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Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the Pertuis Charentais (SW France): A multi-index approach

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Abstract:

The ecological quality status (EcoQ) of intertidal mudflats constrained by Pacific oyster farming was assessed by single (H', AMBI, BENTIX and BOPA) and multimetric (M-AMBI and average score) index approaches in the Pertuis Charentais (SW France). Fifteen sampling stations were monitored seasonally for sedimentological features and macrozoobenthos in 2004. Sediments affected by oyster biodeposits showed organic matter enrichment, and sediments from off-bottom culture sites had higher organic matter contents and lower redox potentials than sediments from on-bottom culture sites. Biotic indices consistently registered responses of macrozoobenthos to organic enrichment but there was only partial agreement between single index-derived EcoQs. The average score was better than M-AMBI and single indices for determining EcoQs. Accordingly, oyster farming alters intertidal macrozoobenthic assemblages moderately, and off-bottom cultures cause more disturbance than on-bottom cultures. Hydrodynamics and seasons may interact with culture practices in smothering/strengthening biodeposition-mediated effects through dispersal/accumulation of biodeposits.

Keywords: Oyster farming practices; Intertidal mudflats; Biotic index; Benthic macrofauna; Seasonal variations; Exposed/sheltered

1. Introduction

Since the pioneering review of Pearson and Rosenberg (1978), numerous attempts have been made to quantify the respective effects of natural and man-induced disturbances on coastal environments. This is a major issue in marine science and has led to the description of new methods to characterise macrozoobenthos responses to organic enrichment. One example is the biotic index of Glémarec and Hily (1981) based on the recognition of five ecological groups within an ecological succession and their various responses to increasing levels of organic matter. As an alternative approach, Warwick (1986) suggested the use of ABC curves as a new tool to discriminate undisturbed from disturbed benthic assemblages. The European Water Framework Directive (WFD) in 1999 emphasised the need to monitor and assess the ecological quality (EcoQ) of coastal, estuarine and continental waters. The overall objective of the WFD is to achieve a “good ecological quality status” for all water bodies in Europe by 2015. The implementation of the WFD has generated a fruitful debate amongst marine scientists about how to establish efficient, reliable bio-assessment tools. As a result, several macrozoobenthos-based biotic indices have recently been proposed as ecological indicators (e.g., Borja et al., 2000; Simboura and Zenetos, 2002; Grall and Glémarec, 2003; Rosenberg et al., 2004; Dauvin and Ruellet, 2007; Muxika et al., 2007). The community succession in a gradient of organic enrichment proposed by Pearson and Rosenberg (1978) is the theoretical background of these indices. AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002) and BOPA (Dauvin and Ruellet, 2007) indices are therefore based on the classification of species (or groups of species) into several ecological groups representing specific sensitivity levels to disturbances. They have been used in a wide range of marine and coastal ecosystems (e.g., Muxika et al., 2005; Labrune et al., 2006; Dauvin et al., 2007; Blanchet et al., 2008) and in situations involving various impact sources including sewage outfalls (Glémarec and Hily, 1981), oil spills (Gomez-Gesteira and Dauvin, 2000) and dredging operations (Grall and Glémarec, 2003).

During the last 30 years, aquaculture in marine waters has greatly increased partly driven by the need for greater self-sufficiency in marine food production. However, it is now stated that aquaculture activities cause environmental disturbances (e.g., Chamberlain et al., 2001; Kaiser, 2001; Carvalho et al., 2006). Numerous studies have demonstrated that mussel culture and fish cage aquaculture modify both sedimentary characteristics and macrofaunal assemblages (e.g. Sauriau et al., 1989; Grant et al., 1995; Drake and Arias, 1997; Chamberlain et al., 2001; Stenton-Dozey et al., 2001; Hartstein and Rowden, 2004; Carvalho et al., 2006; Sanz-Lazaro and Marin, 2006). However, there have been very few studies on the effects of oyster cultures on intertidal habitats (Castel et al., 1989; Nugues et al., 1996; De Grave et al., 1998; Kaiser, 2001; Forrest and Creese, 2006). As a consequence of their filtration capabilities, oysters can reject huge amounts of pseudo-faeces and faeces (Deslous-Paoli et al., 1992). These biodeposits mostly accumulate on sediments beneath oyster cultures (Ottman and Sornin, 1985) and/or in their vicinity (Mitchell, 2006). Oyster biodeposition may thereby elevate intertidal mudflat levels (Ottman and Sornin, 1985; Bertin et al., 2005), enrich seabed sediments with organic matter (Sornin et al., 1983; Mitchell, 2006), and alter the physical structure and geochemical functioning of the sediments involved (Feuillet-Girard et al., 1988; Bouchet et al., 2007). The extent of these effects mainly depend on the hydrodynamic features of the culture site but may also depend on oyster culture methods (Goulletquer and Héral, 1997). In the Pertuis Charentais (SW France), oysters have traditionally been cultivated directly on the sediment, hereafter called on-bottom culture, but currently the commonest technique is on rack culture, hereafter called off-bottom culture: this involved the oysters being placed in plastic mesh bags tied to metal trestles. The presence of trestles arranged in parallel rows in the intertidal area (Goulletquer and Héral, 1997) significantly reduces the strength of tidal currents (Nugues et al., 1996). This limits the dispersal of pseudo-faeces and faeces in the water column and thus increases the natural sedimentation process by several orders of magnitude (Ottman and Sornin, 1985).

The adverse effects of aquaculture-derived organic matter loads on subtidal benthic assemblages are known (e.g., Crawford et al., 2003), so in view of the features of on-bottom and off-bottom culture methods, it is plausible that off-bottom cultures cause more disturbance than on-bottom cultures to intertidal benthic environments. Here, we report an analysis of this issue. We studied macrozoobenthos-based biotic indices and environmental sedimentary variables to determine the effects of oyster culture practices on the ecological quality status of intertidal areas within the Pertuis Charentais (SW France).

Among the six biotic indices commonly used i.e. H’ (Molvaer et al., 1997 in Vincent et al., 2002), AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002), BOPA (Dauvin and Ruellet, 2007), M-AMBI (Muxika et al., 2007) and the average score (Dauvin et al., 2007), four — AMBI, BENTIX, BOPA and M-AMBI — were originally proposed to assess disturbances in marine benthic communities. In this study, their use to describe the EcoQ status of sheltered intertidal mudflats with oyster cultures may lead to biased conclusions due to non-calibrated thresholds and/or difficulties in detecting additional impacts caused by oyster biodeposits on benthic species naturally adapted to high levels of organic matter and hypoxic conditions. Indeed, little is known about their applicability to assessing the potential impact of oyster-processed organic matter accumulated on bottom-sediments. Also, analysis of previous benthic surveys of soft-bottom coastal and transitional waters was hampered by problems of only partial agreement between the various biotic indices of ecological quality status, particularly for intertidal environments in semi-enclosed bays (Blanchet et al., 2008). This appears to constitute a practical limitation for the use of current macrozoobenthos-based biotic indices for implementation of the WFD to various European biotopes.

Therefore, this study aimed to compare single biotic indices and a multi-index approach in order to assess the ecological status of intertidal areas influenced by oyster aquaculture, taking into account the seasonal variability. This will allow determining the more adequate approach to evaluate the ecological status of intertidal mudflats in the Pertuis Charentais.

2. The study area

The Pertuis Charentais are located on the French Atlantic coast north of the Gironde estuary (Fig. 1). The southern part of the Pertuis Charentais, i.e. the Marennes-Oléron Bay, is a semi-enclosed bay with large intertidal areas dominated by muddy and sandy-mud sediments on its eastern and western sides, respectively (Sauriau et al., 1989). Two rivers flow into Marennes-Oléron Bay, the Charente with outputs ranging from 10 to 400 $m^3 s^{-1}$ and the Seudre with flow rates of only 1-40 $m^3 s^{-1}$ (Soletchnik et al., 1998). Oyster parks also extend to the northern part of the Pertuis Charentais, mainly on the northern coast of Ile de Ré, whose sheltered habitats are characterised by muddy and muddy-sand sediments (Faure, 1969).

Since the mid-1970s, most of the intertidal areas have been used for the cultivation of the Pacific oyster *Crassostrea gigas* (Thunberg) after the massive mortalities of the Portuguese oyster *Crassostrea angulata* (Lamarck) (Goulletquer and Héral, 1997). The Pertuis Charentais are the Europe’s largest production area for the Pacific oyster: the standing stock was estimated to be *ca* 125,000 tonnes and the annual production 38,000 tonnes in 2001 (Goulletquer and Le Moine, 2002). Within the Pertuis Charentais, oyster parks are spread over 4,000 hectares of leasing grounds. Less than 10-15% of the intertidal areas occupied by oyster culture is used for on-bottom culture (O. Le Moine, personal communication), the other intertidal areas being dominated by off-bottom culture. Ongrowing oyster parks are located at low tidal levels in the intertidal zone, such that the oysters are subjected to > 50 % immersion time. Biodeposition rates of Pacific oysters, in response to seasonal changes in the amount of suspended particles (Malet et al., 2007), have been estimated to reach a constant mean value of 8.9 mg l^{-1} g DW^{-1} (Deslous-Paoli et al., 1992).

3. Materials and methods

3.1. Sampling strategy

A network of 15 sampling stations was established within the Pertuis Charentais and sampling was carried out at low tide in March, June, September and December 2004. Locations of the sampling stations (Stns) allowed us to take into account the spatial variability occurring in the Pertuis Charentais and to test different environmental conditions and culture practices (Table 1). The sampling stations were located in five intertidal mudflats harbouring oyster parks actively used by shellfish operators: Rivedoux (R), Yves (Y), Charente (CH), Daires (D) and les Traires (LT) (Fig. 1). The use of five intertidal mudflats allowed comparisons of sheltered (R3, R4, R5, CH, D) *vs* exposed (Y, LT) mudflats and off-bottom (R, CH, D, Y1, Y2, Y3) *vs* on-bottom (Y4, LT) culture practices. Differences in the salinity regime between Yves and Charente mudflats are determined by the dispersal of the Charente river plume, therefore Charente mudflats are more subjected than Yves mudflats to low salinities (Fig. 1).

3.2. Sedimentological data

Grain size distributions of sediment collected at each sampling site in March 2004 were analysed using a laser granulometer (Malvern Instruments; grain size from 0 to 800 µm). Grain-size data were processed using the program GRADISTAT® v4.0 (see Blott and Pye, 2001 for details and definitions).
On each sampling date, *in situ* sediment redox potential (*Eh*) was determined using a Cyberscan pH 300 series probe (EUTECH Instruments) at 1 cm below the water-sediment surface of sediment cores sampled at low tide. Measurements were made immediately through 1 cm-spaced holes predrilled into the core tube.
For organic matter analysis, the top first centimetre of three sediment cores (73 mm in diameter) was sliced off and homogenized. The percentage of sedimentary organic matter (SOM) was measured by the method of weight loss upon ignition. Aliquots of sediment were dried at 70 °C for 48 hours to obtain dry weights (DW). Ash dry weights (ADW) were then obtained by incineration at 450 °C for 4 hours; SOM is expressed as follows: SOM (%) = [(DW – ADW) / DW] * 100.

3.3. Biological data

On each sampling date, macrofauna was sampled using three cores (30 cm long, 9.5 cm in diameter). Each core was sieved through a 500 µm mesh and macrofauna specimens were fixed in 4% buffered formalin. Where possible, specimen were identified to the species level and counted. Binomial Latin names of species and authors of the first description were checked according to the European Register of Marine Species hosted at http://www.marbef.org.

3.4. Data analysis

3.4.1. Biotic indices

The Shannon-Wiener index (H', $\log_2$) is the diversity index most commonly used in benthic ecology. It was used as an indicator of EcoQ as described by Molvaer et al. (1997 in Vincent et al., 2002).
The AMBI index (Borja et al., 2000) is based on the proportions of the five ecological groups (EG). The software available at http://www.azti.es and a list that includes >2700 benthic species and their assignments to one EG were used to compute this index. BENTIX (Simboura and Zenetos, 2002) recognises only two EG of the five EGs used by the AMBI index, corresponding to EGs I and II (sensitive species) and EGs III-V (opportunistic species). BENTIX methodology and details concerning species scores are hosted at http://www.hcmr.gr/english_site/services/env_aspects/bentix.html. BOPA (Dauvin and Ruellet, 2007) is not based on the same ecological model of sensitivity/tolerance of species to increasing organic matter input. This index was primarily developed to assess impact of oil spills on benthic communities, as amphipods, the main component of BOPA, are recognised to be sensitive to hydrocarbons (Gomez-Gesteira and Dauvin, 2000). It considers the ratio between opportunistic polychaetes (i.e. polychaetes from EGs IV and V) and amphipods (except those from the genus *Jassa*) as an indicator of environment quality. It was developed to respect the principle of taxonomic sufficiency by using only two well-known zoological groups as indicator species. This consequently limits any misclassification of taxa caused by inclusion of too many ecological groups.
M-AMBI (Muxika et al., 2007) combines AMBI, H' and S (the species richness) in both factor and discriminant analyses. The software available at http://www.azti.es was used for index computation. The reference conditions required for M-AMBI were established using values of S, H' and AMBI from the reference station (R1) at Rivedoux. The highest species richness value (12), the highest H' value (3.34) and the lowest AMBI value (0.64) were used to define High EcoQ conditions, and null values for both H' and S together with and AMBI score of 6 were used to define Bad EcoQ conditions. For calculation of the average score (Dauvin et al., 2007; Ruellet and Dauvin, 2007), a score is attributed between 1 and 5 to each of the EcoQs obtained with the five indices (from High to Bad, respectively). The sum is then divided by the total number of scores.
Threshold values separating the EcoQs given by H', AMBI, BENTIX in both sandy and muddy sediments, BOPA and M-AMBI, and the average score are summarised in Table 2. Replicates data were used for the calculation of biotic indices. The robustness of the AMBI index can be affected when only a very small number of taxa (1-3) and/or individuals (<3 per replicate) are found in a sample (Borja and Muxika, 2005). In such a case, the index was not calculated. To be consistent, we applied this recommendation to the calculation of the other indices used in this study.

3.4.2. Statistical treatment of data

Two-way analyses of variance with replication and fixed effects were performed to test the null hypothesis that seasons (March, June, September and December) and culture practices (off-bottom *vs* on-bottom) did not affect either redox potential (*Eh*) or sedimentary organic matter content (SOM). All data were tested for homoscedasticity (homogeneity of variance) prior to ANOVA using the Bartlett's test (Zar, 1984). The numbers of off-bottom and on-bottom sampled sites were not equal, and this leads to an unbalanced design, which was analysed using the GLM procedure of the MINITAB package release 15. The non-parametric Friedman's test was used to test the null hypothesis that ranks assigned to *Eh*, SOM and biotic index values (replicates data) were ordered in a similar way with seasons irrespective of sampling station; in this non-parametric test, season was considered to be the treatment and sampling station the block. Pearson's correlation tests were used to determine the links between biotic indices and environmental variables (average values per station per season). Correlations between M-AMBI and average score (average values per station per season) were estimated through the non-parametric Kendall's coefficient of rank correlation because it is adapted to ranking data as the average score. The correlation coefficient τ was corrected for ties according to Zar (1984).

Mean annual values of sediment data were used for non-metric multidimensional scaling (nMDS) to assess how stations were distributed relative to each other (normalized Euclidean distance was used for similarity matrix calculation, without any transformation or standardization). Station groups were selected by observations from the dendrogram produced by cluster analysis of the H', AMBI, BENTIX and BOPA annual mean values for each station. Euclidean distance correlation coefficients were used to measure similarities and Ward's linkage method was used to arrange pairs and groups into hierarchic dendrograms. Correlation, nMDS and cluster analyses were performed with PAlaeontological STatistics (PAST v1.80) (Hammer et al., 2001).

An adaptation of the methodology proposed by Blanchet et al. (2008) was used to determine agreement / disagreement between the EcoQs derived from the four biotic indices. "Acceptable" status (i.e. High or Good EcoQ) was scored as '1' and "Not acceptable" (i.e. Moderate to Bad EcoQ) as '0'. The scores given by each biotic index were summed for each station (potentially range from 0 to 4). This sum allowed assessment of the level of agreement/disagreement between the biotic indices (see Blanchet et al., 2008 for more details).

4. Results

4.1. Sediment

Mean grain size was smaller in the stations affected than not affected by oyster cultures (Fig. 2A). Sediments at the sampling stations were classified as mud, except for Stn LT2 (sandy-mud) and Stn R1 (fine sand).

Eh differed significantly according to rearing method (two-way ANOVA, $p < 0.05$) with higher positive values recorded at on-bottom than off-bottom culture stations; off-bottom culture stations which were characterised by hypoxic conditions most of the time. Seasonal variations were highly significant (two-way ANOVA, $p < 0.01$); however, there was no significant interaction between rearing methods and season (two-way ANOVA, $p = 0.21$) indicating similar temporal changes for both off-bottom and on-bottom culture stations. Friedman's test was significant ($p < 0.01$) confirming the seasonal trend at all sampling stations with more oxic conditions in December and more hypoxic conditions in September and June (Fig. 2B).

Sedimentary organic matter contents were not influenced by season (two-way ANOVA, $p = 0.77$) but significant differences were observed between the two rearing methods with lower values in on-bottom than off-bottom sediments (two-way ANOVA, $p < 0.05$). Considering all the sampling stations, organic matter contents were lower in sediments from the reference station (Stn R1; SOM 0.87%) than in sediments from on-bottom (5%) and off-bottom culture stations (8-9%). Stn LT2 had intermediate SOM values (4%) and Stn R2 located at the edge of the oyster park zone of Rivedoux had lower SOM values than the Stns R3, R4 and R5 (i.e. 3% *vs.* 7-8%).

4.2. Macrofauna: composition and ecological groups

Within the sampling stations, 81 macrofaunal species were identified. Sensitive species of the EG I (52%) i.e. *Cyclope neritea* (Linnaeus) and *Urothoe poseidonis* Reibish and both indifferent and tolerant species of the EGs II (24%) and III (18%) dominated at the reference station (Stn R1; Table 2).

Macrofaunal assemblages from on-bottom culture stations (Stns LT1 and Y4) were a mixture of sensitive, tolerant and opportunistic species. At Stn LT1, *Euclymeme oerstedi* (Claparède) of EG I, *Notomastus latericeus* Sars of EG III, *Aphelochaeta marioni* (de Saint-Joseph), *Heteromastus filiformis* (Claparède) and *Pseudopolydora antennata* (Claparède) of EG IV were dominant. At Stn Y4, *Mesopodopsis slabberi* (van Beneden) of EG II, *Prionospio malmgreni* Claparède, and *Tharyx multibranchiis* (Grube) of EG IV were dominant. Macrofaunal assemblages from on-bottom culture stations were thus dominated by species from the EGs III and IV: species from these two ecological groups represented more than 65% of the total abundance (Table 2).

Both tolerant and opportunistic species of crustaceans and annelids from the EGs II to V, i.e. *Mesopodopsis slabberi* of EG II, *Streblospio shrubsolii* (Buchanan) of EG III, *Aphelochaeta marioni*, *Prionospio malmgreni*, *Pseudopolydora antennata* and *Tharyx multibranchiis* of EG IV and oligochaetes including *Tubificoides benedii* (Udekem) of EG V were the dominant species at off-bottom cultures stations (Stns R2 to R5, Y1 to Y3, CH1 to CH3 and D1). Their macrofaunal assemblages were constituted by more than 50% of species from EGs III and IV and up to 12% species from EG V (Table 2).

4.3. EcoQ and comparison between biotic indices

All indices ranked the reference station (Stn R1) as High in 75% of EcoQs. Off-bottom culture stations were classified as Good in 32%, as Moderate in 61% and as Poor in 7% of EcoQs whereas on-bottom culture ones were classified as High in 13%, as Good in 38% and as Moderate in 49% of EcoQs.

Exposed stations exhibited better EcoQs compared to sheltered ones. They were classified as High in 13%, as Good in 50% and as Moderate in 37% of EcoQs whereas biotic indices ranked sheltered ones as Good in 18%, as Moderate in 71% and as Poor in 11% of EcoQs.

Several stations exhibited no or slight seasonal variability in biotic indices, *i.e.* the reference station (Stn R1) and Stns LT1, LT2 and Y4, whereas there were large seasonal changes in the other stations (Fig. 3A, B, C and D). However, there was no significant trend in H' (Fig. 3A), AMBI (Fig. 3B), BENTIX (Fig. 3C) and BOPA (Fig. 3D) values ranked by seasons for all the sampling stations (Friedman's test, $p = 0.48$, $p = 0.07$, $p = 0.59$ and $p = 0.58$, respectively). This may indicate that the variability in biotic indices is driven by the interplay between local environmental conditions rather than by the seasonal cycle alone.

Assessments of EcoQs derived from H', AMBI, BENTIX and BOPA indices partially matched but were in some cases contradictory. EcoQs determined by H' and AMBI coincided for 47% of the sampling stations, for H' and BENTIX for 13% of the stations and for H' and BOPA for 60%. There was 60% of full agreement between AMBI and BENTIX derived-EcoQs, 60% for AMBI and BOPA, and 34% for BOPA and BENTIX EcoQs. Using the method proposed by Blanchet et al. (2008), disagreement in the classification of EcoQs based on the four single biotic indices tested never exceeded 25% of all the sampling stations in any one season, and full agreement rose 68% in June (Fig. 4A). Based on the mean annual values of the single biotic indices, EcoQs disagreed on the status of only 27% of the stations (Fig. 4B); they agreed fully for 27% and partially for 47% of the stations. All these biotic indices agreed that the best EcoQs were at the reference station R1 and the worst at off-bottom culture stations, where they ranged from Bad to Poor depending on the index (Fig. 3).

M-AMBI values from 0.1 to 0.9 were recorded with no seasonal variability for the reference station R1 but a large seasonal variability for several off-bottom culture stations, particularly Stns CH2, CH3 and D1 (Fig. 5A). The EcoQ of the reference station R1 was always High, whereas those of off-bottom culture stations with high seasonal variability ranged from Bad to High. Friedman's test revealed a significant ($p < 0.05$) influence of season on all the sampling stations with highest and lowest ranks, and consequently EcoQs, recorded in September and June, respectively. Consistent with the single biotic indices, no trends in average score values ranked by season were observed between sampling stations (Friedman's test, $p = 0.43$). The average score was the lowest for the reference station (Stn R1) and most of off-bottom culture stations exhibited a large seasonal variability, which induced their respective EcoQs to range from Poor to Good although those of on-bottom culture stations remained

Moderate to Good. M-AMBI and the average score classified 60% of the stations into similar EcoQs (Figs. 5A and 5B). Both indices classified Stn R1 as High, Stns LT2, LT1, Y1, Y3 and CH3 as Good and Stns R5, R4 and D1 as Moderate (Fig. 5C). There were disagreements only for Stns Y4, Y2, R2, R3, CH1 and CH2, which were ranked with a better EcoQ by M-AMBI (Good) than by the average score (Moderate). Results from M-AMBI and average score were, however, highly significantly correlated (Kendall's τ = -0.61, $p < 0.01$). Disagreements between M-AMBI and average score were not influenced by the level of sea exposure; three stations for which there was disagreement were in exposed habitats (Stns Y4, Y2 and R2) and three in sheltered habitats (Stns R3, CH1 and CH2; Fig. 5C).

Three distinct high-level clusters (A, B and C) were identified by the cluster analysis, which is based on single index results at each sampling station (Fig. 6). The reference station at Rivedoux (Stn R1: cluster A) was isolated from the other stations. The second cluster (B) was composed of Stns LT2, LT1, R2, CH2, Y1, Y2 and Y3; all these stations except Stn CH2 are characterised by an exposed habitat, and only Stns LT1 and LT2 are situated in or near on-bottom culture areas. The third cluster (C) contained Stns R3, R4, R5, Y4, CH1, CH3 and D1; all these stations are located in or close to off-bottom culture areas except Stn Y4, which is in an on-bottom culture park. The three clusters given by the cluster analysis almost corresponded to the EcoQ classification given by the average score (Fig. 5B). Stn R1 in cluster A had a High EcoQ, all stations in cluster B had Good to Moderate EcoQs, and stations in cluster C had Moderate to Poor EcoQs.

4.4. Relationship between sedimentological data and biotic indices

We examined correlations between sedimentological data and biotic indices. AMBI, BENTIX, BOPA, M-AMBI and the average score were significantly correlated with the mean grain size, redox potential (*Eh*) and sedimentary organic matter contents whereas H' was not (Table 3). The nMDS plot (Fig. 7) revealed obvious differences in the environmental conditions between the reference station (R1) and other stations under the influence of oyster farming. These other stations were clustered into three groups depending on their sheltered *vs* exposed habitats and off-bottom *vs* on-bottom culture practices. The first group was Stns CH1, D, R5, R4, CH3, R3 and Y2, the most sheltered off-bottom sites. They were all ranked as Moderate or Poor EcoQs by the average score (Fig. 5B). The most open-sea on-bottom culture station (LT1 at les Traires) was clustered in a second group with Stns R2, Y1, CH2 and Y3: all exhibited Good to Moderate EcoQs as defined by the average score (Fig. 5B). The last group, was composed of Stn LT2 (near the on-bottom culture station LT1 at Les Traires) and Stn Y4 (on-bottom culture at Yves) were classified as Good and Moderate EcoQs, respectively. Groups of stations obtained from the nMDS analysis (Fig. 7) were very similar to those given by the Cluster analysis (Fig. 6) and the average score (Fig. 5B).

5. Discussion

5.1. Applicability of biotic indices

In this study, all biotic indices except the average score indicated a large temporal variability particularly for off-bottom oyster culture. At five off-bottom culture stations, derived-EcoQ status even swung across four/five classes such that the range was as large as that observed for all stations sampled including the reference station. Variability in biotic indices has been described elsewhere on both temporal (Labrune et al., 2006; Chainho et al., 2007; Dauvin and Ruellet, 2007) and spatial scales (Quintino et al., 2006). The variability we report here was nevertheless higher than the range of variation across three/four EcoQ classes previously recorded on oyster-free sheltered mudflats of the Marennes-Oléron Bay (see Fig. 4 in Blanchet et al., 2008). In contrast, Bazairi et al. (2005) in the Merja Zerga lagoon (Morocco) and Salas et al. (2004) in the Mondego estuary (Portugal) reported no or only slight time-to-time variability in the AMBI index. Our statistical analyses indicate that the observed temporal variability was not driven by the seasonal cycle alone but may reflect complex interactions between abiotic (including temperature, tidal erosion *vs* deposition periods, emersion/immersion cycle, and mud contents) and biotic factors acting at the species population level (*i.e.* organic matter flux, species competition, and population dynamics).

A classic way to test the applicability of biotic indices is to compare their results in undisturbed and disturbed areas (Warwick, 1986; Borja et al., 2000; Rosenberg et al., 2004; Salas et al., 2004; Labrune et al., 2006). As a background to this study, Blanchet et al. (2008) analysed a large data set collected in 1995 at 262 sampling stations outside oyster-culture areas on both intertidal and subtidal areas of the Marennes-Oléron Bay. The biotic indices H', AMBI and BENTIX showed significant differences in the EcoQ of intertidal oyster-free areas with a highly significant correlation with silt and clay contents of bed sediments: sandy habitats getting a High and muddy habitats Good to Moderate EcoQs (Blanchet et al., 2008). Values of biotic indices and derived EcoQ status we report for the reference station at Rivedoux are consistent with the results of Blanchet et al. (2008) and attributed to this station a better EcoQ than any of the other stations, all in oyster parks. This suggests a higher environmental quality at this reference site despite its very close vicinity to oyster culture areas, particularly for computing the M-AMBI index (see Muxika et al., 2007).

In this study, H' failed to differentiate on-bottom from off-bottom oyster-cultures and was also not correlated with sedimentary variables. This may be due to similar dominance patterns of differing species between sampling stations and/or the lack of ecological considerations in the H' formulae. The difficulties of interpreting H' and associated EcoQs have previously been discussed (Labrune et al., 2006; Blanchet et al., 2008). In contrast, BENTIX overestimated the EcoQ status of most of stations, and appeared less discriminating than AMBI and BOPA. This property has already been described and it was suggested than BENTIX is more effective in Mediterranean ecosystems with high diversity, and AMBI is more suitable for Atlantic ecosystems with lower species richness and higher densities (Dauvin et al., 2007). Also, AMBI and BOPA did not give identical EcoQ classifications, because they are not based on the same ecological model of sensitivity/tolerance to pollution, response of species to organic matter input being considered in AMBI (Borja et al., 2000) whereas BOPA considered their response to hydrocarbons (Gomez-Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007). Moreover, and as suggested by Ruellet and Dauvin (2007) using simulations, even AMBI and BENTIX, both biotic indices based on the same ecological paradigm, did not produce the same assessment of the EcoQ of individual samples. However, even if indices disagreed on the precise level of EcoQ assessed to each station, they ranked stations in the same way, differentiating reference station from the others, off-bottom from on-bottom culture ones and exposed from sheltered ones. To overcome this potential problem due to the inherent mathematical nature of these indices, comparison and intercalibration of indices using a large number of stations is required, and general trends given by mean and/or median values should be used (Dauvin et al., 2007; Ruellet and Dauvin, 2007). This also implies that thresholds between EcoQ classes should be adjusted. The M-AMBI index, which combines H', the species richness and the AMBI in one cumulative index had less discriminating power than the average score, despite the use of local reference conditions. M-AMBI was significantly correlated with environmental variables whereas the average score was very significantly correlated (see Table 3). This may explain high level of similarity in EcoQ status attributed to sampling stations between non-metric multidimensional scaling (nMDS) and the average score. Considering the environmental conditions prevailing in the Pertuis Charentais and the source of disturbances, i.e. organic enrichment acting on naturally tolerant paucispecific macrozoobenthic assemblages, it thus appeared that the use of a single biotic index alone does not allow robust measurement of the EcoQ of affected intertidal mudflats. The average score approach proposed by Dauvin et al. (2007) appeared to be a better compromise because it combines several sources of information concerning both the structural and functional responses of benthic macrozoobenthos. However, combining the use of AMBI and BENTIX and BOPA for the calculation of the average score may overweight the information they contained because these indices are based on the same ecological assumption. To overcome this problem, the use of an average score that account for different type of information, to limit redundancy when combining several indices, appears as an alternative (Chainho et al., 2007; Izsak, 2007; Chainho et al., 2008). It is also consistent both with the previous suggestion made by several authors that a multi-criteria approach should be used (see e.g. Borja et al., 2007; Blanchet et al., 2008 and references therein) and with the recommendations of Raffaeli (2006) and Pranovi et al. (2007), who advised bridging the gap between community ecology and ecosystem ecology to assess effects of community structure changes on ecosystem functioning.

5.2. Oyster farming effects in the Pertuis Charentais

Species richness was the highest at Stn R1, the reference station, where species of the EGs I and II *i.e. Urothoe poseidonis* sensitive to pollution and organic matter (Dauvin and Ruellet, 2007) dominated the macrofaunal assemblages in terms of abundance and characterised the High EcoQ of Stn R1.

Some species of the EG III and in particular the arthropoda *Apseudes latreillii* (Milne-Edwards) and the spionids polychaeta *Pygospio elegans* Claparède were also observed at Stn R1. Species of this ecological group are characteristically found in moderately disturbed sediments or environments during the early stage of perturbation (Glémarec and Hily, 1981; Borja et al., 2000; Grall and Glémarec, 2003). However, Sanz-Lazaro and Marin (2006), in their study on macrofaunal assemblages beneath fish cages, reported *A. latreillii* both at their reference site and at sites from which cages had been removed. Our results are consistent with these observations, and indicate that *A. latreillii* do not characterise disturbed sediments. Survival of *P. elegans* is limited by high sedimentation rate (Nugues et al., 1996) and the species did not appear to be well adapted to oyster culture areas where there are high rates of accumulation of pseudo-faeces and faeces (Sornin et al., 1983; Deslous-Paoli et al., 1992; Mitchell, 2006), as supported by occurrences only at Stn R1. Areas with coarser sediment commonly have better EcoQ than those with finer sediments (Muniz et al., 2005); we confirmed this phenomenon for intertidal macrozoobenthic assemblages of the Marennes-Oléron Bay using H', AMBI and BENTIX indices (Blanchet et al., 2008). Moreover, AMBI values for Stn R1 were consistent with those of other undisturbed areas in Europe (Muxika et al., 2005; Reiss and Kröncke, 2005; Simboura and Reizopoulou, 2007). Muxika et al. (2005) also reported that areas in the vicinity of a source of pollution may not be disturbed if hydrodynamics favour dispersion of the pollution in the opposite direction, and this observation agreed both with our data set and previous descriptions of macrozoobenthic assemblages and sedimentological features by Faure (1969) at Rivedoux.

Contrary to the study of De Grave et al. (1998) on oyster farming in subtidal areas, we find an effect of oyster farming in intertidal areas on macrofaunal diversity. Species richness was lower at the sampling stations harbouring off-bottom cultures, as also observed by Nugues et al. (1996) and Castel et al. (1989) for intertidal oyster culture sites. Biotic index values we found for off-bottom cultures were consistent with those observed in other aquaculture areas (Muxika et al., 2005; Sanz-Lazaro and Marin, 2006; Aguado-Giménez et al., 2007). Opportunistics polychaetes of the families *Cirratulidae*, *Capitellidae* and *Spionidae*, which were observed in the sediments of off-bottom oyster parks, characterise disturbed areas enriched in organic matter (Pearson and Rosenberg, 1978; Samuelson, 2001). These species are commonly reported in sediments beneath oyster cultures (Nugues et al., 1996; Mallet et al., 2006), mussel cultures (Stenton-Dozey et al., 2001; Hartstein and Rowden, 2004), and fish cages (Drake and Arias, 1997; Carvalho et al., 2006). The presence of trestles reduces current velocities by 25% (Nugues et al., 1996) and favours the accumulation of biodeposits enriched in organic matter (Sornin et al., 1983); this in turns leads to the impoverishment of oxygen levels within the sedimentary matrix (Hyland et al., 2005). Driven by seasonal and tidal cycles, simultaneous increases in temperature and availability of accumulated organic biodeposits, plus short-term hypoxic periods can lead to active mineralisation of sedimentary organic matter, inducing production of ammonia and sulphur (Vouvé et al., 2000) as we observed at off-bottom culture sites in the Marennes-Oléron Bay (Bouchet et al., 2007). This phenomenom may enhance the adverse effects of accumulated organic matter on benthic assemblages, particularly in sheltered habitats. On the contrary, in exposed habitat, hydrodynamic processes that favour dispersal of biodeposits enriched in organic matter (Chamberlain et al., 2001) may then interact with the effects of oyster farming by locally limiting its negative consequences on benthic environments. The local topographic and hydrodynamic conditions may thus play a major role in smothering/strengthening biodeposition-mediated effects through dispersal/accumulation of biodeposits.

Macrofaunal assemblages from the on-bottom culture station LT1 were composed of 24% of species of the EG I, characteristic of undisturbed areas (Grall and Glémarec, 2003), associated with species of EG III, e.g. *Notomastus latericeus*. Even if on-bottom cultures less disturbed benthic environments, the presence of tolerant species of the EG III indicates that on-bottom culture also contributes to slightly disturb benthic environments as also observed by Drake and Arias (1997). Opportunistic species of the EGs IV and V were, however, less abundant in sediments from on-bottom sampling stations suggesting lower levels of disturbance than at off-bottom sites. However, as observed for off-bottom culture stations, the more sheltered position of Stn Y4 may have been the cause of its low EcoQ relative to Stn LT1; indeed, only 2% of the macrofaunal assemblages at this site were species of the EG I. On-bottom culture parks do not have trestles, and therefore do not modify tidal current velocities. Consequently, accumulation of mud enriched in organic matter and the resulting impoverishment in oxygen levels within the sediment matrix are not encouraged by on-bottom rearing methods. Indeed, this practice may enhance oxygen and carbon fluxes at the water/sediment interface, as reported by Nizzoli et al (2006) in their study of clam and mussel farming. This view is consistent with our data set, which evidences lower organic matter contents and higher level of oxygenation in on-bottom culture sediments, environmental conditions being less stressful for benthic macrofauna.

Conclusion

The use of a multimetric approach, such as the average score, to determine the EcoQ status of intertidal areas subject to oyster farming in the Pertuis Charentais appeared to be more robust than the use of single biotic approach; single biotic indices resulted in misclassifications of sampling station EcoQs. Average score derived-EcoQs indicated that oyster farming lead to moderate disturbances of intertidal benthic environments, as already reported by a small number of studies for intertidal oyster cultivation areas elsewhere. However, in the Pertuis Charentais area, intertidal off-bottom rearing practice had larger effects than on-bottom practices on benthic macrozoobenthos.

A complex interplay between seasonal changes in abiotic and biotic conditions, dispersal characteristics by tidal hydrodynamics and the fate of oyster biodeposits seem to determine the relative temporal stability and/or instability of intertidal benthic assemblages, which react to oyster farming-induced disturbance by changes in the inherent dynamics of constitutive species populations. However, as observed in the Pertuis Charentais, temporal changes of biotic indices within an oyster farming-affected site may be as large as the variability between sites. As suggested by Chainho et al. (2008) for estuarine communities, this implies that the monitoring sampling procedures recommended in the WFD (every 3 years) seems to be also inappropriate in intertidal areas subjected to high seasonal variability.

In a near future, the establishment of offshore oyster production using subtidal areas as well as oyster long-lines to decrease stocking biomasses on intertidal leasing grounds may have positive feedback on intertidal benthic communities in Pertuis Charentais; however, the potential deleterious effects of these new culture practices on surrounding subtidal areas needs to be further assessed.

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Figures

Figure 1

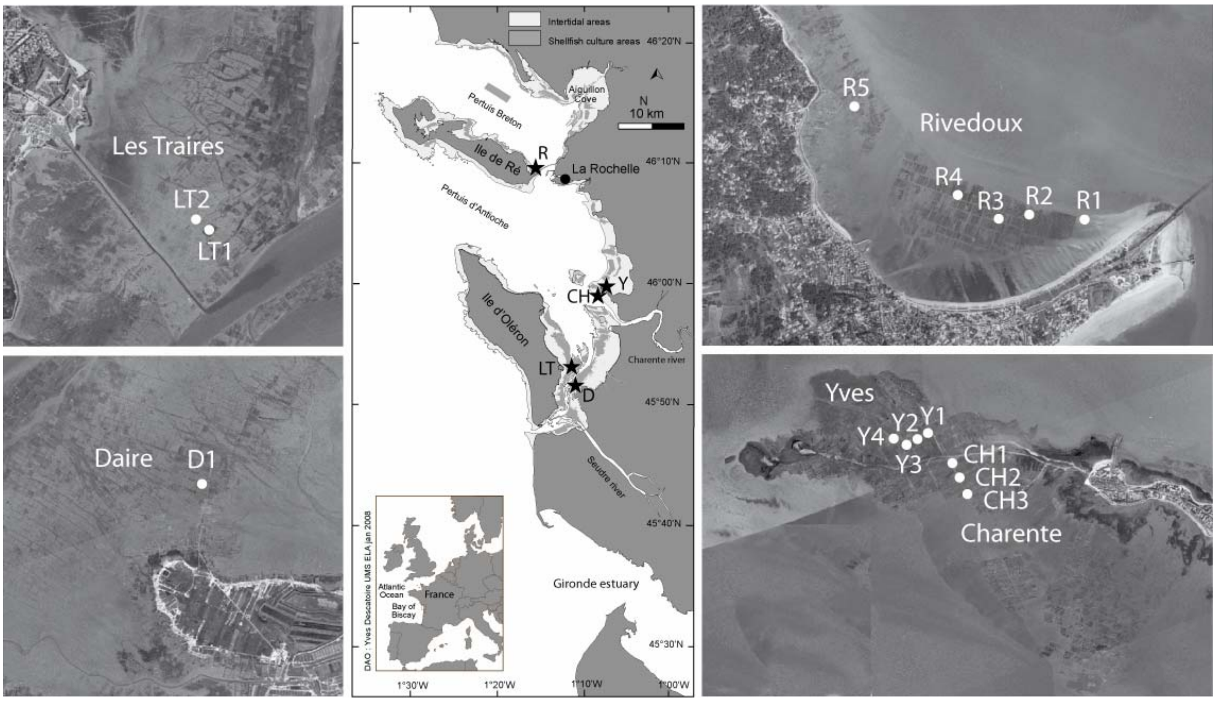
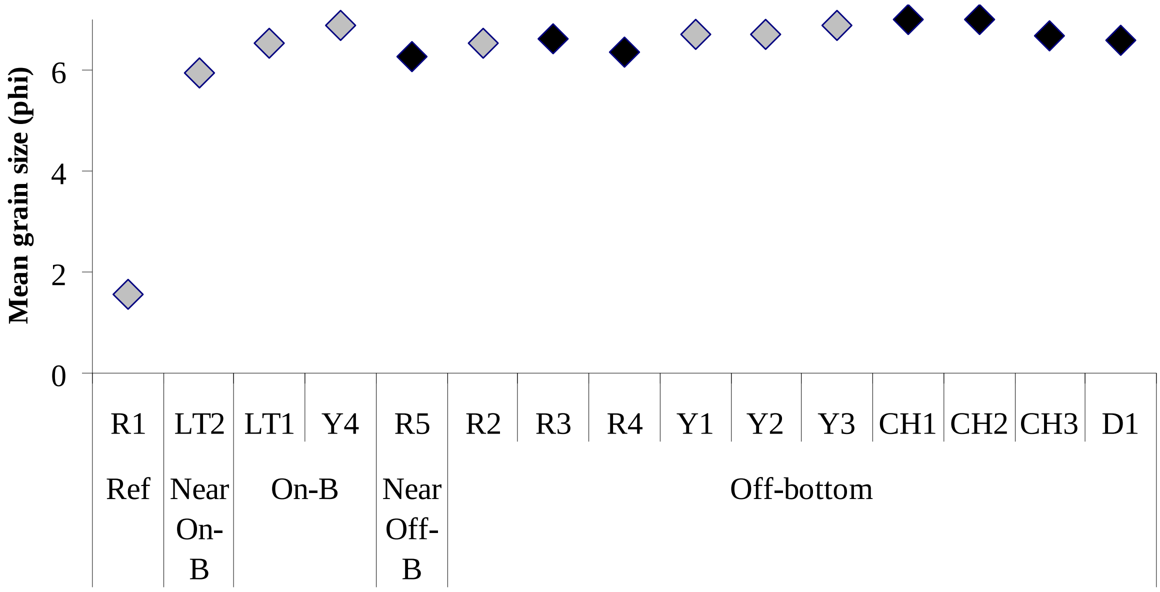


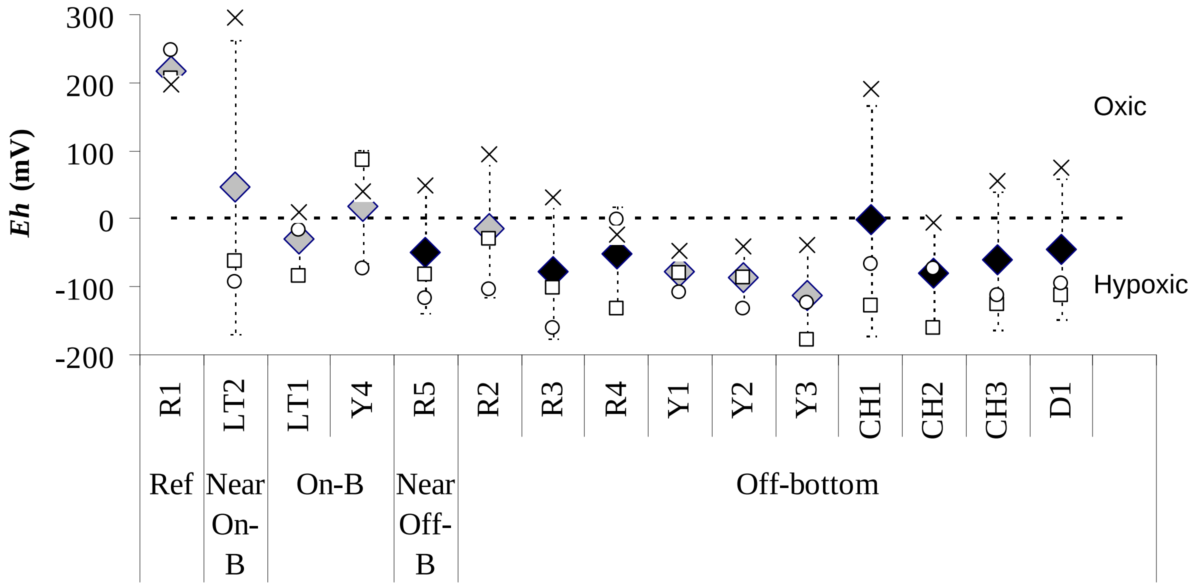
Figure 1: Map of the Pertuis Charentais and location of the five intertidal mudflats (CH: Charente, D: Daires, LT: Les Traires, R: Rivedoux and Y: Yves) with details of the location of their respective sampling stations. Aerial pictures Ortholittoral-2000.

Figure 2



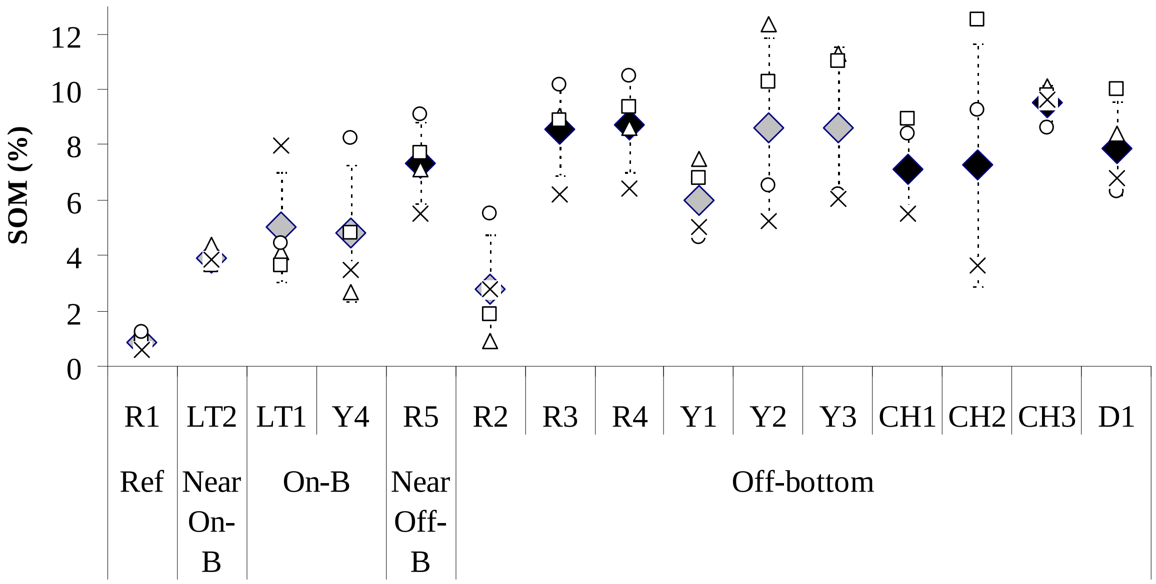
Mean grain size (phi)
0
2
4
6
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

A



Eh (mV)
300
200
100
0
-100
-200
Oxic
Hypoxic
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

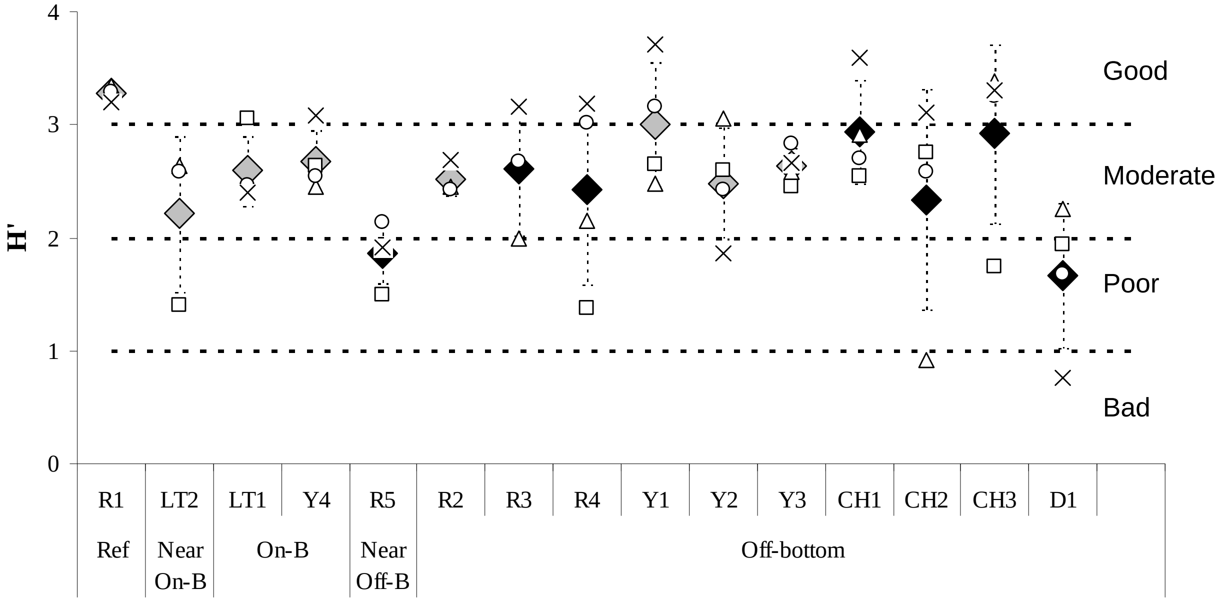
B



C

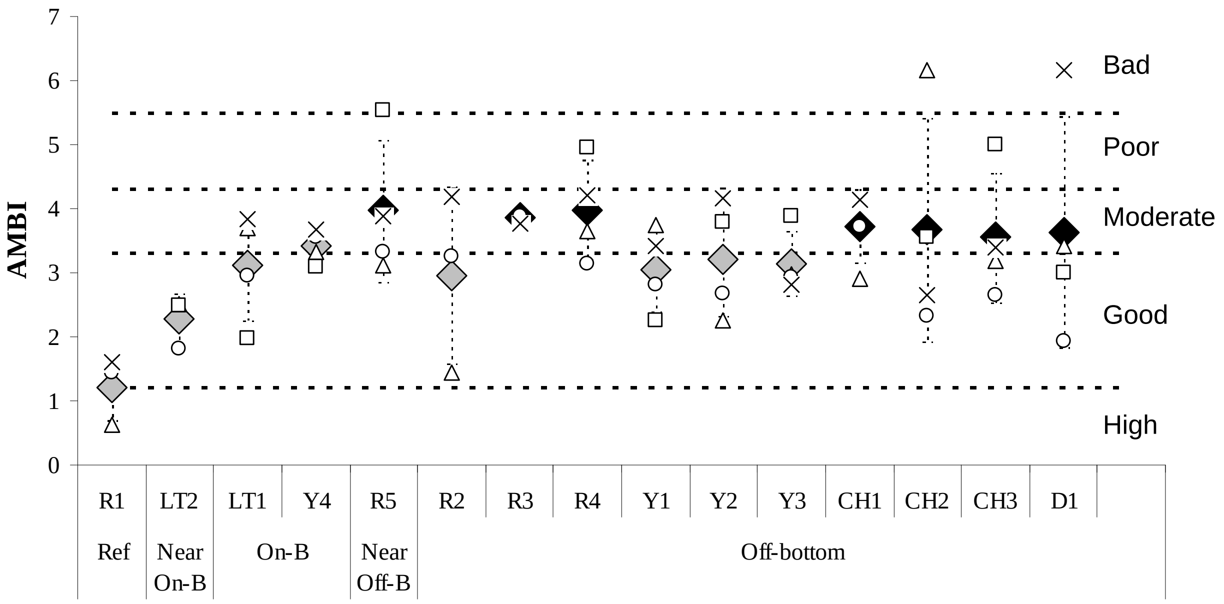
Figure 2: Changes in mean grain size (A), redox potential *Eh* (B) and total organic matter of sediments SOM (C) at each sampling station (bars: standard deviation). Threshold values between oxic/hypoxic conditions for *Eh* were determined according to Teasdale et al. (1998). Triangles: March, squares: June, circles: September, crosses: December and lozenges: mean annual values (grey lozenges: exposed stations and black lozenge: sheltered stations).

Figure 3



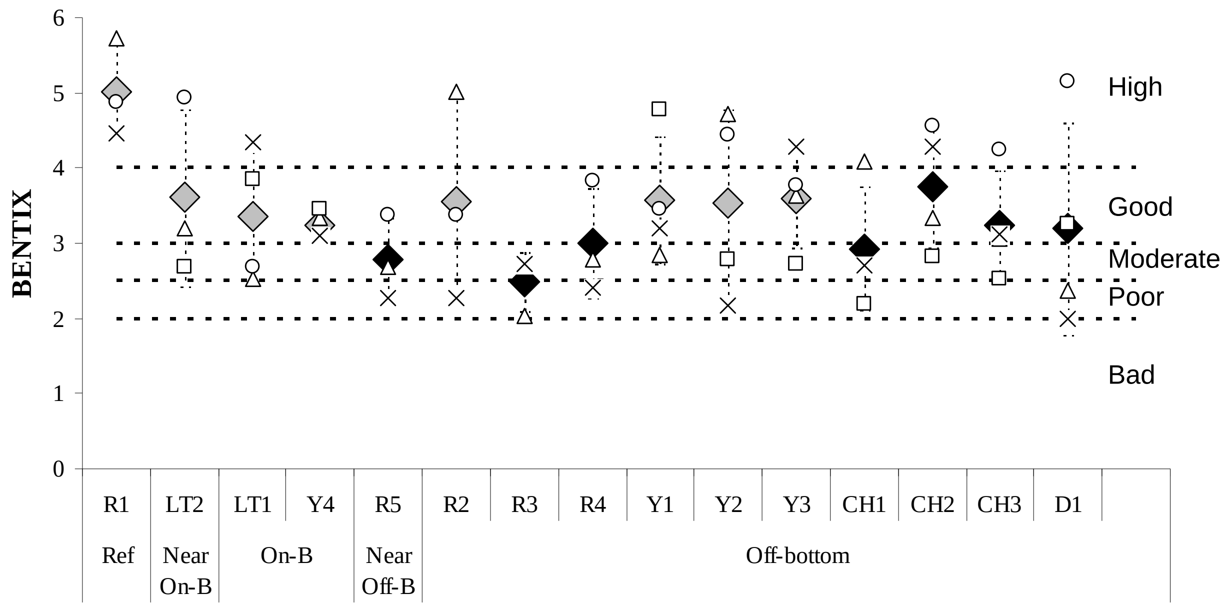
H'
4
3
2
1
0
Good
Moderate
Poor
Bad
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

A

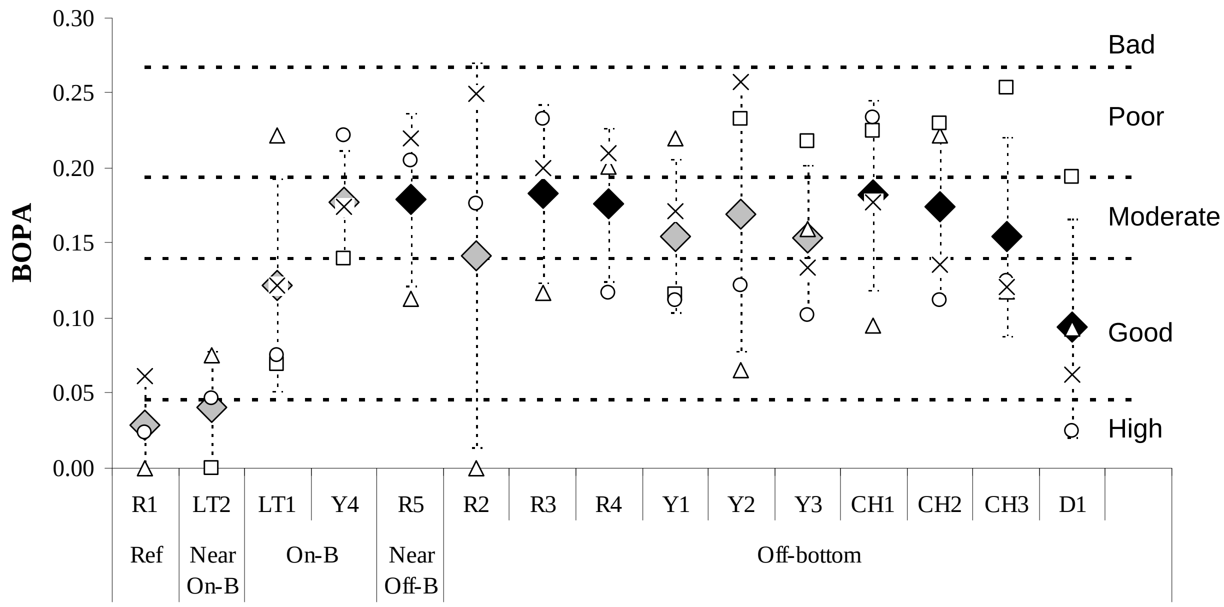


AMBI
7
6
5
4
3
2
1
0
Bad
Poor
Moderate
Good
High
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

B



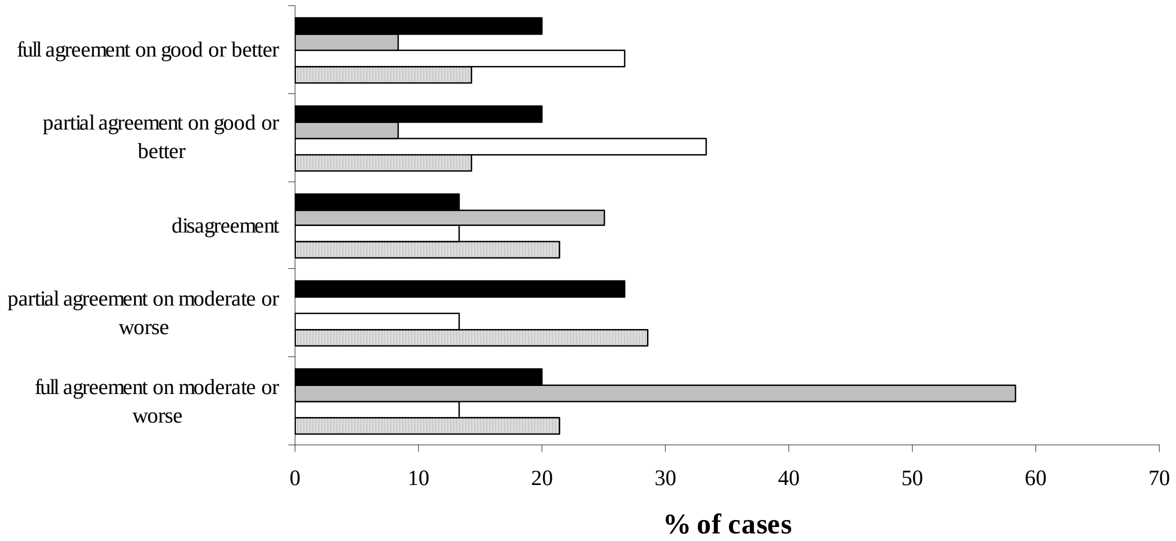
C



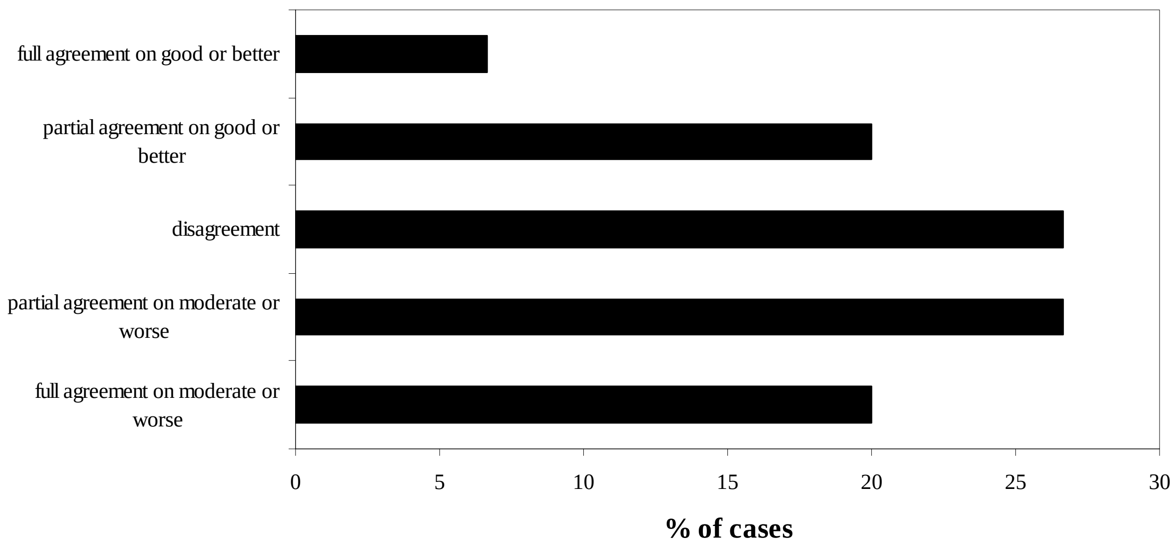
D

Figure 3: Changes in H' (A), AMBI (B), BENTIX (C) and BOPA (D) biotic indices (bars: standard deviation) and ecological quality status (EcoQ) for each sampling station. Triangles: March, squares: June, circles: September, crosses: December and lozenges: mean annual values (grey lozenges: exposed stations and black lozenges: sheltered stations).

Figure 4



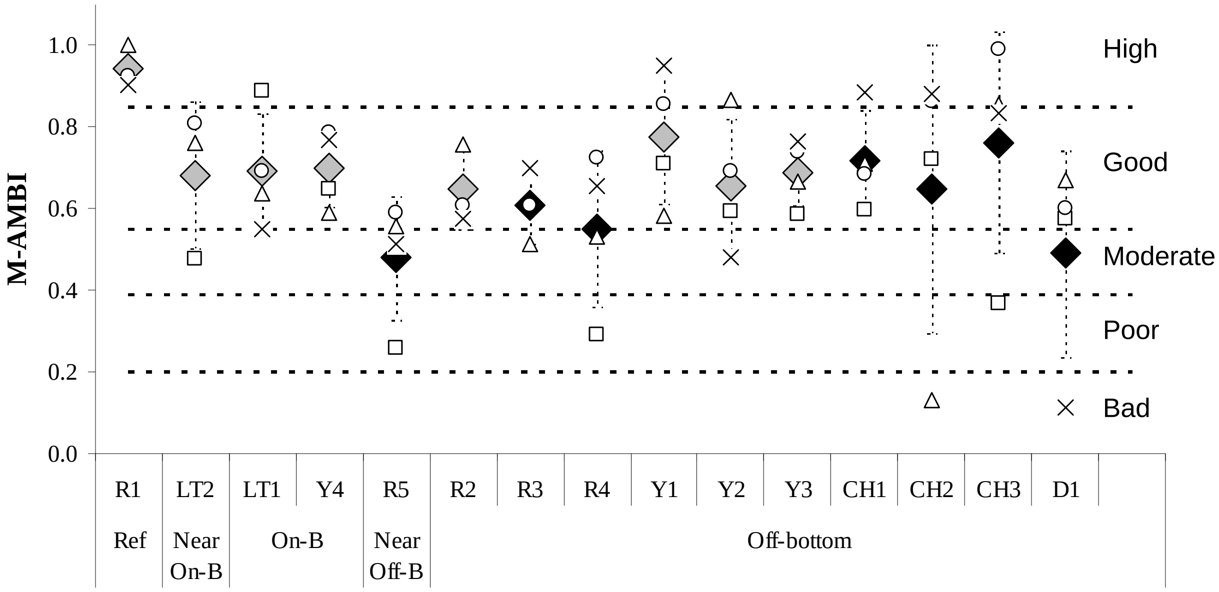
A



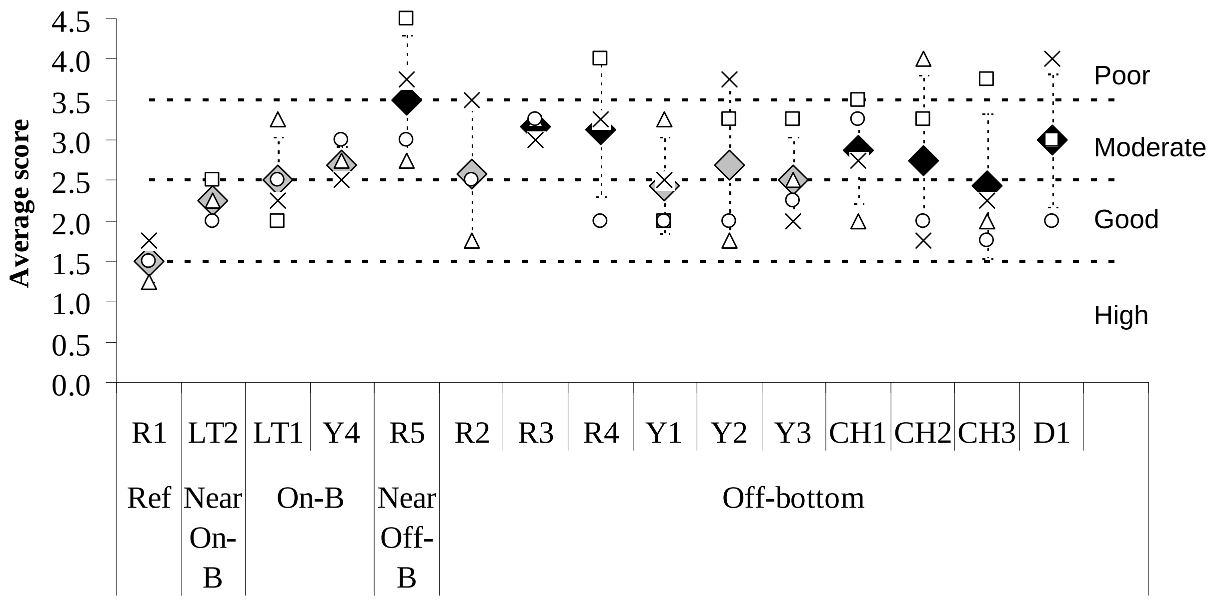
B

Figure 4: Percentage of stations with full agreement, partial agreement and disagreement between EcoQs determined with H', AMBI, BENTIX and BOPA by season (A, black: March, grey: June, white: September and cross-hatched: December) and for the year 2004 (B).

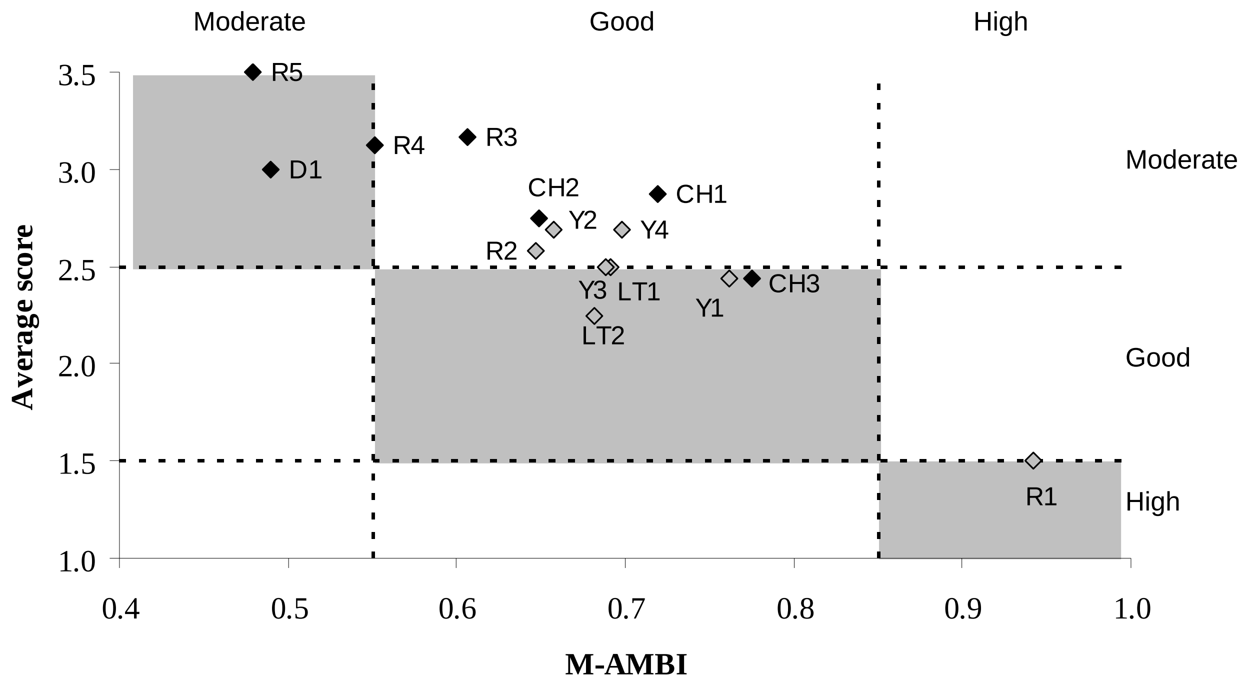
Figure 5


M-AMBI
1.0
0.8
0.6
0.4
0.2
0.0
High
Good
Moderate
Poor
Bad
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

A


Average score
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Poor
Moderate
Good
High
R1
LT2
LT1
Y4
R5
R2
R3
R4
Y1
Y2
Y3
CH1
CH2
CH3
D1
Ref
Near On-B
On-B
Near Off-B
Off-bottom

B



C

Figure 5: Changes in M-AMBI (A) and average score (B) (bars: standard deviation) and linear correlation between average score and M-AMBI (C). Grey symbols: exposed stations and black symbols: sheltered stations.

Figure 6

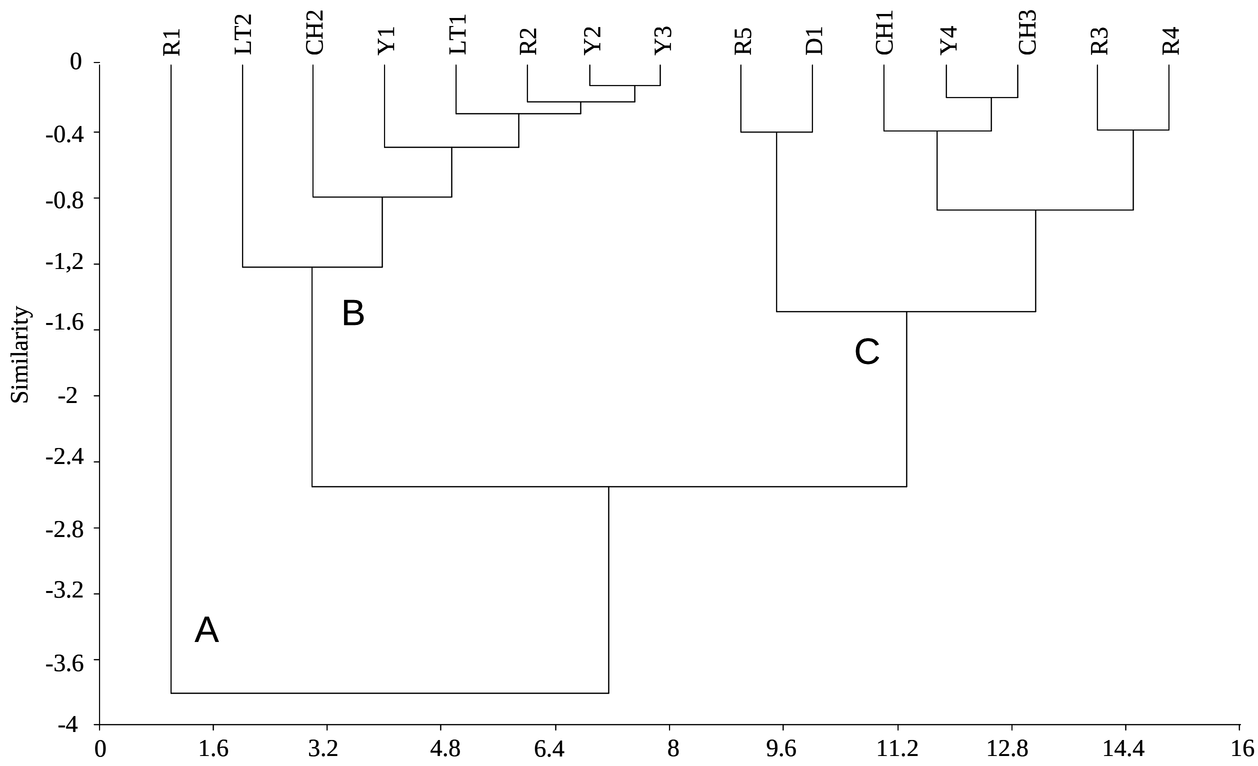


Figure 6: Dendrogram classification of sampling stations produced by cluster analysis using Euclidean distance correlation coefficients and Ward's linkage method (Cut-off value of similarity of -3 and -2 split the stations into three groups of stations).

Figure 7

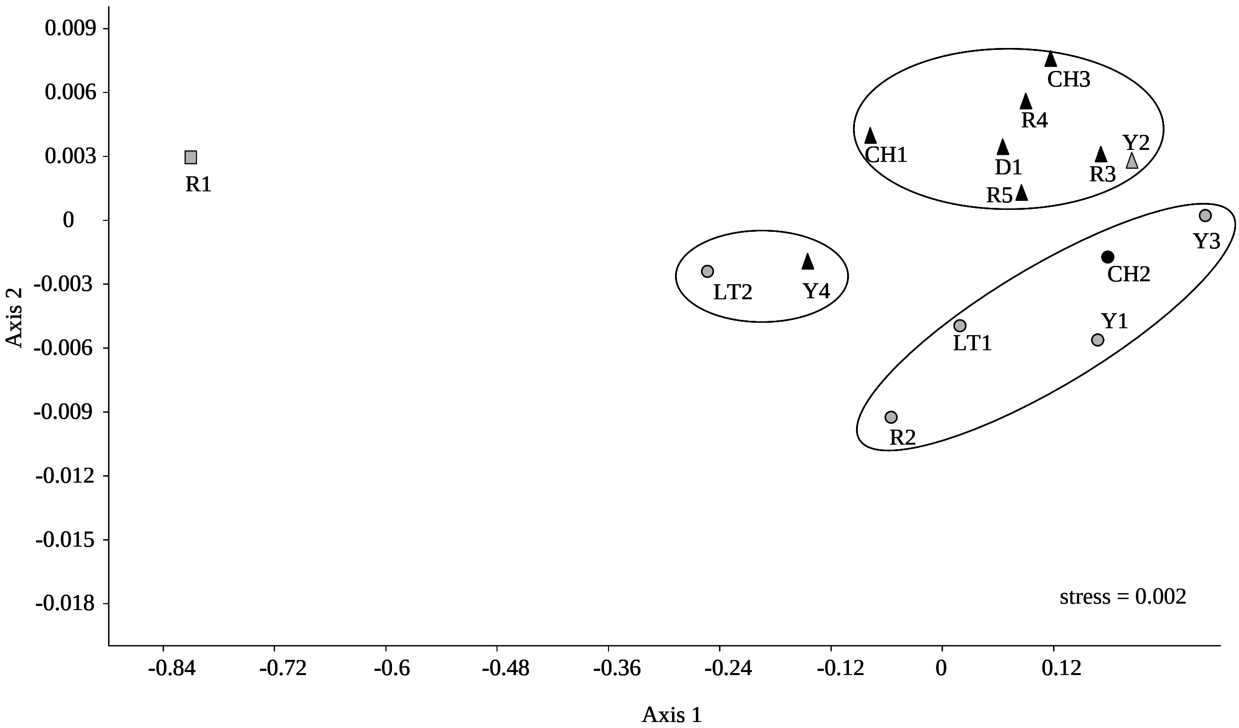


Figure 7: nMDS ordination based on environmental variables (mean grain size, redox potential *Eh* and sedimentary organic matter SOM) recorded at the sampling stations in March, June, September and December 2004. Average score derived-EcoQ for each station is indicated (squares: High EcoQ, circles: Good EcoQ and triangles: Moderate EcoQ). Grey and black symbols identify exposed and sheltered habitats, respectively.

Tables

Table 1: Characteristics of the sampling stations. E: exposed, S: sheltered. Ref: reference, Off-B: off-bottom culture, On-B: on-bottom culture, Near Off-B: near off-bottom culture parks, Stn R5 is located 200 m to the north of nearest Rivedoux oyster parks, hydrodynamics favouring dispersal of oyster biodeposits from Rivedoux oyster parks to Stn R5 (Faure, 1969), Near On-B: near on-bottom culture parks.

Oyster parks	Sampling stations	Exposed/sheltered	Type
Rivedoux	R1	E	Ref
	R2	E	Off-B
	R3	S	Off-B
	R4	S	Off-B
	R5	S	Near Off-B
Yves	Y1	E	Off-B
	Y2	E	Off-B
	Y3	E	Off-B
	Y4	E	On-B
Charente	C1	S	Off-B
	C2	S	Off-B
	C3	S	Off-B
Les Traires	LT1	E	On-B
	LT2	E	Near On-B
Daire	D1	S	Off-B

Table 2: Threshold values separating ecological status (each of High, Good, Moderate, Poor and Bad classifications) for the six selected biotic indices were determined according to Vincent et al. (2002) for H’, Borja et al. (2000) for AMBI, Simboura and Zenetos (2002) for BENTIX, Dauvin and Ruellet (2007) for BOPA, Muxika et al. (2007) for M-AMBI and Ruellet and Dauvin (2007) for average score.

	H'	AMBI	BENTIX		BOPA	M-AMBI	Average score
			for muds	for sands			
	+ ∞	0	6	6	0	1	1
High							
	4	1.2	4	4.5	0.04576	0.85	1.5
Good							
	3	3.3	3	3.5	0.13966	0.55	2.5
Moderate							
	2	4.3	2.5	2.5	0.19382	0.39	3.5
Poor							
	1	5.5	2	2	0.26761	0.2	4.5
Bad							
	0	7	0	0	0.30103	0	5

Table 3: Mean annual abundances (ind m^{-2}) and corresponding ecological groups (EG I to EG V) of the macrobenthic species collected at the 15 sampling stations in the Pertuis Charentais in March, June, September and December 2004.

Species	Ecological group	Stations														
		Ref	Near On-B	On-Bottom		Near Off-B	Off-Bottom									
		R1	LT2	LT1	Y4	R5	R2	R3	R4	Y1	Y2	Y3	CH1	CH2	CH3	D1
Acrocnida brachiata	EG I	13														
Actinia equina										12						
Ampelisca brevicornis			188	26	12	14										26
Ampharete acutifrons										12	12		12	14		
Bathyporeia pilosa		13														
Cereus pedunculatus										12					13	
Crangon crangon		13														
Cyclope neritea		231		26		14	42			12	12			35	24	
Diopatra neapolitana												12		14		
Euclymene oerstedi		51	188	257				14	13							13
Iphinoe tenella			16													13
Leucothoe incisa		38														
Nucula nitidosa						14								14	24	
Palaemon elegans												12	12	14		13
Palaemonidae sp. *(larvae)*			188	103	24					12	35	24		94	24	77
Paphia rhomboides					12											
Phylo foetida atlantica		77														
Pilumnus hirtellus														14		
Rudipapes decussatus						14	14			24			12	14		
Tellina tenuis		51														
Urothoe poseidonis		141		26												
Bodotria scorpioides	EG II														13	
Cumopsis goodsir		38														
Elminius modestus		128				14				12						
Eulalia sp.						14										
Glycera capitata		13			35					24				24	13	
Glycera gigantea			16	13	12											
Glycera unicornis		13		26	12	14		28		12	12	12	35		13	13
Hesione sp.					24					12						
Lanice conchilega														14		
Leptoplana tremellaris														14		
Marphysa bellii				13												
Mesopodopsis slabberi		38	361	51	188	56	56	85	103	129	423	341	118	705	411	218
Nassarius nitidus					35					12		35	12			
Nassarius pygmaeus										24						
Nassarius reticulatus					24					12	24		12	24	24	
Nephtys cirrosa		26				28	14		38							
Nephtys histricis					12							12	12	59	82	13
Nephtys hombergii		38				56	56	14	38		24				13	13
Pomatoceros lamarckii					12		14				12					
Terebellidae sp.										12						
Tubulanus polymorphus			31	13	12	28		14	13	165	35	82	12	24	71	13
Abra alba	EG III				47	14				24		35	24	24		
Abra nitida				13							12		12	14	24	26
Abra tenuis			282													
Aonides oxycephala		26		13	12					106	35	24				
Apseudes latreillii		90														
Cerastoderma edule										12						
Clymenura clypeata		13	470	38												
Corophium volutator			643	56				14								462
Eteone picta					35			14					24			
Hediste diversicolor			31												13	13
Lumbrineris sp.													12			
Macoma balthica						14										
Mediomastus fragilis				13						47	24		24			
Melinna palmata				13								12				
Mysella bidentata		13														
Mytilus edulis															13	13
Neanthes succinea										12						
Notomastus latericeus		13	78	462	12							12	12	24		
Pygospio elegans		38					14						24	14		
Ruditapes philippinarum				13						82	12		47	24		
Scoloplos armiger		13														
Spio decoratus		13					28									
Sternapsis scutata												12				
Streblospio shrubsolii			31	13	12	522	42	607	90	24	71	59	12	47	329	26
Aphelochaeta marioni	EG IV	38	31	475	47	71	183	226	38		12	24	223	71	129	257
Corbula gibba					12				13	12		12	12		24	
Cossura pygodactylata							28	28	26	35	71	24	12	24	59	
Cirriformia tentaculata							14									
Dodecaceria concharum					12											
Heteromastus filiformis			16	141	35	42	14	85	38	35	12		59	35	59	115
Pectinaria koreni					12		14					24	24			
Polydora cornuta									26	24			12			
Prionospio malmgreni			47		129					200	176	223	106	118	94	13
Pseudopolydora antennata			219	128	35	818	42	409	167	35	71	24	71	82	212	180
Tharyx multibranchiis		38		13	588	14	28	113	51	353	482	165	435	494	329	
Capitella capitata	EG V															13
Capitella minima								14								
Oligochaeta spp					35			155	26	35	59	47	106	14	118	90
Tubificoides benedii					12		14	14	26			12	82		24	
Species richness		26	17	23	28	18	17	16	15	31	21	23	29	28	25	21
EG I (%)		52	20	22	3	3	9	1	2	5	4	4	2	10	4	9
EG II (%)		24	14	6	25	12	23	8	27	27	33	39	13	42	30	17
EG III (%)		18	54	33	8	31	14	35	13	20	9	12	12	7	18	33
EG IV (%)		6	11	39	60	54	52	47	51	45	51	40	61	40	42	35
EG V (%)		0	0	0	3	0	2	10	7	2	4	5	12	1	7	6

Table 4: Pearson's correlation tests between biotic indices and sedimentological data (mean grain size, redox potential *Eh* and sedimentary organic mater content SOM). Level of significance is indicated: ^{ns} not significant, $p > 0.05$; * significant, $p < 0.05$; ** very significant, $p < 0.01$ and *** and highly significant, $p < 0.001$.

	Mean Grain size	*Eh*	OM
H'	-0.31 ^{ns}	0.36 ^{ns}	-0.38 ^{ns}
AMBI	0.77 ***	-0.79 ***	0.81 ***
Bentix	-0.65 **	0.66 **	-0.76 **
BOPA	0.62 *	-0.73 **	0.71 **
M-AMBI	-0.52 *	0.58 *	-0.58 *
Average score	0.64 **	-0.66 **	0.67 **