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Impact du creusement de la nouvelle souille de Dispute sur les peuplements benthiques (2012 : + 7 ans)

Herbier recouvert de sable à Dispute - Photo septembre 2007

Juin 2013

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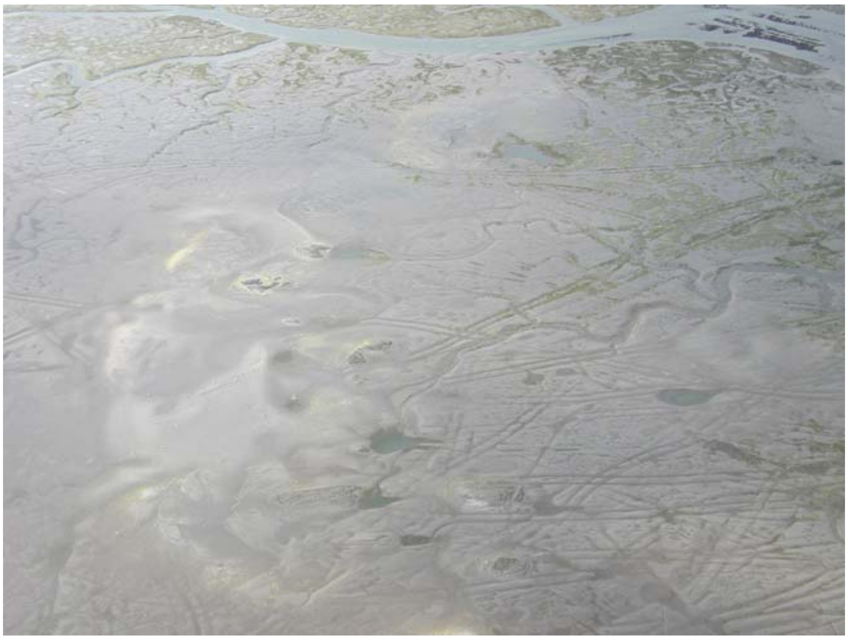

Zone de recouvrement de l'herbier par les sédiments extraits de la nouvelle souille de Dispute (photo août 2005)

Juin 2013

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Résumé

Sept ans après les travaux sur la zone de la Dispute, le constat est le suivant :

- ➪ L'herbier à *Zostera noltei* a été recouvert sur une 30^{aine} d'ha par les sédiments provenant du calibrage de l'Estey du Réservoir et du creusement de la souille de la Dispute, soit le double de la surface prévue. Avec le temps, la zone impactée se subdivise en deux entités, une zone vaseuse (28 ha) et une zone sableuse (2 ha).
- ➪ La superficie de cette zone sableuse n'a pas bougé depuis 2010 (2 ha).
- ➪ L'herbier environnant paraît, en termes de macrofaune, fonctionner 'normalement' et présente des caractéristiques quantitatives (abondance, biomasse, richesse spécifique) similaires à celles de juin 2002 (avant travaux). Cependant, la couverture végétale est très faible, ce site s'inscrivant dans les zones de déclin généralisé de l'herbier.
- ➪ Les peuplements benthiques dans la zone vaseuse ont, au bout de 5 ans, amorcé un vrai retour vers l'état initial, que ce soit en termes de couverture d'herbier qu'en termes de macrofaune associée. Cette tendance est confirmée en 2012 (+ 7 ans).
- ➪ Les peuplements benthiques dans la zone sableuse ont été profondément modifiés et aucun retour à l'état initial n'est noté. Quantitativement, il y a une perte en biomasse qui peut se répercuter par une perte négligeable de mois de 1 t de production annuelle pour les prédateurs. Qualitativement, la baisse de diversité est nette (richesse spécifique divisée par 2) et la structure du peuplement est bouleversée.

- ➪ Nous recommandons la poursuite du suivi, avec un rythme bisannuel.

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1. Introduction

Dans le cadre du Contrat Plan Etat/Région 2000/2006, et dans la continuité du CPER précédent, il était envisagé des travaux dans le Bassin d'Arcachon visant à limiter la prolifération des huîtres dites « sauvages », qui sont en réalité des huîtres japonaises (*Crassostrea gigas*) se développant à l'état naturel. Ces populations se sont principalement installées sur des structures dures d'anciens parcs ostréicoles. Une étude récente a estimé que les tonnages s'élevaient à 16 600 t pour les huîtres en élevage, 65 000 t pour les huîtres en récif et 50 000 t pour les coquilles vides (Scourzic et al. 2011).

L'un des objectifs de ce CPER était d'aménager le domaine conchylicole concédé, notamment en récupérant des surfaces aujourd'hui envahies par les huîtres sauvages pour favoriser l'implantation de jeunes conchyliculteurs. Le site de la Matelle a été envisagé pour mener un projet pilote servant de « base d'expérimentation pour d'autres sites » (de Montaudouin et al. 2002a, de Montaudouin et al. 2005b, de Montaudouin et al. 2006a, de Montaudouin et al. 2009, de Montaudouin et al. 2011).

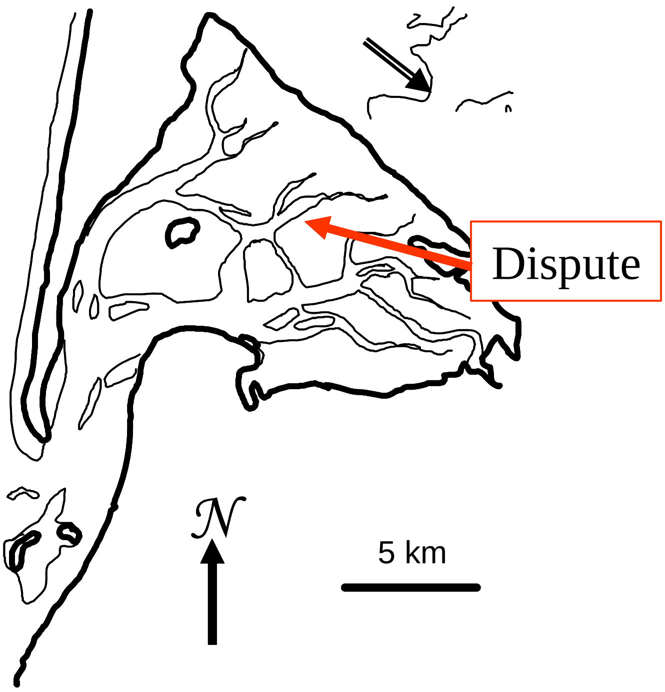

Figure 1 : Position de la souille de Dispute dans le Bassin d'Arcachon

L'un des aspects délicats de ces opérations de nettoyage est le devenir des matériaux. Si l'évacuation à terre des ferrailles, bois, plastiques, etc… est aujourd'hui admise, le devenir des coquilles a suscité plus de débats. Des deux solutions les plus « sérieuses », clapage dans la Passe Nord ou enfouissement dans le Bassin, c'est finalement la seconde qui a été retenue par le Comité Technique, sous réserve des conclusions des études environnementales, et en précisant qu'il s'agirait d'un élargissement d'une zone déjà existante et que cela n'empêchait pas de réfléchir à d'autres solutions à moyen terme (de Montaudouin et al. 2002b, de Montaudouin 2003).

La zone d'enfouissement (« souille ») se situe au milieu du Bassin, au lieu-dit « Dispute », au bout de l'estey du Réservoir (Figures 1 et 2).

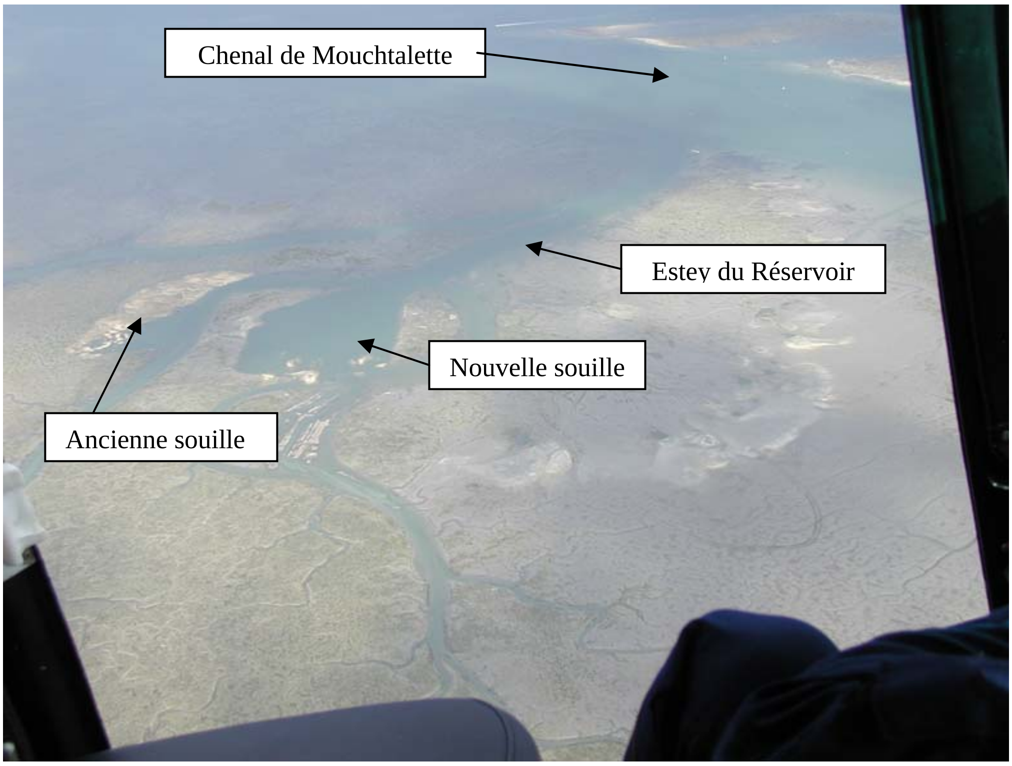

Figure 2 : Souille de Dispute et Estey du Réservoir

Cette souille de 4 ha (200 x 200 m) et de 4 m de profondeur complète une ancienne souille, aujourd'hui pleine, de 0,5 ha. Elle se situe au milieu d'un herbier à *Zostera noltii.* L'objectif est d'y stocker environ 100 000 m^3 de substrat coquillier.

Le Bassin d'Arcachon est caractérisé par la présence d'un immense herbier, le plus grand d'Europe (Auby & Labourg 1996), constitué en grande partie de zostère naine (*Zostera noltii*) en domaine intertidal (70 km^2 avant 2005), et en moindre proportion de grande zostère (*Zostera marina*) sur les talus de certains chenaux (4,3 km^2 avant 2005). Depuis 2005, une régression important de ces herbiers a été constatée, de l'ordre de 40% (Plus et al. 2010). Le Bassin d'Arcachon est aussi une ZNIEFF[3] de type II[4]. Par ailleurs l'Article R. 146-1 de la Loi Littoral stipule que « *sont préservés (...) les milieux abritant des concentrations naturelles d'espèces animales ou végétales telles que les herbiers, les frayères, les nourriceries (...)* ».

En 2002, le Syndicat Mixte du Bassin d'Arcachon (SIBA), maître d'ouvrage des travaux avec la Section Régionale Conchylicole Aquitaine-Arcachon (SRCAA), avait confié au

[3] Zone Naturelle d'Intérêt Ecologique Faunistique et Floristique
[4] Grands ensembles naturels riches et peu modifiés, qui forment des unités de fonctionnement écologique et offrent des potentialités biologiques importantes

Laboratoire EPOC, Station Marine d'Arcachon, une mission d'expertise visant 1) à définir l'état initial des communautés benthiques du site de Dispute dans la perspective d'un agrandissement sur 4 ha, 2) à estimer l'impact des travaux sur ces communautés et 3) à vérifier la présence/absence d'un herbier à *Zostera marina* dans l'Estey[5] du Réservoir accédant à la souille (de Montaudouin et al. 2002b, de Montaudouin 2003, de Montaudouin et al. 2005a, de Montaudouin et al. 2006b, de Montaudouin et al. 2008).

Les travaux de calibrage de l'Estey du Réservoir et le creusement de la nouvelle souille étaient terminés au printemps 2005.

En 2005, le SIBA a demandé au Laboratoire :

- D'estimer l'impact des travaux de refoulement des sédiments sur les herbiers avoisinants (surface prévue 14 ha).
- D'évaluer la restauration de l'ancienne souille, nettoyée des déchets observés en 2002 (de Montaudouin et al. 2002b) et devant être recouverte d'une nappe de sable.

Devant le blocage de la situation concernant le nettoyage de l'ancienne souille qui, en septembre 2005, était toujours en friche, le SIBA et le laboratoire ont convenu qu'il était pour l'instant inutile d'entamer le suivi biologique de ce site. Une visite en août 2008 a permis de constater que la situation n'avait pas évolué.

- L'étude concerne donc le devenir de la zone de refoulement et ses environs proches, et présente aujourd'hui la totalité des résultats des campagnes d'août 2012 en comparaison avec les résultats des campagnes précédentes.

[5] Estey : petit chenal.

2. Nouvelle souille de Dispute

2.1. Matériel et méthode

La première campagne d'échantillonnage sur la souille de Dispute s'était déroulée le 10 juin 2002. Les zones de prélèvements correspondaient aux sites prévus pour l'élargissement de la souille, dans l'herbier, ainsi qu'une station un peu plus éloignée (de Montaudouin et al. 2002b). A partir de photos aériennes prises en juin 2005, six stations ont été choisies, deux dans l'herbier, deux dans la zone recouverte par les sédiments et deux dans la limite herbier/sédiments. Une fois les sédiments stabilisés, il a été décidé à partir de 2008 d'effectuer les comparaisons sur 4 stations : une station impactée par la vase (IM comme Impact Mud), une station impactée par le sable (IS comme Impact Sand), une station témoin proche de la zone impactée (PS comme Proximate Seagrass) et une station témoin éloignée de la zone impactée (RS comme Remote Seagrass) (Figure 3).

En 2010 et 2012, les points GPS de la zone sableuse représentée par la station IS ont été

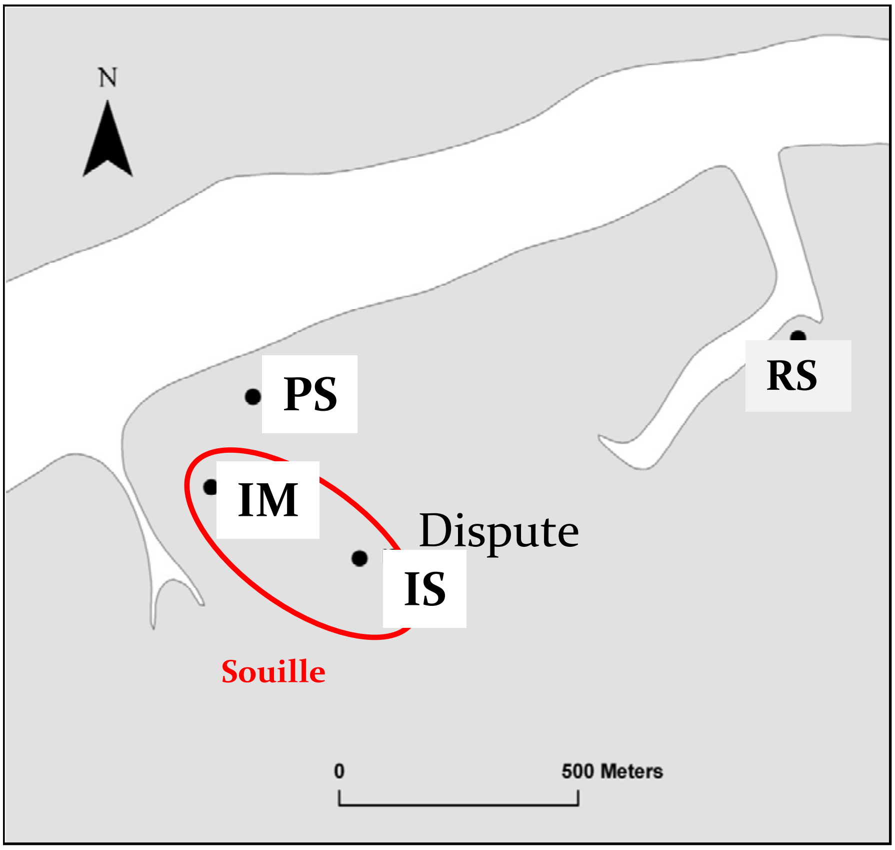

Figure 3 : Localisation des stations d'échantillonnage sur le site de Dispute : site impacté par le sable (IS), par la vase (IM), dans l'herbier à proximité des travaux (PS) et dans l'herber loin des travaux (RS).

relevés pour estimer la surface (Figure 4).

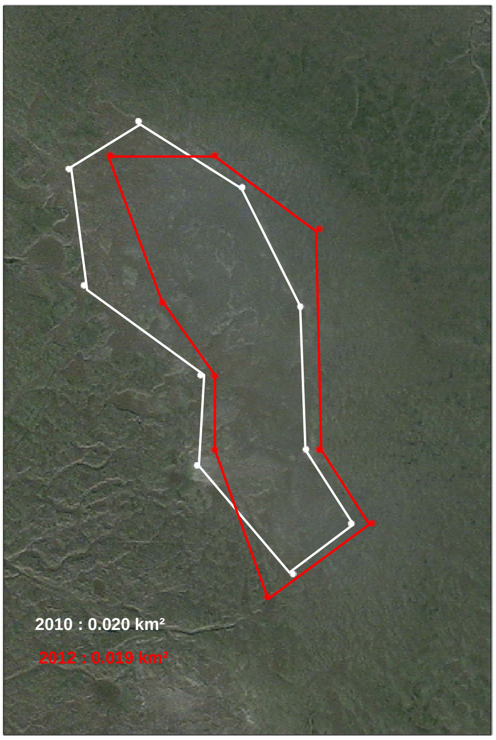

Figure 4 : zone impactée par le sable en 2010 (blanc) et 2012 (rouge) (2 ha).

Les échantillons ont été collectés dans l'herbier de zostère naine (Figure 5) et sur la zone impactée (Figure 6) les mois d'août 2005, 2006, 2008, 2010 et 2012. Les prélèvements ont été réalisés avec un cadre métallique (carottier), chacun consistant en un cube de sédiment de 15 cm de côté. Chaque station a été l'objet de quatre replicats. Chaque unité d'échantillonnage était tamisée sur maille de 1 mm, fixée au formol à 4 % et colorée au Rose Bengal[6]. Le tri des individus a été effectué au laboratoire et l'identification des espèces réalisée à la loupe binoculaire (Annexe 1). Les biomasses ont été estimées en poids sec sans cendre[7] (poids sec - poids des cendres), qui représente le poids sec de matière organique. Le poids sec est atteint après 48 h à l'étuve à 60°C. Les cendres sont obtenues après calcination pendant 4 h à 450°C. Quatre prélèvements supplémentaires ont été récoltés chaque année pour déterminer la granulométrie et la teneur en matière organique du sédiment sur les 5 cm supérieurs. Le cas échéant, le taux de couverture par l'herbier a été évalué par une méthode graphique après prise de 10 clichés par site (Binias et al. In press). Cette méthode permet de calculer un taux de recouvrement à l'échelle décamétrique et une biomasse foliaire au sein des zones d'herbier.

La diversité a été assimilée à la richesse spécifique (= nombre d'espèces) moyenne par échantillon.

[6] Colorant de la matière organique
[7] PSSC dans le reste du texte

Figure 5 : Echantillonnage au carottier dans l'herbier à *Z. noltei.*

Figure 6 : Echantillonnage dans la zone impactée (Station IS).

Par ailleurs, les peuplements benthiques ont été comparés entre 2002 (avant travaux), 2005, 2006, 2008, 2010 et 2012 (après les travaux) au moyen d'une analyse multivariée n-MDS (Logiciel PRIMER®). Cette méthode graphique permet de projeter les stations d'échantillonnage sur un plan, leur positionnement étant calculé d'après la présence des espèces et leur abondance (en racine carrée de x). Ainsi, sur un tel plan, deux stations aux peuplements benthiques similaires seront proches. En d'autres termes, nous étudierons dans le temps le « déplacement » des stations sur ces plans, avec deux cas de figure : soit le nuage de points rejoint celui de 2002 (= état initial) et cela signifie que les peuplements benthiques présentent les caractéristiques initiales, soit le nuage est distinct, signifiant que les peuplements benthiques sont différents. Dans ce dernier cas, il faudra distinguer un nuage de points stable (état d'équilibre), d'un nuage de points en mouvement (colonisation, état transitoire).

Les abondances et les biomasses de la faune ont été comparées en août 2012 entre zone d'herbier (PS et RS poolés) et zone impactée (M et IS) à travers une série de tests statistiques (Cf. § 2.2.4). Il en a été de même entre les herbiers 2002 et les herbiers d'août 2012 (Cf. § 2.2.4.).

Enfin, nous appliquerons l'indice benthique MISS, mis au point sur cet herbier lors d'études précédentes (Lavesque et al. 2009) (Annexe 2) pour estimer l'état de santé de la zone ou du moins son écartement par rapport à un niveau de départ. Cet indice repose sur 16 métriques qu'il est possible de grouper en 3 catégories : 1) les descripteurs de communautés (abondance, biomasse, nombre d'espèces, indice de Shannon, indice d'équitabilité) ; 2) la composition trophique (abondance de brouteurs, déposivores de surface, déposivores de subsurface, filtreurs et carnivores) ; et 3) indicateurs de perturbations (AMBI[8], BOPA[9], abondance d'espèces sensibles, abondance d'espèces opportunistes, W[10]). Pour chacune de ces métriques, un score de 0 ou 1 était attribué selon que la valeur était hors de valeurs seuils définies sur un herbier en bonne santé ou non, puis une moyenne était calculée. L'indice MISS peut donc prendre une valeur allant de 0 (très éloigné des conditions d'un herbier sain) à 1 (herbier sain) (voir Annexe 2 pour détails).

[8] AMBI : Azti Marine Biotic Index
[9] BOPA : Benthic Opportunistic Polychaetes-Amphipods ratio
[10] W : Warwick Statistic

2.2. Résultats

2.2.1. Sédiments et couverture végétale

Les photos aériennes avaient permis en 2005 d'estimer la surface recouverte par les sédiments creusés dans la souille ou issus du calibrage de l'Estey du Réservoir à environ 30 ha (Figure 7) (de Montaudouin et al. 2005a).

En 2010, la tâche de sable (zone écologiquement la plus impactée) mesurait 2 ha et n'a pratiquement pas bougé depuis (Figure 4).

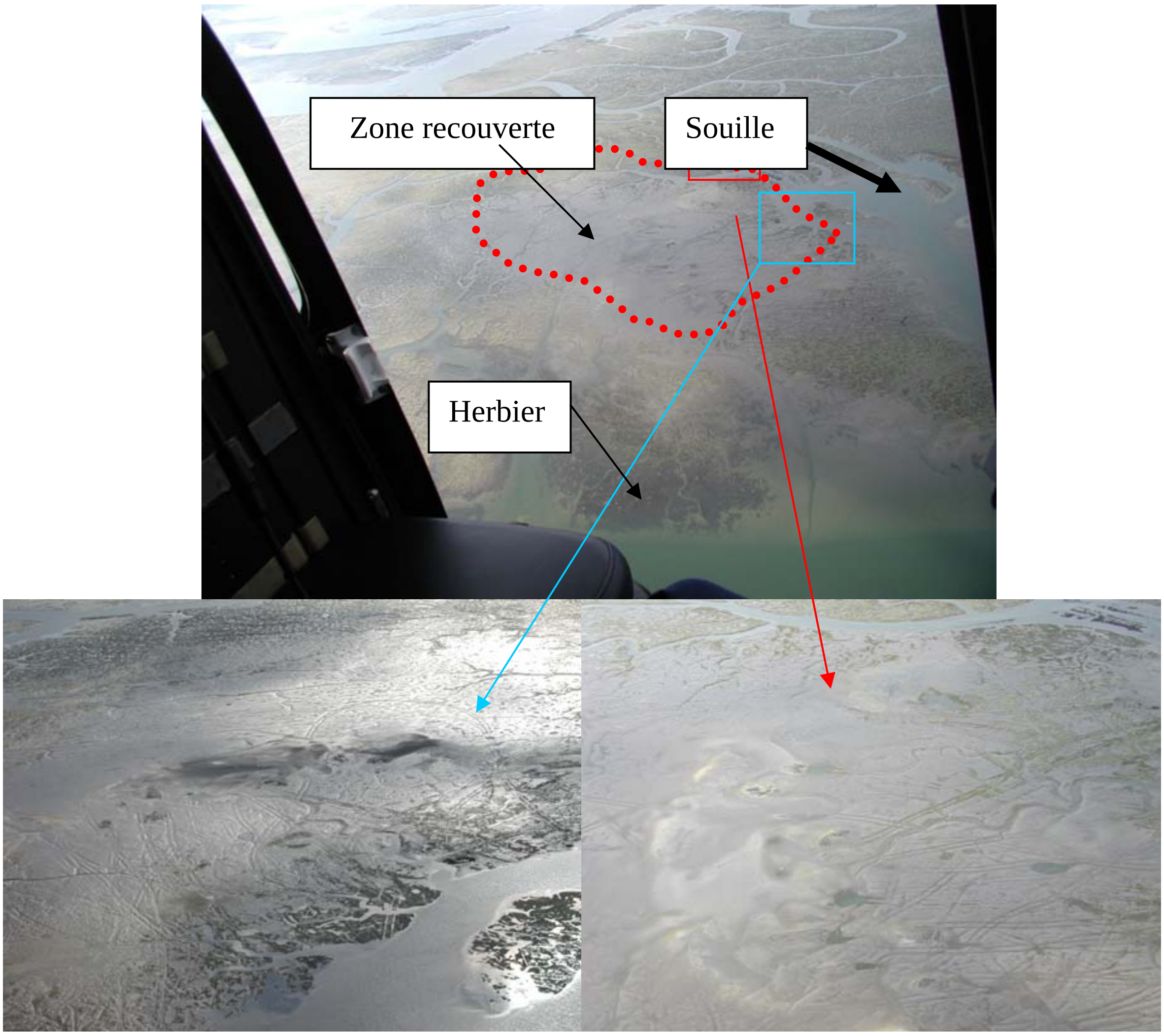

Figure 7 : Zone de la Dispute, de la nouvelle souille et de la zone de rejet

En 2002, l'herbier reposait sur des vases ou des sables fins, la médiane variant autour de 60 µm, le pourcentage de pélites[11] entre 70 et 80% et le pourcentage de matière organique de 5 à 7% (Tableau 1). Les travaux ayant finalement consisté en un brassage de sédiments dans la

[11] Pélites : particules sédimentaires < 63 µm (vases)

même zone, les caractéristiques sédimentaires avaient peu varié en 2005 et étaient semblables dans l'herbier et dans la zone d'impact (médiane entre 70 et 120 µm dans tous les cas ; pélites : 30 à 50% ; matière organique 5 à 9%).

Année	Station	Biomasse feuilles (g PS/m²)	couvert végétal (%)	Médiane granulométrique (µm)	concentration silt et argile (%)	concentration en matière organique (%)
2002	**RS**	80	68	20 (vaso-sableux)	82	5
2005		-	-	90 (sablo-vaseux)	37	8
2006		29	33	100 (sablo-vaseux)	26	10
2008		82	70	30 (vaso-sableux)	73	9
2010		87	72	60 (vaso-sableux)	50	7
2012		10	11	63 (vaso-sableux)	50	8
2002	**PS**	70	62	20 (vaso-sableux)	73	7
2005		103	52	100 (sablo-vaseux)	31	9
2006		9	15	90 (sablo-vaseux)	36	8
2008		-	-	20 (vaso-sableux)	83	10
2010		192	100	40 (vaso-sableux)	57	8
2012		3	4	141 (sablo-vaseux)	21	5
2005	**IM**	0	0	100 (sablo-vaseux)	26	5
2006		0	0	70 (sablo-vaseux)	47	9
2008		0	0	40 (vaso-sableux)	59	6
2010		41	43	40 (vaso-sableux)	60	7
2012		40	38	141 (sablo-vaseux)	21	4
2005	**IS**	0	0	120 (sablo-vaseux)	28	5
2006		0	0	190 (sableux)	5	2
2008		0	0	150 (sablo-vaseux)	23	2
2010		0	0	180 (sableux)	6	1
2012		0	0	243 (sableux)	10	1

Tableau 1 : caractéristiques sédimentaires des stations. RS et PS : zones témoins ; IM et IS, zones recouvertes par la vase et le sable, respectivement. « - » : pas de mesure.

En 2006, les granulométries ont changé dans la zone impactée : les sédiments ont été lessivés et « classés », laissant une composante sableuse (médiane = 190 µm) et une composante vaseuse (médiane = 70 µm). En 2008, les zones d'herbier sont particulièrement vaseuses (médiane entre 20 et 30 µm) tandis que la zone impactée se compose toujours d'une zone « vase » (médiane de 40 µm) et d'une zone « sables » (150 µm). Le taux de recouvrement par l'herbier est de zéro sur les zones impactées, contre plus de 70% sur les zones herbiers (Tableau 1, Figure 8). En 2010, soit plus de 5 ans après les travaux, un virage est remarqué. Si l'herbier reste avec une couverture >70% (sur nos stations témoins), associée à une biomasse >87 gPS/m² de feuilles sur les zones végétalisées et une médiane de vase (44 à 62 µm), la zone impactée a changé. L'importance de ces modifications a dépendu du substrat. La zone de vase (Station IM, médiane de 40 µm) commence à être colonisée par l'herbier avec un taux de recouvrement de 40% et une biomasse en feuilles de 41 gPS/m² (Figure 9). En 2012, une tendance inattendue se dessine avec des stations témoins (RS et PS) moins végétalisées que la station impactée par la vase (8% *vs.* 38%), alors que les sédiments sont partout sablo-vaseux (médiane comprise entre 60 et 140 µm). La station impactée (IS) par le sable conserve une médiane granulométrique élevée (240 µm) et reste pratiquement sans zostère (Figure 10).

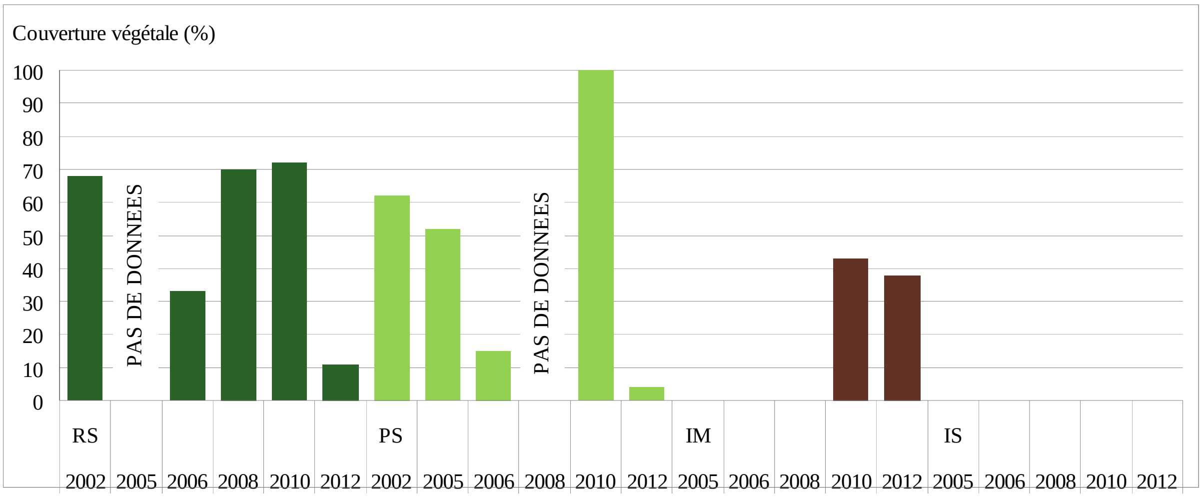

Figure 8 : Couverture végétale (% de sédiment recouvert par l'herbier en fonction des années et des stations. RS et PS : zones témoins ; IM et IS, zones recouvertes par la vase et le sable, respectivement.

Figure 9 : Zone impactée recouverte par la vase (Station IM) et, en 2010 et 2012, colonisée pour la première fois par l'herbier

Figure 10 : Zone impactée recouverte par le sable (Station IS) et, en 2010 et 2012, toujours nue hormis de très rares tâches d'herbier

2.2.2. Communautés benthiques

Il est plus judicieux de comparer les traitements entre eux en août 2012 (herbiers *vs.* impacté) plutôt que de faire référence à l'herbier en 2002, afin de limiter l'influence de la variabilité temporelle. Cependant, il faut souligner que les paramètres quantitatifs de cet herbier (« témoin ») sont restés stables entre 2002 et 2006 (hormis peut-être la biomasse qui est affiche toujours une grande variabilité): abondance de la macrofaune entre 11806 (2002) et 7594 ind./m² (août 2006); biomasse entre 11,1 et 21,7 gPSSC/m² ; et richesse spécifique moyenne entre 26 et 24 espèces (Figure 11). Ensuite, ces paramètres ont évolué. L'abondance et la richesse spécifique moyenne ont légèrement diminué (6567 ind./m² et 15 espèces respectivement, en 2012). En revanche, la biomasse a considérablement augmenté (42 et 64 gPSSC/m² en 2008 et 2010, respectivement, soit 2 à 3 fois plus qu'en 2002) pour retomber en 2012 à un niveau de départ (16 gPSSC/m²) (Figure 11).

Jusqu'à 2006, les mollusques dominaient dans l'herbier (60% de l'abondance). En 2006, le rapport s'équilibre mais les mollusques sont ensuite un peu en recul en 2008 et 2010 (41 et 21%). Cependant, 2010 est marqué par la colonisation par les palourdes japonaises *Ruditapes philippinarum* qui expliquent la dominance des mollusques en termes de biomasse (92%) (Figure 11). En 2012, les mollusques dominent toujours en biomasse mais dans une bien moindre mesure (les palourdes sont plus rares) et l'annélide *Melinna palmata* devient l'espèce la plus abondante (25% de l'abondance totale) (Tableau 2).

En zone impactée, il devient nécessaire depuis 2010 de distinguer les deux stations, vase (IM) et sable (IS5). La station « vase » (6 022 ind./m²) abrite en 2012 une faune assez proche de celle des herbiers avec notamment trois espèces dominantes en commun : *Melinna palmata, Bittium reticulatum* et *Aphelochaeta marioni* (Tableau 2). La biomasse approche comme en 2010 les 14 gPSSC/m² (Figure 11). La richesse spécifique moyenne est de 14 espèces ce qui reste une valeur basse pour un herbier en général mais finalement assez semblable de l'herbier témoin 2012 (entre 13 et 17 espèces pour RS et PS).

La station « sable » reste celle qui est la plus impactée : faible abondance (778 ind./m²), faible biomasse (2,69 gPSSC/m²) et faible richesse spécifique moyenne (10 espèces) (2012). Les espèces sont toutes très différentes de ce qui a été prélevé dans RS, PS ou IM (Tableau 2).

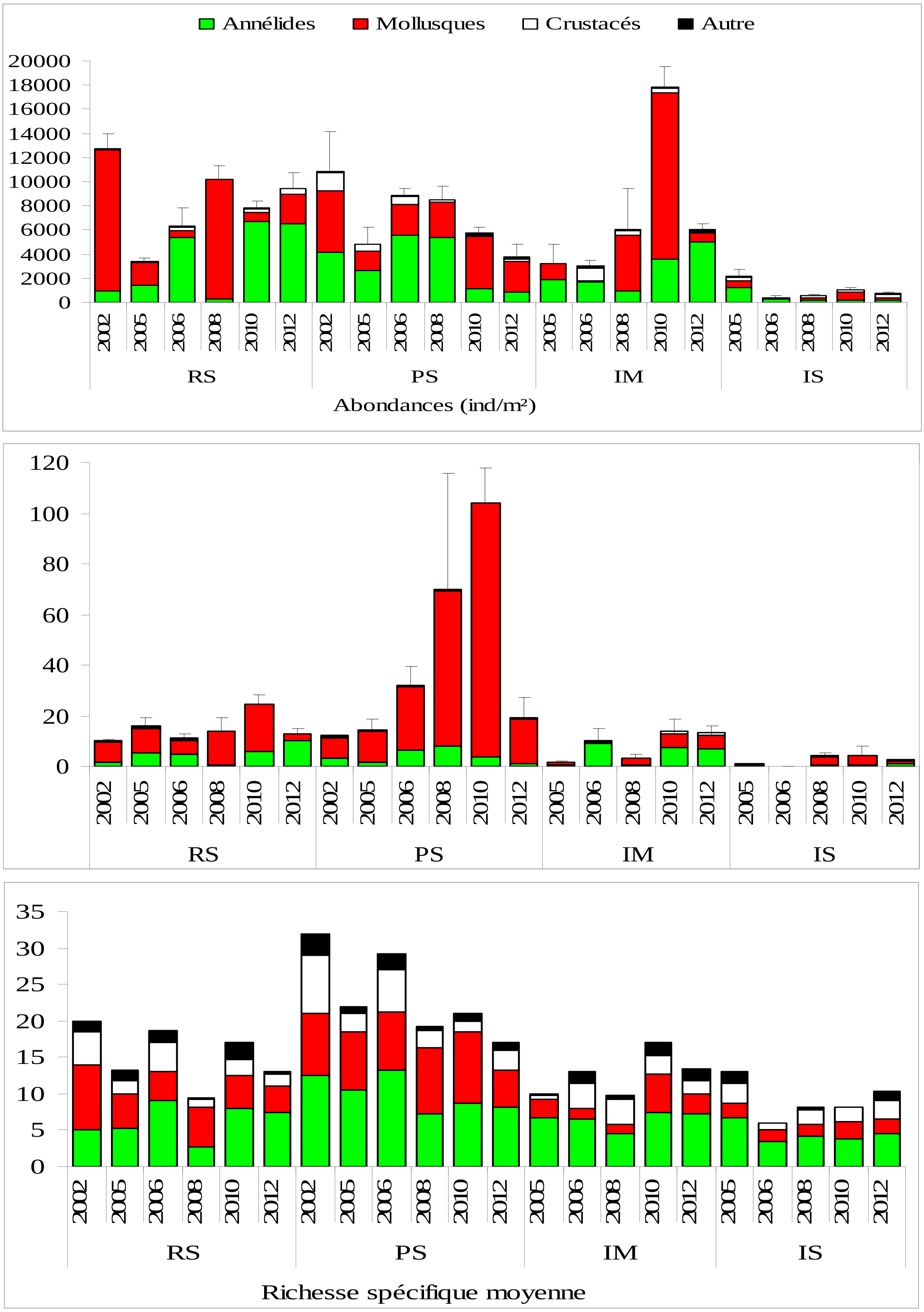

Figure 11 : Synthèse de l'évolution des caractéristiques biocénotiques entre 2002 et 2012, de l'herbier à *Zostera notei*, de la zone limite et de la zone impactée. RS et PS : zones témoins ; IM et IS, zones recouvertes par la vase et le sable, respectivement. Barre d'erreur = erreur standard.

Rang	Espèce	Groupe zoologique	Abondance (ind m^{-2})	%
Herbier 2002				
1	*Hydrobia ulvae*	Mollusque gastéropode	5300	50
2	*Tubificoides benedii*	Annélide oligochète	981	9
3	*Heteromastus filiformis*	Annélide polychète	944	9
4	*Abra segmentum*	Mollusque bivalve	619	6
5	*Melinna palmata*	Annélide polychète	496	5
2005 Herbier				
1	*Hydrobia ulvae*	Mollusque gastéropode	767	19
2	*Heteromastus filiformis*	Annélide polychète	639	16
3	*Tubificoides benedii*	Annélide oligochète	422	10
4	*Bittium reticulatum*	Mollusque gastéropode	378	9
5	*Clymenura clypeata*	Annélide polychète	333	8
Janv-06 Herbier				
1	*Tubificoides benedii*	Annélide oligochète	3267	27
2	*Idotea chelipes*	Crustacé isopode	1522	13
3	*Melinna palmata*	Annélide polychète	1189	10
4	*Microdeutopus gryllotalpa*	Crustacé amphipode	1183	10
5	*Gammarus locusta*	Crustacé amphipode	511	4
Aoû-06 Herbier				
1	*Aphelochaeta marioni*	Annélide polychète	1589	21
2	*Melinna palmata*	Annélide polychète	1283	17
3	*Bittium reticulatum*	Mollusque gastéropode	917	12
4	*Heteromastus filiformis*	Annélide polychète	722	10
5	*Tubificoides benedii*	Annélide oligochète	467	6
Aoû-08 Herbier				
1	*Hydrobia ulvae*	Mollusque gastéropode	5556	60
2	*Melinna palmata*	Annélide polychète	1611	17
3	*Heteromastus filiformis*	Annélide polychète	567	6
4	*Aphelochaeta marioni*	Annélide polychète	289	3
5	*Mytilus edulis*	Mollusque bivalve	250	3
Aoû-10 Herbier				
1	*Melinna palmata*	Annélide polychète	1561	23
2	*Aphelochaeta marioni*	Annélide polychète	1500	22
3	*Hydrobia ulvae*	Mollusque gastéropode	878	13
4	*Bittium reticulatum*	Mollusque gastéropode	800	12
5	*Ruditapes philippinarum*	Mollusque bivalve	317	5
Aoû-12 Herbier				
1	*Melinna palmata*	Annélide polychète	1633	25
2	*Hydrobia ulvae*	Mollusque gastéropode	1283	20
3	*Bittium reticulatum*	Mollusque gastéropode	1017	15
4	*Pygospio eleans*	Annélide polychète	861	13
2	*Aphelochaeta marioni*	Annélide polychète	383	6

Tableau 2 : Liste des 5 espèces les plus communes de la macrofaune benthique de l’herbier à *Zostera noltei* 2002, de l’herbier 2005 à 2012 et de la zone impactée 2005 à 2012, à proximité de la souille de la Dispute. **(Suite page suivante)**

Rang	Espèce	Groupe zoologique	Abondance (ind m^{-2})	%
2005 Zone impactée				
1	*Hydrobia ulvae*	Mollusque gastéropode	833	31
2	*Heteromastus filiformis*	Annélide polychète	439	16
3	*Aphelochaeta marioni*	Annélide polychète	344	13
4	*Streblospio shrubsolii*	Annélide polychète	183	7
5	*Pygospio elegans*	Annélide polychète	133	5
Janv-06 Zone impactée				
1	*Hydrobia ulvae*	Mollusque gastéropode	733	36
2	*Pygospio elegans*	Annélide polychète	261	13
3	*Abra segmentum*	Mollusque bivalve	256	12
4	*Tubificoides benedeni*	Annélide oligochète	128	6
5	*Nephthys hombergii*	Annélide polychète	106	5
Aoû-06 Zone impactée				
1	*Heteromastus filiformis*	Annélide polychète	372	22
1	*Cyathura carinata*	Crustacé isopode	372	22
3	*Nereis diversicolor*	Annélide polychète	367	21
4	*Pygospio elegans*	Annélide polychète	117	7
5	*Microdeutopus gryllotalpa*	Crustacé amphipode	94	6
5	Dolichopodidae	Insecte	94	6
Aoû-08 Zone impactée				
1	*Hydrobia ulvae*	Mollusque gastéropode	2305	70
2	*Heteromastus filiformis*	Annélide polychète	256	8
3	*Cyathura carinata*	Crustacé isopode	139	4
4	*Melinna palmata*	Annélide polychète	111	3
5	*Melita palmata*	Crustacé amphipode	67	2
Aoû-10 Zone impactée VASE				
1	*Hydrobia ulvae*	Mollusque gastéropode	12922	72
2	*Melinna palmata*	Annélide polychète	1978	11
3	*Heteromastus filiformis*	Annélide polychète	689	4
4	*Abra segmentum*	Mollusque bivalve	356	2
5	*Cerastoderma edule*	Mollusque bivalve	267	1
Aoû-10 Zone impactée SABLE				
1	*Hydrobia ulvae*	Mollusque gastéropode	422	41
2	*Cerastoderma edule*	Mollusque bivalve	167	16
3	*Amphithoe* sp.	Crustacé amphipode	133	13
4	*Pygospio elegans*	Annélide polychète	44	4
5	*Melita palmata*	Crustacé amphipode	33	3
Aoû-12 Zone impactée VASE				
1	*Melinna palmata*	Annélide polychète	2667	44
2	*Pygospio elegans*	Annélide polychète	1133	19
3	*Heteromastus filiformis*	Annélide polychète	644	11
4	*Bittium reticulatum*	Mollusque gastéropode	556	9
5	*Aphelochaeta marioni*	Annélide polychète	289	5
Aoû-12 Zone impactée SABLE				
1	*Grandidiriella japonica*	Crustacé amphipode	178	23
2	Anthozoa	Cnidaire	78	10
3	*Glycera unicornis*	Annélide polychète	67	9
4	*Acanthocardia tuberculata*	Mollusque bivalve	67	9
5	*Clymenura clypeata*	Annélide polychète	56	7

Tableau 2 (suite): Liste des 5 espèces les plus communes de la macrofaune benthique de l'herbier à *Zostera noltei* 2002, de l'herbier 2005 à 2012 et de la zone impactée 2005 à 2012, à proximité de la souille de la Dispute.

En termes de structure des peuplements, la n-MDS discrimine trois groupes de « station-date » que nous appellerons « stations » (Figure 12). Un premier regroupe (A) les deux stations de l'herbier (RS, PS), avant (2002) et après travaux, une station impactée sable (IS-2005) et toutes les stations impactées vases hormis IM-2006. Un second groupe (B) réunit toutes les stations impactées sable IS-2005. Enfin un troisième « groupe » (C) isole une station unique impactée par la vase, IM-2006 (Figure 12, Tableau 2, Annexe 1).

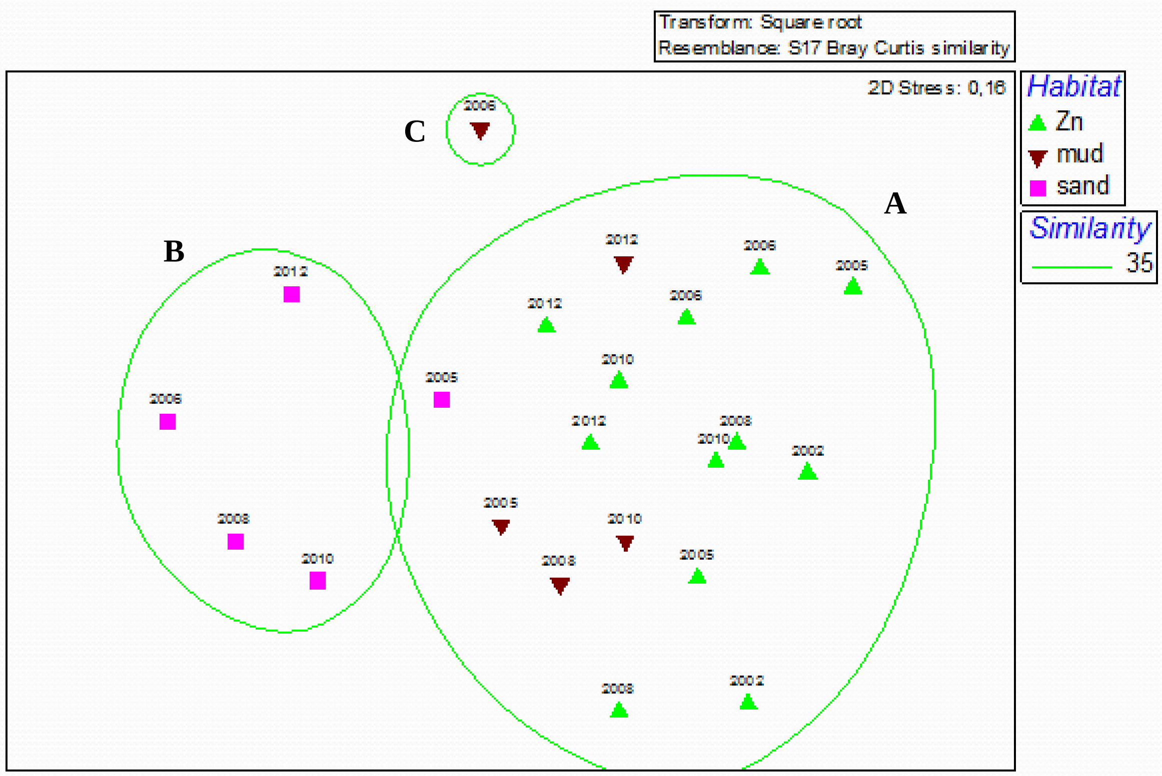

Figure 12 : n-MDS discriminant les peuplements benthiques de la zone intertidale avec un seuil de similarité de 35%. En vert l'herbier à *Zostera noltei* de 2002 à 2012 (RS-PS), en marron la zone impactée « vase » (IM) et en rose la zone impactée « sable » (IS) entre 2005 et 2012.

2.2.3. Indice biotique

L'évaluation de l'état de santé de la zone et son évolution dans le temps ont été évaluées à l'aide de l'indice multi-métrique MISS mis au point sur les herbiers du Bassin d'Arcachon (Figure 13). Cet indice repose sur la composition de la faune benthique à basse mer. Il apparaît globalement que les stations herbiers (RS et PS) répondent au statut écologique « élevé » ou « bon » depuis 2002. Après 2 ans passées dans le statut « modéré », la zone impactée par la vase bascule dans les statuts « bon » ou « élevé » à partir de 2008. La zone couverte de sable demeure depuis 2005 dans un état « modéré » à « mauvais ».

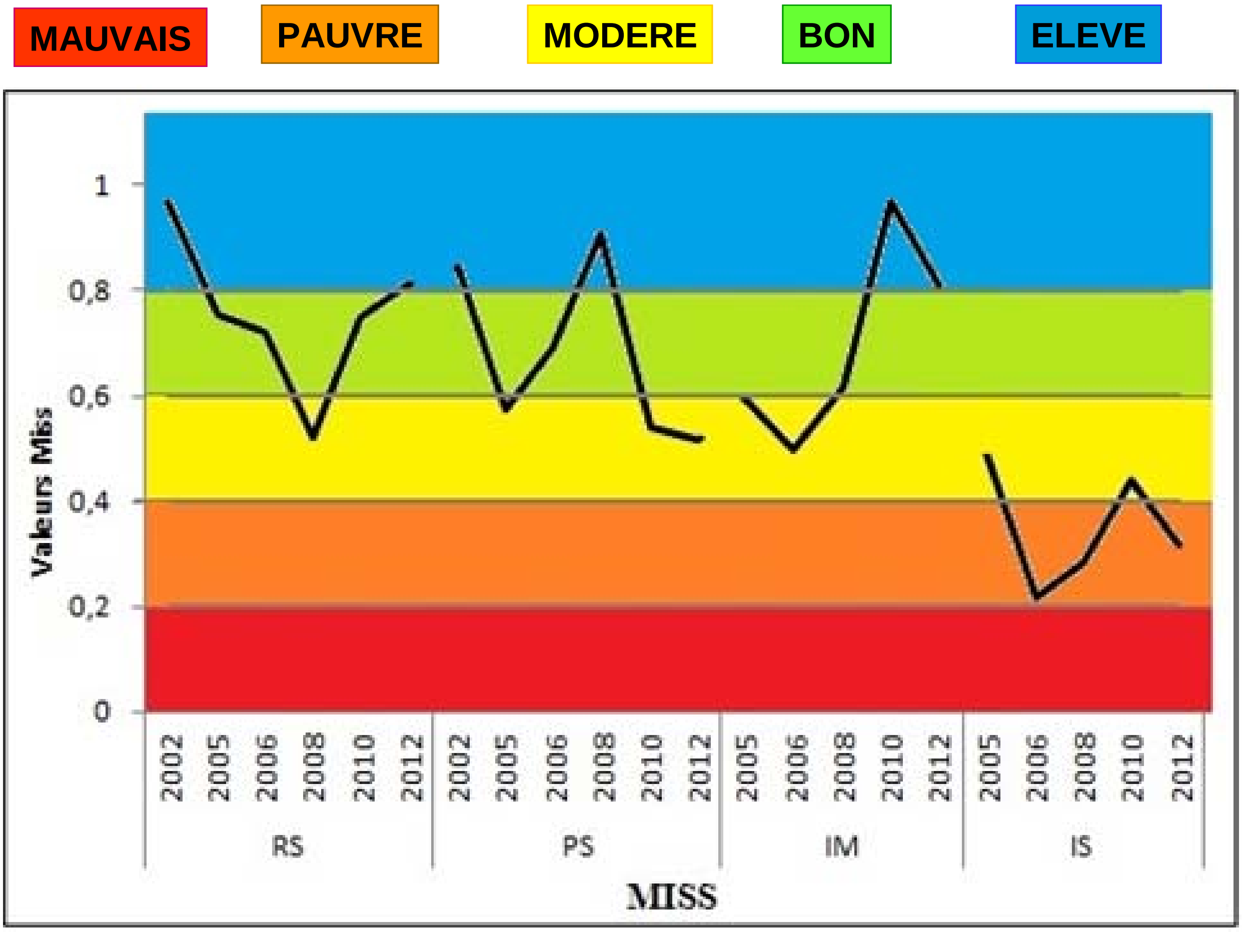

Figure 13 : Evolution de l'indice MISS au cours des années et en fonction des stations (RS et PS = herbiers ; IM = impacté par la vase ; IS = impacté par le sable) et traduction en termes de « qualité écologique ».

2.2.4. Impact des travaux sur les peuplements benthiques et leurs prédateurs

Le suivi *a posteriori* des travaux concerne l'herbier à *Zostera noltii* du site de la Dispute. Les comparaisons seront ici effectuées entre l'herbier de juin 2002 celui d'août 2012, et au sein d'août 2012 entre les prélèvements d'herbier et les prélèvements de sites recouverts par les sédiments, en distinguant recouvrement par vase et recouvrement par sable.

a Critères d'évaluation

Les critères d'appréciation sont très variés, et seront repris pour chaque zone sous forme de tableaux (Tableaux 3 à 5) :

- La nature des travaux (qui dans ce cas est du recouvrement d'herbiers par des sables), la superficie directement affectée, le calendrier d'exécution et la date de l'élaboration de l'état initial sont rappelés.
- Les modifications sédimentaires sont précisées, en mentionnant l'apparition d'herbiers (facteur positif pour l'écosystème) ou l'accumulation d'algues (facteur plutôt négatif).
- Les abondances des peuplements sont rappelées par groupe zoologique et sont comparées. Cette comparaison est faite par deux types de test statistique sur des données log(x+1)-transformées : soit par une Analyse de Variance à un facteur (année) s'il y a homogénéité des variances (test de Cochran), soit par le test de Kolmogorov-Smirnov dans le cas contraire. 'ns', signifie l'absence de différence significative avec un risque de 5 % de se tromper, '*' signifie une différence significative avec un risque de 5 % de se tromper, '**' signifie une différence significative avec un risque de 1 % de se tromper, et '***' signifie une différence significative avec un risque de 0,1 % de se tromper.
- Les biomasses sont traitées comme les abondances. Elles serviront à estimer les pertes en biomasse animale et les répercussions sur les réseaux trophiques supérieurs (production des prédateurs) calculées selon la méthode décrite dans Do et al. (Do et al. 2013).
- L'évolution de la diversité est analysée au travers de la richesse spécifique et des résultats de la n-MDS.
- L'apparition d'espèces exotiques (comme les crépidules) est recherchée.
- Enfin un avis est émis sur l'état de la restauration de l'environnement.

Par ailleurs, les caractères orange soulignent les paramètres encore éloignés des conditions initiales, tandis que les caractères bleus signifient que la restauration (ou l'objectif à atteindre) est (presque) atteinte pour un paramètre donné.

b Herbier non impacté à *Zostera noltei* : comparaison 2002-2012

La comparaison de la faune benthique de l'herbier non impacté entre 2002 et 2012, fait apparaître des situations très similaires, aussi bien quantitativement que qualitativement (Tableau 3). Deux espèces exotiques sont présentes. Le crustacé amphipode *Grandidiriella japonica* (identifié pour la première fois dans le bassin en 2012) et la moule verte (ou moule asiatique) *Musculista senhousia* récemment signalée dans l'ensemble du bassin (Bachelet et al. 2009) et dont la présence ici n'a pas de lien avec les travaux de Dispute. Sa densité reste modérée pour une espèce d'assez petite taille (<50 ind./m²).

Herbier à *Z. noltei* 2002 vs 2012 (Dispute)

TRAVAUX	Type de travaux	Recouvrement par sables fins: hors zone
	Superficie travaux (m^2)	
	Période des travaux	Janvier-Mars 2005
	Etat initial	Jun-02
	Dernière expertise	août 2012
SEDIMENTS	Médiane (µm)	63-141 µm
	Macroalgues	
	Herbiers	Très clairsemé
	Teneur en matière organique (%)	05-Aug
ABONDANCE PEUPLEMENTS	Evolution annélides	2550 -> 2033-> 5506 -> 2833-> 3889 -> 3656 ind. m^{-2}, ns
	Evolution mollusques	8372 -> 1722 -> 1522 -> 6372 -> 2500 ind. m^{-2}, *
	Evolution crustacés	844 -> 333 -> 461 -> 139 -> 233 -> 344 ind. m^{-2}, ns
	Evolution faune totale	11806 -> 4111 -> 7594 -> 9350 -> 6778 -> 6567 ind. m^{-2}, *
BIOMASSE PEUPLEMENTS	Evolution annélides	2,77 -> 3,36 -> 5,53 -> 4,19 -> 4,74 -> 5,71 gpssc m^{-2}, ns
	Evolution mollusques	8,36 -> 11,10 -> 15,18 -> 37,57 -> 59,48 -> 10,20 gpssc m^{-2}, ns
	Evolution crustacés	0,25 -> 0,37 -> 0,20 -> 0,08 -> 0,15 -> 0,08 gpssc m^{-2}, ns
	Evolution faune totale	11,11 -> 15,37 -> 21,66 -> 41,84 -> 64,40 -> 16,02 gpssc m^{-2}, ns
	Perte biomasse (kgPSSC)	sans objet
	Perte biomasse (kgC)	sans objet
	Perte production secondaire annuelle (kgC an^{-1})	sans objet
	Perte production prédateurs annuelle (kgC an-1)	sans objet
	Perte production prédateurs annuelle (tPF an-1)	sans objet
DIVERSITE	Evolution faune totale	47->54-> 57 ->39 ->48 ->41 espèces
	Similarité des communautés (MDS-seuil de 35%)	Oui
	Apparition espèces exotiques	*Musculista senhousia* (2006-08), *Grandidiriella japonica* (2012)
EVOLUTION		PROCHE ETAT INITIAL

Tableau 3 : Synthèse des éléments pris en compte pour estimer l'évolution de l'herbier non impacté. Les valeurs sont comparées (mois d'août uniquement) : 2002 -> 2005-> 2006 -> 2008 -> 2010 ->2012. Les caractères orange soulignent les paramètres encore éloignés des conditions initiales ou d'un état d'équilibre, tandis que les caractères bleus signifient que la situation est restée stable pour un paramètre donné. 'ns' signifie aucune différence significative avec un risque de 5 % de se tromper, '' signifie une différence significative avec 1% de risque de se tromper, '***' signifie une différence significative avec 0,1% de risque de se tromper.**

c Herbier à *Zostera noltei* *vs* zone impactée par vase (août-2012)

Sur les 30 ha impactés au départ, on estime que la partie recouverte par les fractions fines représente (du moins en 2012) environ 93 %, soit 28 ha.

Les résultats de 2012 font apparaître une restauration à un niveau très convenable, que ce soit en termes de couverture végétale par l'herbier ou de faune associée (Tableau 4). Si cette tendance se confirme dans les années à venir, il pourra être considéré qu'un retour à un état initial a été globalement atteint.

Herbier à *Z. noltei* (2012) détruit par VASE (2012) (Dispute)

TRAVAUX	Type de travaux	Recouvrement par sables fins
	Superficie travaux (m^2)	280,000
	Période des travaux	Janvier-Mars 2005
	Etat initial	Jun-02
	Dernière expertise	Août 2012
SEDIMENTS	Médiane (µm)	141 µm
	Macroalgues	
	Herbiers	Colonisation partielle
	Teneur en matière organique (%)	4
ABONDANCE PEUPLEMENTS	Impact sur les annélides	3656 -> 5000 ind. m^{-2}, ns
	Impact sur les mollusques	2500 -> 711 ind. m^{-2}, *
	Impact sur les crustacés	344 -> 78 ind. m^{-2}, ns
	Impact sur la faune totale	6567 -> 6022 ind. m^{-2}, ns
BIOMASSE PEUPLEMENTS	Impact sur les annélides	5,71 -> 6,85 gpssc m^{-2}, ns
	Impact sur les mollusques	10,20 -> 5,48 gpssc m^{-2}, ns
	Impact sur les crustacés	0,08 -> 1,06 gpssc m^{-2}, ns
	Impact sur la faune totale	16,02 -> 13,54 gpssc m^{-2}, ns
	Perte biomasse (kgPSSC)	sans objet
	Perte biomasse (kgC)	sans objet
	Perte production secondaire annuelle (kgC an^{-1})	sans objet
	Perte production prédateurs annuelle (kgC an-1)	sans objet
	Perte production prédateurs annuelle (tPF an-1)	sans objet
DIVERSITE	Impact sur la faune totale	41 -> 26 espèces
	Similarité des communautés (MDS-seuil de 35%)	Oui
	Apparition espèces exotiques	*Grandidiriella japonica* (2012)
RESTAURATION		Proche état initial

Tableau 4 : Synthèse des éléments pris en compte pour estimer l'état de restauration du site. Les valeurs sont comparées : herbier 2012 -> herbier recouvert par vase (2012). Les caractères orange soulignent les paramètres encore éloignés des conditions initiales ou d'un état d'équilibre, tandis que les caractères bleus signifient que la restauration est (presque) atteinte pour un paramètre donné. 'ns' signifie aucune différence significative avec un risque de 5 % de se tromper, '' signifie une différence significative avec 1% de risque de se tromper, '***' signifie une différence significative avec 0,1% de risque de se tromper.**

d Herbier à *Zostera noltii vs* zone impactée par sable (août-2012)

Herbier à *Z. noltii* détruit (2012) par SABLE (2012) (Dispute)

TRAVAUX	Type de travaux	Recouvrement par sables fins
	Superficie travaux (m^2)	20,000
	Période des travaux	Janvier-Mars 2005
	Etat initial	Jun-02
	Dernière expertise	Août 2012
SEDIMENTS	Médiane (µm)	243 µm
	Macroalgues	
	Herbiers	Détruit
	Teneur en matière organique (%)	1
ABONDANCE PEUPLEMENTS	Impact sur les annélides	3656 -> 233 ind. m^{-2}, *
	Impact sur les mollusques	2500 -> 178 ind. m^{-2}, ***
	Impact sur les crustacés	344 -> 277 ind. m^{-2}, ns
	Impact sur la faune totale	6567 -> 778 ind. m^{-2}, ***
BIOMASSE PEUPLEMENTS	Impact sur les annélides	5,71 -> 1,05 gpssc m^{-2}, ns
	Impact sur les mollusques	10,20 -> 1,13 gpssc m^{-2}, *
	Impact sur les crustacés	0,08 -> 0,05 gpssc m^{-2}, ns
	Impact sur la faune totale	16,02 -> 2,70 gpssc m^{-2}, *
	Perte biomasse (kgPSSC)	266
	Perte biomasse (kgC)	133.2
	Perte production secondaire annuelle (kgC an^{-1})	333
	Perte production prédateurs annuelle (kgC an-1)	50
	Perte production prédateurs annuelle (tPF an-1)	0.8
DIVERSITE	Impact sur la faune totale	41 -> 22 espèces
	Similarité des communautés (MDS-seuil de 35%)	Non
	Apparition espèces exotiques	Non
RESTAURATION		NON

Tableau 5 : Synthèse des éléments pris en compte pour estimer l'état de restauration du site. Les valeurs sont comparées : herbier 2012 -> herbier recouvert par sable (2012). Les caractères orange soulignent les paramètres encore éloignés des conditions initiales ou d'un état d'équilibre, tandis que les caractères bleus signifient que la restauration est (presque) atteinte pour un paramètre donné. 'ns' signifie aucune différence significative avec un risque de 5 % de se tromper, '' signifie une différence significative avec 1% de risque de se tromper, '***' signifie une différence significative avec 0,1% de risque de se tromper.**

La surface impactée concerne 2 ha. Cependant, la surface de la souille n'est pas prise en compte ici car aucun espoir de recolonisation n'existe actuellement. Les comparaisons sont effectuées entre l'herbier témoin et la zone sableuse, en août 2012 (Tableau 5). L'impact des travaux sur les peuplements est net. Quantitativement, la perte de production III^{aire} (prédateurs) est de 0,8 tonnes an^{-1} (négligeable).

Qualitativement, la perte de diversité est importante (-50% du nombre d'espèces) et la structure des peuplements est complètement bouleversée.

2.3. Discussion - Conclusions

3. Conclusion

Sept ans après les travaux, la tendance observée en 2010 se confirme, c'est-à-dire un retour à des conditions proches de « la normale » pour la zone impactée par la vase et un statut toujours très perturbé (dans le sens « différent de l'état initial ») dans la petite zone de dépôt de sable.

Cette année, l'observation la plus troublante est finalement le fait que la couverture de l'herbier est plus dense dans la zone « IM » (impactée vase) que dans les zones herbiers témoins (PS et RS). Aucune interprétation n'est proposée, mais il faut préciser que cette étude se fait toujours dans un contexte de dégradation générale de l'herbier dans le bassin d'Arcachon (Plus et al. 2010), ce qui rend globalement plus difficile de discerner l'impact vraiment lié aux travaux. La similarité des résultats (n-MDS) entre la station proche de la zone impactée (PS) et la zone éloignée (RS) confirme que l'impact est bien sur le site de dépôt avec des frontières très nettes. C'est donc vraisemblablement plus la qualité du sédiment que la qualité de l'eau qui a été le facteur clef sur cette zone.

Concernant les 30 ha impactés par le dépôt de sédiments, après un long *statu quo*, on observe donc au bout de 3 ans des modifications qui nous obligent à considérer deux situations bien tranchées : d'une part une zone recouverte par le sable (2 ha) et d'autre part une zone de panache où l'herbier a été recouvert par les éléments fins (28 ha) issus du creusement de la souille. Sur la zone sableuse, l'impact est très fort aussi bien au niveau quantitatif (abondance, biomasse, nombre d'espèces) que qualitatif (identité des espèces, dominances). Quelques très rares tâches d'herbier, de quelques cm de diamètres ont été vues en 2010, sans évolution notable en 2012. La tâche sableuse n'a pas évolué en surface depuis la première mesure chiffrée en 2010.

Il apparaît donc que l'opération Dispute pose finalement plus de problème de par le dépôt de la fraction sableuse. Ce constat a été fait à de nombreuses reprises sur le bassin : à chaque fois qu'il nous a été demandé de qualifier la macrofaune sur ces placage sableux issus d'opération de dragages (de Montaudouin & Gouillieux 2007, de Montaudouin et al. 2010), la conclusion a été que ces habitats restent très pauvres, même avec le temps, comme si la faune avait du mal à s'installer dans cette situation de paradoxe entre un hydrodynamisme faible et un sédiment sableux.

La qualification de la « santé » des habitats de Dispute par des indices biotiques avait fait l'objet d'un programme national (Programme LITEAU 2, Projet QuaLiF). Les publications issues de ses études ont montré que ces indices biotiques classiques (AMBI, BOPA, BENTIX) ne fonctionnaient pas bien dans ce contexte (Annexe 2). D'une part, ces indices calculés sur une même base de données ne répondent pas entre eux de la même manière ; et d'autre part, certains indices ne perçoivent pas les modifications liées au recouvrement par les sédiments (Blanchet et al. 2008). Le message qui peut être rendu est donc brouillé. Un nouvel indice, MISS, avait donc été proposé (Lavesque et al. 2009). Si on ne connaît pas encore sa robustesse hors bassin d'Arcachon, il semble assez fiable pour l'exercice d'estimation des modifications d'habitats au sein du site de Dispute (Do et al. 2012).

Au vu des résultats tranchés entre les différents secteurs et dans un contexte à la fois de gestion de l'ostréiculture et de préservation des habitats, nous recommandons un suivi bisannuel de la zone (avec une prochaine programmation pour l'été 2014).

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Annexe 1

Liste faunistique 2012 (ind/m²). IM : impacté par la vase, IS : impacté par le sable, PS : herbier près de la zone de travaux, RS : herbier loin de la zone de travaux.

Taxon	IM	IS	PS	RS
Abra segmentum	11	44	33	44
Acanthrocardia tuberculata		67		11
Ampelisca brevicornis		44		
Amphithoe rubricata				44
Anthozoa	2	78	133	
Aonides oxycephala			11	
Aphelochaeta marioni	289	11	133	633
Bittium reticulatum	556	44	233	
Carcinus maenas	11		11	
Cerastoderma edule				11
Chaetozone gibber			11	
Clymenura clypeata		56	44	33
Corophium multisetosum		22		11
Cyathura carinata				11
Cyclope neritea			11	
Diopatra biscayensis	56			
Euclymene oesterdi	56		22	
Exogone sp.		11		
Gibbula umbilicalis				11
Glycera unicornis	33	67	11	22
Grandidiriella sp.	33	178	1	389
Heteromastus filiformis	644	11	144	422
Hydrobia ulvae	22	11	267	23
Insecta	11			
Iphinoe trispinosa	11		22	
Lekanesphaera spp			33	11
Leucothoe lilljeborgi		11		
Littorina littorea	89		111	
Melinna palmata	2667			3267
Melita palmata	11		22	
Melitidae	11	22		
Musculista senhousia	11			22
Myriochele oculata			2	
Mytilus edulis	11			
Nassarius reticulatus			33	
Nemertinea			11	22
Nephtys hombergii	44	22	78	33
Nereis diversicolor				256
Notomastus latericeus			44	11
Nudibranchia				22
Opisthobranchia				33
Paraonidae	22			
Perioculodes longimanus			33	
Phoronis psammophila	22			
Phyllodoce mucosa	33		11	11
Phylo foetida		11		
Platyhelminthes		11		
Polyplacophora			11	
Pseudopolydora spp.		11	44	22
Pygospio elegans	1133	22	22	17
Ruditapes decussatus	11			
Ruditapes philippinarum		11	44	
Tubificoides benedii	22	11	56	33

Annexe 2

Publications en relation directe avec l'étude.

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Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats—Implications for the implementation of the European Water Framework Directive

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Abstract

This study deals with the application of macrozoobenthos-based biotic indices (BI) within the frame of the implementation of the European Water Framework Directive. More precisely, this study aimed at assessing the performance of five recently developed methodologies (BI) for the assessment of ecological quality status (EcoQ) in two semi-enclosed, sheltered coastal ecosystems and in one transitional water body situated along the Western French coast, namely Marennes-Oléron Bay, Arcachon Bay, and the Seine Estuary. This study showed that these five indices rarely agreed with each other, describing very different pictures of the overall EcoQ of the three study sites. This work also clearly underlined the limitations of these approaches, notably the dependency of most of these BI and the resulting EcoQ classifications on habitat characteristics, more particularly to natural levels of sediment silt–clay content and the location of stations in the subtidal or the intertidal. The implication of our observations concerning the use of these BI for implementation of the WFD is discussed in terms of definition of habitat-specific reference conditions and necessity to adjust thresholds to the particular habitat occurring in semi-enclosed ecosystems. Meanwhile, the unmodified use of these BI severely impaired accurate assessment of EcoQ and decision-making on the managers' point of view.

Keywords: Biotic index; European Water Framework Directive; Benthic invertebrates; Semi-enclosed coastal ecosystems; Transitional waters

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1. Introduction

Since the publication in 2000 of the European Water Framework Directive (WFD), the interest of European marine ecologists for the bio-assessment of human impact on littoral ecosystems has been renewed (Simboura, 2004; Borja, 2005; Borja and Heinrich, 2005; Dauvin, 2005, 2007). Indeed, European Union countries are now bound to assess and monitor the quality of their surface and ground-water bodies through the survey of a set of physical, chemical and biological quality elements defined in the Annexure V of the WFD. Among these biological quality elements, benthic invertebrates are used for assessing the ecological quality status (EcoQ) of surface water bodies including coastal and transitional (estuaries) water bodies. As a consequence, numerous bio-assessment tools have been developed or adapted to the WFD requirements in recent years (Borja et al., 2000; Simboura and Zenetos, 2002; Rosenberg et al., 2004; Dauvin and Ruellet, 2007), notably in the field of benthic invertebrate ecology (Diaz et al., 2004) because these organisms are generally considered as potentially powerful indicators of aquatic ecosystems health (Beukema and Cadée, 1986; Warwick, 1986; Dauvin, 1993). Indeed, they are situated at the interface between sediment and water column and thus integrate the characteristics of both sub-systems. Moreover, they may give evidence of environmental changes because of their sedentary life preventing them to escape unfavourable conditions and their relatively long lifespan permitting to discriminate between accidental and chronic disturbances (Dauvin, 1993; Reiss and Kröncke, 2005). Finally, in comparison to a chemical approach which consists in measuring pollutant concentrations in water or sediments and comparing them to existing norms, studying benthic invertebrate community can detect real ecological impact of disturbances at the community and ecosystem levels (Fano et al., 2003). Although a large corpus of synecological methodologies has been developed throughout the world to describe community structure and dynamics (Diaz et al., 2004), the current study only concerns a set of univariate biotic indices (BI) supposed to be adapted to fulfil the requirement of the WFD. In this paper, the behaviour of these BIs was tested in semi-sheltered littoral ecosystems. Indeed, most of the BIs proposed to the WFD and addressed in this paper are based on works which concern open marine subtidal areas and their sensitivity to increasing organic matter inputs (Pearson and Rosenberg, 1978; Bellan, 1993; Grall and Glémarec, 1997). Consequently, one can wonder whether these BIs would correctly perform in freshwater-influenced, semi-enclosed environments where sediments are naturally dominated by mud and/or organic carbon, and where intertidal areas can represent a dominant part of the whole area.

The objectives of this study were (1) to test the applicability of a set of currently available univariate BIs for the EcoQ status assessment of three semi-enclosed (two coastal and one estuarine) ecosystems and (2) to evaluate BI dependency on sediment characteristics and immersion/emersion.

2. Materials and methods

2.1. Study sites

The three study sites are situated along the western French coast (Fig. 1). Two sites (Arcachon Bay and Marennes-Oléron Bay) are located in the Bay of Biscay and one (Seine Estuary) in the Eastern English Channel. All sites were characterised by the dominance of soft bottoms, shallow depth and tidal regime.

2.2. Seine Estuary

The Seine Estuary is a 50 km^2 macrotidal estuary (maximum tidal range: 8.5 m). It opens into the English Channel (Fig. 1a). This estuary ranks among the three largest estuaries in France together with the Loire (60 km^2) and the Gironde (625 km^2). Mean flow rate is 410 $m^3\,s^{-1}$ with a maximum of 2000 $m^3\,s^{-1}$ (decennial flood) and a minimum of 81 $m^3\,s^{-1}$ during low river flow (Mouny et al., 1998; Dauvin et al., 2005, 2007). Turbidity reaches up to 100 $g\,L^{-1}$. A salinity gradient can be observed from polyhaline waters (salinity: 30–18) at the opening of the estuary toward oligohaline waters (Desroy and Dauvin, 2003). Sampling stations were situated in the polyhaline and downstream mesohaline zones with a majority of stations (99 out of 111) restricted to the polyhaline zone. Intertidal flats do not reach extended areas in this estuary. This estuary is highly industrialised and

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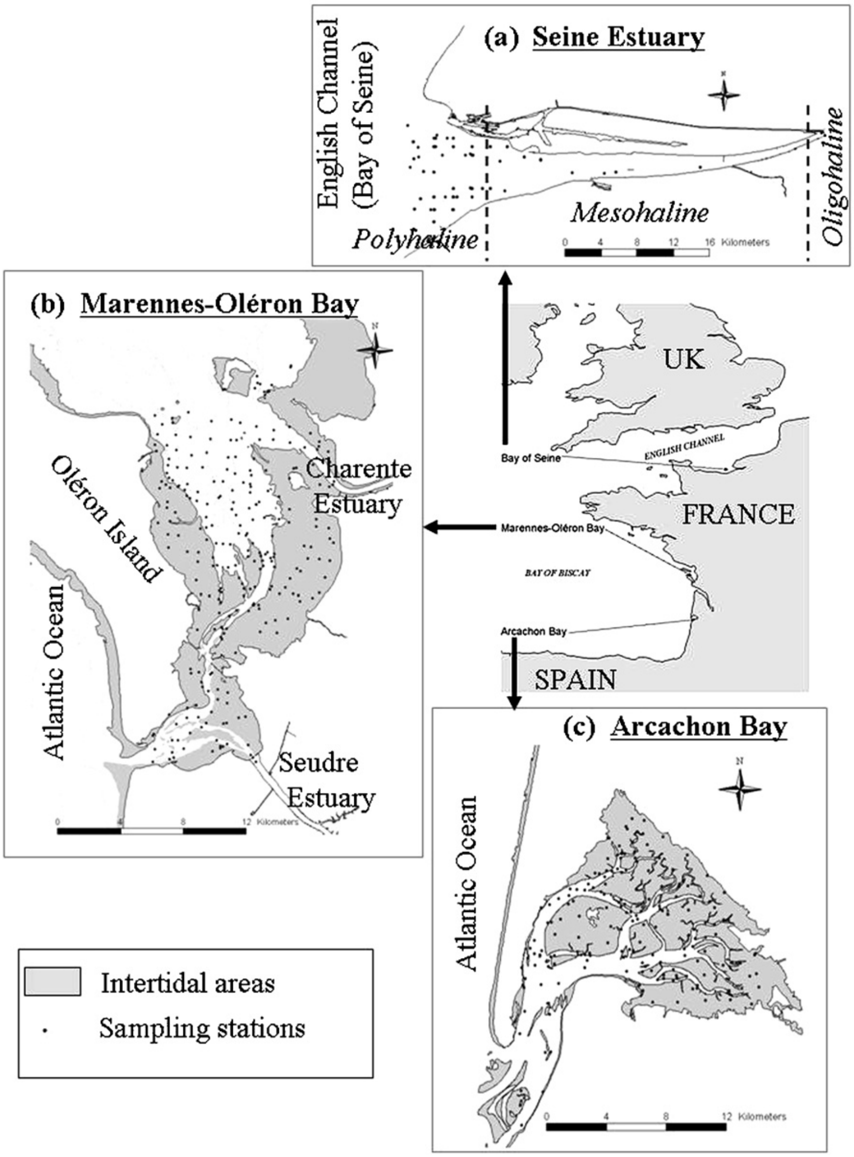

Fig. 1. Map of the studied sites showing their locations along the French west coast and the sampled stations used in the three datasets (a) within the Seine Estuary, (b) within Marennes-Oléron Bay and (c) within Arcachon Bay.

urbanised gathering 26% of the French population and 40% of national industrial activities together with areas of intensive agriculture in its 79,000 km^2 catchment area. Moreover, it has been heavily modified by the development of two major harbours (Le Havre and Rouen) and the estuarine part has been channelled and is regularly dredged (Dauvin et al., 2005). The level of various contaminants is high in water and sediments, classifying this estuary as one of the most contaminated in Europe (Dauvin et al., 2005, 2007).

2.3. Marennes-Oléron Bay

The Marennes-Oléron Bay is a 175 km^2 macrotidal semi-enclosed coastal system which is situated between the Oléron Island to the West and the continent to the East (Fig. 1b). The bay presents shallow depth (<20 m depth) and is characterised by large intertidal mudflats covering 60% of the total area. These flats are mostly unvegetated except on the east coast of the Oléron Island where *Zostera noltii* seagrass beds occur. The bay communicates with the ocean by two openings situated at its southern (Maumusson Pertuis) and northern (Antioche Pertuis) parts. It also receives freshwater inputs (3×10^9 m^3 year^{-1}) by the Charente river which gives 90% of total freshwater inputs (Héral et al., 1978, 1984). Marennes-Oléron Bay is a major French site for oyster and mussel cultures. The level of contamination is relatively low; however, Cd concentrations may be problematic (Pigeot et al., 2006).

2.4. Arcachon Bay

Arcachon Bay is a 180 km^2 meso- to macrotidal (maximum tidal range: 4.9 m) coastal lagoon situated in the south-eastern Bay of Biscay (Bachelet et al., 1996) (Fig. 1c). This triangular-shaped lagoon communicates with the Atlantic Ocean through a natural channel and receives its main freshwater inputs by a small river (L'Eyre) situated on its south-eastern corner (Fig. 1c). The maximum depth reaches about 24 m at the entrance of the lagoon; however, most channels displayed shallower depth (<20 m). Salinity varies from the fully marine waters at the entrance and western part of the Bay to more briny waters (salinity 22–32) toward the inner parts of the lagoon. As the Marennes-Oléron Bay, this lagoon is characterised by large intertidal flats covering 70% of the bay area. The largest and most flourishing *Z. noltii* seagrass bed of Europe (Auby and Labourg, 1996) covers these flats. The lower part of the intertidal is generally devoted to oyster culture, which constitutes a major activity at this site. Owing to the building in the late 1960s of a large sewage collector system that connects the towns and industries situated on its coast, and of the low level of industrialisation of its catchment area, the waters of the lagoon are relatively clean. Despite some signs of moderate eutrophication (e.g. large development of green macroalgae in the early 1990s) the overall water quality of the lagoon is considered as satisfying (Castel et al., 1996; Bachelet et al., 2000).

2.5. Databases

Three databases, each corresponding to one of the study sites, were used in this study. Each database gathered data on soft-bottom macrofauna sampled with a 1-mm mesh sieve during different studies and scientific programs (except some stations sieved on 2-mm mesh in the Seine Estuary). The characteristics of the datasets are shown in Table 1.

Concerning the Seine Estuary, data were extracted form the MABES database which gathers data from the Bay of Seine and the Seine Estuary collected during various sampling campaigns (Dauvin et al., 2007). The dataset consisted of 111 subtidal stations located throughout the estuary and sampled on a single occasion (Table 1). The datasets from Marennes-Oléron Bay and Arcachon Bay consisted of 262 and 177 stations, respectively, each set from one sampling campaign (Table 1). In contrast with the data from the Seine Estuary, these two latter datasets included stations located on intertidal and subtidal areas.

2.6. Biotic indices and derivation of EcoQ

Five different BIs were calculated when possible for each station of the databases, namely the AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002; Simboura et al., 2005; Simboura

Table 1
Characteristics of the three datasets used in this study: number of stations, sampling device, mesh size, and years of sampling

	Arcachon Bay	Marennes-Oléron Bay	Seine Estuary
Number of stations	177	262	111
Sampling device	Ekman grab and box corer (0.045 m^2)	Smith-McIntyre grab and box corer (0.1 m^2)	Various grabs
Mesh size	1 mm	1 mm	1 or 2 mm
Location of stations	89 subtidal, 88 intertidal	135 subtidal, 127 intertidal	111 subtidal
Sampling years	2002	1995	1993–2002

All stations were sampled once.

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and Reizopoulou, 2007), BQI (Rosenberg et al., 2004), Shannon-Wiener diversity (Simboura and Zenetos, 2002; Labrune et al., 2005) and BOPA (Dauvin and Ruellet, in press). These BIs were chosen because they are proposed to be used in the WFD.

AMBI, BENTIX and BOPA indices are based on the classification of species (or groups of species) into several ecological groups representing species level of sensitivity to pollutions. The number of ecological groups varied according to each index (five for the AMBI, two for the BENTIX and the BOPA). AMBI identifies five ecological groups corresponding to most sensitive species (ecological group 1) to most opportunistics/tolerant species (ecological group 5). BENTIX only recognised two groups (sensitive and opportunistic species), corresponding to ecological groups 1 and 2; and ecological groups 3–5, respectively, of the AMBI. BOPA considers the ratio between opportunistic polychaetes (i.e. polychaetes from ecological groups 4 and 5 of the AMBI) and amphipods (except those from the genus *Jassa*) as an indicator of environment quality. Full computational details can be found in Borja et al. (2000), Simboura and Zenetos (2002), Dauvin and Ruellet (2007) and are reported in Table 2.

Shannon index was also used as an indicator of EcoQ by Simboura and Zenetos (2002) and Labrune et al. (2005) and corresponding EcoQ classes from these studies were used. BQI calculation incorporates two measures: (1) the species' specific tolerance value ($ES(50)_{0.05}$) which is a measure of each species sensitivity or tolerance to pollutions, and (2) the diversity of the benthic assemblage estimated through the number of species collected in the sample. The index computes the relative abundance of each species together with their own tolerance value to the sample number of species. Computational details can be found in Rosenberg et al. (2004) and are also reported in Table 2. To apply this index to our study sites, the expected number of species in a random sample of 50 individuals (ES(50); Hurlbert, 1971) was calculated for each sampled station and the tolerance value ($ES(50)_{0.05}$) of each species was determined separately for each of the three study sites as recommended by Rosenberg et al. (2004) and Labrune et al. (2005). The EcoQ assessed by BQI was determined by taking the highest BQI value as a reference value and by defining five classes of equal size between 0 and this reference value (Rosenberg et al., 2004). Due to the difference in the range of index values between intertidal and subtidal stations, a separate scale was used for intertidal and subtidal sites following the same trend than Rosenberg et al. (2004). These separate scales permitted to avoid classifying all intertidal sites as severely degraded. The EcoQ classes in which index values were classified are shown in Table 3.

Table 2
Indices calculated from macrobenthos databases

Biotic index	Algorithms	References
AMBI	$[(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)]/100$	Borja et al. (2000)
BENTIX	$(6 \times \%GS + 2 \times \%GT)/100$	Simboura and Zenetos (2002)
Shannon index	$-\sum\left[\left(\frac{n_i}{N}\right)\log_2\left(\frac{n_i}{N}\right)\right]$	Pielou (1975)
BOPA	${}^{10}\log\left[\frac{fp}{fa+1}+1\right]$	Dauvin and Ruellet (2007)
BQI	$\left(\sum_{i=1}^{s}\left(\frac{A_i}{totA}\,ES(50)_{0.05i}\right)\right)\,{}^{10}\log(S+1)\quad \text{with}\quad ES(50)=1-\sum_{i=1}^{s}\frac{(N-N_i)!(N-50)!}{(N-N_i-50)!N!}$	Rosenberg et al. (2004)

For the AMBI: %GI, relative abundance of disturbance-sensitive species; %GII, relative abundance of disturbance-indifferent species; %GIII, relative abundance of disturbance-tolerant species; %GIV, relative abundance of second-order opportunistic species; %GV, relative abundance of first-order opportunistic species. For the BENTIX: %GS, relative abundance of sensitive species = %GI + %GII; %GT = relative abundance of tolerant species = %GIII + %GIV + %GV. For the Shannon index: n_i, number of individuals belonging to the ith species; N, total number of individuals. For the BOPA: fp, opportunistic polychaetes frequency; fa = amphipods frequency (except *Jassa* sp.). For the BQI: S, number of species in the sample; A_i, total abundance of ith species in the sample; $ES(50)_{0.05i}$, $ES(50)_{0.05}$ of the ith species; tot$A$, total abundance of the individuals belonging to the species for which $ES(50)_{0.05}$ can be computed.

Table 3
Ecological quality (EcoQ) status classes and thresholds used to classify index values in this study

	EcoQ status					References
	High	Good	Moderate	Poor	Bad	
Shannon diversity	>4	4–3	3–2	2–1	<1	Labrune et al. (2005)
AMBI	0–1.2	1.2–3.3	3.3–4.3	4.3–5.5	>5.5	Muxika et al. (2005)
BENTIX for sands	6–4.5	4.5–3.5	3.5–2.5	2.5 – 2	0	Simboura and Zenetos (2002)
BENTIX for muds	6–4	4–3	3.0–2.5	2.5–2	0	Simboura and Zenetos (2002)
BOPA	0–0.04576	0.04576–0.13966	0.13966–0.19382	0.19382–0.26761	0.26761–0.30103	Dauvin and Ruellet (in press)
BQI Seine Estuary	11.5–9.2	9.2–6.9	6.9–4.6	4.6–2.3	2.3–0	This study, Rosenberg et al. (2004)
BQI Marennes-Oléron Bay (intertidal)	10–8	8–6	6–4	4–2	2–0	This study, Rosenberg et al. (2004)
BQI Marennes-Oléron Bay (subtidal)	17.9–14.3	14.3–10.7	10.7–7.1	7.1–3.6	3.6 – 0	This study, Rosenberg et al. (2004)
BQI Arcachon Bay (intertidal)	13–10.4	10.4–7.8	7.8–5.2	5.2–2.6	2.6–0	This study, Rosenberg et al. (2004)
BQI Arcachon Bay (subtidal)	12.8–10.2	10.2–7.6	7.6–5.0	5.0–2.4	2.4–0	This study, Rosenberg et al. (2004)

2.7. Data analysis

Agreement/disagreement between the five BIs was determined by considering only two EcoQ status: ‘Acceptable’ or ‘Not acceptable’. ‘Acceptable’ status was determined for each BI when the derived EcoQ status was ‘High’ or ‘Good’, and scored as ‘1’. This means that, on the managers point of view, no action has to be taken to restore the ecosystem. ‘Not acceptable’ status corresponded to ‘Moderate’, ‘Poor’ or ‘Bad’ EcoQ status, and was scored as ‘0’. When such an EcoQ status is derived from the biotic index, restoration measures are to be taken in order to reach ‘Good’ status by 2015 as stated by the WFD. The scores given to each of the five BIs used were summed for each station (range: 0–5). This sum of scores allowed measuring the level of agreement/disagreement between BIs (Table 4).

A non-parametric sign test was also used to assess agreement or disagreement between the different BIs on the ‘Acceptable’–‘Not acceptable’ status of stations on a statistical basis. This non-parametric test was particularly adapted to our data as it allowed comparing related sample classifications based on nominal data (‘Acceptable’–‘Not acceptable’) (Siegel, 1956).

Correlation between indices-derived classifications of EcoQ was studied in order to assess whether the different indices displayed similar tendency in the classification of stations. In summary, it permitted to assess if two indices ranked the stations from worst to best in the same way regardless of the precise classes of EcoQ. Indeed two given indices may not classify stations along the same range of EcoQ classes: one index may assess a given set of stations in EcoQ ranging from ‘High’ to ‘Moderate’ whereas another may assess the same set along a ‘High’ to ‘Bad’ range. For this test, EcoQ classes were ranked from 1 which corresponded to ‘High’ EcoQ, to 5, corresponding to ‘Bad’ EcoQ. Owing to the nature of data (five EcoQ classes), correlations between indices-based classifications were tested on the basis of ranks through the use of the non-parametric Kendall’s rank-correlation coefficient τ. Ties were taken into account in the computation of τ by using the correction factor recommended by statisticians (Siegel, 1956; Scherrer, 1984). The significance of τ was tested according to Siegel (1956).

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Table 4
Levels used for the measurement of agreement/disagreement between biotic indices for each station

Sum of scores	Interpretation	
0	Full agreement of the five biotic indices on 'Moderate' or worse EcoQ status ('Not acceptable')	[a]
1	Partial agreement (four agreements out of five biotic indices) of the five biotic indices on 'Moderate' or worse EcoQ status ('Not acceptable')	[b]
2	Disagreement between the five biotic indices on the EcoQ status of the station	[c]
3	Disagreement between the five biotic indices on the EcoQ status of the station	[d]
4	Partial agreement (four agreements out of five biotic indices) of the five biotic indices on 'Good' or higher EcoQ status ('Acceptable')	[e]
5	Full agreement of the five biotic indices on 'Good' or better EcoQ status ('Acceptable')	[f]

Overall, full agreement was measured as [a] + [f], partial agreement was measured as [b] + [e] and disagreement as [c] + [d].

For Marennes-Oléron Bay and Arcachon Bay, the non-parametric Kruskal–Wallis test was used to detect significant differences in environmental conditions between stations classified into the different EcoQ classes by the five BIs. Variables used in the analysis were duration of emersion (in number of days per year) and sediment silt–clay content (%). The test was first performed on the full site database to assess significant variations in environmental conditions between EcoQ classes. On a second run, subtidal and intertidal stations were analysed separately to circumvent correlation between sediment silt and clay content and the tidal location of stations. For each tidal location, the linear regression between indices value and silt–clay content was calculated and the significance of the linear coefficient of determination (R^2) was tested. This approach could not be used with the Seine Estuary because sediment characteristics were not systematically studied at all sampled stations.

3. Results

3.1. EcoQ classifications

Use of the different BIs gave a different pattern of the overall EcoQ of the investigated sites (Fig. 2). The BOPA classified a large majority of stations (>97%) as 'Acceptable' in both coastal systems and in the Seine Estuary. In the same way, the AMBI classified the Seine Estuary, Marennes-Oléron Bay and Arcachon Bay stations as 'Acceptable' in 100, 95 and 88% of cases, respectively. However, the AMBI classified a majority of stations as 'Good' (86 and 76% for Marennes-Oléron Bay and Arcachon Bay, respectively) whereas BOPA classified stations predominantly as 'High' (with 65–60% of stations in both coastal systems) (Fig. 2a and b).

The classification of stations by the BENTIX index was more 'Severe' with 43 and 36% of stations considered as 'Not acceptable' (i.e. 'Moderate' EcoQ status or worse) in Marennes-Oléron Bay and Arcachon Bay, respectively. In the Seine Estuary, the percentage of stations considered as 'Not acceptable' was only 10%. 'Bad' and 'Poor' status rarely occurred (Fig. 2).

Shannon diversity classified 37% of the stations in Arcachon Bay, 53% in Marennes-Oléron Bay, and 95% in the Seine Estuary as 'Not acceptable' (Fig. 2). In both coastal ecosystems, the Shannon index identified as 'Poor' or 'Bad' 7 and 12% of the stations in Arcachon Bay and Marennes-Oléron Bay, respectively (Fig. 2a and b). In the Seine Estuary, the Shannon index classified 58% of stations as 'Poor' or 'Bad', whereas BOPA and AMBI never identified such status in this estuary.

The proportion of stations classified as 'Not acceptable' by the BQI was similar to that of the Shannon index, with 57% of the stations in the coastal systems and 95% in the Seine Estuary classified as 'Moderate' or worse. BQI assessed 'Poor' status in 19 and 20% of stations in Marennes-Oléron Bay and in Arcachon Bay, respectively. No station was considered as 'Bad' by the BQI in these two coastal sites whereas 33% of stations of the Seine Estuary were classified as 'Bad' and 40% as 'Poor'.

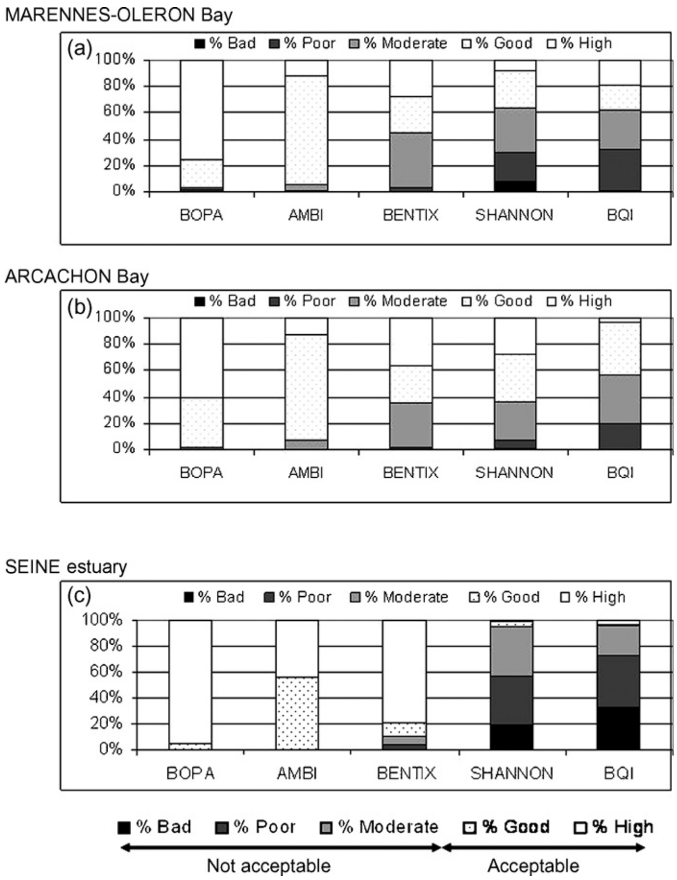

Fig. 2. Percentage of stations of the three study sites (a) Marennes-Oléron Bay; (b) Arcachon Bay; (c) Seine Estuary classified as 'High', 'Good', 'Moderate', 'Poor' and 'Bad' by the five different biotic indices used: BOPA, AMBI, BENTIX, BQI and Shannon indices. The thresholds between 'Acceptable' and 'Not acceptable' ecological quality status is indicated at the bottom of the figure.

3.2. Agreement/disagreement between indices

When considering spatial variations, the different BIs disagreed on the status of 65–90% of the stations (Fig. 3). The different BIs fully agreed on the 'Acceptable' or 'Not acceptable' status in less than 2% of stations. Partial agreement (i.e. four indices out of five agreed on 'Acceptable' or 'Not acceptable') occurred in 33% of stations in Marennes-Oléron Bay, 36% in Arcachon Bay and only 8% in the Seine Estuary (Fig. 3). The general disagreement between indices was confirmed by the sign test (Table 5). Nevertheless, there was no significant disagreement between BOPA and AMBI classifications in Marennes-Oléron Bay and the Seine Estuary and only a significant difference (at a level of significance = 0.05) in Arcachon Bay. BENTIX and BQI moreover significantly agreed in Marennes-Oléron Bay, BENTIX and Shannon in Arcachon Bay and BQI and Shannon in the Seine Estuary (Table 5).

When considering the five EcoQ classes, most indices, with the noteworthy exception of the BOPA, showed significant correlations with each others (Tables 6 and 7). It meant that the BENTIX, BQI, Shannon diversity and AMBI indices basically ranked stations in the same way from worst EcoQ to best EcoQ. However, these results showed that BENTIX, BQI, AMBI and Shannon index basically differed in the range of EcoQ assessed to stations. As an example,

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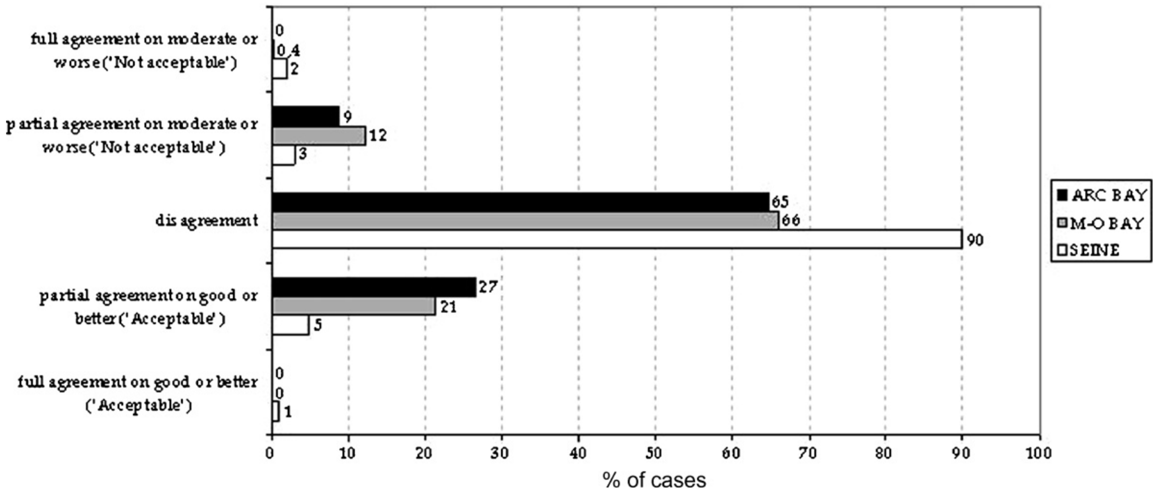

Fig. 3. Percentage of stations of the three study sites (M-O.BAY: Marennes-Oléron Bay; ARC BAY: Arcachon Bay; SEINE: Seine Estuary) where the five biotic indices: (1) fully agreed in assessing 'Good' or better EcoQ status (all five indices classified the station as 'Good' or better); (2) partially agreed on assessing 'Good' or better EcoQ status (four indices over five classified the station as 'Good' or better); (3) fully agreed in assessing 'Moderate' or worse EcoQ status (all five indices classified the station as 'Moderate' or worse); (4) partially agreed in assessing 'Moderate' or worse EcoQ status (four indices over five classified the station as 'Moderate' or worse) or (5) disagreed on the EcoQ classification of the station (three (or two) indices classified the station as 'Good' or better EcoQ status whereas the two (or three) other classified the same station as 'Moderate' or worse).

using the same set of stations, AMBI would classify these stations from 'High' to 'Good' whereas BENTIX, Shannon index or BQI would classify this same set from 'High' to 'Bad' and that stations classified as 'Bad' by the latter BI corresponded to stations classified as 'Moderate' by the first BI. As a result, a manager's decision is highly dependent on the BI used to assess the EcoQ.

Table 5
Significant, very significant and highly significant results of the non-parametric sign test conducted on the datasets of Arcachon Bay, Marennes-Oléron Bay and the Seine Estuary

	BENTIX	BOPA	BQI	Shannon index
Arcachon Bay				
AMBI	***	*	***	***
BENTIX		***	***	ns
BOPA			***	***
BQI				***
Marennes-Oléron Bay				
AMBI	***	ns	***	***
BENTIX		***	ns	***
BOPA			***	***
BQI				***
Seine Estuary				
AMBI	**	ns	***	***
BENTIX		**	***	***
BOPA			***	***
BQI				ns

Level of significance is indicated.
ns Not significant, $p > 0.05$.
* Significant, $p < 0.05$.
** Very significant, $p < 0.01$.
*** Highly significant, $p < 0.001$.

Table 6
Results of the non-parametric Kendall's rank correlation coefficient-test between biotic indices-derived ecological quality (EcoQ) status classifications (with the five EcoQ classes defined by the WFD namely 'High', 'Good', 'Moderate', 'Poor' and 'Bad')

$n = 231$	BENTIX	BOPA	BQI	Shannon
AMBI	$+0.911^{***}$	ns	$+0.458^{***}$	$+0.393^{***}$
BENTIX		ns	$+0.477^{***}$	$+0.709^{***}$
BOPA			ns	-0.365^{***}
BQI				$+0.522^{***}$

These tests were conducted on the pooled data of the two coastal systems (Arcachon and Marennes-Oléron Bays). Level of significance is indicated.
ns Not significant, $p > 0.05$.
*** Highly significant, $p < 0.001$.

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Table 7
Results of the non-parametric Kendall's rank correlation coefficient-test between biotic indices-derived ecological quality (EcoQ) status classifications (with the five EcoQ classes defined by the WFD namely 'High', 'Good', 'Moderate', 'Poor' and 'Bad')

$n = 231$	BENTIX	BOPA	BQI	Shannon
AMBI	+0.999***	ns	ns	ns
BENTIX		ns	ns	+0.325*
BOPA			ns	−0.873**
BQI				+0.679***

These tests were conducted on the data of the Seine Estuary. Level of significance is indicated.
ns Not significant, $p > 0.05$.
* Significant, $p < 0.05$.
** Very significant, $p < 0.01$.
*** Highly significant, $p < 0.001$.

3.3. Sources of variations in semi-enclosed coastal ecosystems

Kruskal–Wallis' test showed that there was a significant difference of both sediment silt–clay content and duration of emersion between EcoQ classes assessed by the AMBI, the BENTIX and the Shannon index (Table 8). There was a significant linear positive correlation ($p < 0.05$) between AMBI values and silt–clay content. However, this hardly modified the decision between 'Acceptable' and 'Not acceptable'. In Arcachon Bay, the index values were also higher and the EcoQ classification worsened (Kruskal–Wallis test, $p > 0.05$, Table 8) in the intertidal compared to the subtidal. As a consequence, stations situated on the muddy sediments associated to *Z. noltii* seagrass beds displayed poorest EcoQ (Fig. 4).

The behaviour of BENTIX was similar to that of AMBI, except that duration of emersion played a significant role in both coastal ecosystems. Intertidal sites were indeed considered as more degraded by this BI than in the subtidal leading to the classification of many intertidal stations as 'Moderate' or worse (Fig. 4). Moreover, BENTIX was more sensitive to vegetation cover, placing the *Z. noltii* stations in a 'Not acceptable' situation (Fig. 4).

Shannon index displayed a non-linear response to sediment silt and clay content (Fig. 4). Indeed, the EcoQ status slightly improved (although R^2 remain low) with silt and clay content but its value dropped with highest sediment silt and clay content (Fig. 4).

Except for the intertidal stations of Marennes-Oléron Bay where sediments did not modify the index value, BQI roughly behaved as BENTIX (and, to a lesser extent, as AMBI) but with this BI generally assessed poorer EcoQ than the two latter BI (Fig. 4).

In contrast with the other indices, BOPA assessed High EcoQ to the majority of stations in both bays, with hardly any correlation with silt–clay content (Fig. 4).

4. Discussion

The overall pattern of ecological quality status was very different according to the biotic index selected. As an example, according to the BOPA, most sites should be considered as displaying 'High' ecological quality status while Shannon index or BQI provided a much more degraded situation in all three sites, especially in the transitional waters of the Seine Estuary when considering the entire databases. With such a simplistic approach, the use of the five different biotic indices to describe the EcoQ added more complexity than clarity, impairing the accurate assessment of the EcoQ status of the benthic invertebrate communities. Such a problem was also identified by Quintino et al. (2006) in a study including three estuarine and coastal areas of the western coast of Portugal and by Labrune et al. (2005) in the Gulf of Lions. Indeed, our data showed that the classifications of EcoQ status derived from each index rarely agreed on a managerial point of view (i.e. 'Acceptable' versus 'Not acceptable' situations). However, when considering the five EcoQ classes of the WFD, correlations were generally significant with the noteworthy exception of the BOPA. It means that the AMBI, BENTIX, Shannon and BQI indices generally ranked stations in the same way but disagreed on the precise level of EcoQ assessed to each station by the different indices. Correlations between AMBI and BENTIX variations could be easily explained by the computational details of these indices. Both indices are based on the classification of species into ecological groups reflecting species sensitivity, tolerance or opportunism. AMBI considered five groups whereas BENTIX considered only two groups with ecological groups 1 and 2 of the AMBI in the first group, and groups 3–5 of the AMBI

Table 8
Results of the non-parametric Kruskal–Wallis test comparing the environmental characteristics of stations (sediment silt and clay content, duration of emersion) between EcoQ classes derived from the five biotic indices for Arcachon Bay and Marennes-Oléron Bay stations

	n	Range of EcoQ	% Silt and clays *p*-level (K–W test)	Emersion *p*-level (K–W test)
Arcachon Bay (whole bay)				
AMBI	176	1–4	***	***
BENTIX	177	1–4	***	***
BOPA	176	1–3	ns	ns
BQI	94	1–5	***	***
Shannon	177	1–5	*	***
Arcachon Bay (intertidal only)				
AMBI	84	2–4	**	
BENTIX	85	1–4	***	
BOPA	85	1–4	ns	
BQI	65	1–5	***	
Shannon	85	1–4	**	
Arcachon Bay (subtidal only)				
AMBI	89	1–3	**	
BENTIX	89	1–4	**	
BOPA	89	1–3	*	
BQI	29	1–4	*	
Shannon	89	1–4	***	
Marennes-Oléron Bay (whole bay)				
AMBI	261	1–4	***	ns
BENTIX	261	1–4	***	***
BOPA	261	1–3	ns	ns
BQI	133	1–5	ns	ns
Shannon	262	1–5	***	***
Marennes-Oléron Bay (intertidal only)				
AMBI	126	1–3	**	
BENTIX	126	1–4	***	
BOPA	125	1–3	ns	
BQI	68	1–4	ns	
Shannon	126	1–5	***	
Marennes-Oléron Bay (subtidal only)				
AMBI	135	1–4	***	
BENTIX	135	1–4	***	
BOPA	133	1–3	ns	
BQI	64	1–5	*	
Shannon	135	1–5	***	

In a first approach the full dataset was used, on a second approach tests were performed dividing the datasets into subtidal and intertidal stations. Range of EcoQ is indicated with '1' corresponding to 'High' EcoQ, 2 to 'Good', 3 to 'Moderate', 4 to 'Poor' and 5 to 'Bad' EcoQ. Level of significance is indicated.

[ns] Not significant, $p > 0.05$.

[*] Significant, $p < 0.05$.

[**] Very significant, $p < 0.01$.

[***] Highly significant, $p < 0.001$.

in the second group (Borja et al., 2000; Simboura and Zenetos, 2002). Conversely, BQI and Shannon index are more or less directly based on alpha diversity measures, namely ES(50) and number of species, and the Shannon-Wiener diversity index, respectively. As a consequence, BQI values are closely related to diversity measures including dominance as stated by Labrune et al. (2005). When using our data, relationships

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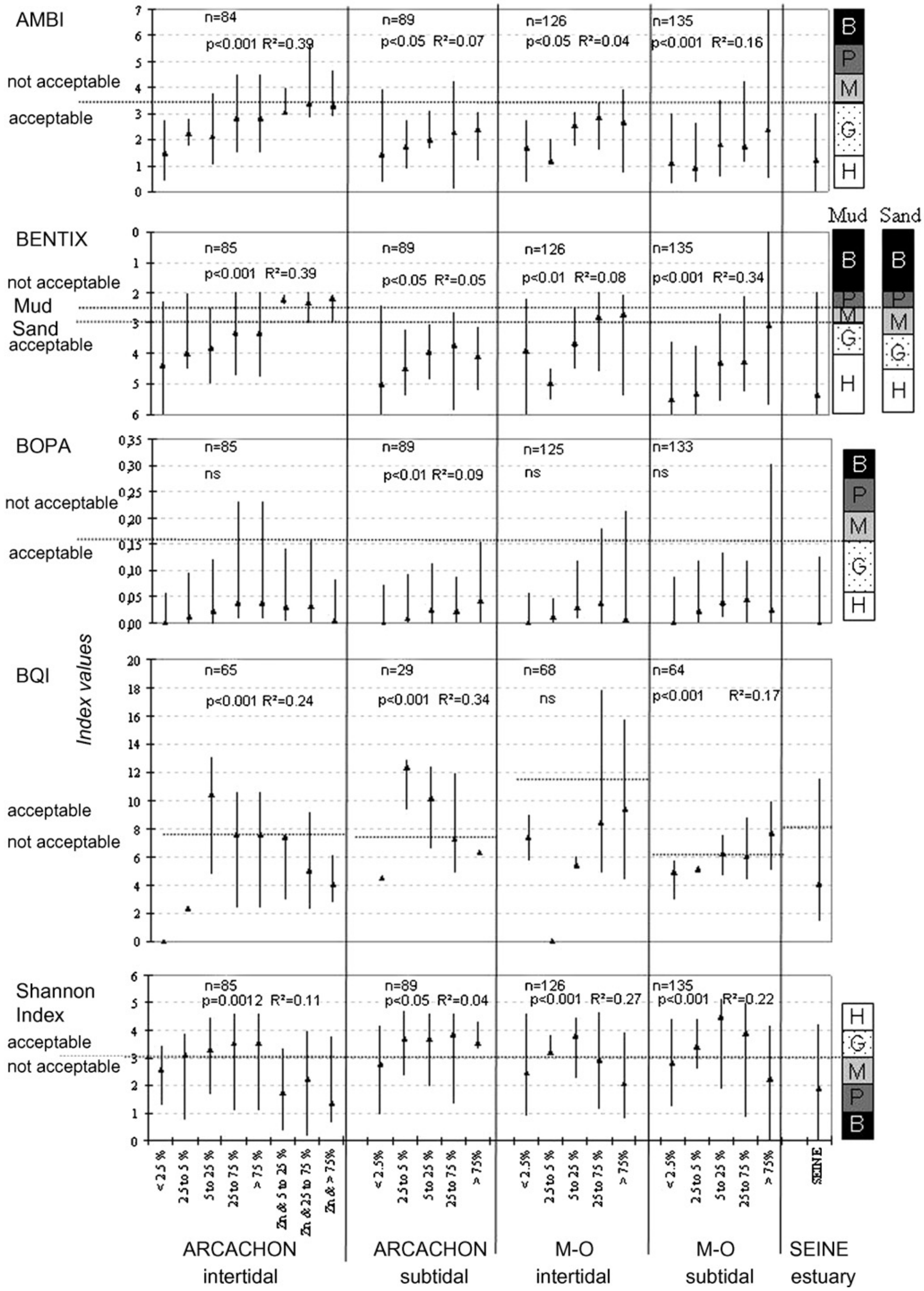

Fig. 4. Median and range (minimum, maximum) of the biotic indices values by location (study site, intertidal, subtidal), sediment silt and clay content (for legibility, silt–clay content was divided into four classes of increasing silt and clay content: <2.5, 2.5–5, 5–25, 25–75 and >75%) and presence of the seagrass *Zostera noltii* (in Arcachon Bay only). Except for the BQI, EcoQ classes boundaries are indicated on the right side of the figure together with the threshold (dashed line) between 'Acceptable' and 'Not acceptable' status (see text for explanations). For the BENTIX,

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between BQI and ES(50) values also proved to be very strong with a highly significant R^2 value of 0.753 between ES(50) and BQI, and a highly significant R^2 value of 0.618 between Shannon index values and BQI (not shown). Disagreement between the BQI and Shannon index mainly consisted into a different definition of thresholds between EcoQ classes. Correlation between BENTIX and Shannon index was more surprising as both indices do not account for the same variables. This correlation could be explained by the numerical dominance of a few species (such as *Hydrobia ulvae*) in intertidal muddy sites. The dominance pattern lowers the value of the Shannon index and the EcoQ derived from BENTIX as these dominant species belong to the tolerant/opportunist species considered by the BENTIX (ecological group 3 of the AMBI).

As a general result our study showed that habitat characteristics such as sediment silt and clay content and the intertidal or subtidal location of stations had significant influence on the EcoQ classification of stations by most of the BI studied here. In particular, intertidal and muddy stations were ranked as more degraded by most indices with the noteworthy exception of the BOPA. These parameters are known as key-factors structuring the benthic macrofauna and should thus be taken into account in any attempt of EcoQ assessment through the use of benthic community structure and composition. This study showed that habitat-related specificity must be taken into account, especially the sediment silt–clay content and the intertidal or subtidal location of habitat. In semi-enclosed environment, biotic index classifications varied according to the silt–clay content of the sediment. This result was not really surprising considering the historical development of the studied indices. BQI, AMBI and BENTIX development was based on the relationship between macrofaunal communities and gradients of increasing organic matter input related to either urban effluents or eutrophication processes (Pearson and Rosenberg, 1978; Glémarec and Hily, 1981; Grall and Glémarec, 1997; Borja et al., 2000; Rosenberg et al., 2004). It was thus not surprising that in muddy environments, where sediment organic matter is naturally high, such indices displayed limitations despite their wide applicability to various sources of impact (Borja et al., 2003; Salas et al., 2004; Muniz et al., 2005; Muxika et al., 2005). As a consequence, these indices express worse quality in naturally muddy environments. The fact that Shannon index had a slight tendency to increase in finer sediments is due to the importance of species richness and to the lack of ecological considerations in the formulae. This index will always increase with species richness, although such tendency is not necessarily correlated with good water quality. This phenomenon was noted by different authors (e.g. Dauvin, 2005; Quintino et al., 2006) but few studies have addressed this particular issue. With regard to these observations, the case of *Z. noltii* beds where the sediment silt–clay content is high (Blanchet et al., 2004) and which were classified as 'Moderate' or even 'Poor' (Fig. 4) by most indices was particularly demonstrating. Indeed, extensive intertidal seagrass beds are considered elsewhere as indicators of a good environmental quality with respect to eutrophication (Tagliapietra et al., 1998; Sfriso et al., 2001; Salas et al., 2004).

Finally, our study evidenced the effect of emersion on these biotic index values and classification (Fig. 4). This environmental factor is a source of natural stress for aquatic species (Cottet et al., in press). Species adapted to emersion usually become dominant in such an environment and biotic indices such as AMBI, BENTIX or BQI classify these communities as of a low EcoQ status. It is also important to highlight that most BI used in this study were originally developed for subtidal communities. For intertidal environments, the thresholds between EcoQ classes should be revised and 'Acceptable' and 'Not acceptable' redefined.

The assessment of the EcoQ status of the Seine Estuary was problematic, as we were unable to determine which part of biotic index variability was attributable to pollution-induced perturbations and which part to habitat characteristics. Moreover, using the BOPA, AMBI and BENTIX classifications, the Seine Estuary appeared in a less degraded condition than the two coastal lagoons studied here. This is in

two scales are shown corresponding to M: muddy sites and S: sandy sites. For each location (intertidal or subtidal) the parameters of the linear regression (n = number of stations, p-level and R^2 coefficient of determination) between sediment silt and clay content and index values are given.

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complete contradiction with what is known about the low pollution levels of these sites compared to that of the Seine Estuary (Dauvin et al., 2005, 2007). In contrast, the BQI and Shannon indices classified the Seine Estuary as of lower ecological quality status, which was more consistent with the pollution level of this site. However, in such transitional waters, the salinity variation effect has to be taken into account as shown by Zettler et al. (2007) in the Southern Baltic Sea.

In contrast with the other BIs tested here, the BOPA showed relative independence to the habitat characteristics studied here. Indeed this index 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 the impact of oil spills on benthic invertebrate communities, as amphipods, the main component of BOPA, are recognised to be sensitive to hydrocarbons (Gomez Gesteira and Dauvin, 2000, 2005; Dauvin and Ruellet, 2007). As a consequence, it did not carry the same bias than the AMBI, BENTIX and BQI for its adaptation to naturally muddy sites.

5. Conclusions and recommendations

This study highlighted some limitations of currently available biotic indices for the implementation of the WFD in particular biotopes occurring in semi-enclosed coastal ecosystems and transitional waters and the need to adapt these biotic indices to habitat specificity. This implies that (1) reference conditions should be determined for each type of habitat and (2) thresholds between EcoQ classes should be adjusted. The definition of reference condition is required by the WFD. The type of habitat and habitat-specific definition of reference conditions is gradually being included in current development of bio-assessment tools. It is the case with the AMBI with the recent development of the Multivariate AMBI (M-AMBI). This tool accounts for reference conditions and includes Shannon-Wiener diversity index, number of species and AMBI for assessing EcoQ (Borja et al., 2007; Muxika et al., 2007). Concerning the definition of thresholds, one main issue deals with the definition of intervals between EcoQ classes. As an example, Rosenberg et al. (2004) used equal sized intervals for the definition of their EcoQ classes based on BQI. This way of defining classes remains highly subjective and cannot be considered as satisfactory because it carries very few ecological meaning. On the other hand, Muxika et al. (2005) defined EcoQ classes on a more ecologically meaningful basis. AMBI was indeed scaled according to the shifts in dominance pattern of the five ecological groups they defined. However, the new M-AMBI defines EcoQ classes in a different way with the risk of losing the ecologically meaning of the former classification of the AMBI (see Muxika et al., 2005; Borja et al., 2007). Moreover, on a more practical approach, scientists have to carefully assess the threshold between what is an ‘Acceptable’ state for benthic communities and what is not (Dauvin, 2007) which should be translated, on a manager point of view, as: where do we need to spend resources to restore the ecosystem and where do we do not? It means that, following the WFD, the threshold between the ‘Good’ EcoQ status and the ‘Moderate’ EcoQ status has to be very carefully defined by the scientists. We think that some of these problems may be partially solved by integrating several of the BIs used here (e.g. the AMBI and the BOPA, which seem to generally perform better in the case of our study sites) into a multi-criteria approach such as those developed in the United States (Weisberg et al., 1997; Eaton, 2001; Llansó et al., 2002a,b; Ranasinghe et al., 2002). These approaches, like the M-AMBI, would better fit the WFD requirements (Muxika et al., 2007) because they include other metrics describing the benthic community integrity (e.g. abundance, biomass, diversity or tropic guilds). In such an approach, one may be able to define, for each metric or BI, what is not significantly different or do not depart from natural background variability and classify it as ‘Acceptable’. ‘Not acceptable’ state would be defined for each metric when measured values would be significantly different or depart from natural background variability. Combining the results for each metrics properly would ease to define than the five-level EcoQ classes of the WFD (Llansó et al., 2002b; Aubry and Elliott, 2006; Dauvin, 2007) based on the EcoQ ratio required by the WFD (Borja et al., 2007). Finally, we think that bimodal response of metrics and BIs have to be considered. This means that one should not always interpret the direction of variation of a given metric (e.g. AMBI, H') as a degradation (if the index

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increases (AMBI)/decreases (H')) or a restoration (if the index decreases (AMBI)/increases (H')) but instead use the different metrics as indicators of change. This last point may allow, in theory, to assess habitat change in a given ecosystem, which is one of the perturbation that has not yet receive much attention despite its importance, particularly in estuarine ecosystems (Dauvin, 2007).

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Development of a multimetric approach to assess perturbation of benthic macrofauna in *Zostera noltii* beds

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ABSTRACT

Biotic indices based on soft-bottom macrozoobenthic communities are currently used throughout Europe to assess the ecological quality of coastal and transitional water bodies according to the European Water Framework Directive. However, the performance of the currently available biotic indices still has to be tested against a variety of different impact sources. In particular, physical perturbations have received much less attention than other kind of disturbances. This study consisted in testing the capacity of currently available uni- (BOPA, AMBI and BENTIX) and multivariate (M-AMBI) Biotic Indices to assess the ecological impact of the destruction of a *Zostera noltii* seagrass bed in Arcachon Bay (France) following sediment deposits. Changes of habitat after this physical perturbation were hardly assessed by any of these Biotic Indices whereas analysis of the benthic community showed drastic changes of structure following the perturbation and no recovery after 15 months. This study demonstrates that these Biotic Indices must be integrated into a multimetric approach which describes better the biological integrity of the benthic community by including a complementary set of metrics. A new multimetric approach, named MISS (Macrobenthic Index of Sheltered Systems) is proposed. MISS correctly highlighted the destruction of the seagrass beds, by using 16 metrics describing the biological integrity of the macrofauna.

Contents

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1. Introduction

Within the European Water Framework Directive (WFD), benthic invertebrates are one of the biological elements to be used for the assessment of ecological quality status (EcoQ) of surface and transitional (estuaries and lagoons) water bodies. Benthic organisms can indeed be considered as potentially powerful indicators of marine ecosystems health because of their position at the sediment-water interface, and their relatively long and sedentary life (Pearson and Rosenberg, 1978; Dauer et al., 2000). Indeed, life at the sediment-water interface makes benthic organisms good integrators of the variations of both sediment and water column (Dauvin, 1993), their sedentary life makes most benthic animals unable to escape unfavourable conditions, and a long lifespan enables them to assess both accidental and chronic perturbations (Dauvin 1993, Reiss and Kröncke, 2005) and allows relatively low-frequency surveys. Finally, benthic macrofauna includes different species displaying different tolerance level to stresses, various feeding guilds and a diversity of life-history traits. In comparison with a purely chemical approach, benthic invertebrate community change testifies real ecological impact of disturbances, at community and ecosystem levels (Fano et al., 2003). As a consequence, benthic macroinvertebrate communities are the most consistently emphasized biotic components to evaluate biological integrity of aquatic systems (Dauvin, 2007).

Several biological indices based on the benthic macrofauna assemblages have been recently developed to assess EcoQ of marine waters within the WFD (Borja et al., 2000; Simboura and Zenetos, 2002; Rosenberg et al., 2004; Dauvin and Ruellet, 2007). These Biotic Indices (BIs) have been tested in a large number of situations (e.g. Salas et al., 2004; Labrune et al., 2006; Marín-Guirao et al., 2005; Muxika et al., 2005; Borja et al., 2006; Dauvin et al., 2007; Bigot et al., 2008; Callier et al., 2008), however a growing number of studies highlights their poor performance (Quintino et al., 2006; Zettler et al., 2007; Blanchet et al., 2008).

A recent study has tested the applicability of BIs for the EcoQ assessment of three French Atlantic coastal semi-enclosed ecosystems (Arcachon Bay, Marennes-Oléron Bay, and the Seine Estuary) (Blanchet et al., 2008). In these particular biotopes, BIs were not adapted. Indeed, sediment silt and clay content and the intertidal or subtidal location of the stations had influence on the EcoQ classification of the stations. The authors showed that in muddy environments, where sediment organic matter is naturally high, such indices displayed limitations and impaired the decision-making (see also Borja et al., 2003; Salas et al., 2004; Muniz et al., 2005; Muxika et al., 2005). More particularly, BIs always expressed worse quality in naturally muddy environment than in coarser sediment, within the same water body (Blanchet et al., 2008). For example, *Zostera noltii* beds were systematically classified in moderate or worse status because of the numerical abundance of species considered as opportunist or tolerant (Blanchet et al., 2008), whereas they were considered healthy and well vegetated (Blanchet et al., 2004), played their ecological key role in the ecosystem (Stoner, 1980; Orth et al., 1984; Edgar, 1990).

In this study, we present the results obtained during the survey of the macrozoobenthic assemblages of a *Zostera noltii* bed following its destruction by sediment deposits. Our objectives were (1) to describe the evolution of benthic assemblages before and after perturbation and (2) to test the performance of uni- and multivariate BIs designed for the implementation of the WFD. On the basis of these results, we proposed a new bio-assessment tool, called MISS (Macrobenthic Index for Sheltered Systems), which includes some of these BIs together with a set of complementary metrics that describe the biological integrity of a benthic assemblage into a multimetric approach.

2. Materials and methods

2.1. Study site

Arcachon Bay (44°40′N, 1°10′W) (Fig. 1) is a 180-km^2 macrotidal (tidal range = 0.9 – 4.9 m) coastal lagoon situated on the South Western coast of France. The lagoon communicates with the Atlantic Ocean by a narrow channel and receives freshwater inputs from a small river (Leyre) situated in the South-Eastern end of the bay. The balance between marine and continental water inputs and the slow renewal of water by tides induces salinity and temperature gradients along a West-East axis (Bouchet, 1993). This lagoon is characterised by large intertidal flats (115 km^2) of which low tide regions are used for oyster farming. Most of these areas (70 km^2) are covered by the largest *Zostera noltii* seagrass bed in Europe (Auby and Labourg, 1996).

The background chemical pollution in Arcachon Bay is low. Its catchment area is dominated by pine forestry (79%) and intensive agriculture occupies only 9% of the surface (de Wit et al., 2005). As a consequence, nutrient inputs to the lagoon are moderate and their concentrations in water remain low (Castel et al., 1996, Bachelet et al., 2000). Some developments of green macroalgae (mainly *Monostroma obscurum* and *Enteromorpha* spp.) occurred in the early 1990s, but these signs of moderate eutrophication have not been observed since. The catchment area is poorly industrialised, and heavy metal contamination is low (Benoit, 2005). Consequently, the overall water quality of the lagoon can be considered as satisfying.

2.2. Perturbation of the Zostera noltii bed and monitoring strategy

In 2004, a small channel was dredged in order to allow free navigation. During the operations, 0.3 km^2 of the nearby *Zostera noltii* bed were covered by sediments (sand and mud) and destroyed. Two “impacted” sites (IS) located in the impacted area and two “control” sites (CS) situated in nearby un-impacted *Zostera noltii* meadow were monitored 3, 8 and 15 months after the operations (Fig. 1). The first 20 cm of the sediment were collected with a 0.0225-m^2 corer (4 replicates per sites). Sediment was sieved through a 1-mm mesh; the remaining fraction was fixed in 4% formalin and stained with Rose Bengal. Macrofauna was sorted, identified when possible to species level, and counted. Biomass was obtained as ash-free dry weight (AFDW) after dessication (60 °C, 48 h) and calcination (550 °C, 2 h). Additional sediment samples were collected at each site. Sediment grain-size was determined after sieving weighted dried sediment through a wet column of sieves with decreasing apertures (1000 µm, 500 µm, 250 µm, 125 µm and 63 µm).

2.3. Multivariate Analysis

Macrofaunal assemblages associated to both impacted (2 sites, 3 surveys: August 2005, January 2006, August 2006) and control (2 sites, 3 surveys: August 2005, January 2006, August 2006) sites were compared with that of 38 sites located in normally vegetated *Zostera noltii* beds sampled in 2002, using Non-Metric Multidimensional

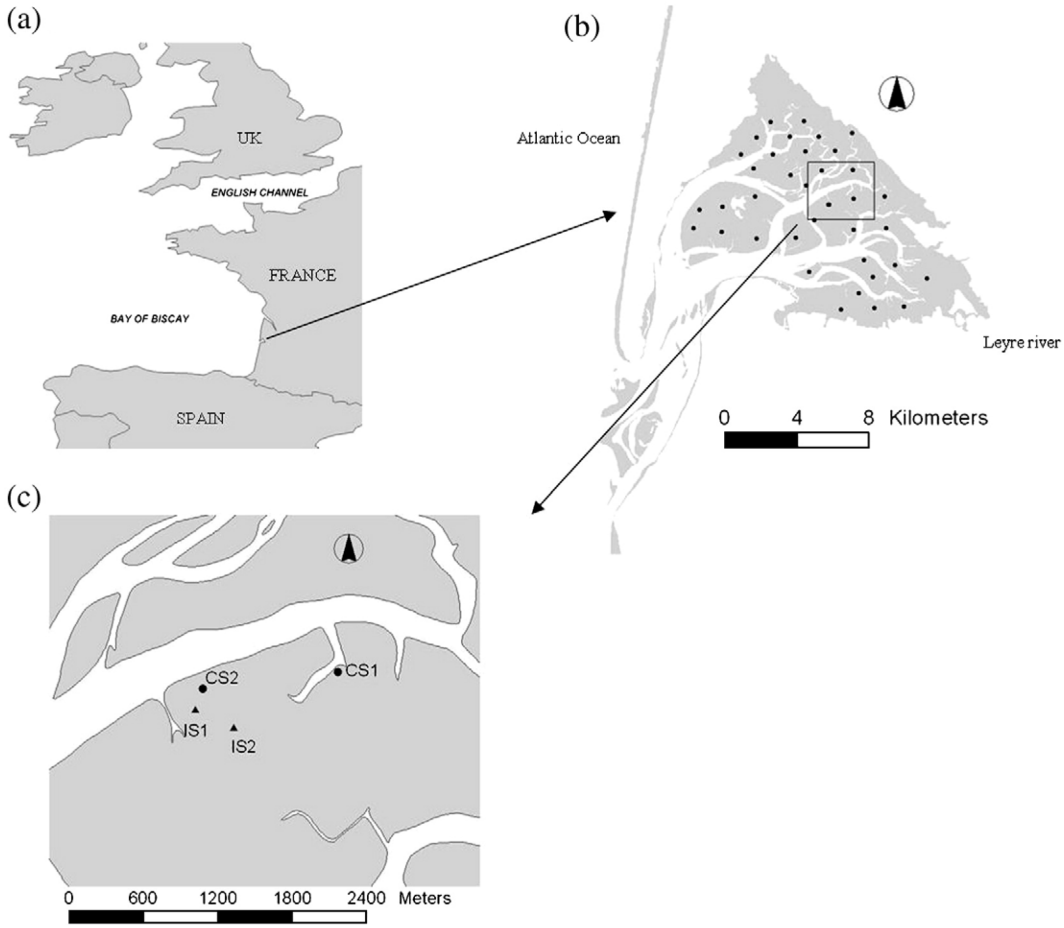

Fig. 1. Maps of (a) Arcachon Bay on the French south west coast, (b) reference conditions sites and (c) the sites in the perturbed area (IS: impacted sites; CS: control sites).

Scaling method (n-MDS). The full database was used, without deleting any species or taxa. Data were previously Log(x+1)-transformed and the Bray-Curtis similarity between sites was computed. The PRIMER® - v6 package was used to perform the analysis (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

2.4. Use of Biotic Indices

Three currently available univariate Biotic Indices (BIs) were tested, namely AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002; Simboura et al., 2005) and BOPA (Dauvin and Ruellet, 2007). These BIs are based on the classification of species into ecological groups according to their level of sensitivity/tolerance to stress. Ecological quality (EcoQ) status and thresholds used to classify index values were reported in Table 1.

AMBI (AZTI Marine Biotic Index) is based on previous work from Grall and Glémarec (1997). It considers five ecological groups (available on web page: http://www.azti.es) ranging from sensitive species (EG_I) to first-order opportunistic species (EG_V) (Borja et al., 2000) (Table 2).

Table 1
Ecological quality (EcoQ) status and thresholds used to classify index values

	EcoQ status		
	Acceptable	Not acceptable	References
AMBI	0 to 3.3	3.3 to 7	Borja et al. (2000)
BENTIX for muds	3 to 6	0 to 3	Simboura and Zenetos (2002)
BOPA	0 to 0.13966	0.13966 to 0.30103	Dauvin and Ruellet (2007)

BENTIX considers only two groups: sensitive (GS) and tolerant species (GT), which correspond to ecological groups I and II, and ecological groups III to V of the AMBI, respectively (Table 2).

The BOPA (Benthic Opportunistic Polychaetes Amphipods index) is based on the ratio of opportunistic polychaetes (i.e. polychaetes of the ecological groups IV and V of the AMBI) and amphipods (except *Jassa* genus) (Table 2).

Table 2
Indices used in this study to assess control and impacted sites

Biotic Indices	Number of ecological groups	Definition of ecological groups	Computation of the indices
AMBI	5	Borja et al. (2000)	$0\ EG_I+1.5\ EG_{II}+3\ EG_{III}+4.5\ EG_{IV}+6EG_V$
		Glémarec and Hily (1981)	based on percentage of ecological groups
BENTIX	2	Borja et al. (2000)	$6\ EG_{I\&II}+2\ EG_{III\text{-}V}$
		Glémarec and Hily (1981)	based on percentage of ecological groups
BOPA	2	Borja et al. (2000)	$\log_{10}[(fp/fa+1)+1]$
		Dauvin and Ruellet (2007)	based on ratio of ecological groups
		Glémarec and Hily (1981)	
M-AMBI	5	Borja et al. (2000)	Multimetric analysis using AMBI, H' and S
		Glémarec and Hily (1981)	based on percentage of ecological groups

EG: ecological groups (see text); fp: opportunistic polychaetes frequency; fa: amphipods frequency.

Table 3
Thresholds derived from *Zostera noltii* meadow reference conditions and used to score each metric

	Thresholds of the different metrics		
	5th percentile	Median	95th percentile
Community			
Abundance (ind. m^{-2})	6206	17078	50922
Biomass (g AFDW m^{-2})	8.1	17.6	44.2
Number of species (0.045 m^2)	16	24	35
H' (Shannon Index)	0.8	1.8	3.6
J' (Pielou's Eveness)	0.19	0.39	0.70
Trophic composition			
Grazer (ind. m^{-2})	639	8933	24772
Selective deposit feeders (ind. m^{-2})	106	1289	6017
Non-selective deposit feeders (ind. m^{-2})	522	4011	31900
Suspension feeders (ind. m^{-2})	44	200	750
Carrion feeders (ind. m^{-2})	67	300	950
Pollution indicators			
AMBI	2.9	3.5	5.1
BOPA	0.001	0.024	0.122
W statistic	−0.111	−0.015	0.098
Sensitive species (ind. m^{-2})	233	844	2794
Tolerant species (ind. m^{-2})	2650	9478	25017
Opportunistic species (ind. m^{-2})	528	4022	31878

The performance of the more recently developed Multivariate AMBI (M-AMBI (Muxika et al., 2007)) was also tested. This method is based on a Factorial Analysis that considers AMBI value, number of species and Shannon-Wiener diversity index, and defines reference conditions (for details, see Borja et al., 2004; Bald et al., 2005; Muxika et al., 2007). M-AMBI was the first method to take into account reference conditions for the assessment of the ecological quality status. Monitoring results were compared with reference conditions, in order to derive an Ecological Quality Ratio (EQR), as specified by the WFD (Table 2). Under “high” status, the reference condition may be regarded as an optimum where the EQR approaches one. Under “bad” status, M-AMBI value approaches zero (Muxika et al., 2007). M-AMBI index was computed using the software provided on the AZTI institute website (http://www.azti.es). Reference conditions were derived by the software from the data collected in the 38 sites located on normally vegetated *Zostera noltii* beds in 2002 (Blanchet et al., 2004).

2.5. Development of a complementary approach

2.5.1. Conception of the approach

In order to accurately assess the benthos modifications, we developed a complementary approach. This approach was called MISS (Macrobenthic Index in Sheltered Systems). MISS basically consisted in including a selection of existing BIs with additional metrics describing the community and its trophic structure. MISS was inspired by the development of Indices of Biological Integrity conducted in North America (e.g. Weisberg et al., 1997; Engle and Summers, 1999; Van Dolah et al., 1999; Llanso et al., 2002a,b).

2.5.2. Definition of reference conditions

The Water framework Directive requires the comparison of data against reference conditions (Muxika et al., 2007). Reference conditions are the physico-chemical (or biological) conditions of the system, reflecting the best physico-chemical (or ecological) status possible and the least anthropogenic impact (Borja, 2005). In this study, reference conditions were defined by using macrofaunal data collected in 38 sites located in *Zostera noltii* beds during late winter-early spring in 2002 (Fig. 1). This database gathered the data of two replicates samples per site, where each sample consisted in two 0.0225-m^2 cores pooled together. In Arcachon Bay, *Zostera noltii* had a significant impact on the structure of the associated macrofauna when the leaf biomass was over 28 g DW.m^{-2} (Blanchet et al., 2004). As a consequence, only sites where the leaf biomass was above that threshold were included in order to define reference conditions. The definition of reference conditions consisted in computing the different metrics describing macrofauna integrity in seagrass meadows considered as not degraded, and in measuring their variability.

2.5.3. Selection of the metrics

At the beginning of this work, forty-five metrics were studied and compared to describe as well as possible the biological integrity of the benthic assemblage. After deleting the most redundant correlated parameters, sixteen parameters were selected and classified into three balanced categories describing the macrofaunal assemblage (Table 3):

- *Community descriptors* (five parameters): abundance per m^2, biomass g AFDW per m^2, number of species (per 0.045 m^2), Shannon Index H', and Pielou's evenness J'. These metrics were computed by using Primer® package.

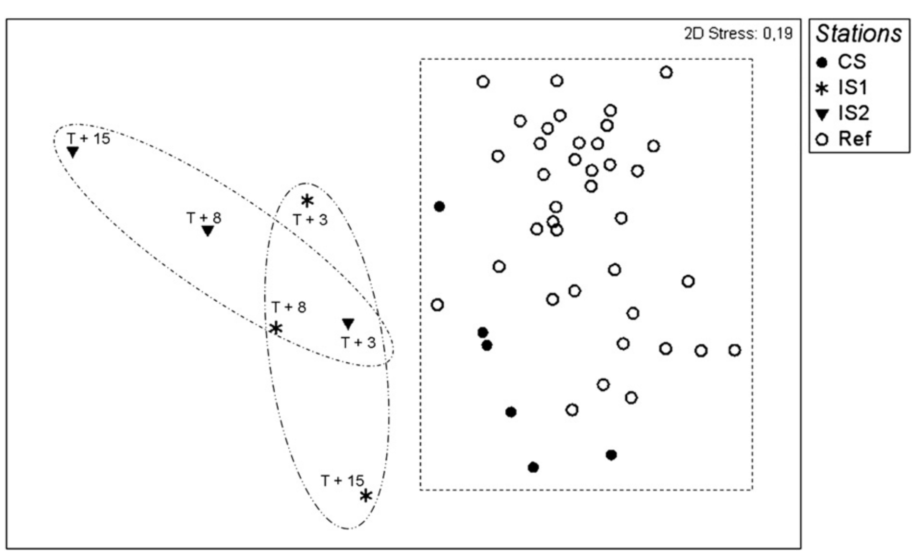

Fig. 2. Results of n-MDS analysis based on species abundances [log (x+1)]. CS: control sites, IS: impacted sites, Ref: reference sites (2002), T+ n: n months after sediment disposal.

Table 4
Comparison of macrofaunal and habitat data between reference conditions, control and impacted sites

	Reference conditions	Control sites	Impacted site 1	Impacted site 2
Main species (mean ± es)	*Hydrobia ulvae* (8933±955) *Tubificoides benedii* (7334±1276) *Heteromastus filiformis* (1155±133) *Abra segmentum* (709±124) *Melinna palmata* (409±81)	*T. benedii* (2915±1305) *Aphelochaeta marioni* (1520±517) *M. palmata* (1476±384) *Idotea chelipes* (552±275) *H. filiformis* (441±60) *Microdeutopus gryllotalpa* (389±133) *H. ulvae* (354±178) *Iphinoe trispinosa* (269±134) *A. segmentum* (239±42)	*H. ulvae* (837±404) *H. filiformis* (515±167) *Cyathura carinata* (278±150) *Nereis diversicolor* (274±217) *A. marioni* (207±173)	*Pygospio elegans* (270±114) *H. ulvae* (222±111)
Median of total abundance	17288 ind.m^{-2}	8856 ind.m^{-2}	3022 ind.m^{-2}	1056 ind.m^{-2}
Median of number of taxa	23	30	17	10
Median of total biomass	18.3 g AFDW.m^{-2}	15 g AFDW.m^{-2}	2.6 g AFDW.m^{-2}	1.05 g AFDW.m^{-2}
Type of sediment	mud (median 43 µm)	mud (median 90 µm)	mud (median 90 µm)	muddy sands (median 170 µm)
Zostera noltii biomass	299±17 g DW m^{-2}	107±15 g DW m^{-2}	5±3 g DW m^{-2}	unvegetated

- *Trophic composition* (five parameters): grazers per m^2, selective deposit feeders per m^2, non-selective deposit feeders per m^2, suspension feeders per m^2, and carrion feeders per m^2 (e.g., carnivorous, omnivorous and scavengers). Species were classified into feeding guilds using literature description (e.g. Fauchald and Jumars, 1979; Bachelet, 1981; Sauriau et al., 1989; Hily and Bouteille, 1999).
- *Pollution indicators* (six parameters)**:** Values of two recently developed Biotic Indices were used (AMBI (Borja et al.; 2000) and BOPA (Dauvin and Ruellet; 2007)), abundance per m^2 of sensitive species (e.g. EG_I and EG_{II}), abundance per m^2 of tolerant species (e.g. EG_{III}), abundance per m^2 of opportunistic species (e.g. EG_{IV} and EG_V). Finally, The W statistic, referring to Abundance-Biomass Comparison (ABC curves) was computed with PRIMER® - v6 package. W measured the extent to which biomass curves lays above the abundance curves (positive values were expected for the undisturbed condition, negative values for impacted samples) (Warwick, 1986). The BOPA and the AMBI indices were included as indicators of, respectively, pollution by hydrocarbons and organic matter inputs.

2.5.4. Scoring method

The scoring method was inspired by Weisberg et al. (1997). Thresholds for the selected metrics were based on the distribution of values at the 38 reference sites (*Zostera noltii* meadow sampled in 2002). For each metric, values below the 5th and above the 95th percentile were scored as 0. Other values (between the 5th and the 95th percentiles) were scored as 1 and considered as natural variations of *Zostera noltii* seagrass beds (Table 3). For each category of metrics (community descriptors, trophic

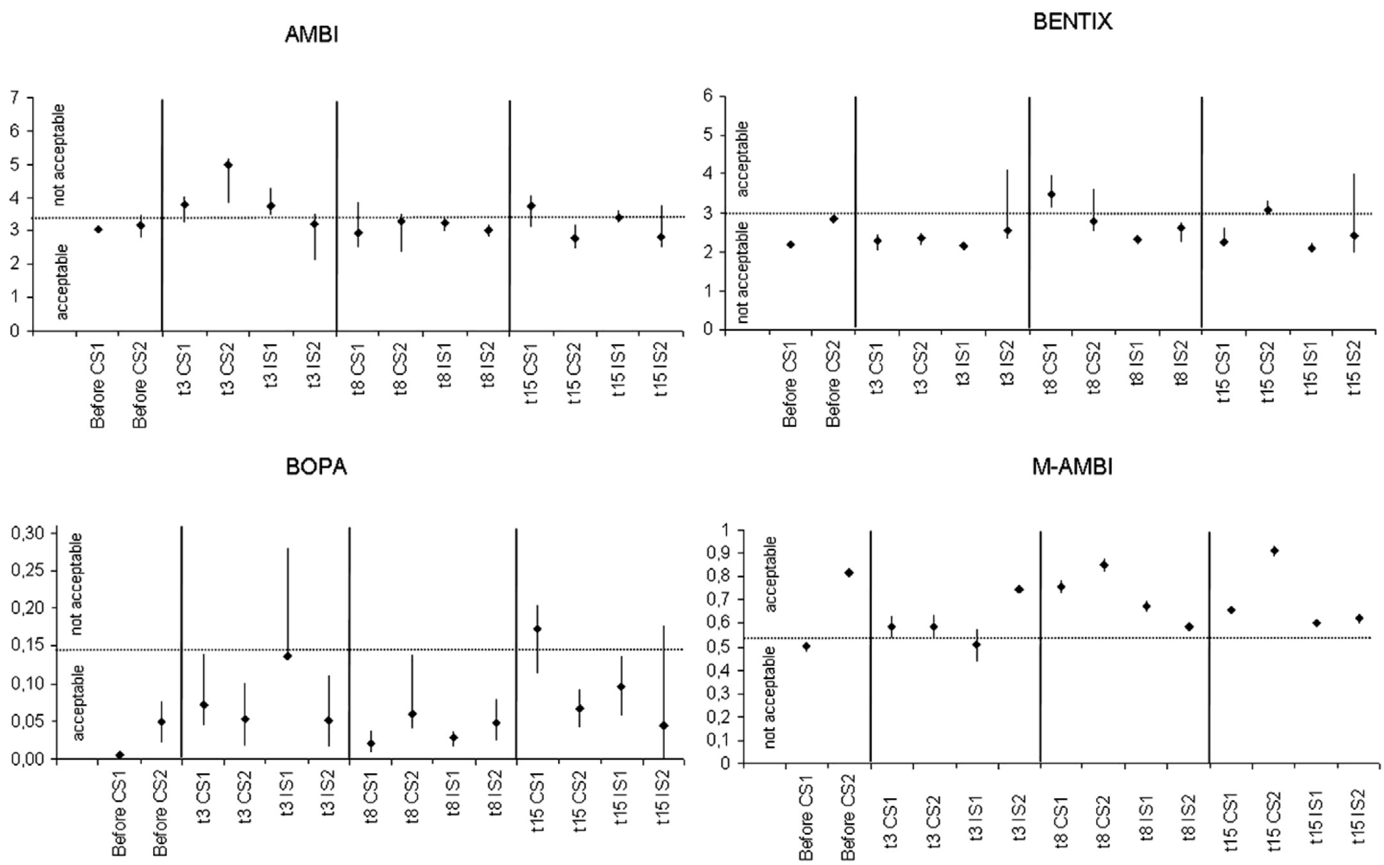

Fig. 3. Median and range (minimum, maximum) of the biotic indices values before and after the perturbation (T+n: n months after sediment disposal). Threshold (dashed line) between 'acceptable' and 'not acceptable' status for each index is indicated on the left side of each graphic. CS: control sites, IS: impacted sites.

Table 5
Significant, very significant, and highly significant results of the non-parametric ANOVA Kruskall-Wallis test conducted on control and impacted sites

	Control site 1	Control site 2	Impacted site 1	Impacted site 2	Significant difference
t+3months					
Abundance (ind. m^{-2})	3489	18956	2222	1933	CS2≠IS2*
Biomass (g AFDW m^{-2})	14.7	12.9	1.2	1	CS1≠IS1*; CS1≠IS2*
Number of species	13–14	25	8–9	9–10	CS2≠IS1*
H' (Shannon Index)	2.4	2.1	2.3	2.9	ns
J' (Pielou's Evenness)	0.67	0.45	0.68	0.84	CS2≠IS2*
Grazer (ind. m^{-2})	1778	578	444	267	ns
Selective deposit feeders (ind. m^{-2})	356	4600	622	1156	CS1≠CS2*
Non-selective deposit feeders (ind. m^{-2})	1022	13333	956	178	CS2≠IS2*
Suspension feeders (ind. m^{-2})	133	667	67	22	CS2≠IS2*
Carrion feeders (ind. m^{-2})	89	778	89	244	CS1≠CS2*;CS2≠IS1*
AMBI	3.8	5	3.8	3.2	CS2≠IS2*
BOPA	0.07	0.05	0.14	0.05	ns
W statistic	0.058	−0.164	0.049	0.131	ns
Sensitive species (ind. m^{-2})	222	1400	89	311	CS2≠IS1**
Tolerant species (ind. m^{-2})	1956	2711	889	1333	ns
Opportunistic species (ind. m^{-2})	1200	15578	1378	356	CS2≠IS2*
t+8months					
Abundance (ind. m^{-2})	9711	14600	3133	800	CS2≠IS2**;CS1≠IS2*
Biomass (g AFDW m^{-2})	9.7	19.1	2.8	1.7	CS2≠IS2**
Number of species	20–21	27	13	7	CS2≠IS2**
H' (Shannon Index)	3.1	3.2	2.8	2.3	ns
J' (Pielou's Evenness)	0.72	0.67	0.76	0.88	ns
Grazer (ind. m^{-2})	4933	1911	1000	222	CS1≠IS2**
Selective deposit feeders (ind. m^{-2})	1089	8822	1000	289	CS2≠IS2*
Non-selective deposit feeders (ind. m^{-2})	2511	2044	289	67	CS1≠IS2*;CS2≠IS2*
Suspension feeders (ind. m^{-2})	67	289	44	0	CS2≠IS2*
Carrion feeders (ind. m^{-2})	244	178	311	111	ns
AMBI	2.9	3.3	3.2	3	ns
BOPA	0.02	0.06	0.03	0.05	ns
W statistic	0.113	0.104	0.134	0.437	ns
Sensitive species (ind. m^{-2})	3644	2956	267	133	CS1≠IS2*;CS2≠IS2*
Tolerant species (ind. m^{-2})	3244	5089	2400	556	CS2≠IS2**
Opportunistic species (ind. m^{-2})	2733	4778	444	111	CS1≠IS2*; CS2≠IS2*
t+15months					
Abundance (ind. m^{-2})	5778	8422	3644	289	CS2≠IS2**
Biomass (g AFDW m^{-2})	11	21.3	7.1	0.2	CS2≠IS2**
Number of species	18	26–27	12	4	CS2≠IS2**
H' (Shannon Index)	2.7	3.5	2.7	1.8	CS2≠IS2**
J' (Pielou's Evenness)	0.66	0.76	0.73	0.91	CS1≠IS2*
Grazer (ind. m^{-2})	311	667	267	22	CS2≠IS2**
Selective deposit feeders (ind. m^{-2})	4400	4556	956	44	CS1≠IS2*; CS2≠IS2*
Non-selective deposit feeders (ind. m^{-2})	622	1689	667	44	CS2≠IS2**
Suspension feeders (ind. m^{-2})	67	667	22	44	CS2≠IS1*
Carrion feeders (ind. m^{-2})	311	467	444	89	ns
AMBI	3.7	2.8	3.4	2.8	ns
BOPA	0.17	0.07	0.1	0.04	ns
W statistic	0.083	0.146	0.219	0.281	ns
Sensitive species (ind. m^{-2})	422	2289	67	67	CS2≠IS1*; CS2≠IS2*
Tolerant species (ind. m^{-2})	2111	3911	2133	200	CS2≠IS2**
Opportunistic species (ind. m^{-2})	3178	2133	711	44	CS1≠IS2*; CS2≠IS2*

Values represent median of each parameters.
Level of significance is indicated.
ns Not significant, $p>0.05$.
* Significant, $p<0.05$.
** Very significant, $p<0.01$.

composition, and pollution indicators) the average score (which, in this case, is equivalent to the computation of a simple matching coefficient (Legendre and Legendre, 1984)) was calculated. Finally the EcoQR (ecological ratio) was the mean (μ) of these three previously calculated scores and represented the distance to the equilibrium status. We also calculated the standard deviation (σ) associated to this mean (μ). The corresponding normal distribution (μ, σ) permitted to determine the level of confidence (reliability) associated to the assessment of the EcoQ status. The EcoQR was divided in 5 equal classes corresponding to the 5 ecological quality status of the WFD ('high': 1–0.8, 'good': 0.8–0.6, 'moderate': 0.6–0.4, 'poor': 0.4–0.2, and 'bad': 0.2–0).

2.5.5. Calibration of the method

This multi-metric method was calibrated by studying the response of other different data sets from Arcachon Bay:

- *Physical perturbation:* we tested MISS against the perturbation monitored in this study.
- *Poorly vegetated meadows:* there's some *Zostera noltii* meadows in the lagoon characterised by a low biomass of leaf (<28 g DW m^{-2}). These seven sites could be considered as perturbed seagrass beds.
- *Unvegetated intertidal habitats:* the method was tested with different unvegetated habitats described by Blanchet (2004),

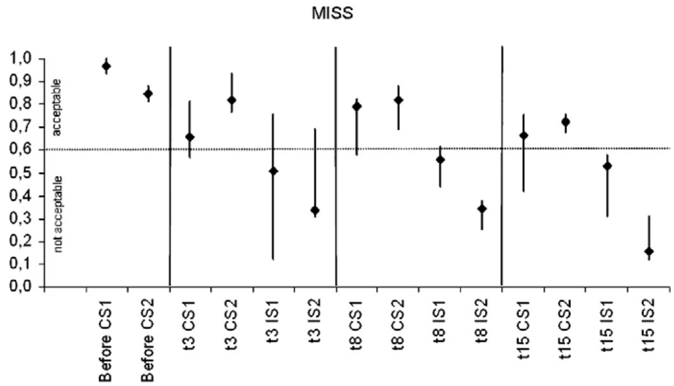

Fig. 4. Median and range (minimum, maximum) of MISS values before and after the perturbation (T+n: n months after sediment disposal). Threshold (dashed line) between 'acceptable' and 'not acceptable' is indicated on the left side of the figure. CS: control sites, IS: impacted sites.

(oceanic sands, muddy-sand and sandy muds, *Scrobicularia plana* muds, *Arenicola marina* sands…).

2.6. Evaluation of the performance of the indices

The performance of the five indices was compared using the following approaches:

(1) Statistically significant differences in the numerical values of each indice between impacted and control sites were tested through a non-parametric Kruskall-Wallis ANOVA and pairwise median test. (2) Statistically significant differences in the ranked values of EcoQ assessed by each index were also compared. For this test, EcoQ classes were ranked from 1, which corresponded to 'high' EcoQ, to 5 corresponding to 'bad' EcoQ. The ranked EcoQ were compared through a non-parametric Kruskall-Wallis ANOVA and pairwise median test. (3) Finally, each index was applied to reference conditions data, and the number of correct classifications ('high' or 'good') was computed. The best index should detect as much significant differences as possible in (1) and (2); and classify sites corresponding to reference conditions as 'high' or 'good' status.

The performance of individual component metrics used in MISS was also tested. For each metric, the ability to correctly detect the perturbation and to correctly assess good or better ecological status of control sites was calculated as the number of occasions where impacted and control sites were correctly classify (based on the score of each metric). The same computation was made for BIs EcoQ and the different categories describing the integrity of macrofauna in MISS: 'community descriptors', 'trophic composition' and 'pollution indicators'.

3. Results

3.1. Consequences of sediment disposal on macrofauna

The MDS analysis (Fig. 2) showed clear differences in the composition of benthic assemblages following the perturbation. The stress value, lower than 0.2, provides useful 2-dimensional picture. The MDS plot clearly distinguished three groups of stations according to their faunal composition.

The first group of stations gathered both the *Zostera noltii* beds sites sampled in 2002 and the control sites, which were situated in, or very close to, some sites of 2002 (Fig. 2). In control sites the structure of the macrofauna was thus similar to that normally encountered in *Zostera noltii* beds in Arcachon Bay. Indeed, the macrofauna was relatively abundant (8856 ind.m^{-2}), well diversified (30 species) and displayed high biomass (15 g AFDW m^{-2}) (Table 4). Consequently, the community of control sites was considered similar to the community in our *Zostera noltii* reference sites.

The two other groups of stations corresponded to impacted site 1 (IS1) and impacted site 2 (IS2) throughout the survey period. MDS separated the two sites and highlighted the growing dissimilarity between the corresponding benthic assemblages through the 15-months survey (Fig. 2).

The samples of impacted site 1 (IS1) consisted in very muddy sands similar to that of control sites (median grain size = 90 µm) (Table 4). However, the impacted site 1 did not display vegetation except in the form of few decaying roots and rhizomes at t + 3 months (mean vegetal biomass over the survey period = 5 g DW m^{-2} *vs* 107 g DW m^{-2} in control sites). In this site, the composition of macrofauna changed throughout the time, but remained different from that of *Zostera noltii* beds (2002 situation and control sites). Macrofaunal assemblages displayed lower abundance (3022 ind.m^{-2}), diversity (17 species) and biomass (2.6 g AFDW m^{-2}) than in control sites. The most abundant species were *Hydrobia ulvae*, *Cyathura carinata* and polychaetes like *Heteromastus filiformis*, *Nereis diversicolor* and *Aphelochaeta marioni*.

The macrofaunal assemblage in impacted site 2 (IS2) was very different from that of the *Zostera noltii* beds. After 15 months, this site displayed a different benthic assemblage compared to that of *Zostera noltii* beds. This site became more sandy after the sediment disposal (median grain size = 170 µm) and the meadow was totally destroyed. The abundance, number of species and biomass of the macrofauna

Table 6
EQR and EcoQ derived from multimetric approach (MISS) before and after perturbation, and associated level of confidence for each status

Treatment	Time	Assessed EcoQ	Mean EQR	Standard deviation	Level of confidence				
					High	Good	Moderate	Poor	Bad
Control site 1	before	high	0.97	0.06	100%	0%	0%	0%	0%
	t + 3	good	0.73	0.23	38%	33%	21%	7%	1%
	t + 8	high	0.85	0.11	68%	31%	1%	0%	0%
	t + 15	good	0.72	0.10	21%	67%	12%	0%	0%
Control site 2	before	high	0.84	0.15	61%	34%	6%	0%	0%
	t + 3	high	0.87	0.12	72%	27%	1%	0%	0%
	t + 8	good	0.75	0.10	31%	63%	7%	0%	0%
	t + 15	good	0.62	0.10	4%	54%	41%	1%	0%
Impacted site 1	t + 3	moderate	0.59	0.10	2%	44%	51%	3%	0%
	t + 8	moderate	0.59	0.25	20%	28%	29%	16%	6%
	t + 15	moderate	0.50	0.21	7%	24%	37%	24%	9%
Impacted site 2	t + 3	moderate	0.53	0.21	10%	27%	36%	21%	6%
	t + 8	poor	0.34	0.04	0%	0%	7%	93%	0%
	t + 15	bad	0.19	0.02	0%	0%	0%	0%	100%

were low, with 1056 ind.m^{-2}, a number of species of 10, and only 1.05 g AFDW m^{-2} of biomass. Only two species displayed abundance levels above 200 individuals per m^{-2}: *Pygospio elegans* and *Hydrobia ulvae* (Table 4).

3.2. Assessment of the perturbation by Biotic Indices

The classification of the sites by AMBI (Fig. 3) was not consistent with *in situ* observations. There were no real differences of AMBI values between control and impacted sites. At t+3 months control sites (CS) were even considered as more perturbed than impacted sites (IS) (Fig. 3). Moreover, ecological status of the IS2 was always ‘acceptable’ (‘Good’ or higher ecological quality status), whereas this site was the most perturbed according to visual observations (disappearance of vegetation and change of sediment type) and MDS (Fig. 2).

BENTIX classified control sites and impacted sites as ‘not acceptable” (‘Moderate’ or worse Ecological quality status) (Fig. 3). Impacted sites did not display lower values of BENTIX (*i.e.* more degraded) than control sites (except at t+8 months). As a result, this index did not detect any perturbation in impacted sites.

In contrast, BOPA considered that the majority of the sites displayed ‘acceptable’ status, even in perturbed sites (Fig. 3).

M-AMBI did not perform better than the three other biotic indices. This index identified reference conditions as corresponding to an AMBI value of 2.78, a Shannon diversity index value equal to 4.08 and determined the maximum number of species as 43 within the 38 reference sites. This method classified most of the situations (sites×dates) as ‘acceptable’.

3.3. Detection of the perturbation with multimetric method

The non-parametric Kruskall-Wallis test was used to detect significant differences in metrics between control sites and impacted sites (Table 5). The three periods were tested separately. Most of the tested parameters reacted at least once to the perturbation (with a significance level of $p<0.05$). The different metrics reacted differently to both kinds of perturbation (mud or sand deposits).

Scores and ecological status obtained with MISS method are represented in Fig. 4. All the control sites were in ‘good’ or ‘high’ ecological status and impacted sites were all classified as ‘not acceptable’. IS1 remained ‘moderate’ after the perturbation, without amelioration. IS2 was ‘poor’ after 3 months and 8 months and finally ‘bad’ after 15 months. These notes reflected the results obtained by the MDS analysis with no observed recolonization. Nevertheless, following situations (sites×dates), confidence levels fluctuated between 29% and 100% (Table 6).

MISS was validated with other sites of Arcachon Bay (Fig. 5). Firstly, MISS was computed for each reference sites. They were all classified as ‘acceptable’ and most of them as ‘high’ (84%). Secondly, MISS was used on poorly vegetated meadows (leave biomass<28 g DW m^{-2}). These scarce meadows were mainly classified as ‘not acceptable’ (83%). Finally, MISS from unvegetated intertidal habitats of the bay was calculated, and 97% of sites were classified as ‘not acceptable’.

Table 7
Comparison of indices performance

	Index values	EcoQ classes	% of correct classifications
AMBI	1/12	0/12	33
BENTIX	2/12	1/12	0
BOPA	0/12	0/12	95
M-AMBI	0/12	0/12	57
MISS	4/12	3/12	100

Ratio of the number of statistically differences between control sites and impacted sites on the basis on (1) index values, (2) EcoQ classification. Percentage of sites corresponding to reference conditions correctly ('High' or 'Good') classified by each index.

3.4. Comparison of indices performance

Kruskall Wallis and median test did not detect any significant difference between impacted and control sites of both index values and EcoQ classes for BOPA and M-AMBI (Table 7). The value of the AMBI was only significantly different at t+3months but this difference indicated that control site 2 was more degraded than impacted site 2. However there was no significant change of EcoQ classification between these two stations (Table 7). There were significant differences of BENTIX values on two occasions which triggered only one significant difference of EcoQ classification. MISS displayed significant differences on four occasions which corresponded to three significant changes of EcoQ (Table 7).

MISS and BOPA correctly classified reference conditions sites (100% and 95% respectively). M-AMBI only provided correct classification in 57% of cases, whereas AMBI failed to correctly classify 67% of sites. Finally, BENTIX misclassified all the reference stations (Table 7).

Metrics and EcoQ derived from BIs were compared on the basis of their capacity to detect impact and assess correctly the control sites. Fig. 6 clearly highlighted a gradient of ‘performance’ for these parameters. BIs were not efficient to assess both perturbation and control situation. BOPA and M-AMBI correctly assess the control sites but not the impact of sediment deposits. AMBI was efficient to detect the perturbation but this index classified as ‘not acceptable’ the control sites. Finally, BENTIX was unable to assess either situation. The best way to observe the differences between control and impacted sites was to use MISS approach (extremity of the gradient). Its component metrics were also distributed along the same gradient. The best descriptors of impact and reference conditions were the abundance and the biomass metrics. However, these parameters were less efficient than the MISS approach (particularly to correctly assess the control sites) The three categories describing the macrofauna integrity (‘community descriptors’, ‘trophic composition’, ‘pollution indicators’) were well represented along the gradient of performance of the metrics. Finally, in the case of a purely physical perturbation, the

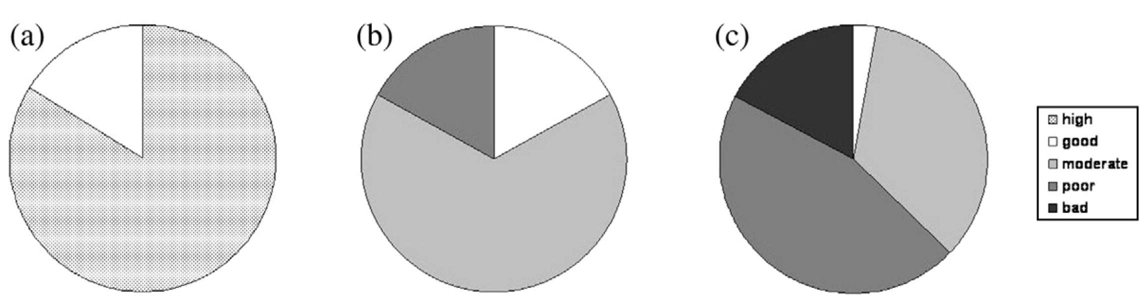

Fig. 5. Relative proportions of ‘high’, ‘good’, ‘moderate’, ‘poor’ and ‘bad’ sites of (a) reference sites, (b) poorly vegetated meadows, and (c) unvegetated intertidal areas. Categorization was made with MISS.

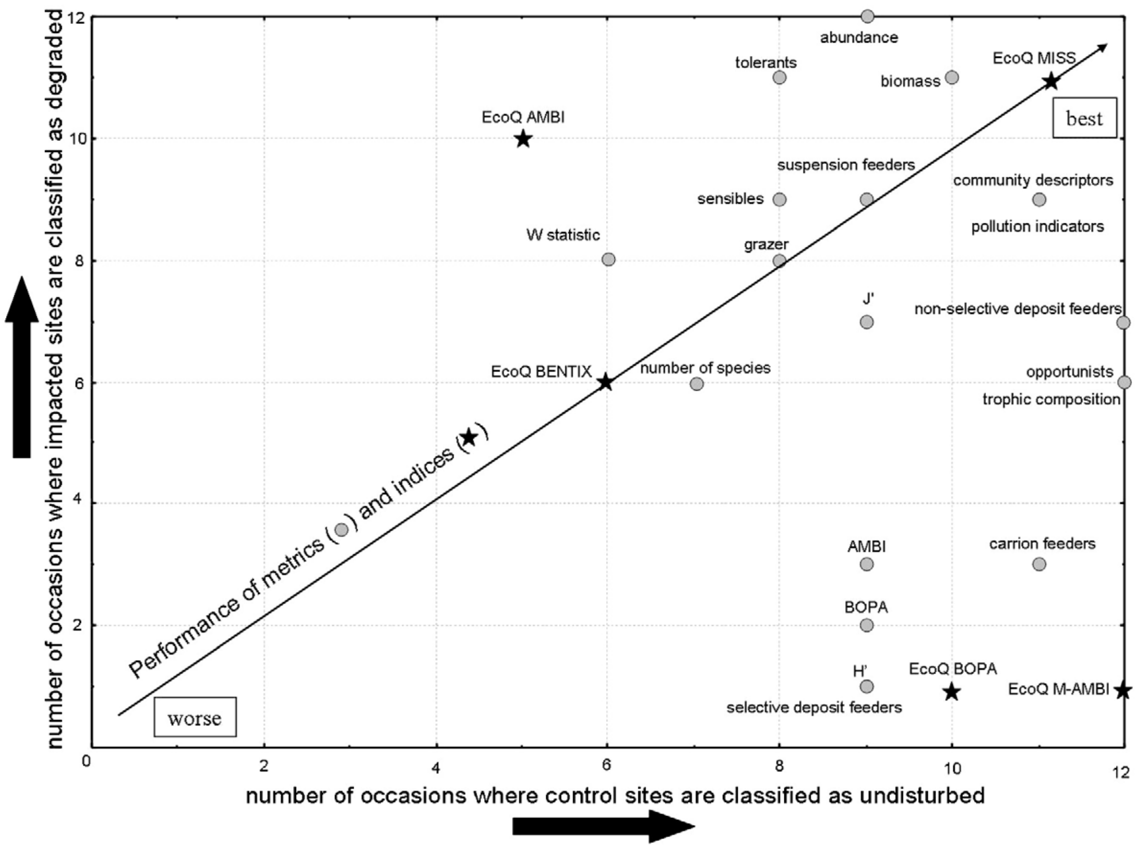

Fig. 6. Graphical representation of the compared performances of BIs and metrics showing their ability in terms of number of occasions where the indices or metrics correctly detect perturbed sites (axis 1) and assess control sites (axis 2). On a total of 24 occasions corresponding to 24 samples, 12 were performed in the control sites and 12 in the impacted sites. As an example, coordinates of EcoQ MISS (11; 11) corresponded to 11 correctly classified occasions for CS out of 12 and 11 occasions out of 12 where impacted sites were classified as degraded. For metrics, values of Table 3 were used in the same way. As an example, abundance for CS should be comprised between 6206 and 50922 ind.m^{-2}: that was the case in 9 replicates out of the 12 replicates in CS. In IS, the abundance was out of this range in all replicates.

others metrics were less represented along the gradient, but these parameters could be more efficient with other kind of pollution (eutrophication, organic matter enrichment).

4. Discussion

The aim of this study was not to assess a perturbation with BIs but to test their performances following a known habitat modification in a naturally muddy environment.

4.1. Perturbation of the macrofauna by sediment disposal

The meadow was completely covered by mud in the impacted site 1 and by sand in the impacted site 2. This perturbation, easily visible on the field, even 15 months after the operations, triggered a considerable change of benthic communities' structures. Such dramatic changes of benthic assemblage after sediment deposits have been reported at several occasions (Harvey et al., 1998; Cruz-Motta and Collins, 2004; Whomersley et al., 2007; Wilber et al., 2007). The two different kinds of perturbation (by sand or mud deposits) had a different impact on the benthic community in terms of abundance, biomass and species richness. Community structure, as revealed by Non-metric Multidimensional Scaling, confirmed that the habitat and the associated fauna were highly modified by the sediment deposits. However these habitat modifications were not highlighted by the BIs designed for the WFD (AMBI, BENTIX, BOPA and M-AMBI) neither after three months nor 15 months later.

Previous studies already showed that most of these Biotic Indices performed badly in semi enclosed ecosystems (Blanchet et al., 2008) where the natural benthic habitat consists in muddy, organic matter-enriched sediments. The species adapted to this habitat typically occur in high numbers and are listed as tolerant or opportunistic (Glémarec and Hily, 1981; Borja et al., 2000). Consequently the ratio between [tolerant+opportunistic species] *vs* [sensitive or indifferent species] is high and induces a low EcoQ. It could be argued that the bad performance of the BIs used in this study would be solved by adapting the scale of conversion (the threshold between EcoQ classes) into ecological quality status to each situation. Our study however showed that different kinds of perturbation did not trigger the same response from the benthic community. For example, three months after the sediment deposition, AMBI revealed better values in perturbed sites compared to control sites. In this case, changing the scale of conversion from BI value to ecological quality status would not solve the problem. It was thus not surprising that such indices were unable to detect the particular changes that occurred in the impacted sites following sediment disposal. In the same way, Muxika et al. (2005) admitted that AMBI was not a good indicator of physical perturbations including sand deposits. This limitation has to be taken into account especially in the case of littoral or estuarine water bodies where dredging operations are regular and may significantly affect the ecological functioning of these systems (Cruz-Motta and Collins, 2004; Dauvin, 2007).

Finally it is probable that the response of a given species is dependant on the kind of perturbation. For example, the estuarine amphipod *Corophium volutator* can be considered as sensitive to metal contamination (Warwick, 2001). Conversely, Norkko et al. (2006) described the opportunistic behaviour of this species following experimental defaunation on intertidal location of the Swedish west coast. The latter authors concluded that the magnitude of opportunistic response is scale- and intensity- dependant. The response of a given species could also depend on the nature of the perturbation (see also Bustos-Baez and Frid, 2003). The classification of a given species along a sensitivity-tolerance continuum is thus a very difficult task and still a matter of debate (e.g. Labrune et al., 2006). Consequently, a given Biotic Index based on his own unique classification of species into sensitive to opportunistic will only consider the level of sensitivity/tolerance to a particular kind of perturbation. Such an index will only be able to assess the perturbation for which it was designed for.

Given the large set of different kinds of perturbations that may affect coastal and transitional water aquatic ecosystems (Ellis et al., 2000), it is a necessity to include in an integrated approach as many measures of disturbance as possible or, at least, provide as many measure of the biological integrity of a community as possible in order to detect a potential perturbation. This simple observation and the results obtained in this study justified the development of the MISS approach.

4.2. The MISS (Macrobenthic Index for Sheltered Systems) approach

The lack of coherence between Biotic Indices challenged to a strong physical perturbation motivated to propose a complementary method based on a wider panel of metrics. Indeed, BIs constitute an extreme in terms of data reduction from the species × abundance tables to a single numerical value. As a consequence, they were unable to assess the drastic changes that occurred following sediment disposal. Given the poor performance of these indices in a variety of highly productive ecosystems undergoing anthropogenic pressure (Quintino et al., 2006; Zettler et al., 2007; Blanchet et al., 2008), it was necessary to develop complementary bio-assessment tools reliable for the WFD. As a consequence, we developed a complementary approach, called MISS (Macrobenthic Index for Sheltered Systems), that includes some of the existing BIs, namely BOPA and AMBI, together with an additional set of metrics. The MISS approach uses a set of 16 metrics gathered into three categories: community descriptors, trophic composition, and pollution indicators. This approach was mainly inspired by the different Indices of Biological Integrity developed in North American estuarine and coastal ecosystems (Weisberg et al., 1997; Engle and Summers, 1999; Van Dolah et al., 1999; Llanso et al., 2002a,b). The MISS approach however differs from this approach on two main points.

MISS is basically an index for the evaluation of habitat modification but can be interpreted as an EQR if the reference conditions are considered as 'Good' or 'High' in the sense of the WFD. As a tool to compare a given sample to a reference condition, MISS provides lower and higher limit values of a given metric with respect to natural variability. A comparable scoring method was used by Weisberg et al. (1997) but only in the case of abundance and biomass metrics. We however applied this method to all metrics considering that, consistently with Pearson - Rosenberg model of secondary succession, the number of species, abundance and biomass will increase at the beginning of the process of organic enrichment (Pearson and Rosenberg, 1978).

In contrast to Weisberg et al. (1997), we decided to keep as much metrics as possible because these metrics will respond differently according to the nature of the perturbation. For example, we kept the now classical Biotic Indices (AMBI, BOPA) for the computation of MISS even if they did not respond correctly in our case study. Indeed, these indices performed well when challenged to organic matter inputs and in the case of pollution by hydrocarbons (Borja et al., 2003; Muxika et al., 2005; Gomez Gesteira and Dauvin, 2000, 2005; Dauvin and Ruellet, 2007).

Indices of Biological Integrity mainly differ from those designed for European coasts by taking a larger set of metrics which describe the biological integrity of the benthic community in terms of abundance, diversity and dominance of sensitive or opportunist species but also includes the functional aspect of the community in terms of feeding guilds. Moreover, in contrast to what has been designed in Europe, it takes into account the natural variability associated to the reference conditions.

MISS is highly dependant on the reference conditions used. In the present case the data was issued from the study of Blanchet et al. (2004) gathering a large amount of samples within well vegetated *Zostera noltii* bed. We considered that it is a well vegetated meadow compared to other studies (Blanchet et al., 2004) but we cannot certify that it reflects the 'pristine' conditions of the WFD. Sliding references are a major problem when trying to assess current community health status by comparing to a reference condition based on previous sampling campaign (Dayton et al., 1998). Indeed, there are no other quantitative or qualitative data to rely on. Should such qualitative data exist, they would probably not represent pristine conditions. Indeed no sewage collector existed before 1971 in Arcachon Bay. As a consequence, the ecological status of the bay was probably not better than today, and most probably worse. Arcachon Bay has a long history of oyster culture and exploitation. We can't estimate the situation of *Zostera noltii* bed fauna prior the colonization of intertidal flats by oyster parks in the 1860s. There are unfortunately no ways to credibly detect what these seagrass beds looked like prior to this period. However, considering the known pollution background of Arcachon Bay, we considered that the 2002 situation corresponds to the conditions of an at least 'Good' Ecological Status.

Finally MISS proposes an estimate of the incertitude to the results it provides. It gives to the stakeholder the opportunity to consider how far the ecosystem is from the original state in the light of the incertitude associated to the ecological status assessment by the index.

The computation of MISS can be performed with the same database that was previously used to calculate the other Biotic Indices. However biomass was included because it is an important parameter in terms of whole ecosystem functioning and it may permit to take into account specific perturbations such as fishing, dredging, or the occurrence of invasive species. This parameter is essential to describe benthic community health and react very well under disturbed conditions (Pearson and Rosenberg, 1978; Warwick, 1986; Lampadariou et al., 2008). At the equilibrium status, biomasses of soft-bottom macrobenthos are dominated by large bodied species, in low density (k-strategists). These species are the most susceptible to be eliminated after perturbation (especially from organic enrichment). They are replaced by large number of individuals of small-bodied "opportunist" species (r-strategists). Indeed, r-dominated assemblages only need few weeks to recolonized sediments after dredged material disposal (Cruz-Motta and Collins, 2004).

It is still however necessary to test the index we proposed on other set of *Zostera noltii* communities in order to test its validity. One should notice that our reference conditions should not be applied in the case where the seagrass is present but display naturally low coverage in high turbidity conditions. An Excel file is available on the EPOC laboratory website (http://www.epoc.u-bordeaux.fr/indiv/Blanchet/MISS_computation.xls) to help users to compute MISS. Users could test their own *Zostera noltii* data with this file, or test other data with different reference conditions.

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Seagrass burial by dredged sediments: Benthic community alteration, secondary production loss, biotic index reaction and recovery possibility

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ABSTRACT

In 2005, dredging activities in Arcachon Bay (France) led in burying 320,000 m^2 of *Zostera noltii* intertidal seagrass. Recovery by macrobenthos and seagrass was monitored. Six months after works, seagrass was absent and macrobenthos drastically different from surrounding vegetated stations. Rapidly and due to sediment dispersal, disposal area was divided into a sandflat with a specific benthic community which maintained its difference until the end of the survey (2010), and a mudflat where associated fauna became similar to those in adjacent seagrass. Macrobenthic community needs 3 years to recover while seagrass needs 5 years to recover in the station impacted by mud. The secondary production loss due to works was low. In this naturally carbon enriched system, univariate biotic indices did not perform well to detect seagrass destruction and recovery. Multivariate index MISS gave more relevant conclusions and a simplified version was tested with success, at this local scale.

1. Introduction

The disposal of maintenance dredged material constitutes one of the most important problems in coastal zone management (Bolam and Rees, 2003; Bolam and Whomersley, 2005; Van Dolah et al., 1984). Coastal works (e.g. harbors, docks, breakwaters), beach stabilization, dredging and excess siltation from changes in land catchments, are examples of anthropogenic activities that result in changes of the sedimentary dynamics and consequent seagrass loss. Frequently, such human-induced activities result in complete, perhaps irreversible, disappearance of seagrass meadows from coastal areas (Cabaco et al., 2008).

Many studies concerning the effects of dredged material deposition on benthic macroinvertebrates and physical environment have been carried out. The effects of dredge material relocation are smothering (Stronkhorst et al., 2003), chemical contamination (Bolam et al., 2006), changes in sedimentology (Essink, 1999; Harvey et al., 1998), increased levels of organic carbon, reduction in abundance, number of species and diversity (Cruz-Motta and Collins, 2004; Van Dolah et al., 1984; Wildish and Thomas, 1985), increased dominance of tolerant and opportunistic species (Rees et al., 1992). Impacts of burial are the most obvious effects of dredged material placement on benthic organisms in the short term, both at intertidal and subtidal placement sites (Bolam, 2011; Powilleit et al., 2006; Roberts et al., 1998). Since different types of effects were identified in these studies, it is impossible to draw a general conclusion about the impact of dredged material deposition on the benthic community structure (Harvey et al., 1998). In addition, benthic community recovery after dredged material deposition has not been well studied, particularly with reference to seagrass habitats (Sheridan, 2004).

A long term and large-spatial scale study on effects of seagrass bed burial was initiated in Arcachon Bay, following sediment disposal. Arcachon Bay harbours the largest *Zostera noltii* seagrass bed in Europe (Auby and Labourg, 1996), which occupies most of intertidal areas. Arcachon Bay is also an important site for oyster-farming which implies regular cleaning of oysterparks that are rapidly invaded by non-exploited, "wild" oysters (*Crassostrea gigas*). Empty shells and live animals are traditionally buried in large holes ("souille") dug in remote areas, within the lagoon. In 2002, these "souille" were full and stakeholders decided to dig another one, nearby the previous one, in a seagrass area. It consisted in a 5000-m^2 pit able to receive 100,000 m^3 of shells. Works were implemented during 2004-winter and extracted sediments were disposed round over a 20,000 m^2 seagrass surface, rapidly dispersing within a total impacted area of 300,000 m^2 ($\times$10 cm thickness). Between 2002 and 2010, a benthic survey was performed in the primary disposal area, in the secondary sediment spreading area and in controlled stations. Our aim was: (1) to monitor macrobenthic communities in and out of impacted area in terms of biomass, abundance, diversity, structure and trophic groups. (2) To assess the loss of secondary production related to seagrass destruction, *Z. noltii* biomass being a major component influencing the overall macrobenthic production (Dolbeth et al., 2011). Besides, secondary

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production is one of the most comprehensive measurements of ecosystem health (Dolbeth et al., 2005). It may reveal greater insights into ecosystem change than static parameters such as diversity, density or biomass. Combining production with long-term datasets could increase our level of understating system functioning (see for instance, Dolbeth et al., 2007; Pranovi et al., 2008). (3) To compare different biological indicators implemented or not in the Water Framework Directive (WFD) and based on macrofauna communities structure and to observe how they responded to this physical stress and potential seagrass recovery. Indeed, benthic macrofauna is a powerful tool to detect even slight environment changes (Blanchet et al., 2005). It may locally detect the level of stress and integrate the recent history of stress, constituting a sort of memory for the system (Patricio et al., 2009). The composition and structure of benthic macrofauna is one of the indicated biological quality elements to be used in transitional (estuaries and lagoons) and coastal waters for ecological status assessment. Several biological indices (AZTI's Marine Biotic Index (AMBI), Benthic Opportunistic Polychaetes Amphipods Index (BOPA), BENTIX and Macrobenthic Index of Sheltered System (MISS)) based on the benthic macrofauna assemblages have been recently developed to assess ecological status (ES) of marine waters (Borja et al., 2000; Dauvin and Ruellet, 2007; Lavesque et al., 2009; Simboura and Zenetos, 2002).

A particular attention will be devoted to a newly developed multi-metric index, MISS (Lavesque et al., 2009). The implementation of this index in a routine monitoring exposes two major problems. Firstly, two out of 16 metrics are based on biomass (total biomass and the W statistic). Assessing biomass is time consuming and requires samples destruction. Secondary, five metrics are related to the identification of trophic groups which is often a hazardous task, except maybe for suspension feeders. Then, we will test MISS in different derived versions to check whether we can simplify its calculation without degrading the information.

2. Materials and methods

2.1. Study site

Study site is an intertidal mudflat called "Dispute" in the middle of Arcachon Bay (Fig. 1). Arcachon Bay is a triangular-shaped macro-tidal lagoon (180 km^2), located on the southwest French Atlantic coast (44°40′N, 1°10′W). It communicates with the Atlantic Ocean through a narrow (2-km wide) entrance. The tide is semi-diurnal and the tidal amplitude varies from 0.8 to 4.6 m. The average temperatures vary seasonally between 6 and 22.5 °C. Fluctuations in freshwater contributions from rivers and rainwater influence water salinity, ranging between 22 and 35. Many small streams run into the lagoon, but the two main rivers, the Leyre and Canal des Etangs, contribute 73% and 24%, respectively, of the total annual freshwater inflow (813 million m^3 y^{-1}). The total lagoon surface (180 km^2) can be divided in two parts: the subtidal channels (63 km^2), and the intertidal areas (117 km^2) (Plus et al., 2010). The main channels have a maximum depth of 25 m and are extended by a secondary network of shallower channels. The intertidal area comprises sandy to sandy-mud flat (Plus et al., 2010). Most of these flats (61 km^2 in 2005 are covered a *Z. noltii* seagrass bed (Plus et al., 2010). The lower part of the intertidal is generally devoted to Japanese oyster (*C. gigas*) culture, which constitutes a major activity at this site. Adjacent to the mudflats, and lining the channels, eelgrass (*Zostera marina*) occupies the subtidal sector. Sediment temperatures annually varies between −1 and 35.4 °C (average = 15.8 °C), salinity varies between 18.5 and 34.5 (average = 30).

Around Dispute, four stations were monitored. Two "impacted" stations (IS: impacted by sand and IM: impacted by mud) located in the impacted area and two "un-impacted" *Z. noltii* stations, with one situated nearby impacted stations (PS: proximate seagrass) and one situated far from impacted stations (RS: remote seagrass), were monitored after the operations in August 2005, 2006, 2008 and 2010 (Fig. 1). Before the works, in August 2002, two stations corresponding to RS and PS were sampled.

2.2. Macrofauna sampling

At low tide, the top 20 cm of the sediment was collected with a 0.0225-m^2 corer, with four replicates per station. Sediment was sieved through a 1-mm mesh; the remaining fraction was fixed in 4% buffered formalin and stained with Rose Bengal. In the laboratory, macrofauna was sorted, identified when possible to the species level, and counted. Biomass was determined as ash-free dry weight (AFDW) after desiccation (60 °C, 48 h) and calcination (450 °C, 4 h).

2.3. Sediment and seagrass leaves analysis

The top 3-cm sediment layer was also sampled for granulometric analysis. Sediment grain-size characteristics (median grain-size, percentage of silt and clays) were determined after sieving weighted dried sediment through a wet column of sieves with decreasing apertures (1000, 500, 250, 125 and 63 μm). Percentage of organic matter in the sediment was assessed after ignition (450 °C, 4 h) of a dried aliquot of sediment.

In 2002 and 2006, *Z. noltii* leaves were cut in each macrofauna sample and desiccated (60 °C) until a constant dry weight was obtained. In 2010, a new method of assessing *Z. noltii* leaves biomass was developed. Fourteen [15 × 15 cm]-quadrats were delicately laid over the sediment surface, at low tide. For each quadrat, a numeric photograph was taken perpendicularly, one meter above the surface. The method consisted of drawing three equidistant lines across each numerical image and counting the intersections between lines and leaves. Then biomass and percentage of coverage could be obtained, using the following relationships: $\log_e(\text{DW}) = 1.514 \times \log_e(\text{mean number of intercepts per line}) - 1.911$, with $R = 0.98$ ($n = 14$ pictures); $\log_e(S) = 0.690 \times \log_e(\text{DW}) + 1.195$ with $R = 0.98$ ($n = 10$ pictures); where DW is *Z. noltii* leaves dry weight in g m^{-2} and S is the percentage of sediment surface covered by *Z. noltii*. Ten pictures per station were analysed.

2.4. Estimated loss of secondary production

Species were gathered in five trophic groups based on the feeding types (Bachelet, 1981; Fauchald and Jumars, 1979; Hily and Bouteille, 1999; Sauriau et al., 1989): (1) deposit feeders, (2) grazers, (3) predators, (4) scavengers and (5) suspension feeders. Then, biomass of each group was calculated. A P/B ratio was assigned for each trophic group using values calculated by Blanchet (2004). Then at each date we subtracted the secondary production in the impacted area from the secondary production in the seagrass to obtain a gross estimation of secondary production loss. This value was multiplied by the surface of destroyed seagrass and the time elapsed from the previous sampling date.

2.5. Data analysis

2.5.1. Multivariate analysis

Multivariate analysis was performed to compare macrozoobenthic communities structure between areas. Abundances were square root-transformed to minimize the influence of the most dominant taxa. A non-metric multidimensional scaling (n-MDS) based on Bray-Curtis similarity coefficient was carried out to obtain an ordination plot. A Cluster Analysis was used to determine

Fig. 1. Location of Arcachon Bay on the southwest French coast (A), the study site in the lagoon (B), different stations: RS: remote seagrass; PS: proximate seagrass; IM: impacted by mud; IS: impacted by sand (C).

groups of stations × dates that were homogenerous in terms of benthic community. SIMPER analysis was performed to determine which species contributed to between-group dissimilarity. These analysis were performed using PRIMER® – v6 package (Clarke and Gorley, 2006; Clarke and Warwick, 2001).

In order to investigate the pattern of change of numerical descriptor of the benthic assemblage such as total biomass, total number of species, total abundance, number of species, abundance and biomass of epi- and infauna, biomass of the different trophic groups, a Principal Coordinate Analysis (PCO) was performed on the matrix of Euclidean distances among stations based on fourth-root transformed and normalized data. This analysis was performed using PRIMER PERMANOVA package (Anderson et al., 2008).

2.5.2. Biotic Indices

Three currently available univariate Biotic Indices (BIs), namely AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002), BOPA (Dauvin and Ruellet, 2007) and one mutilmetric approach called MISS (Lavesque et al., 2009) were tested. Ecological quality status and thresholds used to classify index values were reported in Table 1.

AMBI is based on previous work from Grall and Glémarec (1997). It considers five ecological groups (available on web page: http://www.azti.es) ranging from sensitive species (EGI) to first-order opportunistic species (EGV) (Borja et al., 2000) (Table 1).

BENTIX considers only two groups: sensitive (GS) and tolerant species (GT), which correspond to ecological groups I and II, and ecological groups III to V of the AMBI, respectively (Table 1).

BOPA is based on the ratio of opportunistic polychaetes (i.e. polychaetes of ecological groups IV and V of the AMBI) and amphipods (except *Jassa* genus) (Dauvin and Ruellet, 2007) (Table 1).

MISS basically consisted in including a selection of existing BIs with additional metrics describing the community (five parameters): abundance per m^2, biomass g AFDW per m^2, number of species (per 0.045 m^2), Shannon Index H', and Pielou's evenness J'; trophic composition (five parameters): grazers per m^2, selective deposit feeders per m^2, non-selective deposit feeders per m^2, suspension feeders per m^2, carrion feeders per m^2 (e.g. carnivorous, omnivorous and scavengers); and pollution indicators (six parameters): values of two Biotic Indices were used (AMBI and BOPA), abundance per m^2 of sensitive species (e.g. EGI and EGII), abundance per m^2 of tolerant species (e.g. EGIII), abundance per m^2 of opportunistic species (e.g. EGIV and EGV). Finally, the W statistic, referring to Abundance-Biomass Comparison (ABC curves) was computed with PRIMER® – v6 package. W measured the extent to which biomass curves lays above the abundance curves (positive values were expected for the undisturbed conditions, negative val-

Table 1
Indices used in this study to assess Ecological Status (ES) and thresholds used to classify index values.

Biotic Indices	Number of ecological groups	Computation of the indices	Ecological Status (ES)		References
			Acceptable	Not acceptable	
AMBI	5	$0\ EG_I + 1.5\ EG_{II} + 3\ EG_{III} + 4.5\ EG_{IV} + 6\ EG_V$ based on percentage of ecological groups	0–3.3	3.3–7	Borja et al. (2000)
BENTIX	2	$6\ EG_{I\&II} + 2\ EG_{III\text{-}V}$ based on percentage of ecological groups	3.5–6 (for sand) 3.0–6 (for mud)	0–3.5 (for sand) 0–3.0 (for mud)	Simboura and Zenetos (2002)
BOPA	2	$\log_{10}[(fp/fa + 1) + 1]$ based on ratio of ecological groups	0–0.13966	0.13966–0.30103	Dauvin and Ruellet (2007)
MISS		Sixteen parameters were classified in three categories describing the macrofauna assemblages	0.6–1	0–0.6	Lavesque et al. (2009)

EG: ecological groups as determined by Borja et al. (2000); fp: opportunistic polychaetes frequency; fa: amphipods frequency (except *Jassa* sp.).

ues for impacted samples) (Warwick, 1986). The BOPA and the AMBI indices were included as indicators of, respectively, pollution by hydrocarbons and organic matter inputs (Lavesque et al., 2009). MISS was inspired by the development of Indices of Biological Integrity conducted in North America (Engle and Summers, 1999; Llanso et al., 2002a,b; Van Dolah et al., 1999; Weisberg et al., 1997). Monitoring results were compared with reference conditions, in order to derive an Ecological Status (ES) (Table 1).

The reference condition for a water body type is a description of the biological elements, which corresponds totally, or almost totally, to undisturbed (pristine) conditions, i.e. with no, or only a very minimal, impact from human activities (Borja and Muxika, 2005; Muxika et al., 2007). In this study, reference conditions were derived by the software from the data collected in the 38 stations located on normally vegetated *Z. noltii* beds in 2002 (Blanchet, 2004; Lavesque et al., 2009).

In addition, derived calculations of MISS were investigated. Firstly, we deleted biomass related parameter (biomass and W values) from calculation because biomass assessment is time consuming and it can be useful to keep samples in laboratory. Secondly, we did not consider trophic groups because definition is often controversial (except for suspension feeders), and many species have mixed trophic habits. Thirdly, different combinations of the two first attempts (without 'biomass + W + trophic groups except suspension feeders') were performed.

3. Results

3.1. Seagrass and sediment disposal

Before sediments disposal (in 2002), seagrass covered 62–68% of sediments surface with biomass ranging from 70 to 80 g DW m^{-2} (Table 2). Six months after works, in August 2005, seagrass was completely destroyed in both impacted stations (IS and IM) and remained absent until 2008, included. In 2010, seagrass only recovered in IM stations (41 g DW m^{-2} or 43% coverage total surface) while it was still absent in IS station (Table 2). In un-impacted stations PS and RS, seagrass were always present though its cover varied according to year (Table 2).

Sediments consisted in sandy muds to muddy sands in un-impacted stations with median grain-size varying from 20 to 100 according to date and small-scale heterogeneity (Table 2). In both stations submitted to sediments deposit (IM and IS) sediments in 2005 (i.e. just after works) were not still sorted and consisted in muddy sands (median: 100–120 μm; silt and clay: 26–28%; organic matter: 5%). With time, finer sediments were washed out. Near the discharge place (IS) only grosser material remained while fine sediments accumulated around IM station. Hence, sediment evolved toward sands with low silt and clay content at IS station (median: 150–190 μm; silt and clay fraction ⩽23%; organic matter ⩽2%) whereas sediments consisted in muddy sand to sandy mud at

Table 2
Seagrass leaves biomass (g DW m^{-2}), seagrass cover (% of sediment surface), sediment median particle size (μm), silt and clay and organic matter content in the sediment (%) before dredging (August 2002) and after (August 2005-2006-2008-2010), "–": missing data.

Status	Date	Station	Seagrass leaves biomass (g DW m^{-2})	Seagrass coverage (%)	Median grain-size (μm)	Silt and clay content (%)	Organic matter content (%)
Remote seagrass	Aug. 2002	RS	80	68	20 (sandy mud)	82	5
	Aug. 2005		–	–	90 (muddy sand)	37	8
	Aug. 2006		29	33	100 (muddy sand)	26	10
	Aug. 2008		82	70	30 (sandy mud)	73	9
	Aug. 2010		87	72	60 (sandy mud)	50	7
Proximate seagrass	Aug. 2002	PS	70	62	20 (sandy mud)	73	7
	Aug. 2005		103	52	100 (muddy sand)	31	9
	Aug. 2006		9	15	90 (muddy sand)	36	8
	Aug. 2008		–	–	20 (sandy mud)	83	10
	Aug. 2010		192	100	40 (sandy mud)	57	8
Impacted by mud	Aug. 2005	IM	0	0	100 (muddy sand)	26	5
	Aug. 2006		0	0	70 (muddy sand)	47	9
	Aug. 2008		0	0	40 (sandy mud)	59	6
	Aug. 2010		41	43	40 (sandy mud)	60	7
Impacted by sand	Aug. 2005	IS	0	0	120 (muddy sand)	28	5
	Aug. 2006		0	0	190 (sand)	5	2
	Aug. 2008		0	0	150 (muddy sand)	23	2
	Aug. 2010		0	0	180 (sand)	6	1

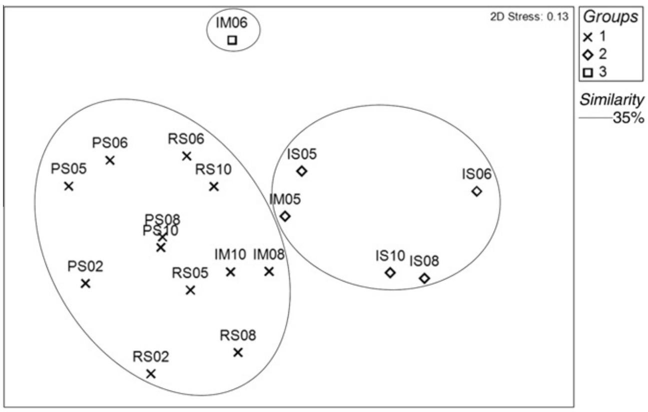

Fig. 2. Non metric multidimensional scaling (n-MDS) of stations based on Bray–Curtis similarity matrix after square root-transformed abundance data. RS: remote seagrass; PS: proximate seagrass; IM: impacted by mud; IS: impacted by sand. Groups of stations identified by the Cluster Analysis at a 35% similarity level are identified (Group 1: ×; Group 2: ◇; Group 3: □).

station IM (median: 40–70 μm; silt and clay: ⩽60%; organic matter: 6–9%) (Table 2).

3.2. Main macrozoobenthic assemblages identified in the dataset

Multivariate analysis performed on the '18 stations-dates × 110 species' data matrix showed, at a 35% similarity level, that three different groups of stations-dates could be identified (Cluster Analysis) (Fig. 2). The first main group (Group 1) included all un-impacted stations (PS and RS) as well as two IM stations from the two last dates of the monitoring, 2008 and 2010. The second group of stations (Group 2) gathered all stations which were impacted by sands (IS) as well as the IM station just after the sediments deposits (in 2005) (Fig. 2). Finally, the latter station (IM station in 2006) displayed a benthic community differing from that of all other situations the first year after sediment disposal (Group 3, Fig. 2).

According to the SIMPER analysis, the dissimilarity between groups 1 and 2 was mainly due to: (1) a much lower abundance or disappearance of several taxa of grazing epifauna such as *Hydrobia ulvae* (4193 ± 951 ind. m^{-2} *vs* 444 ± 208 ind. m^{-2}), *Bittium reticulatum*, *Idotea chelipes* or *Littorina littorea* in Group 2 (Table 3); (2) a decrease of the abundance level of many infaunal polychaete taxa such as *Melinna palmata*, *Aphelochaeta marioni*, *Heteromastus filiformis* or *Clymenura clypeata* as well as other infaunal taxa such as the bivalves *Abra segmentum* and *Loripes lacteus* (which disappeared from stations of Group 2); (3) nevertheless, few species were found at higher abundance level in Group 2 such as the infaunal polychaetes *Nephtys hombergii* and *Streblospio shrubsolii*, the bivalve *Cerastoderma edule* and the amphipod *Ampelisca* sp. (Table 3).

3.3. Trend in the numerical descriptor of the macrofauna assemblages

The first axis of the principal coordinates analysis (PCO) extracted more than 58% of total variation. Together with axis two, 72.5% of total variation was represented (Fig. 3). The ordination

Table 3
List of the main species contributing to contribution to dissimilarity (SIMPER analysis) between groups identified by Cluster Analysis (Group 1: RS and PS stations from 2002 to 2010 and IM stations from 2008 to 2010; Group 2: IS stations from 2005 to 2010 and IM station in 2005; Group 3: IM station in 2006, see Fig. 2). Top five dominant species in each group and top five contributed species for dissimilarity between groups are in bold.

Zoological group	Position	Trophic group	Taxon	Mean abundance ind. m^{-2} ± standard deviation			% Contribution to dissimilarity between groups		
				Group 1	Group 2	Group 3	1 *vs* 2	1 *vs* 3	2 *vs* 3
Nemertea	Infauna	predators	Nemertinea	69 ± 20	4 ± 3	11 ± 11	1.6	1.2	0.9
Mollusca Gastropoda	Epifauna	Grazers	*Hydrobia ulvae*	**4193 ± 951**	**444 ± 208**	0	**13.5**	**14.1**	**7.1**
			Bittium reticulatum	405 ± 130	0	11 ± 11	3.7	2.6	1.4
			Littorina littorea	53 ± 16	2 ± 2	0	1.4	1.3	0.3
		Scavengers	*Nassarius reticulatus*	37 ± 8	2 ± 2	0	1.2	1.1	0.3
Mollusca Bivalvia	Infauna	Deposit feeders	*Abra segmentum*	219 ± 62	22 ± 20	56 ± 21	3.6	1.6	2.8
			Abra tenuis	3 ± 2	11 ± 9	22 ± 13	0.6	1.2	1.5
		Suspension feeders	*Cerastoderma edule*	45 ± 20	53 ± 18	0	1.5	1.2	2.6
			Loripes lacteus	97 ± 26	0	0	1.9	1.7	0
			Ruditapes philippinarum	104 ± 27	7 ± 4	0	2.0	1.9	0.8
		Suspension feeders	*Mytilus edulis*	67 ± 42	0	0	1.3	1.1	0
Annelida Polychaeta	Infauna	Deposit feeders	*Aonides oxycephala*	76 ± 29	0	0	1.2	1.1	0
			Aphelochaeta marioni	**682 ± 173**	**147 ± 93**	0	**4.7**	**4.8**	3.2
			Clymenura clypeata	136 ± 41	9 ± 5	0	1.8	1.6	0.9
			Euclymene oerstedi	47 ± 13	2 ± 2	0	1.2	1.0	0.2
			Heteromastus filiformis	**609 ± 78**	**191 ± 91**	**700 ± 204**	**4.7**	1.9	**7.3**
			Melinna palmata	**1099 ± 220**	9 ± 9	78 ± 21	**6.7**	**5.2**	3.2
			Polydora spp.	0	2 ± 2	22 ± 13	0.2	1.2	1.7
			Pseudopolydora spp.	67 ± 21	38 ± 22	33 ± 11	1.6	1.4	1.7
			Pygospio elegans	46 ± 31	**89 ± 45**	100 ± 71	2.1	2.0	1.8
			Streblospio shrubsolii	27 ± 18	**73 ± 37**	0	1.6	0.6	1.8
		Predators	*Glycera* spp.	33 ± 8	27 ± 7	0	1.0	1.0	2.1
			Nereis diversicolor	0	4 ± 3	**733 ± 456**	0.4	**7.1**	**10.8**
			Nephtys hombergii	38 ± 9	51 ± 12	0	1.0	1.3	2.6
Annelida Oligochaeta	Infauna	Deposit feeders	*Tubificoides benedii*	**412 ± 110**	31 ± 16	44 ± 44	**4.4**	3.2	2.1
Phoronida	Infauna	Suspension feeders	*Phoronis psammophila*	35 ± 12	0	0	1.2	1.1	0
Crustacea Amphipoda	Infauna	Deposit feeders	*Ampelisca* sp.	7 ± 4	42 ± 23	0	1.4	0.4	2.3
			Corophium urdaibaiense	1 ± 1	0	22 ± 13	0.1	1.1	2.0
			Melita palmata	40 ± 17	33 ± 15	22 ± 22	1.4	1.1	1.4
		Grazers	*Microdeutopus gryllotalpa*	57 ± 35	11 ± 8	**167 ± 38**	1.2	3.0	4.5
Crustacea Isopoda	Infauna	Deposit feeders	*Cyathura carinata*	40 ± 14	7 ± 4	**744 ± 286**	1.2	**6.2**	**10.5**
	Epifauna	Deposit feeders	*Lekanesphaera* spp.	2 ± 1	24 ± 10	0	1.2	0.1	1.6
		Grazers	*Idotea chelipes*	105 ± 33	0	0	2.1	1.9	0
Insecta Diptera	Infauna	Grazers	Dolichopodidae	15 ± 6	2 ± 2	**189 ± 90**	0.7	3.1	**5.5**

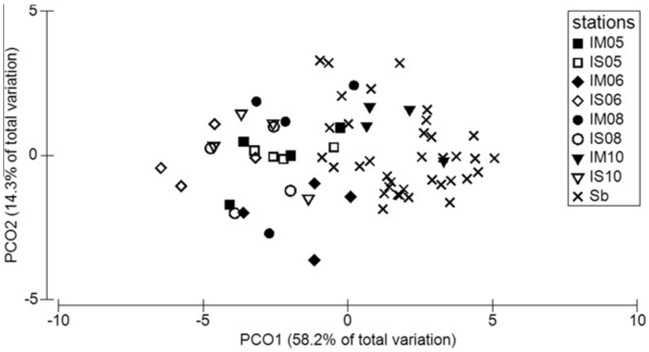

Fig. 3. Ordination of samples obtained by the principal coordinates analysis (PCO) performed on the matrix of numerical descriptors of macrofauna assemblages. Sb: samples retrieved in un-impacted seagrass beds (RS and PS from all dates from 2002 to 2010); IM: samples retrieved in the stations with mud deposits; IS: samples retrieved in the station with sand deposits. The number indicates the year of collection from 2005 (05) to 2010 (10).

of samples along the first axis of the PCO clearly separated samples retrieved within un-impacted seagrass beds (positive values) from samples collected in both impacted areas (negative values). Nevertheless there was a noteworthy exception with samples from 2010 collected at the IM station which gathered within the data cloud corresponding to un-impacted seagrass (Fig. 3).

The ordination of samples obtained through the first PCO axis was positively correlated with all numerical descriptors showing that samples collected in impacted sites (except at station IM in 2010) displayed lower values for almost all of these descriptors (Table 4). However the best correlations (Spearman coefficient of correlation >0.8) were obtained with total, epifauna and infauna biomass, total number of species and abundance of epifauna (Table 4). This analysis shows that, on a purely numeric point of view, only station IM in 2010 displayed values comparable to un-impacted seagrass beds.

3.4. Dynamic of impact and recovery of macrobenthic community

Six months after sediments disposal (in August 2005), both impacted areas were covered by a mixture of sand and mud. Seagrasses were destroyed and the macrofauna was dramatically changed in a same way at both impacted stations (IM and IS) (Figs. 2 and 4). Compared to un-impacted seagrass beds, benthic macrofauna assemblage was characterized by very low biomass

Table 4
Correlations (Spearman Rank Correlation Coefficient) of each numeric descriptor values with the first two axes of the principal coordinates analysis. In bold: $R > 0.8$.

Variables	PCO axis 1 (58.2% of total variation)	PCO axis 2 (14.3% of total variation)
Total biomass	**0.91**	−0.11
Total number of species	**0.90**	−0.24
Biomass of epifauna	**0.88**	0.27
Abundance of epifauna	**0.85**	0.22
Biomass of infauna	**0.82**	−0.32
Total abundance	0.80	0.27
Deposit feeders biomass	0.79	−0.29
Abundance of infauna	0.78	−0.33
Biomass of infauna	0.78	−0.45
Grazers biomass	0.77	0.50
Suspension feeders biomass	0.70	−0.14
Scavengers biomass	0.64	−0.27
Abundance of epifauna	0.63	0.68
Carnivores biomass	0.50	−0.42

(1.3 ± 0.4 g AFDW m^{-2} *vs* 15.4 ± 2.9 g AFDW m^{-2}) in impacted stations of both epifauna and infauna (Table 5). The number of species was also drastically reduced with 11 ± 2 taxa per station in impacted sites and 17 ± 2 taxa per station in un-impacted areas (Table 5).

With time, the perturbed area divided into two distinct habitats. A 2.10^4 m^2-sandflat (IS) nearby the place where sediments were disposed at the origin (2005) and a larger bare mudflat (IM, 28.10^4 m^2), due to fine sediment migration. In August 2006, benthic communities at both impacted sites were still very different from that of seagrass beds. In the mudflat (IM), biomass, abundance and species richness were half those of un-impacted seagrass bed (Table 5). In the sandflat, the benthic community was drastically different with a particularly low abundance (400 ind. m^{-2} i.e. 13% of mudflat abundance and 5% of seagrass abundance), low biomass (0.2 g AFDW m^{-2} i.e. 2% of biomass in bare mudflat and 1% of biomass in seagrass bed), and very low number of species (5 species).

More than 3 years after the perturbation, in August 2008, the macrozoobenthic assemblage from the mudflat (IM) was similar to that of seagrass beds according to Cluster Analysis (Fig. 2) while no seagrass recovery was observed at that time. Though benthic assemblage was similar in terms of species composition and dominance pattern, the benthic assemblage from station IM still displayed reduced biomass and rather low abundance and diversity compared to seagrass beds assemblage (Fig. 4). In the meantime, the macrobenhic assemblage within sandflat remained very different from that of all other studied stations (Fig. 2). In this station, the benthic assemblage was still characterized by reduced diversity, abundance and biomass (Fig. 4).

In 2010, the full recovery of the benthic assemblage in terms of species composition, dominance pattern, abundance, biomass and diversity patterns was observed at IM station (Figs. 2 and 4). In the meantime, *Z. noltii* shoots had returned at this site, 4–5 years after its destruction. However, the IS site remained devoid of any seagrass cover and still displayed clearly different benthic community (Figs. 2–4).

3.5. Loss of secondary production

The loss of secondary production increased between 2005 and 2010 in impacted sites, from 20 to 51 g AFDW m^{-2} y^{-1} and from 20 to 75 g AFDW m^{-2} y^{-1} in mud and in sand respectively (Table 6). This increase was mostly due to suspension feeders (mussels, clams) that progressively settled in un-impacted stations and not in impacted stations (see Table 3). Indeed, the part of secondary production loss due to suspension feeders was of 30% in 2005, against more than 70% in 2010. The global loss of production was $\times$ 10–16 times higher in area impacted by mud than area impacted by sand, mainly due to difference of surface ($\times$14 times). Over 5.5 years and a total surface of 30×10^4 m^2, secondary production loss reached 74 t AFDW.

3.6. Biotic Indices

3.6.1. Assessment of perturbation by univariate Biotic Indices

AMBI classified all stations as "good" or "moderate" without any relation with sediment disposal (Table 7). As well, BENTIX classified almost all stations as "moderate" and "poor" without any relation with sediment disposal (Table 6). BOPA achieved more contrasted results, from "poor" to "high". This index increased the quality status of IM along time. Conversely, BOPA gave fluctuating ecological status (ES) to control stations (RS, PS) and observed strong amelioration of station IS that was however considered as the most impacted during the whole monitoring (Table 7). In general, there was a disagreement between the classification of uni-

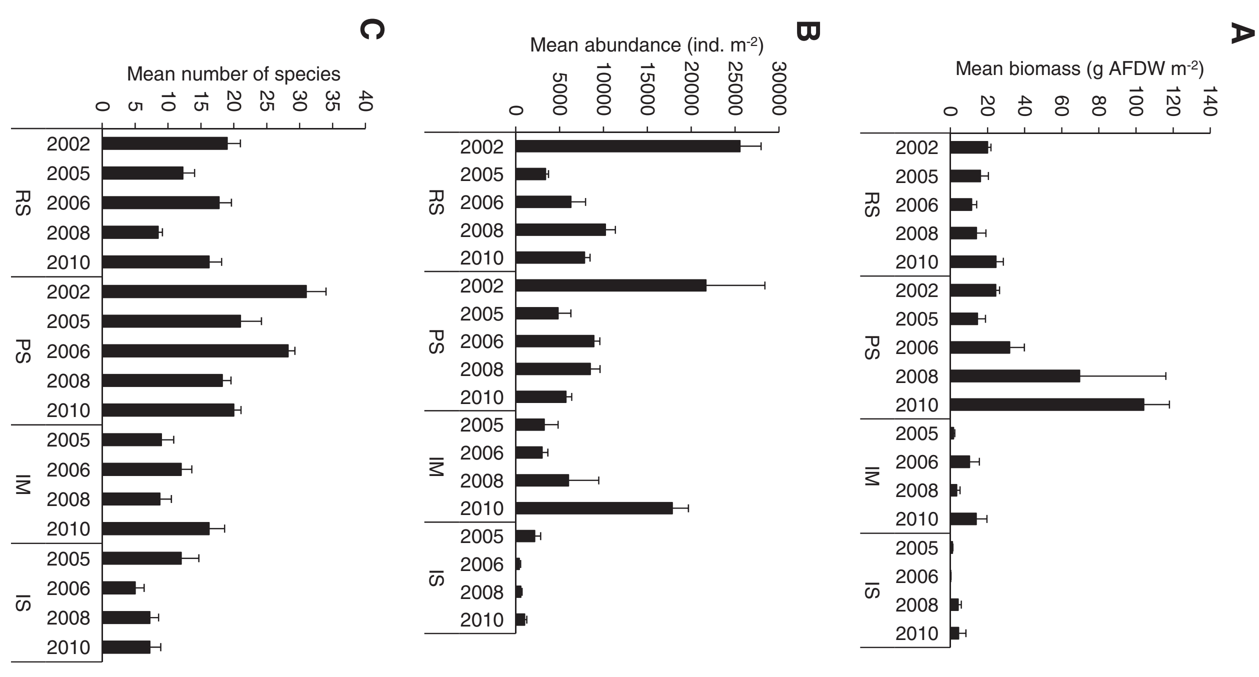

Fig. 4. (A) Mean biomass (g AFDW m^{-2}), (B) abundance (ind. m^{-2}), (C) species richness of the benthic macrofauna. RS: remote seagrass; PS: proximate seagrass; IM: impacted by mud; IS: impacted by sand (Fig. 1).

variate Biotic Indices (AMBI, BENTIX, BOPA) and they did not detect the perturbation related to sediment disposal.

3.6.2. Assessment of perturbation by multimetric approach (MISS) and derived-MISS

In seagrass, ES from MISS was comprised between "moderate" and "high" according to years and positions (RS, PS). In site impacted by mud (IM), ES remained at "moderate" level until 2010 when it achieved "good". In site impacted by sand (IS), ES was "poor" until 2006, and reached moderate between 2008 and 2010 (Table 7). When removing biomass-related parameters (i.e. total biomass and W), ES from MISS changed in one situation out of 18 (IS in 2005). Conversely, when MISS was calculated without trophic groups parameters, it modified 1/3 of ES estimations, mostly by degrading them (Table 7).

Table 5
Mean values (± standard deviation) of abundance, biomass, number species richness of macrofauna, biomass of epifauna and infauna and biomass of each trophic groups in un-impacted (RS, PS), IM and IS stations from 2005 to 2010.

Parameters	August 2005		August 2006			August 2008			August 2010		
	RS, PS	IM, IS	RS, PS	IM	IS	RS, PS	IM	IS	RS, PS	IM	IS
Abundance (ind. m^{-2}, ±SD)	4111 ± 744	2700 ± 817	7594 ± 974	3011 ± 648	400 ± 151	9350 ± 806	6011 ± 3450	567 ± 150	6777 ± 580	17844 ± 1844	1022 ± 243
Abundance epifauna (ind. m^{-2}, ±SD)	1611 ± 408	933 ± 494	1239 ± 502	56 ± 33	67 ± 43	6183 ± 1490	4589 ± 3430	133 ± 91	2161 ± 664	13289 ± 2382	489 ± 252
Abundance infauna (ind. m^{-2}, ±SD)	2483 ± 460	1750 ± 360	6339 ± 795	2956 ± 630	333 ± 143	3156 ± 1082	1422 ± 489	411 ± 102	4611 ± 993	4556 ± 1016	533 ± 189
Biomass (g AFDW m^{-2}, ±SD)	15.4 ± 2.9	1.3 ± 0.4	21.7 ± 5.5	10.3 ± 5.4	0.2 ± 0.1	41.8 ± 24.0	3.3 ± 1.8	4.2 ± 1.7	64.4 ± 16.5	13.9 ± 5.8	4.4 ± 3.9
Biomass epifauna (g AFDW m^{-2}, ±SD)	5.8 ± 2.2	0.6 ± 0.3	7.3 ± 2.2	0.4 ± 0.4	0.05 ± 0.03	30.6 ± 23.0	2.4 ± 1.8	2.4 ± 2.0	15.4 ± 3.8	5.6 ± 2.3	0.2 ± 0.1
Biomass infauna (g AFDW m^{-2}, ±SD)	9.0 ± 2.3	0.7 ± 0.1	14.2 ± 3.8	9.9 ± 5.4	0.1 ± 0.1	11.2 ± 3.3	0.9 ± 0.3	1.6 ± 1.1	49.0 ± 13.0	8.3 ± 4.5	4.2 ± 3.9
Number species (±SD)	17 ± 2	11 ± 2	23 ± 2	12 ± 2	5 ± 1	13 ± 2	9 ± 2	7 ± 1	18 ± 1	16 ± 2	7 ± 2
Deposit feeders (g AFDW m^{-2}, ±SD)	2.4 ± 0.6	0.4 ± 0.1	4.9 ± 0.7	0.8 ± 0.2	0.05 ± 0.0	4.2 ± 1.7	0.8 ± 0.2	0.4 ± 0.2	3.9 ± 0.8	7.3 ± 4.1	0.9 ± 0.8
Grazers (g AFDW m^{-2}, ±SD)	2.9 ± 1.9	0.6 ± 0.3	5.1 ± 2.3	0.3 ± 0.1	0.03 ± 0.02	4.6 ± 0.9	2.2 ± 1.7	0.1 ± 0.1	4.4 ± 1.4	4.4 ± 1.4	0.1 ± 0.1
Predators (g AFDW m^{-2}, ±SD)	1.7 ± 1.0	0.3 ± 0.1	1.9 ± 0.7	9.2 ± 5.5	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.4	1.6 ± 0.6	1.8 ± 0.8	0.05 ± 0.02
Scavengers (g AFDW m^{-2}, ±SD)	2.5 ± 1.2	0	1.6 ± 0.4	0	0	1.9 ± 1.0	0	2.0 ± 2.0	3.2 ± 1.0	0	0
Suspension feeders (g AFDW m^{-2}, ±SD)	5.5 ± 1.8	0.05 ± 0.05	8.1 ± 3.1	0	0.03 ± 0.02	30.7 ± 23.1	0.01 ± 0.01	1.3 ± 1.0	51.3 ± 15.7	0.4 ± 0.2	3.3 ± 3.2

Table 6
Loss of secondary production by trophic group (g AFDW m^{-2} y^{-1}). The total secondary production loss (total area and total duration from the previous date. For 2005, elapsed time from the works was 0.5 year) is given on last line. RS, PS, IM and IS: see Fig. 1.

Trophic group	P/B y^{-1}	2005	2006		2008		2010	
		IM, IS g AFDW m^{-2} y^{-1}	IM g AFDW m^{-2} y^{-1}	IS g AFDW m^{-2} y^{-1}	IM g AFDW m^{-2} y^{-1}	IS g AFDW m^{-2} y^{-1}	IM g AFDW m^{-2} y^{-1}	IS g AFDW m^{-2} y^{-1}
Deposit feeders	2.4	4.8	9.8	11.8	8.2	9.1	−8.2	7.2
Grazers	2.4	5.5	11.5	12.2	10.8	5.8	0.0	10.3
Predators	0.9	1.3	−6.6	1.6	0.0	−0.1	−0.2	1.4
Scavengers	0.9	2.3	1.4	1.4	1.7	−0.1	2.9	2.9
Suspension feeders	1.1	6.0	8.9	8.9	33.8	32.3	56.0	52.8
Total production		19.9	25.0	35.9	54.5	47.0	50.5	74.6
Total production loss g AFDW		2.99×10^6	7.04×10^6	0.72×10^6	30.49×10^6	1.90×10^6	28.30×10^6	2.99×10^6

Table 7
Biotic indices (AMBI, BENTIX, BOPA, MISS and d-MISS) and ecological status (ES) according to Table 1. For d-MISS: values different from MISS are in bold. RS, PS, IM and IS: see Fig. 1.

Station	Year	AMBI		BENTIX		BOPA		MISS (with 16 indices)		d-MISS (without biomass + W)		d-MISS (without trophic groups except suspension feeders)		d-MISS (without W + biomass + trophic groups except suspension-feeders)	
		Score	ES	Score	ES	Score	ES	Score	ES	Score	ES	Score	ES	Score	ES
RS	2002	3.1	Good	2.53	Moderate	0.00662	High	0.9	High	0.90	High	1.00	High	1.00	High
	2005	3.7	Moderate	2.78	Moderate	0.09607	Good	0.54	Moderate	0.50	Moderate	0.57	Moderate	0.53	Moderate
	2006	3.7	Moderate	2.46	Poor	0.24761	Poor	0.76	Good	0.66	Good	0.51	**Moderate**	0.51	**Moderate**
	2008	3.0	Good	2.46	Poor	0.00619	High	0.60	Moderate	0.56	Moderate	0.43	Moderate	0.39	**Poor**
	2010	3.5	Moderate	2.66	Moderate	0.25719	Poor	0.69	Good	0.69	Good	0.52	**Moderate**	0.52	**Moderate**
PS	2002	3.2	Good	2.48	Poor	0.08911	Good	0.66	Good	0.62	Good	0.49	**Moderate**	0.45	**Moderate**
	2005	2.4	Good	2.51	Moderate	0.18368	Moderate	0.49	Moderate	0.47	Moderate	0.36	**Poor**	0.44	**Poor**
	2006	2.5	Good	2.54	Moderate	0.18309	Moderate	0.56	Moderate	0.57	Moderate	0.56	Moderate	0.57	Moderate
	2008	3.1	Good	2.54	Moderate	0.20392	Poor	0.73	Good	0.74	Good	0.53	**Moderate**	0.54	**Moderate**
	2010	1.9	Good	2.90	Moderate	0.07912	Good	0.52	Moderate	0.60	Moderate	0.42	Moderate	0.50	Moderate
IM	2005	3.8	Moderate	2.98	Moderate	0.21241	Poor	0.47	Moderate	0.50	Moderate	0.42	Moderate	0.45	Moderate
	2006	3.5	Moderate	3.09	Good	0.17760	Moderate	0.52	Moderate	0.55	Moderate	0.52	Moderate	0.55	Moderate
	2008	3.1	Good	3.01	Good	0.08949	Good	0.54	Moderate	0.52	Moderate	0.67	**Good**	0.66	**Good**
	2010	3.0	Good	2.87	Moderate	0.08095	Good	0.72	Good	0.77	Good	0.62	Good	0.67	Good
IS	2005	3.0	Good	2.69	Moderate	0.15609	Moderate	0.38	Poor	0.42	**Moderate**	0.36	Poor	0.40	**Moderate**
	2006	3.0	Good	2.48	Poor	0.19562	Poor	0.34	Poor	0.35	Poor	0.37	Poor	0.38	Poor
	2008	1.9	Good	2.63	Moderate	0.10574	Good	0.42	Moderate	0.43	Moderate	0.42	Moderate	0.43	Moderate
	2010	2.6	Good	2.72	Moderate	0.09314	Good	0.44	Moderate	0.42	Moderate	0.38	**Poor**	0.35	**Poor**

4. Discussion

4.1. Seagrass destruction and recolonization

Six months after the sediments disposal, seagrass totally disappeared in the sites that were covered by sediment (sand and mud). The responses of seagrass to sediment burial have been assessed in many studies (Cabaco and Santos, 2007; Cabaco et al., 2008; Duarte et al., 1997; Han et al., 2012; Marba and Duarte, 1994) and is species specific and strongly size-dependent (Cabaco et al., 2008). According to Cabaco and Santos (2007), *Z. noltii* is highly sensitive to burial and erosion disturbances due to the small size of this species and the lack of vertical rhizomes. Hence, most *Z. noltii* plants under complete experimental burial died between the 1st and the 2nd week (Cabaco and Santos, 2007). Whereas the mortality caused by burial increased with decreasing seagrass size, the potential to recover from disturbances by growth is enhanced with decreasing seagrass size (Duarte et al., 1997; Peralta et al., 2005). A trade-off related to seagrass size exists, in terms of recovery time versus resistance to stresses, such as sediment disturbance (Han et al., 2012).

Duarte et al. (1997) found that small seagrass species, such as *Halophila ovalis* and *Halodule uninervis* were able to recover within 4 months after burial disturbance, while Cabaco and Santos (2007) did not observe any recovery of *Z. noltii* within 2 months after experimental burial. In fact, *Z. noltii* is well adapted to cope with sediment disturbances of limited amplitude (i.e. ±6 cm) and with continuous events by rapidly relocating their rhizomes to the preferential depth (Han et al., 2012). However, in our study, seagrass partly recovered in IM station only 5 years (from 2005 to 2010) after burial. This long delay could be related to the thick layer of sediment (⩾10 cm) massively discharged at a single occasion (Han et al., 2012). Characteristics of sediment were also an important factor, since we observed that areas covered by sand remained free of seagrass after these 5 years. The reason is certainly not directly linked to the sediment grain-size since Do et al. (2011) showed that *Z. noltii* could colonize a sand flat within 4 years.

4.2. Benthic community alteration and recovery possibility

Seagrass destruction and the changes due to sediment disposal altered benthic community. The impact depends on the amount of discharged sediment, disposal time, water depth, currents, particle size, and other abiotic parameters (see review in Witt et al., 2004). One of the main effects of dumping of dredged sediments relates to burial of benthos at dump sites (Essink, 1999). Local benthos has to cope with deposition of sediments which are in many cases strongly anaerobic. Sensitivity of benthos to being covered by dredged sediments is strongly dependent on the thickness of sediments and their ability to restore contact with the overlying water (Essink, 1999). Mortalities generally increase with increased sediment depth, exotic sediment and burial time (Harvey et al., 1998; Maurer et al., 1981a,b, 1982). Decreases in macrofaunal abundances, biomasses and species richness as a consequence of the disposal have been reported in several studies (Cruz-Motta and Collins, 2004; Harvey et al., 1998; Ware et al., 2010; Witt et al., 2004). Our study confirmed that benthic macrofauna community structure at the disposal sites had changed substantially following deposition. Indeed, 6 months after work (in August 2005), macrofauna assemblages showed a decrease of biomass, abundance and species number in impacted (IM, IS) stations. After 18 months (in August 2006), the macrofauna assemblages displayed a clear difference between impacted (IM, IS) and un-impacted stations (PS, RS). Biomass, abundance and diversity were especially lower in the station affected by sand disposal. Faunal differences between the disposal sites and the reference areas were indeed correlated with changes in the sediment composition depending on impact types. The disposal sites had a higher proportion of mud or sand, which influenced species composition (Witt et al., 2004). Sediment disposal affected differently benthic macrofaunal species according to their specific feeding behavior, mobility or morphology (Pearson and Rosenberg, 1978; Van Dolah et al., 1984; Witt et al., 2004).

Following burial, macrobenthic invertebrate recovery can occur by a combination of three main mechanisms: planktonic recruitment of larvae, lateral migration of juveniles/adults from adjacent un-impacted areas and/or vertical migration through the deposited material (Bolam et al., 2011; Bolam and Whomersley, 2005). The relative importance of these mechanisms will depend on a number of factors such as spatial scale timing, rate and depth of placement (Bolam et al., 2006). Which mechanism ultimately predominates has important implications for the rate and successional sequences of invertebrate recovery (Bolam et al., 2010). For example, in cases where material is deposited thinly over a large area, a relatively rapid recovery through vertical migration may occur. If the sediments are deposited at a depth which exceeds the organisms' burrowing and migration ability, total elimination of the ambient community will occur (although some species may successfully be transported within the dredged material) in the short term due to smothering; a slower recovery will then ensue through lateral migration (days/weeks) and/or planktonic settlement (weeks/months/years) (Bolam, 2011). Because of thick disposal sediment layer, in our case, few or no species were able to recolonize the disposal sites by vertical migration. On the other hand, migration of adults from undisturbed areas and reproduction and larval recruitment from undisturbed areas could explain the gradual re-establishment of macrofauna (Harvey et al., 1998).

Invertebrate recovery following dredged material disposal in relatively unstressed marine environments generally takes between 1 and 4 years, while in more naturally stressed areas, recovery is generally achieved within 9 months, although deeper polyhaline habitats can take up to 2 years to recover (Bolam and Rees, 2003). Differences in recovery times are attributed to the number of successional stages required to regain the original community composition that depend on their life-history traits (Bolam and Rees, 2003). In Upper Laguna Madre, mollusc and polychaete species compositions and densities in seagrasses that had colonized dredging deposits required at least 10 years to become similar to communities in adjacent natural seagrass beds (see review in Sheridan, 2004). In our study, benthic community showed a recovery 3 years after sediment disposal. At that time, sediment consisted of mud, like in 2002, but seagrass had not yet recovered. This observation highlights the importance of sediment type for benthic organisms, especially concerning infauna which is more independent to seagrass presence (Cottet et al., 2007).

On other hand, the recovery of seagrass after 6 years in IM station also explained the increase of species richness, abundance and biomass. The benefits of seagrass habitats for ecosystems' diversity, health and functioning were broadly documented (Blanchet et al., 2004; Do et al., 2011; Edgar, 1990; Orth et al., 1984). Sheridan (2004) reported that, once seagrasses start to cover dredged sediments, increases in densities of the associated mobile macrofauna would be expected. The presence of intertidal seagrasses potentially increases food availability for both infauna and epibenthic organisms by acting as a sink for organic matter (Asmus and Asmus, 2000). Seagrass meadows reduce water movement and increase sedimentation rates of fine particles and detritus. The above-ground vegetation provides habitats and substrates for free-living animals and epiphytic animals and algae also, the below-ground rhizome network offers sediment stability, creating favorable living conditions, including shelter from predation, for a wide range of infaunal organisms (Fredriksen et al., 2010).

4.3. Secondary production loss

Increasing percentages of plant burial significantly increase mortality and consequently decrease secondary production (Mills and Fonseca, 2003). Since secondary production responds quickly following the disposal of dredged material, the response of benthic production to disposal is more predictable than community (Bolam, 2011; Wilber et al., 2008). In fact, our results showed that both approaches differ. While benthic macrofauna tended to recover in terms of structure since 2008 (at least in mud), secondary production loss reached the highest values in 2008 and kept similar in 2010.

A gross calculation however, tends to show that this production loss has small consequence on higher trophic level. Indeed, we calculated a total loss of 74 t AFDW, over the whole area and in 5.5 years. With a production/consumption rate of 15%, this would consist in a loss of predator production of 11 t AFDW over 5.5 years, i.e. 1.5 t AFDW per year (i.e. 15.2 t Fresh Weight per year) which is insignificant at the scale of the lagoon. However this calculation is only based on trophic pathways and does not take in consideration the effect of seagrass destruction as an habitat loss for potential predators (Boström et al., 2006; Irlandi, 1994; Summerson and Peterson, 1984).

4.4. Biotic indices reaction

Previous studies already stated that some Biotic Indices (AMBI, BENTIX, BOPA) may perform badly in semi-enclosed ecosystems that are naturally enriched in organic carbon (Blanchet et al., 2008; Lavesque et al., 2009). The present study confirmed that these BIs did not detect both the seagrass burial and its recovery.

Some previous studies have attributed a poor performance of AMBI to highlight anthropogenic pressures (Albayrak et al., 2006; Labrune et al., 2006; Quintino et al., 2006; Simonini et al., 2009; Zettler et al., 2007), especially when the disturbance agent is not related to organic enrichment (Sampaio et al., 2011). AMBI has ability to detect different anthropogenic impacts worldwide, including anoxia and hypoxia, eutrophication, nutrient loads, sediment toxicity (metals, PAH), and aquaculture (see review in Borja and Tunberg (2011)). However, when using AMBI, reference conditions must be assessed independently for each habitat (Borja et al., 2012; Muxika et al., 2007). In addition, our results showed that the AMBI classified "Good" ES whereas the BENTIX assigned 'Moderate' ES for most times/sites. The disagreements between both indices were already reported in some previous studies (Do et al., 2011;

Simonini et al., 2009). It was suggested that the discrepancy in the AMBI and BENTIX results could be ascribed to differences in: (i) the weighting of sensitive and tolerant groups of species in the formulae; (ii) the scaling of boundary limits among classes; (iii) the arrangement of the 'tolerant' species, which are weighted separately in the AMBI, whereas the BENTIX method required all tolerant species to be weighted equally; and (iv) the attribution of the species to the ecological groups (see reviewed in Simonini et al., 2009).

BIs constitute an extreme in terms of data reduction from the species × abundance tables to a single numerical value. As a consequence, they are unable to assess the drastic changes that occur following sediment disposal. Our result showed that MISS (Macrobenthic Index for Sheltered Systems), that includes some of the existing BIs, namely BOPA and AMBI, together with an additional set of metrics showed better results in assessing the seagrass burial and its recovery. However, MISS requires biomass which is destructive and time-consuming. We showed that it was possible to calculate a derived MISS (d-MISS) without biomass that provided very similar conclusions. This BI should be now tested in other conditions. Conversely, we recommend to keep considering trophic group separation, even though it is often uneasy to classify species according to a clear trophic regime.

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