Table 4
Description of the Fish-based Estuarine Biotic Index (EBI), after Breine et al. (2007)

No.	Metric	Scores				
		0	0.25	0.5	0.75	1
1	Number of species (N)	⩽7	>7	>9	>10	>11
2	*Osmerus eperlanus* individuals (%)	⩽0.33		>0.33	>1.12	>2.68
3	Marine juvenile migrating individuals (%)	⩽33.0	>33.0	>54.2	>73.1	>82.0
4	Omnivorous species (%)	⩾16.44	<16.44	<7.90	<3.37	<1.17
5	Piscivorous species (%)	⩽12.84	>12.84	>19.44	>27.23	>41.19

2.3.5. Transitional Fish Classification Index (TFCI) (Coates et al., 2007)

The Transitional Fish Classification Index (TFCI) (Table 5) was developed for the Thames River estuary, and compared to a reference estuarine fish community, derived from a number of estuaries of the same typology as the Thames. Each metric has an associated score of 1, 2, 3, 4, or 5, and the TFCI is calculated as the total score for each sampling date divided by the maximum possible score. The EQS thresholds are the same as in the methodology by Borja et al. (2004). Sampling was carried out based on a multi-method approach: in the upper to mid estuary a 45 · 3.5 m seine net with a 5 mm knotless mesh centre and 20 mm wings was deployed from the shore; a 1.52 m wide beam trawl with a 20 mm knotless outer mesh and 5 mm knotless cod end was trawled for 250 m parallel to the seining site; in the mid and lower estuary were also used paired 8 m wide otter trawls with a 40 mm outer mesh with a 5 mm knotless 'cod end' mesh (Coates et al., 2007). According to the authors, and for the TFCI only, the mean number of taxa within the upper quintile (top 20%) was determined and used as the boundary value between RS4 and RS5. Percentages of this value were used to calculate the boundaries for each metric (see Table 5).

2.4. Reference data

An ideal reference community is derived from the same site at the same time of year using the same methods, during a period when the environment is pristine and no anthropogenic changes have occurred (Coates et al., 2007). Since reference data was not available for the Mondego estuary (or any other Portuguese A2 type estuary – Mesotidal well-mixed estuaries with irregular river discharge; Bettencourt et al., 2004) to use in the Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004) and in the Transitional Fish Classification Index (TFCI) (Coates et al., 2007), it was determined that the average densities from June 2003 to May 2004 would be used as reference, since it was the period when environmental conditions (namely precipitation and freshwater runoff) were within regular values. This decision was taken in accordance with Martinho et al. (2007b), who outlined that the fish community is sensitive (to some degree) to variations in precipitation and freshwater runoff regimes.

Table 5
Description of the Transitional Fish Classification Index (TFCI), after Coates et al. (2007) (Metrics 4, 5, 6, 8 and 9 scores defined according to the Mondego estuary fish community)

No.	Metric	Scores				
		1	2	3	4	5
1	Species composition (% similarity)	<19.9	20–39.9	40–59.9	6–79.9	80–100
2	Presence of indicator species	Presence				
3	Species relative abundance (% similarity)	<19.9	20–39.9	40–59.9	6–79.9	80–100
4	Taxa that make up 90% of the abundance (N)	<0.6	0.6–1.19	1.2–1.79	1.8–2.39	⩾2.4
5	Estuarine resident species (N)	<0.6	0.6–1.19	1.2–1.79	1.8–2.39	⩾2.4
6	Estuarine-dependent marine species (N)	<0.6	0.6–1.19	1.2–1.79	1.8–2.39	⩾2.4
7	Functional guild composition (N)	0–1	2	3		
8	Benthic invertebrate feeding species (N)	<0.6	0.6–1.19	1.2–1.79	1.8–2.39	⩾2.4
9	Piscivorous species (N)	<0.6	0.6–1.19	1.2–1.79	1.8–2.39	⩾2.4
10	Feeding guild composition (N)	0	1	2	3	4

2.5. Data analysis

The structure of the fish community was analyzed based on ecological guilds, derived from habitat usage patterns (adapted from Elliott and Dewailly, 1995): marine adventitious species (MA), marine juvenile migrant species (MJ; occurring usually in low densities in estuaries as an alternative habitat), marine species that use the estuary as nursery grounds (NU; occurring in clear seasonal patterns, higher densities and remaining longer periods in estuaries), estuarine resident species (ER), catadromous adventitious species (CA) (no anadromous species were found) and freshwater adventitious species (FW), and feeding guilds: planktivorous (PLANK), benthic invertebrate feeders (INVV), piscivorous (PISV), omnivorous (OMN) (adapted from Elliott and Dewailly (1995), Breine et al. (2007) and Coates et al. (2007)). Fish densities were estimated as the number of individuals per 1000 m^{-2}. Whenever needed, data was transformed according to the procedures proposed by each index.

Results from the indices were compared based on Kendall's coefficient of correlation. This correlation coefficient varies between ·1 (total disagreement) and 1 (perfect agreement), and if the correlation equals zero, the rankings are completely independent. Differences between seasonal results were tested with an ANOVA and Tukey-type a posteriori tests were used whenever the null hypotheses were rejected. A significance level of 0.05 was considered in all test procedures.

3. Results

3.1. Environmental background

Within the study period, a severe drought occurred in 2004/2005, with associated reduction in precipitation and freshwater runoff (Fig. 2A). In fact, only in 2003/2004 were recorded precipitation values above the 1931–2006 average, and freshwater runoff to the estuary was reduced almost 10-fold from the highest value in 2003 to the lowest value in 2005. According to the Portuguese Weather Institute (http://web.meteo.pt/pt/clima/clima.jsp), the 2005 drought was the harshest since 1931.

Temperature showed a typical pattern for temperate latitudes, ranging from 8.8 ± 1.7 to 22.7 ± 2.6 °C, while salinity showed a clear increase during the drought (Fig. 2B). For further detailed information on the drought conditions and its main effects on estuarine planktonic and fish communities see Dolbeth et al. (2007b), Marques et al. (2007) and Martinho et al. (2007b).

3.2. Estuarine fish community

The Mondego estuary fish community was studied from June 2003 to August 2006 on a monthly basis, being so far identified 42 species, belonging to 23 Families (Leitão et al., 2007; Martinho et al., 2007a,b) (Table 6). As a general pattern, the fish commu-

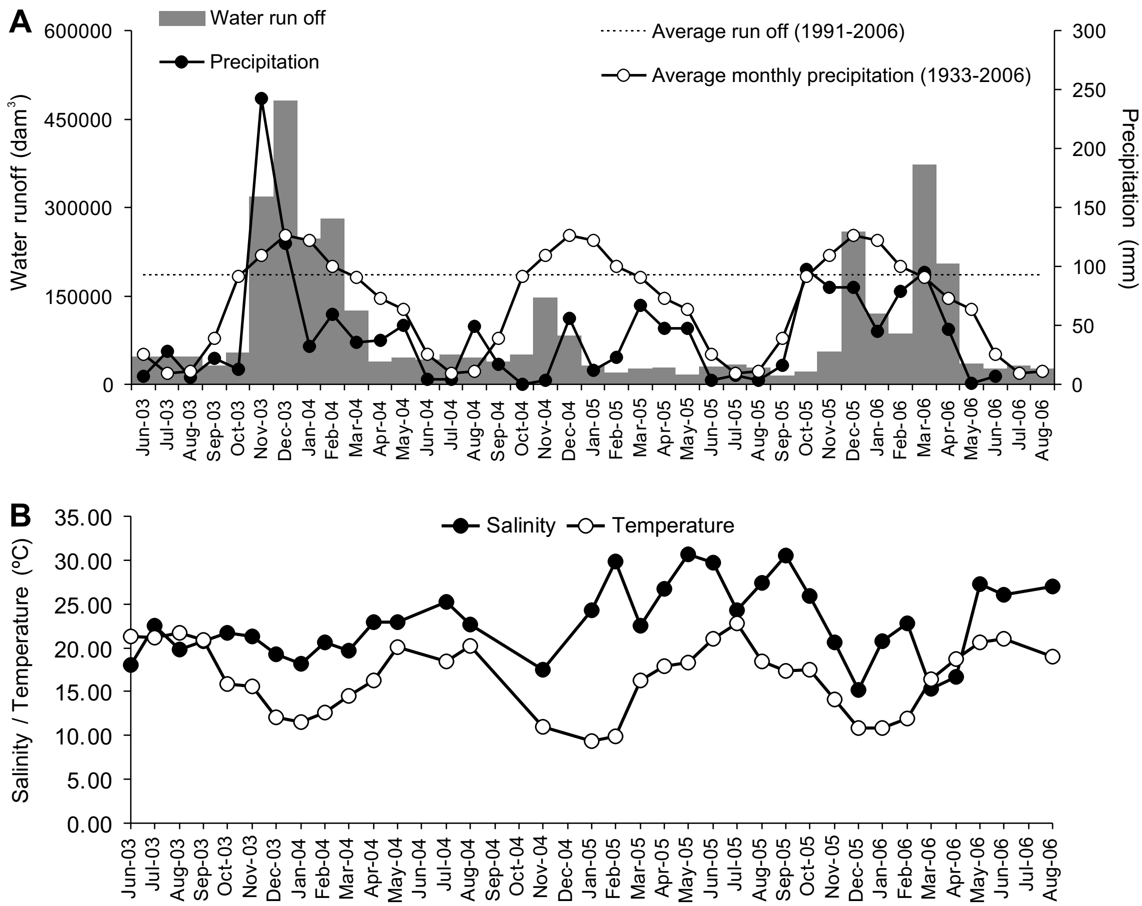

Fig. 2. Monthly variation of (A) precipitation (mm) and freshwater runoff (dam^3) and (B) average temperature (°C) and salinity from June 2003 to August 2006. Dashed line indicates the average value of freshwater runoff for the period 1931–2006.

nity was dominated by the estuarine residents (ER) *Pomatoschistus microps* and *Pomatoschistus minutus*, the marine species that use the estuary as nursery area (NU) *Dicentrarchus labrax*, *Solea solea* and *Platichthys flesus*, and the marine juvenile (MJ) *Diplodus vulgaris*. Freshwater adventitious species (FW) (*Barbus bocagei*, *Carassius auratus* and *Gambusia holbrooki*) were only occasionally caught until the winter of 2004, and marine adventitious species (MA) such as *Arnoglossus laterna*, *Buglossidium luteum*, *Gaidropsarus mediterraneus*, *Solea lascaris* and *Symphodus bailloni* only appeared after the summer of 2004. In fact, *A. laterna*, *B. luteum*, *S. lascaris* and *Trisopterus luscus* were only captured inside the estuary in 2005.

Regarding the total number of species (Fig 3A), throughout the study period were captured an average of 15 (±3) per month; the highest species number was collected in January 2005 (22 spp). Total fish densities (Fig. 3B) were higher in the beginning of the study (·140 ind. 1000 m^{-2}), with an average value of 27.4 ± 25.1 ind. 1000 m^{-2} throughout the study period. No clear seasonal patterns were identified both for the number of species and total densities.

3.3. Ecological quality: metrics results

The monthly evaluation by the Estuarine Biotic Integrity Index (EBI) is shown in Fig. 4A. The index exhibited a constant value over the study period, corresponding to a Medium Ecological Quality Status (EQS). The exception was December 2005, in which the index presented a Low EQS. The Estuarine Demersal Indicators (EDI) proposed by Borja et al. (2004) (Fig. 4B) classified in general as Good status, with the highest amplitude of values during the drought period: in May 2005, the EDI classified as Moderate status and in August 2005 as High status. In May 2006, the lowest EQR was obtained (0.44 – Moderate) and in the winter of 2004 this index classified as High status.

Although quite constant, the Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004) (Fig. 4C) showed a slight decreasing tendency regarding the EQS of the Mondego estuary. As a general pattern, this index classified as Good status from 2003 to 2005 (with few exceptions), while all 2006 was classified as Moderate status. The Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007) was the only index that evidenced a clear decrease in the EQS (Fig. 4D), particularly during the drought period (from mid-2004 to 2005). As a result, during 2003 a Good status was obtained, while in 2004/2005 the values decreased and the estuary was classified in Moderate and Poor status. In 2006, the index values increased, and a Good status was obtained in the end of the study period. Fig. 4E reports the classification of the EQS according to the Transitional Fish Classification Index (TFCI) (Coates et al., 2007). This index evidenced the highest and more constant results, classifying as High status almost all the sampling situations, with the exception of August 2004, December 2005 and June 2006.

3.4. Comparison of Indices

Table 7 shows the results of the Kendall tau rank correlation coefficient between the selected indices ($P < 0.05$). Significant positive correlations were found between Borja et al. (2004) and Breine et al. (2007) ($T = 0.41$), Coates et al. (2007) and Breine et

Table 6
Species list of the Mondego estuary fish community, with respective family, ecological guild, feeding guild, indicator status and average density (number of individuals per 1000 m^2) trhoughout the study period; CA – catadromous; ER – estuarine resident; MA – marine adventitious; FW – freshwater; MJ – marine juvenile; NU – nursery; PLANK – planktivorous; INVV – benthic invertebrate feeder; PISV – piscivorous; OMN – omnivorous

Species	Family	Ecological guild	Feeding guild	Indicator species	Average N ind. 1000 m^{-2}
Ammodytes tobianus	Ammodytidae	MA	PLANK	N	0.115 ± 0.33
Anguilla anguilla	Anguillidae	CA	INVV/OMN	Y	0.614 ± 0.93
Aphia minuta	Gobiidae	MA	PLANK	N	0.060 ± 0.22
Arnoglossus laterna	Scophthalmidae	MA	PISV	N	0.015 ± 0.05
Atherina boyeri	Atherinidae	ER	PLANK/OMN	N	0.768 ± 1.27
Atherina presbyter	Atherinidae	ER	INVV/OMN	N	0.096 ± 0.21
Barbus bocagei	Cyprinidae	FW	INVV/OMN	N	0.007 ± 0.04
Buglossidium luteum	Soleidae	MA	INVV	N	0.003 ± 0.02
Callionymus lyra	Callionymidae	MA	INVV/OMN	N	0.143 ± 0.26
Carassius auratus	Cyprinidae	FW	INVV/OMN	Y	0.002 ± 0.01
Chelon labrosus	Mugilidae	MJ	DETR/OMN	N	0.009 ± 0.04
Ciliata mustela	Gadidae	MJ	INVV	N	0.126 ± 0.21
Conger conger	Congridae	MA	PISV	N	0.018 ± 0.04
Dicentrarchus labrax	Moronidae	NU	PISV	N	7.540 ± 7.82
Dicologlossa hexophthalma	Soleidae	MJ	INVV/OMN	N	0.002 ± 0.01
Diplodus vulgaris	Sparidae	MJ	INVV/OMN	N	1.394 ± 1.81
Echiichthys vipera	Trachinidae	MA	INVV/OMN	N	0.026 ± 0,07
Engraulis encrasicolus	Engraulidae	MA	PLANK/OMN	N	0.050 ± 0.16
Gaidropsarus mediterraneus	Gadidae	MA	INVV	N	0.002 ± 0.01
Gambusia holbrooki	Poeciliidae	FW	INVV/OMN	N	0.011 ± 0.06
Gobius niger	Gobiidae	ER	INVV	N	0.121 ± 0.14
Liza aurata	Mugilidae	MJ	DETR/OMN	N	0.014 ± 0.04
Liza ramada	Mugilidae	CA	DETR/OMN	N	0.242 ± 0.57
Mugil cephalus	Mugilidae	MJ	DETR/OMN	N	0.005 ± 0.02
Mullus surmuletus	Mullidae	MJ	INVV	N	0.106 ± 0.17
Nerophis lumbriciformis	Syngnathidae	ER	INVV	N	0.008 ± 0.05
Parablennius gattorugine	Blenniidae	MA	INVV	N	0.003 ± 0.02
Platichthys flesus	Pleuronectidae	NU	INVV	N	1.473 ± 1.63
Pomatoschistus microps	Gobiidae	ER	INVV	N	8.061 ± 11.41
Pomatoschistus minutus	Gobiidae	ER	INVV	N	3.623 ± 5.96
Sardina pilchardus	Clupeidae	MJ	PLANK	N	0.267 ± 1.08
Scophthalmus rhombus	Scophthalmidae	MJ	PISV	N	0.053 ± 0.08
Solea lascaris	Soleidae	MA	INVV	N	0.024 ± 0.07
Solea senegalensis	Soleidae	MJ	INVV	N	0.096 ± 0.15
Solea solea	Soleidae	NU	INVV	N	1.621 ± 1.42
Sparus aurata	Sparidae	MJ	INVV/OMN	N	0.019 ± 0.04
Spondyliosoma cantharus	Sparidae	MA	INVV/OMN	N	0.018 ± 0.06
Symphodus bailloni	Labridae	MA	INVV/OMN	N	0.053 ± 0.12
Syngnathus abaster	Syngnathidae	ER	INVV/OMN	N	0.161 ± 0.25
Syngnathus acus	Syngnathidae	ER	INVV	N	0.251 ± 0.52
Trigla lucerna	Triglidae	MJ	PISV	N	0.117 ± 0.25
Trisopterus luscus	Gadidae	MA	INVV/OMN	N	0.099 ± 0.24

al. (2007) ($T = 0.31$), Harrison and Whitfield (2004) and Coates et al. (2007) ($T = 0.64$); the only significant negative correlation was found between Deegan et al. (1997) and Borja et al. (2004) ($T = \cdot 0.30$). The conformity between the methods tested was in general low (Table 7), as the relative number of cases when one index classified a sampling occasion as "High" or "Good" and the other as "Moderate", "Poor" or "Bad" (mismatch) was high. The lowest mismatch value was obtained between the indices by Borja et al. (2004) and Coates et al. (2007) (6%).

Concerning the seasonal variation of the ecological status of the system, only EBI (Breine et al. 2007) found significant seasonal differences ($F = 0.982$; $P < 0.05$), and in particular, between the Autumn 2003 and Autumn 2004 ($q = 0.04$; $P < 0.05$) and between the Winter 2004 and Winter 2005 ($q = 0.048$; $P < 0.05$). For the other indices, no significant seasonal variations were found.

4. Discussion

4.1. Assessing the EQS and its relation with drought events

An ecologically parsimonious approach dictates that investigators should place greater emphasis on evaluating the suitability of indices that already exist prior to developing new ones (Diaz et al., 2004). In agreement, this work aimed at evaluating the performance of five selected multimetric indices to assess the Ecological Quality Status of transitional waters using fish data and their response to an extreme drought event that occurred in 2005. Testing of indices is an exercise aiming not only to select the best appropriate index for each case, but also to assure that results are comparable among two or more indices (Simboura and Reizopoulou, 2008). One of the major concerns when undertaking this exercise was the lack of publications in this subject, in opposition with the benthic component of transitional waters, which has generated a large debate and accordingly, several multimetric indices are being tested by European member states (e.g. AMBI – Borja et al., 2000; BENTIX – Simboura and Zenetos, 2002; BQI – Rosenberg et al., 2004).

In general, all tested methodologies gave constant results throughout the study period, particularly the indices proposed by Harrison and Whitfield (2004) and Coates et al. (2007) (the metrics with the highest correlation values between them). This would be expected, since the metric by Coates et al. (2007) is an adaptation of the South African index developed by Harrison and Whitfield (2004) to European transitional waters, particularly to the Thames River. In the particular case of the work due to Coates et al. (2007), the system was almost always classified as High status, possibly due to the type of data used as reference condition (the first year of the study). This will be a common issue in implementing the WFD,

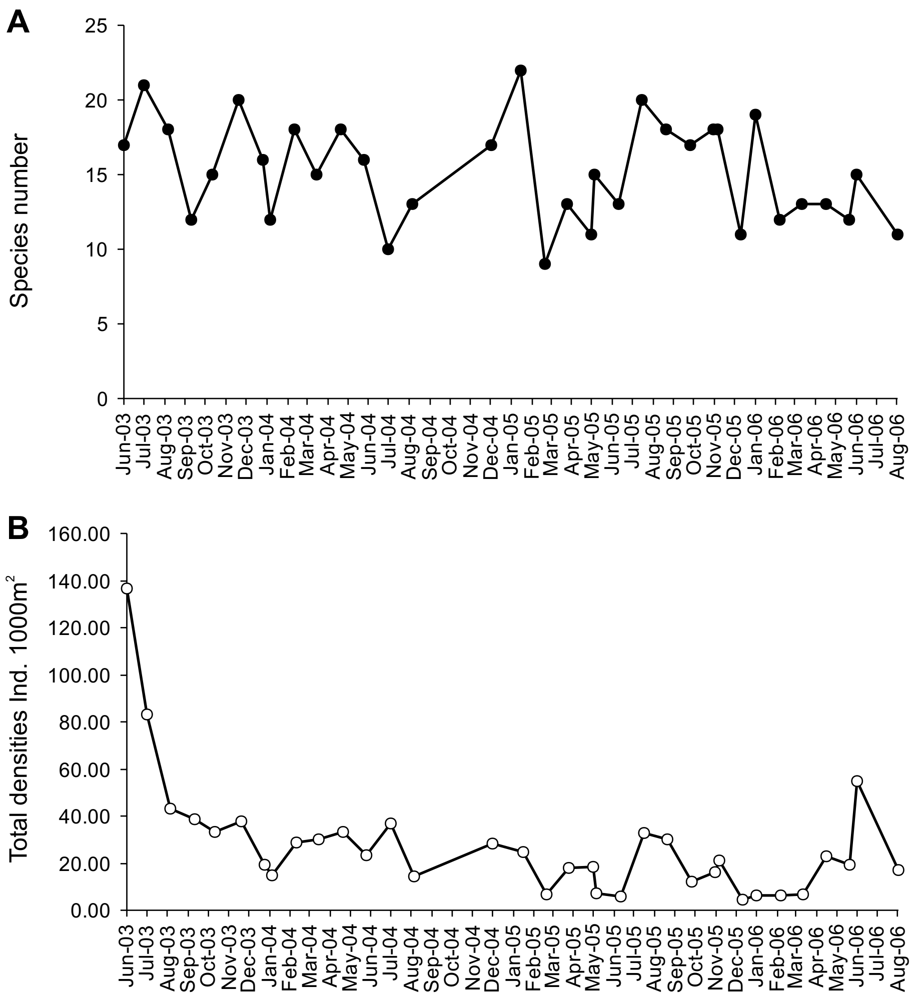

Fig. 3. Monthly variation of the (A) number of species and (B) total densities (N ind. 1000 m^{-2}) of the fish community of the Mondego estuary during the study period.

since quality reference data will not be available for most estuaries, thus becoming an important source of bias when determining the EQS. In terms of status classification, the indices proposed by Borja et al. (2004) and Coates et al. (2007) classified the estuary with the highest status, evidencing the lowest level of mismatch among all the indices tested (Table 7).

The only index that presented clear interannual and seasonal variations between year fluctuations was the EBI by Breine et al. (2007), which could be the result of two aspects: (a) the elimination of the metric correspondent to the abundance of *Osmerus eperlanus* (due to the inexistence of this species in Portuguese and southern European waters, since the southern limit of distribution is the Gironde estuary, France (Rochard and Elie, 1994) and (b) this is the index that has the lowest number of metrics (4) within the indices compared in this study. Thus, the use of a larger number of metrics seems more adequate for the fish component of transitional waters (acting as a buffer) and also the use of metrics based on single species should be discouraged, since there is a small number of species that could be considered as indicator and present in all European estuaries. The disadvantage of relying on one single species had already been stated by Breine et al. (2007).

When comparing the classification status of all indices for the same period, considerable variations existed (Fig. 4). The diverse or not consistent responses of the different indices may lead to doubt in managers' minds regarding the value of the methods (Quintino et al., 2006). According to the same authors, the outcome of the use of the indicators has a financial dimension, such that areas misclassified as being in "Poor status" will then require expensive remediation measures. This was quite evident when analyzing the level of mismatch between the indices tested, induced by the different background of sampling methods, geographical areas, seasons, pressures and determination of metrics and thresholds for the EQS ranges. In particular, the index by Deegan et al. (1997) gave consistently the lowest results, possibly due to the determination of only the thresholds between "Low" and "Medium" status. It would also have been the case of the EBI by Breine et al. (2007), since reference conditions could not be attained and the boundaries between the highest statuses could not be defined. However, the use of quintile methods and the EQS scale from 0 to 1 allowed defining the thresholds between "Moderate" and "Good" and between "Good" and "High" statuses.

One of the aspects highlighted by this work was the seasonal constancy of the indices (except in the EBI), evidencing that the changes induced by the drought in the fish community, namely the increase in marine adventitious species and a decrease of the estuarine residents, mainly *P. minutus* (Dolbeth et al., 2007b; Martinho et al., 2007b) were not reflected at other guild levels, which are the main components of the indices tested. A characteristic of a good ecological indicator is that it should reflect changes in the ecosystem, while taking into account the natural variability inherent of natural processes, in agreement with the Estuarine Quality Paradox (Elliott and Quintino, 2007), which was verified for all indices except for the EBI (Breine et al., 2007). Thus, it is recommended

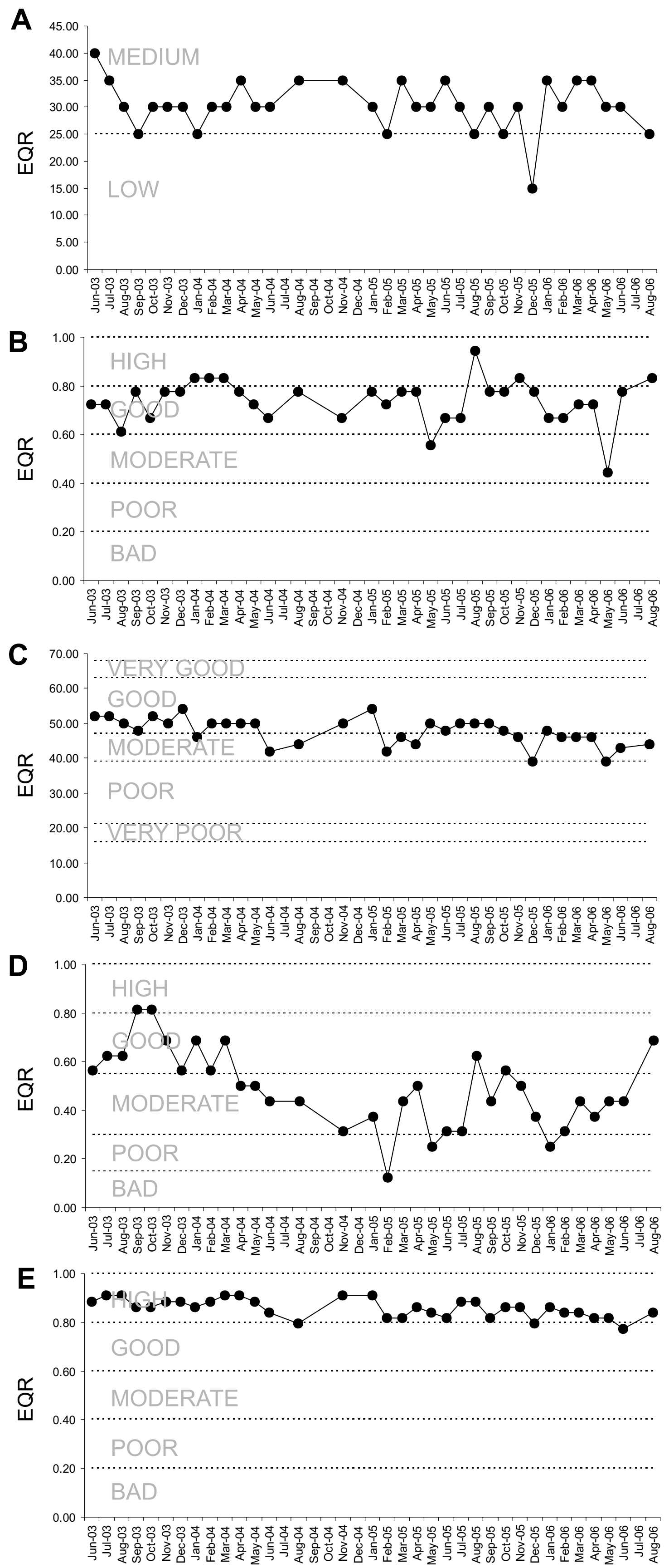

Fig. 4. Classification results of the selected indices (EQR) and correspondent Ecological Quality Status (EQS): (A) EBI – Deegan et al. (1997), (B) EDI – Borja et al. (2004), (C) EFCI – Harrison and Whitfield (2004), (D) EBI – Breine et al. (2007), (E) TFCI – Coates et al. (2007).

Table 7
Kendall tau rank correlation coefficient between the tested indices and conformity between the different methods, given by the percentage of mismatch (relative number of cases in which one of the methods classified a sampling occasion as "High" or "Good" and the other as "Moderate", "Poor" or "Bad" (after Borja et al., 2007)

	EBI Deegan et al. (1997)	EDI Borja et al. (2004)	EFCI Harrison and Whitfield (2004)	EBI Breine et al. (2007)	TFCI Coates et al. (2007)
EBI Deegan et al. (1997)	–	91.18%	73.53%	41.18%	97.06%
EDI Borja et al. (2004)	·0.30*	–	23.53%	55.88%	5.88%
EFCI Harrison and Whitfield (2004)	0.21	·0.01	–	44.12%	23.53%
EBI Breine et al. (2007)	·0.23	0.41*	0.22	–	61.76%
TFCI Coates et al. (2007)	0.09	0.06	0.64*	0.31*	–

* Significant values for $P < 0.05$.

that this last index should be used with cautiously, considering the Mondego estuary fish database.

4.2. Sampling methodologies

One of the main problems in applying and comparing the selected indices is the discrepancy in sampling methodologies, which included gillnetting, beam and otter trawling, deployment of fyke and seine nets, all with different efficiencies, catch rates and sampling efforts. An ideal approach for the implementation of the WFD would be a multi-method sampling regime, in agreement with Coates et al. (2007), since the particular limitations of one sampling gear would be surpassed by other. This, however, would have high costs implicated for the EU Member States, in terms of sampling gears and facilities, human and time resources.

In the specific case of the Mondego estuary, Leitão et al. (2007) found that otter trawl samples did not collect as many species as beam trawl samples, due to restrictions in operating the otter trawl imposed by the lower depths of the upstream areas. Also, the beam trawl is one of the most extensively methods used for scientific sampling of estuarine fish assemblages (Hemingway and Elliott, 2002), being possible to estimate the area covered by each trawl, in opposition to seine nets. However, and according to Coates et al. (2007), the beam trawl is likely to produce samples with lower relative scores than the seine net and otter trawl because it targets benthic fish communities, since it is a much more discriminative technique than the other methods, capturing lower species diversity. In spite of the sampling method used (or a combination of more), it should be standardized the units in which data is converted (e.g. catch per unit effort (CPUE), number of individuals per unit area), enabling to test and compare different methods in the future.

4.3. Definition of guilds

An important component of fish-based indices is the functional guild analysis and classifications. Nonetheless, and due to different classification schemes, some variation occurred between indices, with some species being differently classified in the various approaches and others not assigned to particular guilds. This could be corrected by building an European database of fish species allocated to respective functional guilds (ecological, feeding, vertical preferences, among others), using the recently reviewed and generally accepted concepts of the guild approach for categorizing fish assemblages by Elliott et al. (2007).

Also, it is known that some species can have ontogenic variations at different latitudes, thus being included in different guilds, which should be taken into account. As an example, flounder (*P. flesus*) is classified as a resident species in the UK (Elliott and Dewailly, 1995), while in southern Europe is classified as species that uses estuaries as nursery areas (Leitão et al., 2007; Martinho et al., 2007a). Thus, it is recommended that a standardized guild approach should take place, reducing the variability between indices and according to Elliott et al. (2007), presenting an opportunity to compare and contrast estuarine and other transitional habitats worldwide.

4.4. Monitoring

According to the WFD guidance, the minimal monitoring frequency for the fish component of transitional waters should be once every three years, which may have little biological relevance, being probably inconsistent in terms of natural spatial and temporal variability, management actions or decision making (de Jonge et al., 2006). In agreement, and despite that the majority of the selected methodologies showed a good tolerance to the changes induced by an extreme drought event, such a long time period will probably miss important events that can take place in the highly variable estuarine environment (such as sudden pollution and eutrophication, disease outbreaks or even a synergistic effect of extreme climatic episodes). For the fish component, and since no significant variations were found between seasons, the minimal monitoring frequency should be reduced to once every year. The challenging aspect of the WFD is its holistic approach (de Jonge et al., 2006), by assessing the river basin as a whole (ecosystem-based management); thus, the biological and chemical elements that are being used to assess the Ecological Quality Status of water bodies should ideally have shorter minimal monitoring frequencies (which would certainly also imply a higher effort by the EU member states in terms of budget, time and human resources).

4.5. Conclusions

As a main conclusion it can be stated that despite some variation, all the indices gave consistent results throughout the study period. However, the ones that seem more adequate for an immediate application and assessment of the EQS are the indices by Borja et al. (2004) and by Coates et al. (2007), given the available dataset for the Mondego estuary. Since there is no reference data available, the index by Borja et al. (2004), with a few modifications, adjusting it to a larger size of estuaries, since it was built for small sized estuaries (Borja et al., 2004), is the one that can could be readily used and validated. One of the modifications that would enable this index to be used in a broader scale would be changing the number of species in the metric concerning the total number of species to a percentage of the maximum number of species ever caught in a given estuary, since the number of species in the Basque estuaries (fish + crustaceans or fish only) is quite low, when compared to other transitional waters.

Nevertheless, the high level of mismatch between the selected indices indicates that there is still a great amount of work to be done in the intercalibration process, and concurrently, further comparisons of different indices for the fish component of transitional waters throughout European member states should be encouraged, in order to test their responses in different water body typologies, time series, sampling methods and designs. Furthermore, the determination of the EQS in transitional waters using fish data will be a challenging task, due to the high mobility of fish species, coupled with the unstable environment that characterizes estuaries.

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