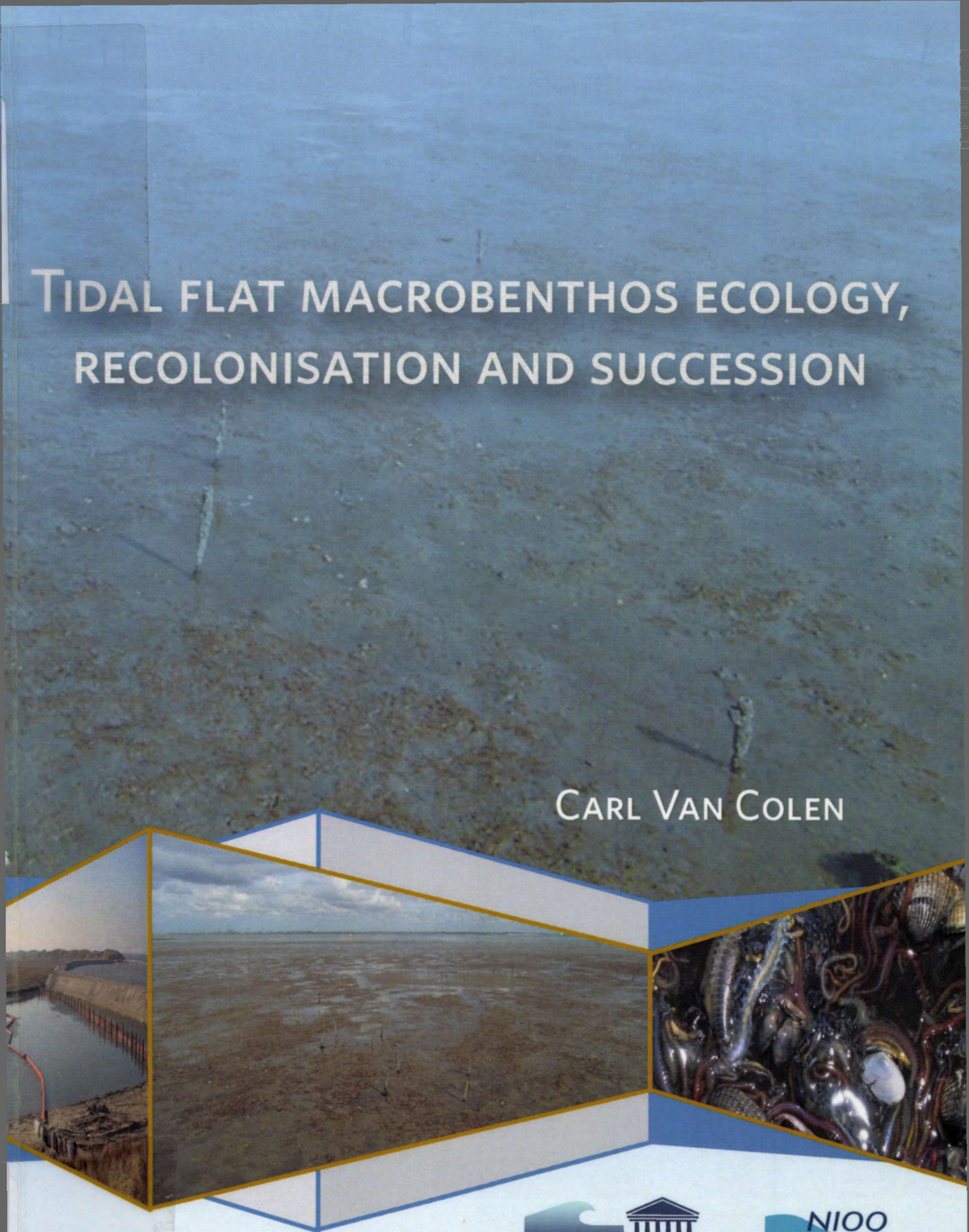


TIDAL FLAT MACROBENTHOS ECOLOGY, RECOLONISATION AND SUCCESSION

CARL VAN COLEN

Mariene Biologie
Universiteit Gent

NIOO

UNIVERSITEIT
GENT

FACULTEIT WETENSCHAPPEN

Tidal flat macrobenthos ecology, recolonisation and succession

UNIVERSITEIT
GENT

FACULTY OF SCIENCES

Marine Biology
Ghent University

NIOO

Tidal flat macrobenthos ecology, recolonisation and succession

Ecologie, herkolonisatie en successie van het macrobenthos in slikken

Carl Van Colen

Promotor:	Prof. Dr. Magda Vincx
Co-promotors:	Dr. Steven Degraer
	Dr. Tom Ysebaert

Academic year 2008-2009

ISBN: 978-90-902416-6-1

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor in Science
(Biology)

Members of the reading committee

Prof. Dr. Peter M.J. Herman (NIOO, Yerseke, the Netherlands)
Dr. Simon F. Thrush (NIWA, Hamilton, New Zealand)
Prof. Dr. Patrick Meire (Antwerp University)
Prof. Dr. Tom Moens (Ghent University)

Members of the examination committee

Prof. Dr. Wim Vyverman, Chairman (Ghent University)
Prof. Dr. Magda Vincx (Ghent University)
Dr. Steven Degraer (MUMM, Brussels, Belgium)
Dr. Tom Ysebaert (NIOO, Yerseke, the Netherlands)
Prof. Dr. Ann Vanreusel (Ghent University)
Prof. Dr. Peter M.J. Herman (NIOO, Yerseke, the Netherlands)
Dr. Simon F. Thrush (NIWA, Hamilton, New Zealand)
Prof. Dr. Patrick Meire (Antwerp University)
Prof. Dr. Tom Moens (Ghent University)

CONTENTS

Dankwoord 6

Chapter 1: General introduction 9

Chapter 2: Benthic community structure, diversity and trait displacement across an estuarine tidal flat hydrodynamic stress gradient 17

Chapter 3: Macrobenthic recovery from hypoxia in an estuarine tidal mudflat 35

Chapter 4: Benthic community-mediated sediment dynamics 59

Chapter 5: Benthos-mediated sediment dynamics: implications for growth and production of juvenile recruits 93

Chapter 6: Tidal flat nematode responses to hypoxia and subsequent macrofauna-mediated altered sediment properties 105

Chapter 7: A long-term study of the macrobenthos recovery following hypoxia-induced mass mortality 125

Chapter 8: Sediment segregation by biodiffusing bivalves 145

Chapter 9: Settlement of *Macoma balthica* larvae in response to benthic diatom films ... 177

Chapter 10: General discussion: Weaving ecological theory with tidal flat restoration. 199

Appendix 1: Macrobenthic community structure and distribution in the Zwin nature reserve (Belgium and the Netherlands) 211

Appendix 2: Does medium-term emersion cause a mass extinction of tidal flat macrobenthos? - The case of the Tricolor oil pollution prevention in the Zwin nature reserve (Belgium and The Netherlands) 229

Appendix 3: Supplementary material 241

Cited Literature 253

Summary – Samenvatting 291

Publication list 299

DANKWOORD

Dit proefschrift was er niet geweest zonder de hulp van vele anderen. Ik wil iedereen voor de geboden hulp en steun, of het nu vanzelfsprekend, of onverwacht was, of omdat ik er om gevraagd had, heel erg bedanken.

Heel veel heb ik te danken aan mijn promotor Prof. Dr. Magda Vincx en co-promotoren Dr. Steven Degraer en Dr. Tom Ysebaert. Steeds heb ik een enorme vrijheid ervaren waarin ik eigen accenten mocht leggen en deze met jullie kritisch kon bediscussiëren. Bedankt voor dit vertrouwen, het kritisch nalezen en het voorzien van commentaar van talloze teksten – in welk stadium dan ook- en me in te tomen wanneer ik weer eens té enthousiast veel te veel ineens wou onderzoeken.

I would like to express my gratitude to all the people who were involved in the Paulina defaunation experiment: Prof. Dr. Peter Herman, Prof. Dr. Jack Middelburg, Dr. Francesca Rossi, Dr. Britta Gribsholdt, Dr. Maria Andersson, Dr. Matthijs Vos and Drs. Francesc Montserrat. Our cooperation has resulted in a nice piece of multidisciplinary scientific work which will definitely result in a nice scientific output. In het bijzonder, wil ik Prof. Dr. Peter Herman bedanken voor de meegegeven kennis over en inzichten in estuariene ecologie, de uitleg over diverse statistische analyses en het brainstormen over verschillende experimentele set-ups. Een bijzonder woord van dank wil ik richten tot Francesc Montserrat. Met gebundelde kracht hebben we heel wat gegevens verzameld, verwerkt, bediscussieerd en gepubliceerd. Naast de vele uren gezamenlijk geploeter en gezwoeg in de modder heb ik bovenal ook enorm genoten van dit veldwerk en heel wat opgestoken uit onze samenwerking.

Voor heel wat veld –en labowerk kon ik beroep doen op de enthousiaste hulp van Dirk Van Gansbeke, Danielle Schram, Annick Vankenhove, Bart Beuselinck, Danny Peelaers, Guy Desmet, Bas Koutstaal, Jos Van Soelen, Dr. Oscar Bos, Dr.

Tjeerd Bouma, Yves Israel, Jurgen Verstraeten en zowat elke marbiol-collega. In het bijzonder wil ik de 'macrobiol' collega's bedanken voor de vele fijne overleg –en discussiemomenten en mijn thesis –en maandwerk studenten Joke Lenoir, Katleen Verbist, Greet Meulepas, Clio Cnudde, Els Heynssens en Ine Pauwels voor het mee helpen generen en analyseren van heel wat benthos data. Verder wil ik Ed Stikvoort, Kirsten Wolfstein, Fred Schenk en René Beijersbergen bedanken voor het verlenen van de nodige vergunningen voor het veldwerk op het Paulinaschor.

Een gezonde geest in een gezond en ontspannen lichaam...de Romeinen wisten het al. Ik ben dan ook veel verschuldigd aan mijn vrienden voor de vele ontspannende momenten en hun steun – niet tegenstaande velen het vaak vreemd vonden waarom ik het zo plezant en interessant vond om met modder te spelen -. In het bijzonder wil ik de skipterippers, de joskes, de bende van Torhout en de biologen '2004'-bende bedanken.

Tenslotte wil ik graag mijn ouders, pepé en Eline bedanken voor hun steun en hulp (ook als ik bijvoorbeeld per se weer eens vroeg in de morgen een proefopstelling wou gaan checken en best wel wat hulp kon gebruiken...), om elke dag van mijn leven specialer te maken. Heel erg merci! Dit doctoraat is zeker ook jullie verdienste.

Carl
maart 2009

De financiële steun voor dit doctoraatsonderzoek werd verschaft door het Instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen (IWT, SB/43483), het Fonds Wetenschappelijk Onderzoek Vlaanderen (FWO-Kiss, 3G048005) en de Universiteit Gent.

CHAPTER 1
GENERAL INTRODUCTION

Rationale

Ecosystems provide essential services that maintain the functioning of the biosphere, and thus contribute to human welfare (Lant *et al.* 2008 and references therein). By covering about 70 % of the planet's surface, benthic marine ecosystems are arguably one of the largest ecosystems on Earth. Consequently marine benthic ecosystem processes have important local and global effects (Snelgrove 1998, 1999). For instance, Costanza *et al.* (1997) suggested that the oceans account for approximately two-thirds of the value of global ecosystem services. However, along with the expanding human population, the diversity and intensity of anthropogenic stressors (i.e. a factor that impacts on the fitness of individuals; Thrush *et al.* 2008) increasingly impact these essential services that we take for granted, both directly (e.g. declining fish stocks due to fisheries) and/or indirectly (e.g. declining water quality) (Worm *et al.* 2006 and references therein). To date, no marine area is unaffected by human disturbances (i.e. temporally discrete events which remove biomass from a system through killing or displacement of individuals; Grime 1977) (Halpern *et al.* 2008) and a large fraction is strongly affected by multiple anthropogenic drivers (e.g. Crain *et al.* 2008, Thrush *et al.* 2008). Historically, habitat loss and over-exploitation of key species were the main human impacts on the marine ecosystems (Jackson *et al.* 2001), whereas climate change (Easterling *et al.* 2000), destructive fishing (Thrush & Dayton 2002, Hiddink *et al.* 2006, Kaiser *et al.* 2006), organic enrichment and pollution (Hyland *et al.* 2005), sediment deposition (Lohrer *et al.* 2006) and hypoxia (Diaz & Rosenberg 2008) are now judged as the most severe threats to marine ecosystems (Halpern *et al.* 2007). Furthermore, because approximately 40 % of the human population lives within 100 km from the coast (Cohen *et al.* 1997), coastal areas, in particular, are impacted by anthropogenic threats (Worm *et al.* 2006, Halpern *et al.* 2008). For example, Airoldi & Beck (2007) recently stated that anthropogenic stressors have affected about 85 % of the European coastline.

Scientists stress the increasing need for habitat restoration in coastal areas (e.g. Madgwick & Jones 2002) and it is now generally accepted, and in many cases legally demanded (e.g. the Water Framework Directive, Anon. 2000), that anthropogenic activities that damage ecosystems require mitigation measures (Elliott & Cutts 2004) that return the damaged ecosystem to a dynamic equilibrium state which functions properly with the present and future and which is ecologically, economically and socially acceptable (Hobbs & Norton 1996). Hence, in addition to the documentation of the effects of various anthropogenic stressors on species and marine ecosystems, there is a burning need to gather knowledge on the recovery of disturbed marine habitats and its inhabitants (i.e. communities). Bio(logical)diversity - ecosystem function relationships provide an essential baseline to enhance our understanding of such marine habitat recovery dynamics because recovering communities may be expected to affect their habitat and vice versa (e.g. see pioneering work on succession dynamics and community recovery by Connell & Slatyer (1977) and Pearson & Rosenberg (1978)). Redundant, linear and idiosyncratic mechanisms through which increased biodiversity may have a positive (facilitation, insurance and complementarity) or negative effect (competition and inhibition) on ecosystem functioning, as well as insensitive functional relations to any changes in diversity have been suggested (Naeem *et al.* 2002). Within coastal habitats, it is perhaps in shallow habitats such as estuarine tidal flats, marshes and coastal lagoons that relationships between (recovering) diversity and ecosystem processes/functions need to be assessed most urgently (Bolam *et al.* 2002). These habitats play a major role in biogeochemical cycles because they receive mass inputs of terrestrial organic matter and nutrients (Snelgrove *et al.* 1997), and are among the most geochemically and biologically active and productive areas of the biosphere (Gattuso *et al.* 1998). Consequently many of these habitats are incorporated into nature reserves and designated as Special Areas of Conservation (SACs) according to the EU Habitats Directive (Anon. 1992).

Moreover, restoration of estuarine habitats through management has been proven feasible (e.g. waste water treatments in the Delaware estuary (US), Thames, Mersey, Forth estuary (UK) and Scheldt estuary (B, NL)(Albert 1988, Jones 2006, Pomfret *et al.* 1991, McLuskey & Elliott 2004, Soetaert *et al.* 2006) and habitat creation in the Humber, Tees and Orwell estuary (UK), Newport River and Tijuana estuary (US) and Scheldt and Yser estuary (NL, B) (Bolam & Whomersley 2005, Evans *et al.* 1998, Hampel *et al.* 2003, Hoffman *et al.* 2005, Levin *et al.* 1996, Mazik *et al.* 2007, Mander *et al.* 2007, Meire *et al.* 2002, Maris *et al.* 2007, Zedler 2001)). Such restoration projects thus provide an opportunity to regain human-induced ecosystem function losses and also offer a gain for society (e.g. decreasing flooding risk, water quality improvement) and economics (e.g. fisheries, aquaculture and nursery habitats) (Gilbert & Janssen 1998).

As an integral part of management practice, monitoring should occur in order to detect change and to determine whether management is having a desired effect (McLusky & Elliott 2004). The macrobenthos (i.e. bottom fauna commonly defined as organisms retained on a 0.5 or 1 mm mesh sized sieve) of estuarine tidal flats recycles nutrients (Lohrer *et al.* 2004), alters sediment transport processes considerably (Solan *et al.* 2008) and represents an important trophic linkage, being a food source for epibenthic crustaceans, fish and birds (Hampel et al 2004) and feeding on benthic algae and bacteria (Herman *et al.* 2000, Van Oevelen *et al.* 2006). Further, the macrobenthos reflects the integrity of its immediate environment (i.e. bottom)(Alden *et al.* 1997 and references therein) and macrobenthos population metrics are therefore incorporated in several environmental quality indices (e.g. Borja *et al.* 2008, Pinto *et al.* 2009). Given its central role in the functioning and its representation of the ecosystem quality (Nillson & Rosenberg 1997), monitoring of the macrobenthos should be part of an integrated evaluation of the progress of marine and estuarine restoration projects (Franco *et al.* 2007).

Macrobenthic bioturbation (i.e. sediment reworking by burrowing animals) and bio-irrigation (i.e. enhanced solute transport that results from bioturbation and burrow flushing) can strongly alter the benthic physical environment, i.e. ecosystem engineering (Jones *et al.* 1994). Typically, ecosystem engineering species exert a strong influence on ecosystem properties that exceeds what may be expected based on their abundance alone (Hooper *et al.* 2005) and tend to be most dominant in stressful habitats (e.g. tidal flats) (Jones *et al.* 1997). For instance, tidal flat reef-building filter feeders affect local hydrodynamics and particle trapping by their epibenthic structure (i.e. autogenic engineering) (Widdows *et al.* 1998, 2002). Further, the combination of bioturbation and bio-irrigation activities of tidal flat macrobenthic organisms may considerably affect sediment stability and resource flows (i.e. allogenic engineering) (de Deckere *et al.* 2000, Thrush *et al.* 2006, Volkenborn *et al.* 2007). Consequently, ecosystem engineers may strongly affect other organisms and ecosystem functioning (Meysman *et al.* 2006, Lohrer *et al.* 2008). Understanding the relationships between the functionality of a recovering macrobenthic community and tidal flat ecosystem processes will therefore represent an added value to restoration projects. Such information will enable a proper interpretation of the systems' recovery status and may also enhance restoration success by the application of well-founded management actions (e.g. the use of appropriate soils and tidal elevation, introduction of key species). Moreover, such knowledge will enhance the understanding of the role of marine biodiversity in maintaining ecosystem services, in general.

Aims and outline of the thesis

The principal aim of this thesis was to study the ecology of macrobenthic organisms in estuarine tidal flat habitats with emphasis on recolonisation and succession mechanisms. Specifically, it was aimed to delineate insights in the importance of macrobenthos-environmental interactions (i.e. ecosystem engineering) in determining benthic settlement, community recovery dynamics and benthic community structure, in general. This was achieved by gathering information from monitoring (Chapter 2, Appendices 1 & 2), manipulative field experiments (Chapters 3-7) and mesocosm experiments (Chapters 8 & 9).

In **Chapter 2**, the reader is introduced to the tidal mudflat and its macrobenthic inhabitants where most of the fieldwork, involved in this thesis is conducted. The macrobenthos at the Paulina polder tidal flat, located in the lower part of the turbid, nutrient-rich and heterotrophic Westerschelde estuary, is studied in relation to the intertidal gradient. Underpinning mechanisms from the macrobenthic diversity and community structure distribution along the prevailing intertidal hydrodynamic stress gradient are deduced from a community-wide functional group investigation.

In the following part, different aspects of benthic ecology following hypoxia-induced macrobenthos mass mortality and subsequent recovery are presented and discussed based on a large-scale, multidisciplinary field experiment. Hypoxia occurs when dissolved oxygen levels falls below 2 mg l^{-1} and is considered among the most widespread deleterious anthropogenic influences on estuarine and marine environments (Halpern *et al.* 2007). At present, about 500.000 metric tons of benthic biomass is missing over a total area of 245.000 km^2 as a result of hypoxia and global change is expected to expand these "dead" zones due to the predicted increase of water-column stratification, warming, change of rainfall patterns and enhanced discharges of fresh water and agricultural nutrients (Diaz & Rosenberg 2008). Based on the information deduced in Chapter 2, the experimental study site was allocated at a tidal elevation which enables the investigation of a diverse set of ecological interactions.

Chapter 3 describes the macrobenthos progressive change in community composition and dynamics (i.e. succession) during the first recruitment season and discusses the potential role of facilitative and inhibitory biological-physical interactions as driving mechanisms, whereas these benthos-mediated sediments dynamics are analyzed into more detail in **Chapter 4**. Furthermore, the role of benthos-mediated sediments dynamics on the growth and production of four common tidal flat macrobentic species, *Pygospio elegans*, *Nereis diversicolor*, *Macoma balthica* and *Cerastoderma edule*, is shown and discussed in **Chapter 5**, whereas the effects of these benthos-sediment interactions on nematode community temporal patterns after hypoxia is discussed in **Chapter 6**. **Chapter 7**

deals with the 3-year observations of the macrobenthic community and investigates whether this long-term recovery pattern corresponds to the generalized Pearson-Rosenberg model of recovering benthic communities (Pearson and Rosenberg 1978).

In a third part, two experiments are presented in which one aspect of the ecology of a particular species is studied, which became relevant to the topic of this thesis through the analyses of the abovementioned investigations. **Chapter 8** depicts the specific effect of the common cockle, *Cerastoderma edule,* on sediment segregation and **Chapter 9** deals with the settlement of *Macoma batlhica* larvae in response to benthic diatom films.

Key issues and considerations, deduced from the main results of the different chapters and additional work on biogeochemical fluxes and carbon cycling (Andersson 2007, Rossi *et al.* in press), are discussed with reference to biodiversity-ecosystem function relationships and restoration ecology in the General discussion (**Chapter 10**).

Finally, two papers describing the macrobenthic community structure in the Zwin coastal lagoon and the impact of prolonged emersion on the macrobenthos resulting from an oil pollution protection of the nature reserve by damming are presented in **Appendix 1 and 2**. Furthermore, supplementary material to the Chapters 3 and 5 is provided in **Appendix 3**.

Each chapter is intented to be an autonomous part, which can be read separately from the other chapters. Inevitably, there is some overlap between the introductions and material and methods sections of the different chapters. Cited literature is compiled in a single list at the end of the thesis. Besides the general introduction, general discussion and the two papers compiled in the appendices, the candidate is first author of chapters 2, 3, 5, 6, 7 and 9. Further, the candidate provided data and input for Chapters 4 and 8.

148075

CHAPTER 2

BENTHIC COMMUNITY STRUCTURE, DIVERSITY AND TRAIT DISPLACEMENT ACROSS AN ESTUARINE TIDAL FLAT HYDRODYNAMIC STRESS GRADIENT

Van Colen C., Meulepas G., De Backer A., Van der Wal D., Vincx M., Degraer S., Ysebaert T.

Abstract

The intermediate disturbance hypothesis (IDH, Connell 1978) states that maximal diversity is maintained at intermediate disturbance or stress levels, and is perhaps one of the best accepted principles in ecology. However, recent research (Mackey & Currie 2001) highlights the lack of generality of the principle, indicating prudence in its overall application, e.g. in the development of ecosystem management plans. We investigated whether tidal mudflat macrobenthos community diversity and structure performed a unimodal response to the prevailing hydrodynamic stress gradient and whether this response is related to community-wide biological trait displacements.

Principal Component Analysis on the environmental characteristics of the sampling stations revealed the prevalence of one main 'hydrodynamic stress' axis (PC_{stress}: eigenvalue = 8.99, explanatory value = 74.9 %) which strongly correlated with cross-shore tidal current aspects and sediment composition. Sampling stations scores from PC_{stress} were retained to analyze diversity and community-wide patterns of biological traits in relation to the intertidal hydrodynamic stress gradient. Community structure differed significantly between stress levels and community species richness, diversity and evenness peaked at low hydrodynamic stress and were lowest at high hydrodynamic stress. Hence, our data did not support the IDH and suggest that competitive exclusion is not a main driver of macrobenthos diversity-stress relationships in highly stressed tidal flat habitats. Further, because additional stress components (e.g. dessication, predation, access to food) may point in different directions and be different between functional groups, the detection of a unimodal community-wide diversity-stress response may be hampered and the IDH principle may therefore not be appropriate within the hydrodynamic stress range of our study site.

Suspension feeding peaked at intermediate hydrodynamically stressed stations, located in the mid intertidal where bottom shear stress is drastically lower as compared to the lower intertidal and submersion time, and consequently suspension feeding time, is slightly longer as compared to the high intertidal zone. In contrast, the proportional biomass and abundance of surface deposit feeders and the proportional abundance of species with a lecitotrophic larval development were lowest at intermediate hydrodynamically stressed stations. *Cerastoderma edule* is the most important suspension feeder, both in terms of biomass, biovolume and abundance at our study site and has been shown to affect benthic community structure negatively through disturbance of the benthic boundary layer. Given the surface dwelling life habit of surface deposit feeders and lecitotrophic non-pelagic larvae, these organisms may be expected as most vulnerable to the *C. edule* mediated disturbance in the upper sediment layer. This indicates that inhibitory benthos-physical interactions on ecosystem diversity, such as interference by bioturbation, should be incorporated in tidal flat biodiversity-stress models.

Keywords: *Intermediate disturbance hypothesis, Biological traits, Macrobenthos – environmental stress models, Bioturbation, Tidal mudflat, Westerschelde estuary*

Introduction

Understanding the factors and mechanisms that control patterns of diversity at a local scale is important since many ecosystems increasingly experience human disturbances which affect their species diversity (Hughes *et al.* 1997) and hence, ecosystem functioning and the resilience of ecosystems to environmental change

(Elliott *et al.* 2007, Naeem *et al.* 1994). The intermediate disturbance hypothesis (IDH, Connell 1978) predicts that sessile community diversity is maximized at intermediate levels of disturbance intensity and/or frequency because this state enables the coexistence of stress tolerant species and potential competitors, whereas competitively dominant species may exclude subordinate species at low disturbance and less tolerant species may directly be eliminated at high disturbance.

In marine benthic ecosystems, taxonomic community composition derived diversity measurements, such as species richness, diversity indexes (e.g. Shannon-Wiener) and taxonomic distinctness have traditionally been used to describe diversity in relation to different or changing environments or stress (e.g. Somerfield & Clarke 1995, Warwick & Clarke 1993). Species-specific ecological or functional characteristics (e.g. body size, feeding habit, mobility) have often, subsequently, been linked in order to determine indirectly the processes that underpin the observed diversity patterns. However, because the same two species may share characteristics but also differ in others, these species may interfere or not and react differently to stress (Ramsay *et al.* 1998), thereby hampering the detection of the driving ecological function of the stress response (Bremner *et al.* 2003). Therefore, regarding the two main drivers of the IDH, i.e. competitive exclusion and stress tolerance, a functional group approach, assigning species sharing the same biological traits into groups, regardless of the species composition, is expected to provide a more appropriate approach to unravel stress-diversity relations (e.g. Bremner *et al.* 2003).

Intertidal habitats experience pronounced gradients in environmental stress (Raffaelli & Hawkins 1996) and, hence, provide a good opportunity to test hypotheses on local scale community diversity in relation to stress or disturbance. Most of the knowledge in this field has accumulated by using rocky shore habitats as model systems, since the relatively small size of the intertidal organisms, together with the stress gradients that occur across short distances, facilitate observational and experimental research (Menge & Branch 2001, Scrosati & Heaven 2007). In the soft-sediment intertidal, diversity tends to peak at intermediate (Beukema 1976) to low intertidal levels (Degraer *et al.* 1999, Armonies & Reise 2000, Giménez *et al.* 2005, 2006, Harriague & Albertelli 2007, Honkoop *et al.* 2006) on sandy beaches and sandflats, whereas comparable detailed information on cross-shore species diversity is limited for sheltered intertidal mudflats.

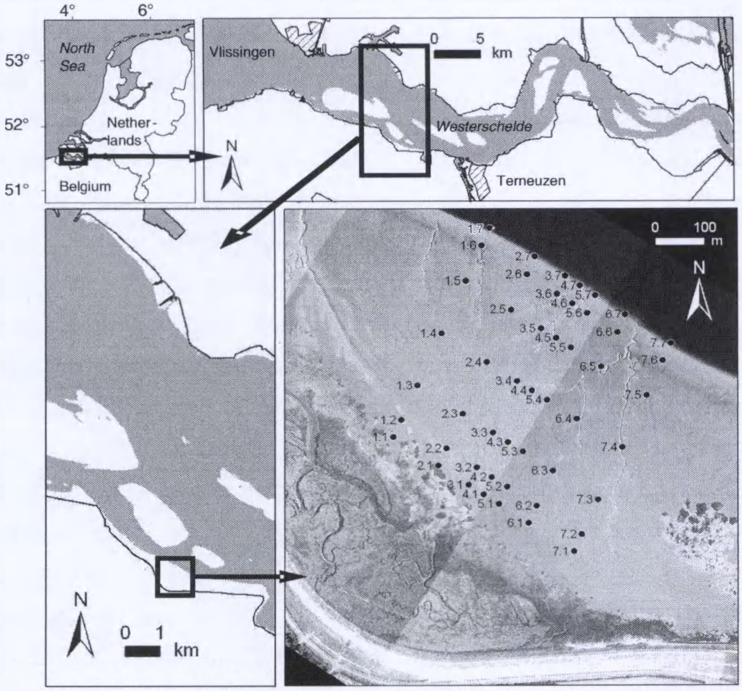


Fig. 1. *Location of the intertidal study site with indication of the seven cross-shore transects. Sampling stations are depicted as bullets with their label.*

In this study, the macrobenthic community across a full intertidal gradient on a single tidal mudflat was used to investigate how diversity, biological traits and community structure vary in relation to the prevailing hydrodynamic stress gradient. Given the general idea that sets of traits are related to species abilities to cope with stress, a functional group approach was used to give insight in the underlying drivers of the community-wide responses to changing hydrodynamical stress.

In particular, we addressed the following questions:

1. Does benthic species diversity exhibit a unimodal hydrodynamic stress response, congruous the IDH?
2. Does the macrobenthos community structure differ in relation to hydrodynamic stress?
3. Do community-wide biological trait shifts occur along the hydrodynamic stress gradient, which may explain the observed stress response?

Materials & methods

Site description and sampling

Samples were collected on 17, 19 and 23 April 2002 at Paulinapolder tidal flat, which is located along the south bank of the lower, polyhaline part of the Westerschelde estuary (SW the Netherlands) (Fig. 1). The flat has a semi-diurnal tidal regime with a mean tidal range of 3.9 m and an average salinity of 24 PSU (Ysebaert *et al.* 2003). The sediment material generally consists of mud (average median particle size = 65 µm, average mud content = 51 %) but the sand fraction increases linearly down the flat, associated with the prevailing stronger currents towards the estuarine main channel (Table 1). The established macrofauna community is rich, consisting of 21 species characterized by a wide variety of biological traits (Table 2).

Macrobenthos samples were collected with a stainless corer (inner ø 11 cm) to a depth of 40 cm along seven intertidal transects. Each transect consisted of seven sampling stations, covering a complete cross-shore gradient from the marsh edge towards the subtidal estuarine main channel (Fig. 1). The macrobenthos samples (n = 1 sampling station^{-1}) were washed over a 0.5 mm mesh-sized sieve and the residual organisms were fixed in a buffered 4% formalin-seawater solution. In the laboratory, the retained organisms were sorted, counted and identified to species level (except for oligochaetes), after staining with Rose Bengal. At each sampling station, the first sediment layer was sampled, freezedried and stored at –20°C, and subsequently processed in the laboratory for granulometry using Malvern laser diffraction analysis. Further environmental characteristics were extracted at the sampling stations in a Geographical Information System, based on maps from Rijkswaterstaat, the Dutch Ministry of Transport, Public Works and Water Management (Graveland 2005). Elevation data were obtained from a laseraltimetry survey of 2001 for the intertidal zone, combined with a multibeam survey for the subtidal zone. Spatial information on current velocity, i.e. the maximum current velocity during flood and ebb on spring and mean tides, were derived from simulations (using the 2001 elevation data) with SCALWEST, a numerical flow model developed for the Westerschelde. The 2001 elevation data were also combined with water level information from 2002 from the nearby tide gauge station Terneuzen (Fig. 1) to calculate inundation duration at the sampling stations at spring and mean tide, respectively.

Parameter	Minimum-maximum	PC_{stress}		Tidal elevation	
		r	p	r	p
Maximal ebb current velocity mean tide ($cm.s^{-1}$)	6 - 45	-0.98	< 0.001	-0.97	< 0.001
Maximal flood current velocity mean tide ($cm.s^{-1}$)	11 - 49	-0.95	< 0.001	-0.93	< 0.001
Maximal ebb current velocity spring tide ($cm.s^{-1}$)	11 - 51	-0.98	< 0.001	-0.97	< 0.001
Maximal flood current velocity spring tide ($cm.s^{-1}$)	16 - 58	-0.98	< 0.001	-0.97	< 0.001
Median particle size (µm)	37.4 – 131.8	-0.88	< 0.001	-0.80	< 0.001
Mud content (% < 63 µm)	14.4 – 75.2	0.87	< 0.001	0.79	< 0.001
Very fine sand fraction (% 63-125 µm)	19.7 – 42.6	-0.84	< 0.001	-0.74	< 0.001
Fine sand fraction (% 125-250 µm)	3.0 – 44.8	-0.87	< 0.001	-0.80	< 0.001
Medium sand fraction (% 250-500 µm)	0 – 10.9	-0.28	0.057	-0.29	0.040
Sorting coefficient (φ)	1.1 – 3.3	0.68	< 0.001	0.55	< 0.001
Submersion duration at spring tide (hrs)	4.8 – 10.8	-0.96	< 0.001	-1.00	< 0.001
Submersion duration at mean tide (hrs)	4.6 – 12.0	-0.96	< 0.001	-1.00	< 0.001

Table 1. *Minimum and maximum values for each parameter and Spearman rank correlations with the first principal component (PC_{stress}) and elevation (cm above Dutch Ordnance Datum NAP) of sampled variables across the intertidal flat at Paulinapolder. Relations with PC_{stress} are significant at $p < 0.004$ after Bonferroni correction.*

	Feeding habit	**Development mode**
Arenicola marina	Subsurface deposit feeder	Direct
Aphelochaeta marioni	Surface deposit feeder	Lecitotrophic
Anaitides mucosa	Omnivore/Scavenger	Planktotrophic
Abra tenuis	Surface deposit feeder	Direct
Cerastoderma edule	Suspension feeder	Planktotrophic
Capitella capitata	Subsurface deposit feeder	Lecitotrophic
Corophium volutator	Surface deposit feeder	Direct
Eteone longa	Omnivore/Scavenger	Planktotrophic
Heteromastus filiformis	Subsurface deposit feeder	Planktotrophic
Hydrobia ulvae	Surface deposit feeder	Planktotrophic
Macoma balthica	Surface deposit feeder	Planktotrophic
Mysella bidentata	Suspension feeder	Planktotrophic
Nephtys cirrosa	Omnivore/Scavenger	Planktotrophic
Nereis diversicolor	Omnivore/Scavenger	Lecitotrophic
Oligochaeta	Subsurface deposit feeder	Direct
Polydora cornuta	Surface deposit feeder	Planktotrophic
Pygopsio elegans	Surface deposit feeder	Planktotrophic
Retusa obtusa	Omnivore/Scavenger	Planktotrophic
Streblospio benedicti	Surface deposit feeder	Lecitotrophic
Scrobicularia plana	Surface deposit feeder	Planktotrophic
Spio sp.	Surface deposit feeder	Planktotrophic

Table 2. *List of macrobenthic species of the Paulinapolder mudflat, recorded in this study with indication of their biological traits. Feeding habits are based on Fauchauld & Jumars 1979 and Van Colen et al. 2008, development modes are based on Eckert 2003, Dekker & Beukema 1999 and <https://www.marlin.ac.uk/>.*

Characterization of the environmental stress gradient

Principal Component Analysis (PCA, Goodall 1954) was carried out on the environmental characteristics of the sampling stations (Table 1). PCA revealed the prevalence of one main 'hydrodynamic stress' axis (PC_{stress}; eigenvalue = 8.99; explanatory value = 74.9 %), which strongly correlated with tidal current aspects and sediment composition after Bonferroni correction (Table 1). Decreasing values of PC_{stress} indicate a lower intertidal position with an increased submersion time, increased current velocity during ebb and flood and a substrate composed of less sorted, less muddy sediment. Because we aimed to study tidal flat macrobenthos diversity, community structure and community-wide patterns of biological traits in relation to hydrodynamical stress *sensu lato,* we retained sampling stations scores from the first PCA axis for further analysis. Stations were characterized as low, medium and highly stressed according to their distribution within the upper ($PC_{stress} > 0.4$), middle ($-2.9 > PC_{stress} < 0.4$) and lower ($PC_{stress} < -2.9$) third of the PC_{stress} axis.

Data analysis: species diversity and biological traits

Species richness and diversity have often been used as synonyms in past studies and in the context of the IDH, both terms have been used interchangeably (e.g. Menge & Sutherland 1987, Bertness 2007). However, since diversity depends on the number of species in the community and the degree of similarity in abundance among species (i.e. evenness), their patterns in relation to environmental stress may differ (e.g. Kimbro & Grosholz 2006). Hence, we calculated species richness (i.e. total number of species), species diversity (Shannon-Wiener diversity index, H'(e)) and evenness (Pielou's evenness, J') for each sampling station. Further, to delineate insights in the underlying drivers of the diversity and community structure stress response, the influence of hydrodynamical stress on the community-wide proportional biomass and abundance of the feeding habits (surface deposit feeder, subsurface deposit feeding, filter feeding, omnivore/scavenging) and the community-wide proportional abundance of the species' development modes (lecitotrophic, planktotrophic, benthic development) was analyzed. Species-specific traits and the respective references, upon which they are based, are presented in Table 2. The influence of hydrodynamical stress on community-wide diversity and biological traits was assessed using linear and quadratic models (GLMs, Statistica 7.0). The first PCA axis was used as the continuous factor, reflecting hydrodynamic stress *sensu lato,* whereas species richness, diversity, evenness and the arcsine transformed proportional abundance and biomass data were used as dependent variables. Extreme outliers were excluded from the analysis (casewise plot of residuals ± 3 sigma, Statistica 7.0) to achieve normality of residuals. Akaike's information criterion (AIC)(Johnson & Omland 2004) revealed that quadratic models were, overall, more reliable as compared to the linear regressions, and consequently these models were retained and presented in this manuscript. One sampling station (5.1), which contained only three individuals of two different species, was omitted from all analyses because it consistently behaved as an outlier in the GLM. One-way analysis of similarity (Anosim, Clarke & Gorley 2001) was applied to investigate difference in community structure based on log (x+1) transformed, non-standardized abundance data and the species contributing most to the dissimilarities were identified using similarity of percentages analysis (Simper, Clarke 1993).

Results

Species diversity and community structure

Overall species richness, diversity and evenness peaked at low hydrodynamic stress levels (Fig. 2, Table 3). Fig. 2 shows that the peak of the latter variable is less pronounced as compared to the species richness and diversity. Anosim revealed a significant difference between low, medium and highly stressed stations (low-medium; $R = 0.478$ $p < 0.001$, low-high; $R = 0.897$ $p < 0.001$, medium-high; $R = 0.639$ $p < 0.001$), with different species comprising the majority of the community (Table 4). *Pygospio elegans*, Oligochaeta spp. and *Cerastoderma edule* contributed most to the dissimilarity between highly stressed and low - medium stressed communities, whereas differences between low and medium stressed communities were attributable to contributions of the surface deposit feeders *Aphelochaeta marioni*, *Hydrobia ulvae*, *Streblospio benedicti* and *P. elegans* (Table 4).

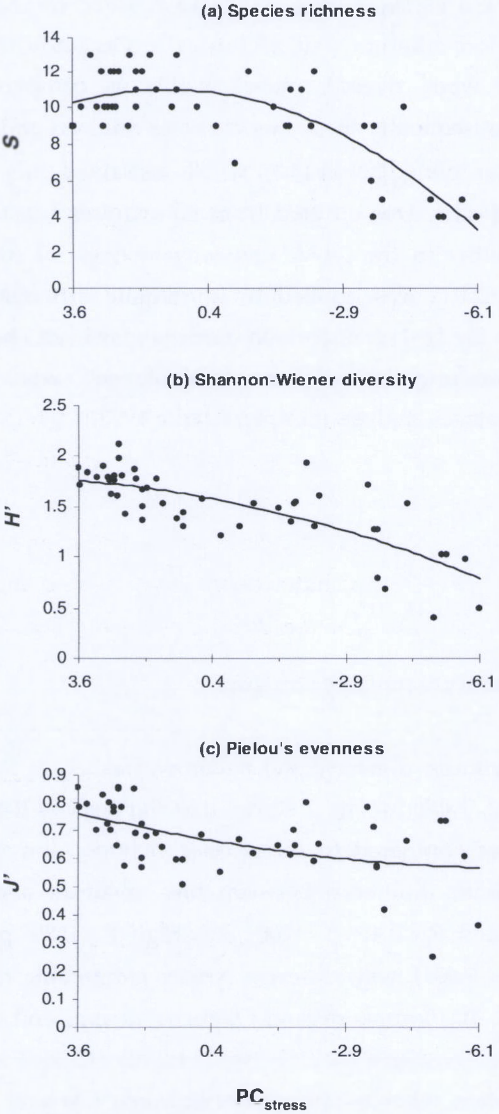


Fig. 2. Relationship between overall (a) species richness, (b) Shannon-Wiener diversity, (c) Pielou's evenness and the degree of hydrodynamic stress across the intertidal gradient. The principal component scores derived from a principal component analysis of station-specific habitat characteristics (PC_{stress}) are used to indicate the degree of hydrodynamic stress along the x-axis.

Community-wide biological traits

Statistics of the relationships between PC_{stress} and community-wide feeding and development traits are shown in Table 5 and the significant relationships are presented in Fig. 3. The relation between PC_{stress} and proportional suspension feeding abundance and biomass revealed an intermediate optimum across the hydrodynamic stress gradient. The proportional subsurface deposit feeder abundance and biomass and the proportional biomass of omnivore/scavengers increased with increasing hydrodynamic stress (i.e. lower PC_{stress} values), whereas the opposite pattern was found for the proportional surface deposit feeding abundance and biomass (Fig. 3). Furthermore, a differing trait pattern was also apparent for development mode: lecitotrophic development showed a U-shaped distribution pattern across the hydrodynamic stress gradient, whereas direct benthic development peaked at medium stress levels and the proportional abundance of species with a planktotrophic larval development mode slightly increased towards less hydrodynamically stressed environments (Fig. 3).

	Df numerator	Df denumerator	F	p	Adjusted R^2
Species richness	2	45	39.167	< 0.001	0.62
Shannon-Wiener diversity	2	45	31.616	< 0.001	0.57
Pielou's evenness	2	45	7.866	0.001	0.23

Table 3. *Quadratic regression statistics of the relation between* PC_{stress} *and total species richness, Shannon-Wiener diversity and Pielou's evenness.*

Discussion

The present study primarily aimed to test whether tidal mudflat macrobenthos diversity performs a unimodal response to hydrodynamic stress, as predicted by the IDH (Connell 1978). In agreement with the IDH, low overall species richness, diversity and evenness was found at high hydrodynamically stressed stations in the low intertidal. However, diversity, evenness and species richness peaked at low hydrodynamic stress occurring in the high intertidal, and hence, no diversity optimum at intermedium hydrodynamical stress was found. Consequently, overall, our data provide no support for the IDH.

Species	**Average Abundance**	**Average Abundance**	**Contribution to the dissimilarity**	**Cumulative percentage**
	Low stressed	Medium stressed		
Aphelochaeta marioni	3065 ± 639	453 ± 120	13.97	13.97
Hydrobia. ulvae	745 ± 195	53 ± 24	11.46	25.43
Streblospio benedicti	487 ± 93	53 ± 28	10.26	35.68
Pygospio elegans	4207 ± 1415	1358 ± 195	9.88	45.56
Oligochaeta	9491 ± 2443	5740 ± 1405	8.61	54.17
Cerastdorma edule	663 ± 88	1000 ± 215	8.53	62.7
	Low stressed	High stressed		
Pygospio elegans	4207 ± 1415	278 ± 153	15.15	15.15
Oligochaeta	9491 ± 2443	5790 ± 1405	14.06	29.21
Cerastoderma edule	663 ± 88	0 ± 0	10.34	39.55
Streblospio benedicti	487 ± 93	0 ± 0	8.51	48.06
Macoma balthica	550 ± 44	67 ± 33	8.41	56.46
Aphelochaeta marioni	3065 ± 639	1158 ± 354	8.31	64.77
	Medium stressed	High stressed		
Oligochaeta	5740 ± 1405	5790 ± 1405	15.85	15.85
Cerastoderma edule	1000 ± 215	0 ± 0	14	29.85
Pygospio elegans	1358 ± 195	278 ± 153	13.37	43.22
Macoma balthica	463 ± 83	67 ± 33	8.94	52.16
Aphelochaeta marioni	453 ± 120	1158 ± 354	8.93	61.09

Table 4. *Contribution of species to > 60 % dissimilarity between low, medium and high hydrodynamically stressed sampling stations using Simper analysis based on Bray-Curtis similarity of log (x+1) species abundance data. Presented abundances ± standard errors (ind.m^{-2}) are not transformed.*

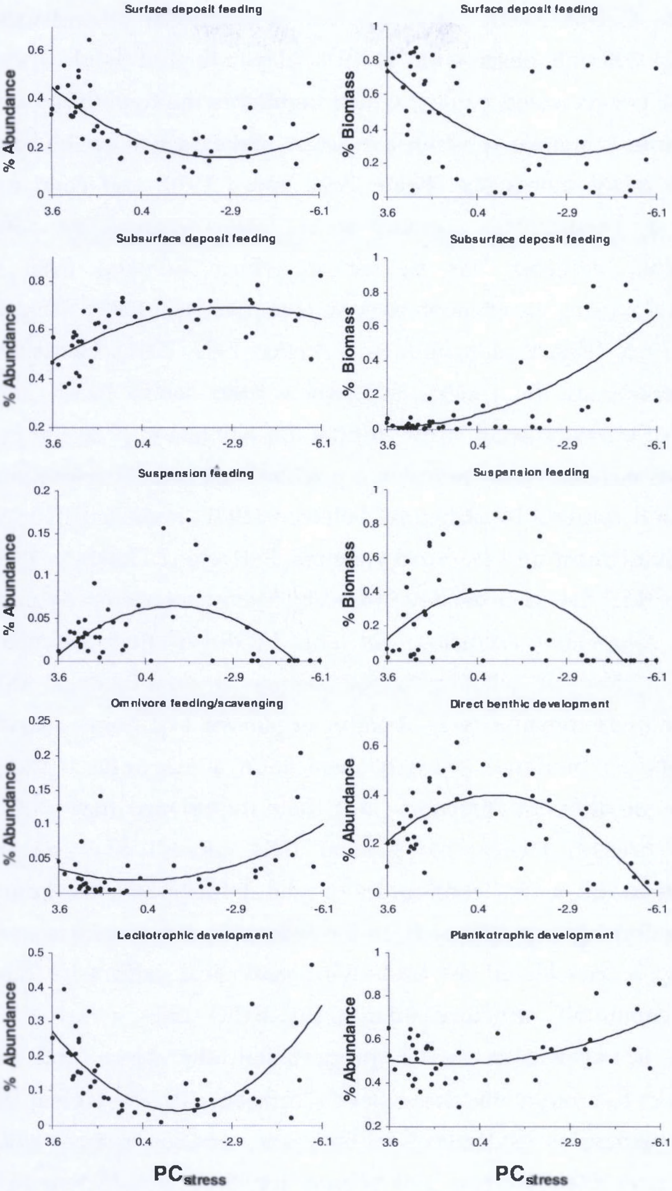


Fig. 3. Relationship between the proportional community biomass and/or abundance of species exhibiting surface deposit feeding, subsurface deposit feeding, suspension feeding, omnivore feeding/scavenging, lecitotrophic development, direct benthic development, planktotrophic development and the degree of hydrodynamic stress along the x-axis (i.e. PC$_{stress}$).

Mackey & Currie (2001) revealed that a unimodal disturbance-diversity relationship was only observed in 11-19 % (depending on the diversity measure applied) of 116 reviewed papers, which highlights the hypothesis status of the IDH principle. In marine substrates, experimental evidence for the IDH has been shown for rocky shores (e.g. Paine 1966, Sousa 1979) and coral reef studies (Aronson & Precht 1995, Connell *et al.* 2004) whereas for soft-sediment environments, evidence has so far only been invoked from mesocosm disturbance/organic enrichment experiments (Austen 1998, Schratzberger & Warwick 1998, 1999, Widdicombe and Austen 1999, 2001). Hence, the present study corroborates the general suggestion from sandy beach and sandflat surveys that environmental stress in tidal flat habitats may be too high so that competitive exclusion does not play a predominant role in determining species diversity and community structure. Peterson (1991) reviewed that, contrary to intertidal hard substrates (e.g. rocky shores, Raffaelli & Hawkins 1996), benthic zonation in tidal flats is rarely determined by intense interference competition for space (i.e. competitive exclusion) but more by direct effects of changes in the physical environment, whether or not mediated by the benthos. Moreover, in addition to hydrodynamic stress, benthic organisms experience also other stress components, e.g. predation pressure, dessication, access to food. These stressors may point in different directions and their importance may differ between functional groups. Hence, the general IDH concept may simply not be appropriate to the benthic community of tidal flat habitats. Based on the results of the functional group approach, in the following, we present some processes which may be considered the underlying ecological drivers for the observed pattern in community structure and diversity in this study.

The peak in suspension feeder proportional abundance and biomass at intermediate hydrodynamic stress levels corroborates the typical distribution pattern of suspension feeders in tidal flats: concentration in areas where bottom shear stress is relatively low and submersion time is sufficient to feed long enough to meet energetic requirements (Beukema 1976, Herman *et al.* 1999). These conditions are best in the mid intertidal region of our study site, where tidal currents, and thus bottom shear stress, are drastically lower as compared to the lower intertidal and submersion time is slightly longer as compared to the highest intertidal region. The common edible cockle, *Cerastoderma edule*, is the most important suspension feeder, both in terms of biomass, biovolume and abundance in the upper 5 cm at our study site (Ysebaert & Herman 2002, Van Colen *et al.* 2008, Montserrat *et al.* 2008, this study) and has been shown to affect the benthic boundary layer significantly by increasing bottom shear stress due to the physical structure of its shell and by biodiffusion due to its movement (Ciutat *et al.* 2007, Montserrat *et al.* 2008). Interference by bioturbation of large macrofauna adversely affect juvenile recruitment success, and benthic community stucture in general, directly (e.g. by predation; Hiddink *et al.* 2002) and/or indirectly (e.g. by destabilisation of the sediment, inhibition of microphytobenthos development; Ólafsson 1989, Ólafsson *et al.* 1994, Flach 2003). Consequently, given the feeding habit and surface dwelling life habit of surface deposit feeders and lecitotrophic non-pelagic larvae, these organisms may be expected as most vulnerable to the cockle mediated disturbance in the upper sediment layer. The low proportional abundance of surface deposit feeders and species with a lecitotrophic development in this study support and generalize the findings of Flach (1996), who showed a severe negative effect of *C. edule* densities on juvenile recruitment of the surface deposit feeders *P. elegans*, *M. balthica* and the lecitotrophic surface deposit feeder *A. marioni*. Therefore, the suspension feeding optimal stress response at intermediate hydrodynamic stress presumably enhances the overall surface dwelling organisms' stress due to interference by bioturbation, which may have contributed to the overall community diversity peak at low hydrodynamically stressed habitats in the high intertidal. Additionally, at muddier high intertidal habitats, overall benthic diversity, and surface dwelling species in particular, may benefit from (1) lower hyperbenthic predation pressure due to shorter submersion times and (2) higher benthic primary production (de Jong & de Jonge 1995, van de Koppel *et al.* 2001), despite some possible adverse effects due to enhanced desiccation stress and avian predation pressure.

Increased awareness of the importance of benthos-physical interactions, determining overall benthic community structure and diversity (e.g. Kimbro &

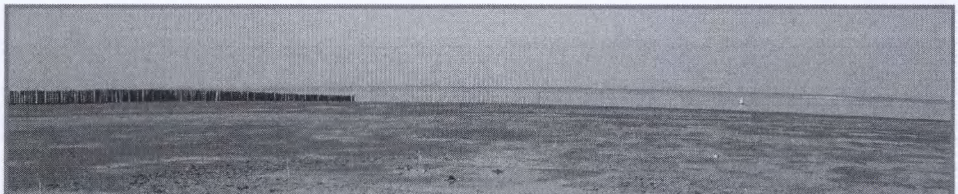
Grosholz 2006, Rabaut *et al.* 2007, Thrush *et al.* 2008, Van Colen *et al.* 2008), have led to the incorporation of facilitative interactions into recently updated environmental stress models (Bruno *et al.* 2003, Scrosati & Heaven 2007). Additionally, the results of the present study indicate that inhibitory benthos-physical interactions on overall ecosystem diversity such as interference by bioturbation (this study, Solan *et al.* 2008) should be incorporated in tidal flat biodiversity-environmental stress models, as well.

	Df numerator	Df denumerator	F	p	Adjusted R^2
% Abundance surface deposit feeders	2	45	17.826	< 0.001	0.41
% Abundance subsurface deposit feeders	2	44	14.805	< 0.001	0.38
% Abundance suspension feeders	2	44	8.839	< 0.001	0.26
% Abundance omnivore/scavengers	2	42	1.432	0.250	0.02
% Biomass surface deposit feeders	2	45	8.362	< 0.001	0.24
% Biomass subsurface deposit feeders	2	45	27.602	< 0.001	0.53
% Biomass suspension feeders	2	44	7.730	0.002	0.23
% Biomass omnivore/scavengers	2	41	4.843	0.012	0.15
% Abundance planktotrophic development	2	45	5.801	0.006	0.17
% Abundance lecitotrophic development	2	44	18.856	< 0.001	0.44
% Abundance direct benthic development	2	45	14.004	< 0.001	0.36

Table 5. *Quadratic regression statistics of the relation between* PC_{stress} *and the arcsine transformed proportional community biomass and/or abundance of the biological traits surface deposit feeding, subsurface deposit feeding, suspension feeding, omnivore/scavenger feeding habit, planktotrophic development, lecitotrophic development and direct benthic development.*

Acknowledgements

This study is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen). The authors want to thank J. van Soelen and B. Koutstaal for their help during sampling and processing of the samples. K. Lambeets and W. Willems are acknowledged for their advice on statistical models. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446).



-148079

CHAPTER 3

MACROBENTHIC RECOVERY FROM HYPOXIA IN AN ESTUARINE TIDAL MUDFLAT

Adapted from:

Van Colen C., Montserrat F., Vincx M., Herman P.M.J., Ysebaert T., Degraer S. (2008)
Macrobenthic recovery from hypoxia in an estuarine tidal mudflat
Marine Ecology Progress Series 372: 31-42

Abstract

Macrobenthic recolonisation patterns after complete defaunation resulting from experimentally induced hypoxia were investigated in a polyhaline, estuarine mudflat. Based on simultaneous sampling of biotic and environmental variables in replicated 16 m^2 control and defaunated plots, with a high resolution in time during 6 mo, the ecological interactions related to the macrobenthos reassembly were elucidated. Colonisation was predominantly determined by juvenile recruitment, and 3 successional stages were identified, each characterised by different species assemblages and environmental characteristics. During recovery, a shift in functional group dominance from mobile surface deposit feeders to tube-dwelling surface deposit feeders to biodestabilising taxa occurred, while their proportional dominance remained quite stable in the control plots throughout the experiment. Species colonisation patterns of later colonists revealed positive interactions with early colonising opportunistic tube-building polychaetes *Pygospio elegans,* while later successional species (*Heteromastus filiformis, Macoma balthica*) adversely affected the stable, favourable conditions created by the tube-building infauna. Transitions between different successional stages were related to recruitment of species, changes in environmental characteristics (oxygenation state of the sediment), direct and indirect ecological interactions (bio[de]stabilisation, exploitation competition for food). In general, our study suggests that macrobenthic reassembly after hypoxia is related to different types of interactions, all acting in a unique manner. Hence, macrobenthic successional dynamics in a tidal mudflat habitat should be considered as a dynamic process, related to resource availability, natural temporal variation, life history traits (e.g. opportunistic behaviour) and bio-engineering capacities of the colonising species.

Keywords: *Macrobenthos (re)colonisation, Succession, Physical–biological interactions, Exploitation competition for food, Tidal mudflat, Westerschelde estuary*

Introduction

Habitat loss and degradation result in an alarming decrease of biodiversity and constitute a major, widespread environmental problem. Since ~60% of the world population lives along estuaries and coasts (Lindeboom 2002), marine benthic habitats are extremely vulnerable to different anthropogenic disturbance pressures, such as dredging and dredge disposal (e.g. Newell *et al.* 1998), bottom trawling fisheries (Thrush & Dayton 2002), altered tidal regimes (Van Colen *et al.* 2006), toxic chemicals (Lenihan *et al.* 2003) and eutrophication (Cloern 2001). These disturbances can lead to partial or even complete macrobenthic mortality in marine sediments (Thrush & Dayton 2002, references therein). Such mortalities are particularly frequent in coastal seas, tidal flats and estuaries (Beukema *et al.* 1999), where the defaunated areas can cover several km^2 (Diaz & Rosenberg 1995). In estuarine tidal flat habitats, macrobenthic organisms fulfil several key roles in benthic remineralisation processes (Herman *et al.* 1999), sediment transport processes (Paterson & Black 1999) and pelagic food chains, being an important food source for epibenthic crustaceans, fish and birds (Hampel *et al.* 2004). Due to their essential role in the estuarine ecosystem functioning, even local extinction of the benthos can have dramatic consequences (e.g. Beukema & Cadée 1996). Once the disturbance(s) that cause mortality abate or disappear, macrobenthic recovery may occur.

Pearson & Rosenberg (1978) found a gradual succession of macrobenthic community recovery along gradients of decreasing disturbance from a peak in abundance dominated by superficially living opportunistic species to a community with stable abundances of deeper burrowing species. The Pearson-Rosenberg model was developed using data from macrofaunal community dynamics after organic enrichment in subtidal, stable (i.e. low hydrodynamical stress) muddy sediments. However, this model is now widely considered as a general qualitative model of macrobenthic recovery from severe disturbances. Connell & Slatyer (1977) postulated 3 generalised models of recovering communities based on interactions between early (i.e. pioneering) and later colonists. Early colonists can promote the establishment of later colonists (facilitation model), reduce the establishment of later colonists (inhibition model) or have little or no effect on the establishment of later colonists (tolerance model). These interactions can be direct and/or indirect (e.g. predation, interference competition, exploitation competition; e.g. Benedetti-Cecchi 2000, Wootton 2002). Additionally, as the spatial scale of disturbance will influence recovery dynamics (Norkko *et al.* 2006, references therein), recolonisation and succession mechanisms are scale-dependent processes.

A Pearson-Rosenberg type of macrobenthic recovery, consisting of different successional stages and clear opportunistic responses, has not always been observed in the few large-scale recolonisation studies in tidal flat habitats (e.g. Thrush *et al.* 1996, Beukema *et al.* 1999). Furthermore, the mechanisms of succession are not fully understood. Facilitative, inhibitory, as well as no interactions between earlier and later colonists have been reported and suggested from smaller-scale manipulative experiments (e.g. Gallagher *et al.* 1983, Whitlatch & Zajac 1985, Thrush *et al.* 1992, Bolam *et al.* 2004). Distribution of macrobenthos in estuaries strongly depends on physical characteristics such as grain size, bed level and hydrodynamics (e.g. Herman *et al.* 2001, Ysebaert *et al.* 2003). Moreover, physical–biological interactions can change these characteristics, e.g. due to biodestablisation (by bioturbators, e.g. adult bivalves, large polychaetes) or biostabilisation (e.g. by microphytobenthos, reef-forming epifauna) of the sediment. This, in turn, has been shown to structure benthic populations (Bolam & Fernandes 2003, van Wesenbeeck *et al.* 2007, Volkenborn & Reise 2007). It can therefore be assumed that physical–biological interactions will also affect macrobenthic recolonisation dynamics, but the role of such interactions in macrobenthic reassembly remains poorly understood.

In this study, we examined macrofaunal recolonisation dynamics during 1 recruitment season based on sampling of both environmental and biotic changes in large experimentally replicated defaunated plots with a high temporal resolution. Based on this integrative approach, we investigated (1) whether macrobenthic recovery patterns in an intertidal mudflat resemble the Pearson-Rosenberg model, and (2) whether the macrobenthic recovery pattern is related to biological and physical–biological interactions between earlier and subsequent successional assemblages.

Materials & methods

Study site

The experiment was conducted at Paulinapolder, a tidal flat habitat located along the southern shore of the polyhaline part of the Westerschelde estuary, The Netherlands (51° 21' 24" N, 3° 42' 51" E). The mudflat has a gentle slope and a mean tidal range of 3.9 m, with a semidiurnal regime. A homogeneous study site (45 × 45 m), sheltered from waves and strong tidal currents associated with the main tidal channel at the mudflat, was selected based on environmental and biotic data collected in December 2004 (Table 1 in Appendix 3). The macrofaunal community at the study site consists of 18 macrobenthic species (~30000 ind. m^{-2}, 68 g ash-free dry weight [AFDW] m^{-2}, Shannon-Wiener diversity index $H' = 1.87$) and is numerically dominated by 6 species, comprising ~85% of the total macrobenthic abundance: the polychaetes *Heteromastus filiformis*, *Pygospio elegans*, *Aphelochaeta marioni* and *Malacoceros tetracerus*, tubificid oligochaetes and the bivalve *Macoma balthica*. However, due to their relatively small size, these polychaetes and oligochaetes do not contribute greatly to the total biomass (~23%); the bivalves *Cerastoderma edule* and *Macoma balthica* account for ~76% of the total biomass (Table 1 in Appendix 3).

Experimental design, sampling and laboratory treatment

Three replicate 4 × 4 m defaunated and control plots were randomly positioned within the study site, at least 5 m from each other. Based on scale-dependent colonisation studies in tidal flats (e.g. Günther 1992) this scale was considered efficient (1) to minimise bias in successional dynamics through direct immigration by adult and juvenile organisms from the surroundings and (2) to allow frequent monitoring without disturbance of the experimental plots (see below). Plots were defaunated by covering the sediment with a water-proof polyethylene sheet (0.1 mm thick) and a tarpaulin sheet (140 g m^{-2}) and digging in the edges up to 30 cm, following Beukema *et al.* (1999) and Dittmann *et al.* (1999). On 30 March 2005, 40 d after covering, both layers were removed but the 30 cm deep lining of the trenches was left in place to prevent horizontal subsurface migration of adult infauna and to minimise disturbance. In this experiment, no survival of macrobenthic organisms was detected immediately after removal of the sheets, indicating complete defaunation of the macrofauna. The sediment was anoxic, containing high NH_4^+ (51338 μg l^{-1}) and low NO_3^- (2 μg l^{-1}) and NO_2^- (26 μg l^{-1}) pore water concentrations in the upper cm (14×, 8.4×, 17.9×, respectively, compared to the control sediment) and was characterised by a black surface.

Because recruitment by (post)larval macrobenthos was hypothesised to be the dominant colonisation mechanism at the experimental scale (Günther 1992), this experiment was conducted from 30 March until 30 September 2005, covering the macrobenthic peak recruitment period during spring and early summer. Environmental and biotic recovery was monitored 17 times (i.e. daily during the first 3 d, then weekly during the first month and then biweekly until the end of the experiment). Moreover, to avoid disturbance in the plots due to sampling, samples were collected from a bridge, and sampling holes were filled with closed

PVC tubes, pushed flush with the sediment surface. Furthermore, to minimise possible edge effects, sampling only occurred in the inner 3 × 3 m.

At each sampling day, 2 replicate subquadrats (37.5 × 37.5 cm) per plot, never located next to each other, were randomly chosen beforehand. Depending on the response variable, 1 (macrofauna, organic matter, mud content and erosion threshold) or 2 (bed level, surface chlorophyll [chl] a, oxygen concentration, water content and nutrient pore water concentration) subquadrats were sampled in each plot.

Macrobenthos was sampled with a core (inner ø 12.5 cm) to a depth of 40 cm and fixed with a neutralised 8% formalin solution. In the laboratory, the samples were sieved through a 0.5 mm mesh, and the residual was fixed and preserved using a neutralised 4% formalin solution with 0.01% Rose Bengal until processing. All macrofauna was sorted, counted and identified to the species level, except for nematodes and tubificid oligochaetes. To distinguish between juvenile and adult individuals, population size-frequency analysis was carried out for species present in all replicate samples after recruitment and with a mean abundance of >30 ind. sample^{-1} for either control or defaunated plots. This criterion was chosen to include only populations in which a representative size distribution and comparison between populations of control and defaunated plots could be ascertained. All size measurements were conducted using a stereo microscope fitted with an eyepiece graticule, except for larger bivalves, which were measured to the nearest 0.01 mm using a Vernier calliper.

The upper 1 cm of the sediment was sampled with a core (inner ø 6.3 cm for nutrient determination; inner ø 3.6 cm for all other variables), and immediately frozen (–80°C for samples used for pigment analysis, –20°C for the other samples) awaiting analysis. These samples were analysed in the laboratory for granulometry using laser diffraction (Malvern Mastersizer 2000), water content (loss of mass after lyophilisation), total organic matter (loss on ignition at 500°C for 2 h), total organic carbon and nitrogen (Element Analyser N1500, Carlo Erba) and photopigment concentration (HPLC analysis of the supernatant, extracted from the lyophilised sediment by adding 10 ml 90% acetone). The chl a concentration (µg g^{-1} dry sediment) of the upper 3 mm of the sediment was used as a proxy for the microphytobenthos biomass (MPB biomass; Jeffrey *et al.* 1997). Separate cores (inner ø 3.6 cm) containing 15 cm of sediment overlaid with seawater were carefully transported in a cold, dark container to the laboratory and incubated for 1 d in a controlled climate chamber (with approximately the same temperature as in the field) to measure a vertical sediment oxygen profile, using microelectrodes (OX 25, Unisense; n = 1 sample^{-1}). Ammonium pore water concentrations were determined (SANplus segmented flow analyser, SKALAR) and together with the oxygen measurements used to evaluate the oxidation status of the sediments. The sediment erosion threshold was measured in situ with a cohesive strength meter (CSM Mk III) and used as a proxy for sediment stability (Tolhurst *et al.* 1999). According to de Deckere *et al.* (2001), the critical erosion threshold was defined as the pressure at which transmission in the test chamber dropped below 90%. Bed level elevation was determined (n = 3 for each subquadrat) relative to a fixed reference point in the vicinity (i.e. measurement site by the Dutch National Institute for Coastal and Marine Management; RIKZ) and set to the Dutch Ordinance Level (NAP), using a rotating laser and receiving unit on a measuring pole. At the end of the experiment, ~50% of the surface was disturbed in both plot types due to sampling and subsequent scouring around the PVC tubes. However, additional sampling of the surrounding sediment did not indicate significant differences from the control plot sediments. As such, temporal variation of macrobenthos in the experimental plots was not largely affected by the repeated sampling.

Data analyses

Rare species (average maximum < 1 ind. sample^{-1} in both plot types) and typical meio-, hyper- and epibenthic species were not taken into account for further analysis. Species were classified into functional groups according to their mobility, feeding guilds and burrowing activity (tube-building versus biodestabiliser) based on literature (Fauchauld & Jumars 1979, Gerino *et al.* 2003, Volkenborn & Reise 2007) and our own observations (Table 1 in Appendix 3).

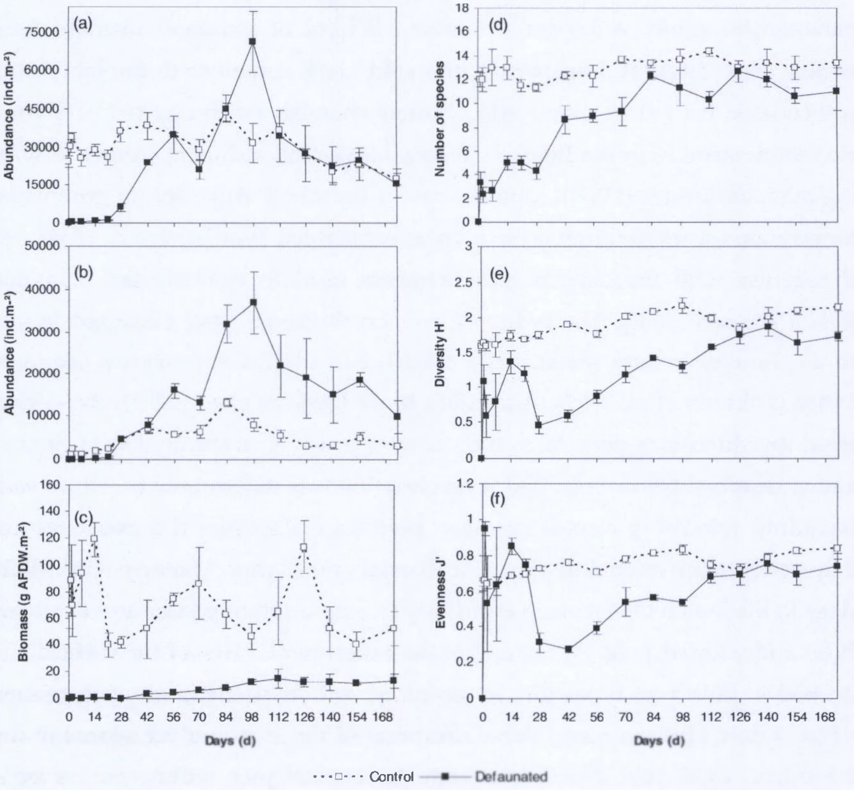


Fig. 1. Temporal variation of (a) total abundance, (b) total juvenile abundance, (c) total biomass, (d) number of species, (e) Shannon-Wiener diversity and (f) Pielou's evenness in defaunated and control plots. Values are means (n = 3) ± 1 SE.

Repeated measures analyses of variance (ANOVA; Green 1993) were conducted, in which Treatment and Time were fixed factors, to test the effect of Treatment (defaunated versus control) and Time (days after the start of the experiment) on community variables (macrobenthic abundance and biomass, juvenile abundance, species richness, Shannon-Wiener diversity index *H'* and Pielou's evenness index *J'*). The same analysis was performed on the abundances of the species with an averaged abundance >12 ind. sample^{-1} over both plot types during the experiment and the environmental variables MPB biomass and NH_4^+ pore water concentration. Prior to analyses, the homogeneity of the variance-covariance structure (i.e. compound symmetry assumption) was analysed using the Mauchley test of sphericity, and Bartlett's and Cochran's tests were used to verify homogeneity of variances. Data not meeting these criteria were appropriately transformed. Replicated samples of variables per plot were pooled to avoid pseudoreplication (Hurlbert 1984). To determine relations in the temporal variation of biotic and environmental variables during succession and to relate temporal variation to the time of recovery, Pearson product-moment correlations were performed after the data were normalised. For variables that did not conform to a normal distribution, a non-parametric Spearman-rank correlation test was used.

To investigate species reassembly and recovery status, dissimilarities between and within species assemblages of control and defaunated plots for each sampling time were assessed by the similarities of percentage procedure (SIMPER, Clarke 1993). Prior to analysis the community abundance data were standardised square-root transformed using the Bray-Curtis index of similarity and visualised by non-metric multi-dimensional scaling (MDS). Different assemblages, characterised by distinct changes in species and functional group dominance, were defined as successional assemblages (SAs), and species indicative of these SAs were determined by calculation of their indicator value (IV) using the IndVal program (Dufrêne & Legendre 1997). Further, relations between community reassembly and the environmental variables were assessed using the BIO-ENV routine (Clarke & Gorley 2001). Prior to the BIO-ENV, environmental variables were appropriately transformed to gain normality and normalised to put them on a common, dimensionless measurement scale. Environmental variables that were not measured for each sampling time (oxygen penetration depth, sediment stability and bed level) were omitted for univariate and multivariate correlative analyses concerning the complete duration of the experiment. Statistical analyses were performed using Statistica 7.0 (Statsoft 1984 to 2004), the Plymouth routines in multivariate ecological research (PRIMER) package, version 5.2.9 (Clarke & Gorley 2001). A significance level of $p < 0.05$ was used in all tests.

Results

Recolonisation and successional stages

Repeated measures ANOVA revealed a significant Treatment × Time effect for the macrobenthic abundance and biomass, species richness, juvenile abundance, H' and J' (Fig. 1, Table 1). Both macrobenthic biomass and species richness were significantly related to the total time of recovery ($r^2 = 0.85$, $p < 0.001$; $r^2 = 0.80$, $p < 0.001$, respectively). However, only species richness returned to control level at the end of the experiment, while biomass remained much lower. Diversity and evenness were high during the first 3 wk of recovery, followed by a significant decrease. Both variables converged linearly towards control values at the end of the experiment ($r^2 = 0.84$, $p < 0.001$; $r^2 = 0.84$, $p < 0.001$, respectively). Both total abundance and juvenile abundance first increased linearly ($r^2 = 0.92$, $p < 0.001$; $r^2 = 0.99$, $p < 0.001$, respectively), achieving a peak abundance overshoot in the defaunated plots at 98 d after defaunation, followed by a linear decrease towards the end of the experiment ($r^2 = 0.89$, $p < 0.001$; $r^2 = 0.89$, $p < 0.001$, respectively). Whereas macrobenthic abundance converged to control values after Day 98, juvenile abundance remained significantly higher than the controls.

Six species were considered sufficiently abundant for statistical analysis: in order of overall abundance, the annelids *Pygospio elegans, Heteromastus filiformis, Aphelochaeta marioni,* tubificid oligochaetes, the bivalve *Macoma balthica* and the annelid *Nereis diversicolor,* which, in total, comprised ~85% of all individuals. A significant Treatment × Time effect was found for the abundances of all of these species (Fig. 2, Table 1).

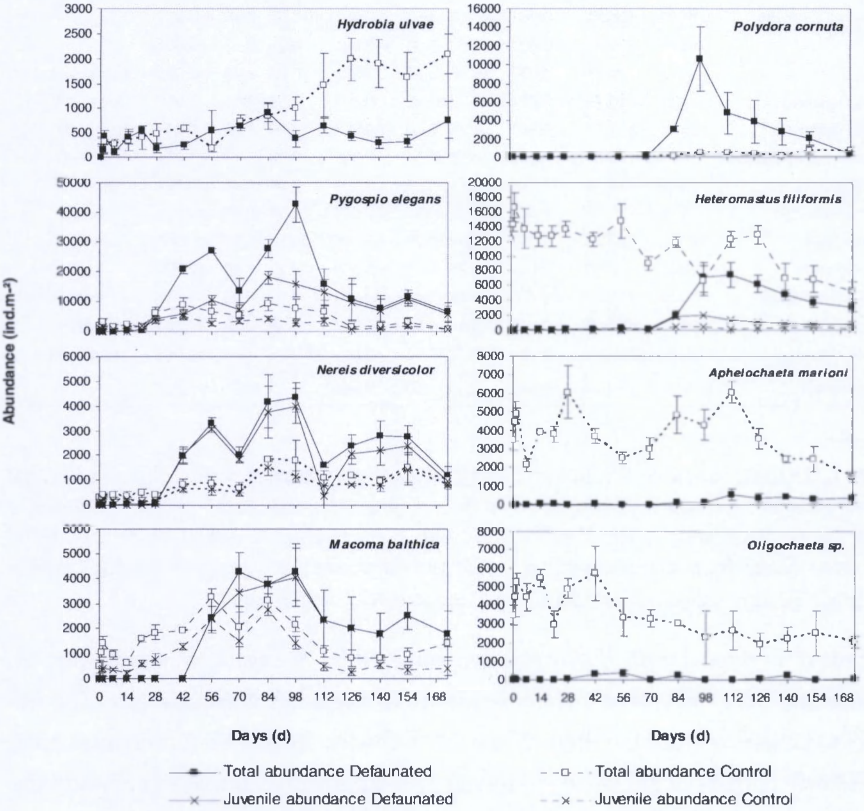


Fig. 2. Hydrobia ulvae, Pygospio elegans, Nereis diversicolor, Macoma balthica, Polydora cornuta, Heteromastus filiformis, Aphelochaeta marioni *and* Oligochaeta. *Temporal variation in mean abundance (n = 3) ± 1 SE in defaunated and control plots.*

	Treatment			Time			Treatment x Time			Transformation
	MS	F	p	MS	F	p	MS	F	p	
Species richness	516.02	136.71	0.001	26.64	12.60	< 0.001	15.97	7.55	< 0.001	-
H'	10.45	500.42	< 0.001	0.57	8.88	< 0.001	0.21	3.33	0.001	-
J'	0.72	20.97	0.020	0.06	3.14	0.001	0.05	2.43	0.009	arcsin-sqrt
Total abundance	13.17	219.70	0.001	1.87	63.79	< 0.001	1.83	62.61	< 0.001	log (x+1)
Total biomass	28.36	514.01	< 0.001	0.26	7.73	< 0.001	0.29	8.77	< 0.001	log (x+1)
Juvenile abundance	3.16	56.03	0.005	4.00	22.51	< 0.001	2.08	11.73	< 0.001	log (x+1)
P. elegans	2.66	11.22	0.044	3.71	16.65	< 0.001	2.64	11.85	< 0.001	log (x+1)
N. diversicolor	12.36	19.27	0.022	20.28	34.14	< 0.001	9.17	15.44	< 0.001	sqrt
M. balthica	107.62	64.07	0.004	18.00	30.39	< 0.001	15.04	25.38	< 0.001	sqrt
H. filiformis	648.52	526.41	< 0.001	9.24	8.24	< 0.001	19.32	17.23	< 0.001	sqrt
Oligochaeta spp.	708.60	435.74	< 0.001	4.37	3.15	0.001	3.42	2.46	0.008	sqrt
A. marioni	656.60	364.92	< 0.001	3.18	1.94	0.039	4.55	2.78	0.003	sqrt
MPB biomass	299.52	10971.50	0.020	0.1870	11.73	< 0.001	0.1216	7.63	< 0.001	arcsin-sqrt
Ammonium	8.86×10^8	241.18	0.041	1.51×10^8	14.09	< 0.001	1.57×10^8	14.71	< 0.001	-

Table 1. *Repeated measures ANOVA results for the general univariate community variables, the 6 most abundant macrobenthic species, MPB biomass and NH_4^+ pore water concentrations (df = 1, 16, 16 for Treatment, Time, Treatment × Time, respectively). Assumptions for compound symmetry (Mauchley test of sphericity; $p > 0.05$) and homogeneity of variances (Bartlett-Cochran test; $p > 0.05$) were met for all variables. –: no transformation performed*

Colonisation started with *Hydrobia ulvae,* followed by *P. elegans, N. diversicolor, M. balthica* and *Polydora cornuta.* The abundance of the latter 4 species exceeded the control values within the first 2 mo of recovery, reaching a maximal total abundance overshoot at Day 98, followed by a significant decrease in abundance. Distinct colonisation of *H. filiformis* and *A. marioni* in the defaunated plots was only noticed from Day 98 onwards. Abundances of these 2 species never exceeded the control values during the experiment. Tubificid oligochaetes hardly colonised the defaunated plots at all. Colonisation of the defaunated plots was largely determined by juvenile recruitment, and, except for the first 3 wk of recovery, juvenile abundance was higher in the defaunated plots than in the controls (Fig. 1).

Multivariate analyses based on species abundances revealed that the species assemblage in the defaunated plots evolved towards the control assemblages during the experiment (Fig. 3), but a dissimilarity of ~27% between the species assemblages in both plot types still remained at the end of the experiment. Based on biomass, the dissimilarity was ~49% at the end of the experiment (Table 2 in Appendix 3). Three successional stages, characterised by clear shifts in proportional abundance of species and functional group dominance were identified: 0 to 21, 28 to 98 and 112 to 175 d after defaunation (Fig. 1 and Table 3 in Appendix 3). During recovery, a shift in functional group dominance from mobile surface deposit feeders (Stage 1) to tube-dwelling surface deposit feeders (Stage 2) to biodestabilising taxa (Stage 3) occurred, while their proportional dominance remained more or less stable in the control plots throughout the experiment (Fig. 1 in Appendix 3). Indicator species for the different species assemblages were *Hydrobia ulvae* (SA I, IV = 53), *Pygospio elegans* (SA II, IV = 39.4), *Macoma balthica* (SA III, IV = 59.4) and *Heteromastus filiformis* (SA III, IV = 45.3). Furthermore, the steep decrease in total macrobenthic abundance, characterising the transition between successional Stages 2 and 3, was numerically determined by the decrease of *P. elegans*, *M. balthica* and *Nereis diversicolor*; their decline accounted for ~88% of the decrease in total abundance.

Relationships between macrofauna and environmental variables

Repeated measures ANOVA revealed a significant Treatment × Time effect for MPB biomass and NH_4^+ concentration. Both variables also best explained the macrobenthic recolonisation pattern (BIO-ENV; $\rho = 0.651$). Adding other variables resulted in a lower correlation (e.g. mud content, water content, MPB biomass and NH_4^+ concentration: $\rho = 0.606$). Ammonium pore water concentrations, which were extremely high during Stage 1, recovered during successional Stage 3, while MPB biomass achieved control values already after 1 wk. MPB biomass further increased exponentially to Day 28, followed by a decrease towards Day 98, where control values were reached again (Fig. 4). During the period of exponential MPB growth, co-occurring with the first successional stage, the first macrobenthic species appeared in the defaunated plots. During the second successional stage, the increase of the later successional species *Heteromastus filiformis* and juvenile abundance were significantly positively related to the abundance of the indicator species *Pygospio elegans* (r =

0.89, p = 0.017; r = 0.87, p = 0.021, respectively). MPB biomass decreased during Stage 2 with the colonisation of surface deposit feeding populations and biodestabilising fauna (r = –0.82, p = 0.042; r = –0.83, p = 0.040, respectively). Furthermore, the sediment was more stable during Stage 2 as compared to Stage 3 (Fig. 5, Montserrat *et al.* 2008). The decrease in sediment stability content during the second and third stages was correlated with the colonisation of *Heteromastus filiformis*, an indicator species for SA III (r = –0.77, p = 0.006) and increase in the biomass of the biodestabilising infauna in general (r = –0.66, p = 0.033). Successional Stage 3 was characterised by more oxygenated sediments, comparable to control values, indicated by deeper oxygen penetration and lower ammonium concentrations in the upper sediment layer (Figs. 4 & 5). The decline in NH_4^+ pore water concentrations during recovery was related to the increase in biomass of biodestabilising macrobenthos (r = –0.91, p < 0.001) and *H. filiformis* in particular (r = –0.97, p < 0.001).

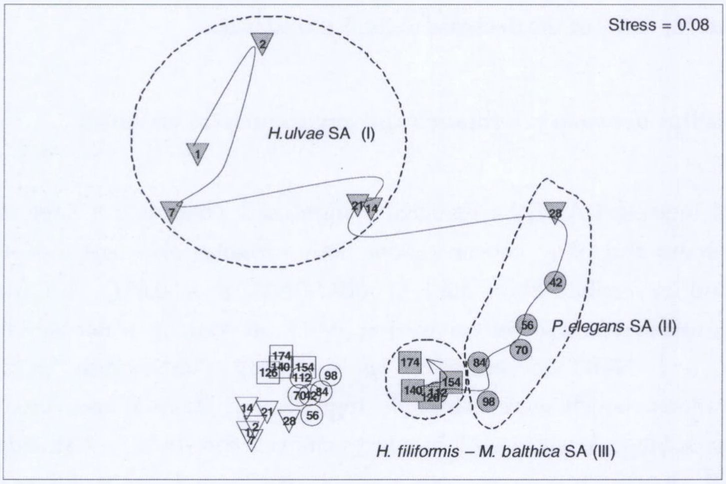


Fig. 3. Multi-dimensional scaling plots of species assemblages (SA) in defaunated and control plots over time based on Bray-Curtis similarities of standardised square-root abundance species data. Shading indicates treatments (grey = defaunated; white = control) and symbols indicate seasonality (triangles = March–April; circles = May–July; squares = August– September). Numbers are days after opening of the plots. The dashed lines indicate the defined successional assemblages.

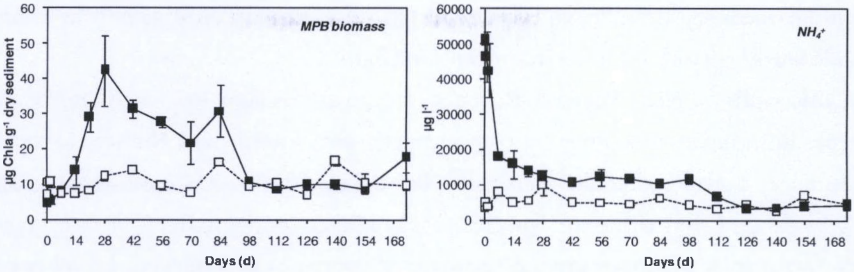


Fig. 4. Temporal variation in (a) microphytobenthos (MPB) biomass (µg chl a g^{-1} dry sediment) and (b) NH$_4$$^+$ pore water concentration. Means (n = 3) ± 1 SE in both defaunated and control plots.

Discussion

Recovery

Our study did not demonstrate complete recovery or 'steady state' of succession, which would have required a high similarity to the control assemblage in terms of species abundance and biomass. Recovery rate is dependent on the scale of disturbance in intertidal habitats (Zajac *et al.* 1998, Norkko *et al.* 2006). Fast adult migration is the predominant recovery mechanism at smaller scales (<1 m^2), while colonisation of large-scale disturbed habitats is initiated and dominated by post-larval and juvenile recruitment (Günther 1992). Due to the scale of disturbance, and the careful avoiding of lateral subsurface movement of adults in the plots, our study therefore focused on recovery mechanisms determined by juvenile recruitment. According to Beukema *et al.* (1999), complete recovery at the community level at larger scales can last several years. Furthermore, any estimate of the recovery time depends on the criteria used. In this study, the total macrobenthic density was restored within 42 d after defaunation, while diversity measurements (species richness, *H'*, *J'*) required a longer period to recover.

Similar recovery times have been reported by Dittmann *et al.* (1999) in a large-scale study performed in an intertidal sandflat.

In this study, a clear Pearson–Rosenberg type succession sequence, including a peak abundance overshoot by opportunists, was found, and the macrobenthos recovery trajectory could generally be divided into 3 successional stages, characterised by different species assemblages and distinct environmental characteristics. Furthermore, no turnover of species was observed, i.e. all species remained in the communities throughout the recorded successional period. Hence, early succession in intertidal mudflats should be interpreted in terms of increases and decreases of species dominance.

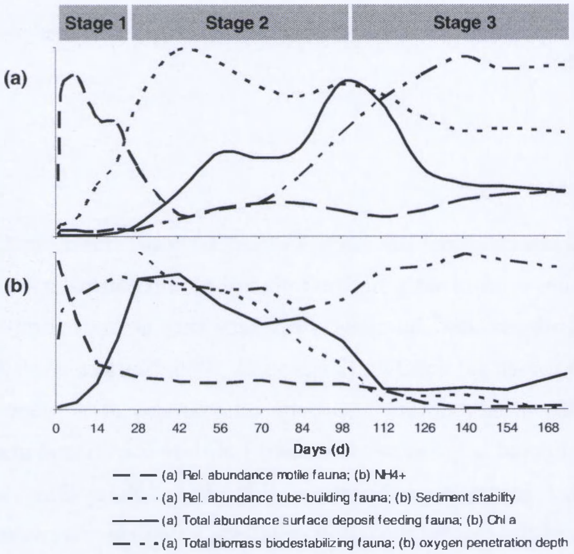


Fig. 5. *(a) Temporal variation of surface deposit feeding population abundance, biomass of biodestabilising populations, relative abundance of tube-building populations and motile infauna populations during succession. (b) Temporal variation of microphytobenthos biomass, sediment stability, oxygen penetration depth and ammonium concentration. Curve fits are running averages (period = 2 sampling occasions) through normalised data. Exact data for the variables presented are given in Fig. 4, Fig. 1 in Appendix 3) and Montserrat et al. (2008).*

Succession stages and transitions

A conceptual scheme presenting the hypothesised interactions during this recovery study is given in Fig. 6. These interactions are based on significant relationships of both environmental and biotic variables and often contemporaneous shifts of these variables between the successional stages. However, we want to point out that relationships do not necessarily imply causality. Therefore, manipulative species interactions are required to investigate the driving processes of succession. The interactions presented in Fig. 6 enable targeted research regarding the driving interactions of macrobenthos succession in tidal mudflats.

Stage 1

Hydrobia ulvae dominated the community during the first stage, but abundance overshoots were not observed. This species is a mobile, grazing mudsnail that can cover large distances by crawling over the sediment and passive 'rafting' during flooding (Haubois *et al.* 2002). Consequently, this species may be considered a good coloniser. Colonisation by *H. ulvae* was determined by adult immigration. In general, juvenile recruitment did not occur until Day 28 in the defaunated plots, although some juvenile recruitment occurred in the controls during the same period. The more anoxic situation during the first 4 wk of recovery likely inhibited juvenile recruitment of later successional species and opportunistic behaviour of *H. ulvae*. Especially, ammonium pore water concentrations in the upper sediment layer were high during the first week of recovery compared to the control values. This high level of reduced nitrogen resulted from the defaunation technique, in which bio-irrigation of the bottom was inhibited. Decreased juvenile macrobenthic recruitment and settling success have been found in anoxic and suboxic sediments (Marinelli & Woodin 2002, Engstrom & Marinelli 2005). Further, successional Stage 1 was determined by the exponential development of a microphytobenthic mat. This is in accordance with Daborn *et al.* (1993) and is suggested to be the result of the low abundance of grazers and the high pore water nutrient concentrations during Stage 1 compared to the control plots. Furthermore, *H. ulvae* abundance and biomass were not significantly related to abiotic or biotic variables during the first stage, suggesting that this early coloniser, which is present in relatively low numbers, has no clear effect on subsequent colonisers or changing environmental conditions. Therefore, the transition between successional Stages 1 and 2 is presumably related to the changes in oxygen characteristics of the sediment and peak recruitment of *Pygospio elegans*, an indicator species for SA II.

Stage 2 and 3

Successional Stage 2 was characterised by the dense microphytobenthic mat and by abundant immigration of juveniles of several macrobenthic species. Enhanced juvenile recruitment has often been noted in marine biofilms and is hypothesised to be the result of both inductive species-specific responses to the bacterial composition of the biofilm (reviewed by Rodriguez *et al.* 1993) and a lower post-settlement mortality due to food limitation (Ólafsson *et al.* 1994, Gosselin & Qian 1997). The surface deposit feeding species *Pygospio elegans, Macoma balthica, Polydora cornuta* and the omnivore *Nereis diversicolor* showed an opportunistic response, i.e. abundance overshoot followed by a steep decline and faster growth (Chapter 5) compared to the ambient sediments. The higher recruitment success of juveniles in the defaunated plots compared to the controls was related to the higher abundance of tube-building polychaetes (*P. elegans* and *P. cornuta). P. elegans* and *P. cornuta* are small, sedentary, tube-building polychaetes with a wide habitat tolerance, a variety of feeding mechanisms and a remarkable diversity of reproductive strategies (Anger *et al.* 1986, Zajac 1991, Bolam & Fernandes 2002). Therefore, these species are capable of rapidly colonising disturbed areas and using new resources. Both species reached high abundances (i.e. 5.6× and 19.5× control values on Day 98, respectively, for *P. elegans* and *P. cornuta*) during Stage 2. Adverse effects of the dense tube aggregations on juvenile settling, either indirectly or through predation, are possible (Cummings *et al.* 1996) but are presumably covered up by their facilitative effects in the defaunated plots. Polychaete tubes exert profound effects on near-bed flow, which above a certain threshold abundance leads to sediment stabilisation where passive deposition of larvae or juveniles is enhanced (Eckman 1983, Friedrichs *et* *al.* 2000). The sediment stability data confirm the higher stability of the sediments in the defaunated plots during the period of high *P. elegans* abundance (i.e. successional Stage 2; Fig. 5, F. Montserrat *et al.* 2008). Furthermore, polychaete tubes have been suggested to provide a refuge from disturbance and predation to larvae and juveniles (Gallagher *et al.* 1983). In this study, the control sediments experienced a net erosion of 3.4 cm during successional Stage 2 while sediment bed level elevation remained stable in the defaunated plots. This was related to the abundance of *P. elegans* and their enhanced effect of mud particle retainment (Montserrat *et al.* 2008). Moreover, dense aggregations of polychaete tubes have been found to increase food availability indirectly, which is hypothesised to result from biogeochemical bio-irrigation impacts of the tubes (Brey 1991, Bolam & Fernandes 2002 and references therein).

Inhibitory adult-juvenile interactions structure juvenile recruitment in marine soft-bottom habitats. For instance, large biodestabilising macrofauna adversely effect juvenile recruitment success directly (e.g. by predation; Hiddink *et al.* 2002) and/or indirectly (e.g. by destabilisation of the sediment, inhibition of microphytobenthos development, competition for space; Ólafsson 1989, Ólafsson *et al.* 1994, Flach 2003). Therefore, enhanced juvenile recruitment to the defaunated plots is presumably also related to the lower biomass of destabilising infauna in the defaunated plots. One species, the common cockle *Cerastoderma edule,* largely contributed to the average difference in biomass between the defaunated and control species assemblage throughout the experiment (Table 3 in Appendix 3). Flach (1996) showed a severe negative effect of *C. edule* densities on juvenile recruitment, thereby largely influencing the macrobenthic community in tidal flats in the Wadden Sea. The absence of large *C. edule* in the defaunated plots in this study is in accordance with the study of Beukema *et al.* (1999). The time to complete restoration of ambient age distributions of *C. edule,* and thus biomass, may be expected to be as long as the life-span of the cockle. In summary, the enhanced macrobenthic recruitment of later successional species (i.e. *Nereis diversicolor, Polydora ligni, Macoma balthica*) in the defaunated plots during Stage 2 is presumably related to (1) the increased passive larval settling, lower post-settlement mortality and dispersal due to the created favourable conditions within the dense *Pygospio elegans* patches and (2) the low biomass of large biodestabilising organisms (i.e. *C. edule*).

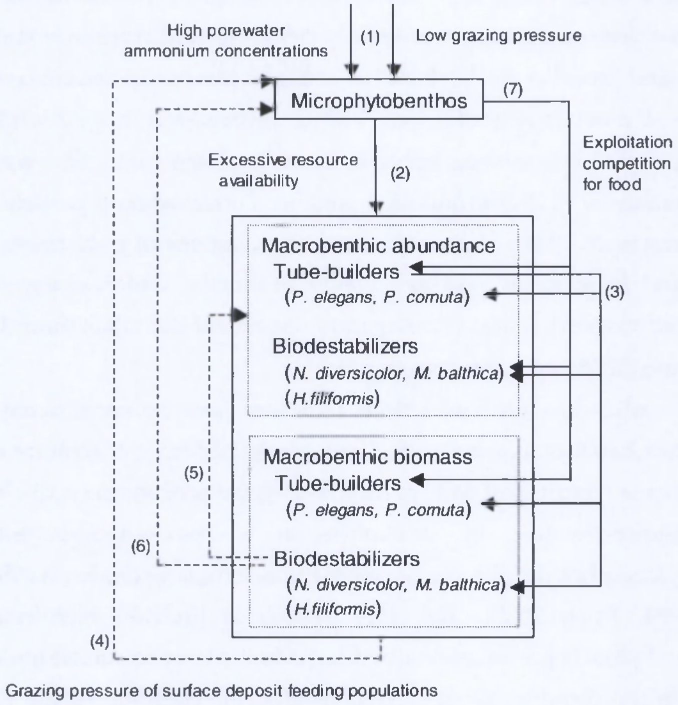


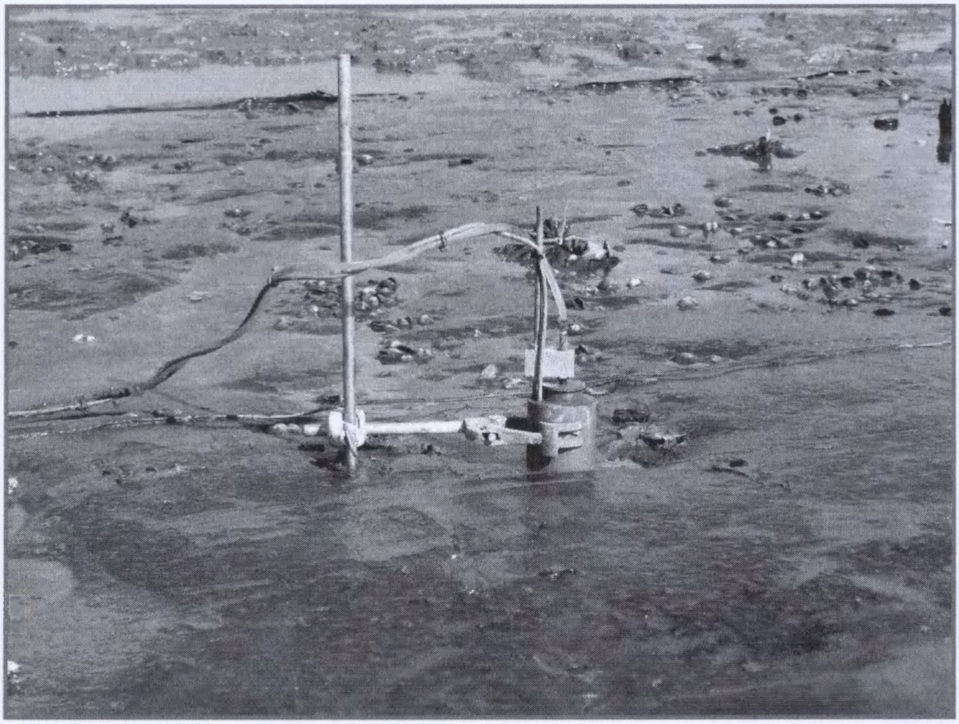
Fig. 6. *Schematic representation of the hypothesised facilitative (solid lines) and inhibitory (dashed lines) interactions during this succession study. Following defaunation resulting from hypoxia, the low grazing pressure of surface deposit feeding populations and the high pore water ammonium concentrations enhance microphytobenthic development at early successional stages (1; excessive resource availability), which, in turn, facilitates the recruitment of juvenile tubebuilding and biodestabilising macrofauna during successional Stage 2 (2). Tube-builders enhance macrobenthic recruitment success by protecting the sediment from erosion due to the production of skimming flow (3). This results in an enhanced grazing pressure of surface deposit feeders, consuming the microphytobenthos (4). At later succession stages, biomass of biodestabilising fauna has increased, enhancing their bioturbation impact, which, in turn, counteracts the favourable conditions provided by the tube-builders (5) and microphytobenthic development (6). Exploitation competition for food results in the demise of surface deposit feeding population abundances and the biomass of tube-builders (7). Key species governing the interactions are presented in parentheses (see Fig. 2 for full species names).*

The transition between successional Stages 2 and 3 coincided with (1) the decline in resource availability, i.e. MPB biomass, immediately followed by the decrease in surface deposit feeder abundance and (2) the take-over in biomass dominance of destabilising omnivores/scavengers (i.e. *Nereis diversicolor*), surface deposit feeders (i.e. *Macoma balthica*) and subsurface deposit feeders (*Heteromastus filiformis*). The decrease in MPB biomass was related to the increase of surface deposit feeders, suggesting that the take-over is regulated by direct exploitation competition for food. This trophic group achieved a maximal abundance overshoot of 58481 ind. m^{-2} on Day 98 (i.e. 6.5× control values) and thereby possibly reached the ecological carrying capacity for its populations. However, indirect effects on resource availability caused by *H. filiformis*, an indicator species for SA III, cannot be excluded. *H. filiformis* is a subsurface deposit conveyer belt feeding polychaete that produces very resistant faecal pellets on the sediment surface (Cadée 1979, authors' pers. obs.). At high densities these pellets may decrease primary productivity by covering the sediment surface, resulting in a lowered MPB biomass. Furthermore, the contemporaneous decrease in sediment stability along with the increase in biomass of biodestabilising species suggest that the colonisation of *H. filiformis*, together with the growth of *N. diverscicolor* and *M. balthica*, counteracted the favourable, stable conditions provided by the tube-dwelling infauna during successional Stage 2. These results are consistent with Bolam & Fernandes (2002), who found that the demise of dense *Pygospio elegans* patches coincided with a dramatic increase in the abundance of 2 biodestabilising bivalve species (*M. balthica* and *Cerastoderma edule*). Taken together, we suggest that the transition in species assemblages between successional Stages 2 and 3 is triggered both by exploitation competition for food and the bioengineering impact on the sediment characteristics of *H. filiformis*, and by biodestabilising fauna in general, during successional Stage 3.

In general, our study suggests that macrobenthic reassembly after hypoxia is related to different types of interactions. Macrobenthic successional dynamics in a defaunated tidal mudflat habitat should be considered as a dynamic process, related to resource availability, natural temporal variation, life history traits (e.g. opportunistic behaviour) and bio-engineering capacities of the colonising species.

Acknowledgements

This research was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen), the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministery of Economic Affairs. We thank D. Van Gansbeke, D. Schram, M. Houtekamer, C. Kleppe, H. Francke and Y. van der Maas for their assistance during the processing of the samples, as well as B. Beuselinck, D. Peelaers, J. van Soelen and B. Koutstaal for their help during sampling. M. Vos and 3 anonymous reviewers provided valuable comments and advice on the manuscript. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446).



-148080

CHAPTER 4

BENTHIC COMMUNITY-MEDIATED SEDIMENT DYNAMICS

Adapted from:

Montserrat F., Van Colen C., Degraer S., Ysebaert T., Herman P.M.J. (2008)
Benthic community-mediated sediment dynamics
Marine Ecology Progress Series 372: 43-59

Abstract

We assessed the influence of benthic communities on sediment properties in large defaunation experiments in replicated 16 m^2 plots on a tidal flat in the Westerschelde estuary (SW Netherlands). We compared microphytobenthos and benthic macrofauna recovery and recolonisation between control and defaunated sediments during 8 mo following the macrobenthic defaunation, focussing on how the temporal scale of biological responses interact with the temporal scale of sedimentological developments (grain size, bed level, erosion threshold). In the first month, microphytobenthos (surface chl *a* content) increased to >3 times the control values and remained elevated until 3 mo after the start of the experiment. Macrofaunal recovery started with mobile mudsnails after only a few days. Tube-building macrofauna dominated first, followed by surface-disrupting species. Both groups became much more dominant in defaunated than in control plots. Surface pelletisers almost recovered to control levels after 4 mo, while biodiffusing bivalves did not recover during the course of the experiment. Mud content of the sediment surface first increased with chl a, but started to decrease, concomitant with an over-representation of surface disruptors. A similar trend was observed for critical erosion threshold. Bed elevation of experimental plots exceeded controls by several cm after 1 mo, and remained higher through summer. The time scales of changes in microphytobenthos and in abiotic characteristics of the sediment were largely set by the time scale of macrofauna recovery. Macrobenthos plays a critical, but complex role in the dynamics of intertidal sediments.

Keywords: *Intertidal, Sediment, Erosion, Deposition, Bio-physical interactions, Macrobenthos, Microphytobenthos, Grain size*

Introduction

Biogenically mediated sediment (in)stability in intertidal cohesive sediments of estuaries and coastal areas has been well studied over the past decades (Rhoads 1967, Daborn *et al.* 1993, Underwood & Paterson 1993, Widdows *et al.* 2004). It has become increasingly clear that biotic components of the ecosystem can exert significant influence on the erosion and deposition processes in sediments (Woodin & Jackson 1979). For example, Rhoads & Young (1970) demonstrated that the presence of bioturbating, deposit-feeding polychaetes in a US East Coast tidal embayment altered sediment variables in the top layer. Widdows *et al.* (2000) found no significant relations between erosion and physical sediment variables (e.g. mud content, porosity), whereas those between erosion and biological variables (e.g. chl *a* and colloidal carbon concentrations, and macrobenthic density and biomass) were highly significant.

Research on sediment-biota relationships in intertidal soft sediments recognises 2 counteracting forces: (1) the sediment cohesion-inducing and sediment surface-armouring effect of bacteria (e.g. cyanobacteria) and benthic microalgae (microphytobenthos; MPB), such as epipelic diatom mats (de Boer 1981, Krumbein *et al.* 1994, de Brouwer *et al.* 2000) and (2) the sediment cohesion-disrupting, destabilising or bioturbatory effects of benthic macrofauna (Rhoads & Young 1970, Grant & Daborn 1994, de Deckere *et al.* 2001, Orvain *et al.* 2006). The main effect of microphytobenthic aggregates is on the sediment surface strength, which results in an increase of the critical erosion threshold (τ_{crit}). Within the aggregates the cells and sediment particles alike are bound together by extracellular polymeric substances (EPS; Hoagland *et al.* 1993, de Brouwer *et al.* 2003a) and promote cohesiveness and resistance. This was demonstrated in the classical field experiment of de Boer (1981) where MPB poisoning was shown to increase erodability of the sediment.

The influences of benthic macrofauna on erodability of fine-grained and muddy sediments seem ambiguous at first glance as both stabilisation and destabilisation can be expected (Woodin & Jackson 1979, Jumars & Nowell 1984a, Snelgrove & Butman 1994). Sediment characteristics can be altered in various ways, depending on the feeding mode, locomotory behaviour and sediment processing by the organism in question. Also, certain effects may be density-dependent (Eckman *et al.* 1981, Bolam & Fernandes 2002, Ciutat *et al.* 2007). Reef-forming epifauna (e.g. oysters, mussels) protect the sediment from erosion by shielding it from the current and by modifying the turbulent benthic boundary layer responsible for erosion (van Duren *et al.* 2006). The suspension feeding behaviour of these organisms can have profound effects on the immediate environment. They reduce turbidity through clearance of the water column, which may promote MPB primary production. The excretion of faeces and/or pseudo-faeces is thought to have an enhancing effect on the cohesiveness of the surrounding sediment and to facilitate dispersal, deposition and even growth of resuspended MPB (Barille & Cognie 2000, Widdows *et al.* 2000). Motile infauna (e.g. bivalves, crustaceans, gastropods, polychaetes) are generally considered to have a destabilising effect on the sediment surface, either directly or through grazing on MPB aggregates. (Blanchard *et al.* 2000, de Deckere *et al.* 2001, Andersen *et al.* 2002, Orvain *et al.* 2004). In some cases, both stabilisation as well as destabilisation of the sediment by the same species has been observed. Probably the macrobenthic soft sediment species with the most diverse effects is *Nereis* (= *Hediste*) *diversicolor*, which displays a whole array of modes of locomotion and foraging, thereby processing the sediment in very different manners and exerting equally various influences on it, both stabilising and destabilising (Gerino & Stora 1991, Banta *et al.* 1999, Palomo & Iribarne 2000).

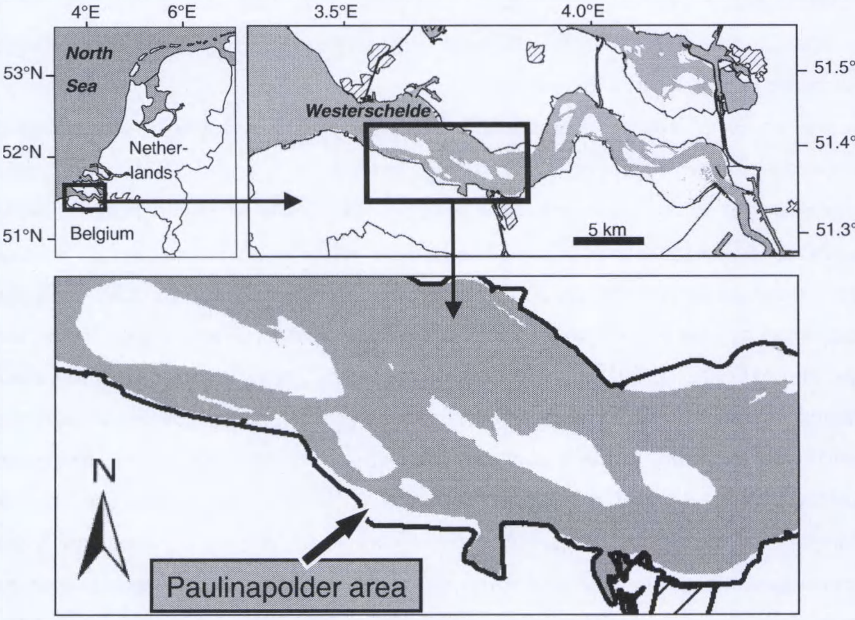


Fig. 1. Location of the intertidal area used in this study. Plots were located on the south shore, within the polyhaline part of the Westerschelde estuary.

Estuarine sediments consist of mixtures of sand and mud (van Ledden *et al.* 2004, Winterwerp & van Kesteren 2004). Mud, in turn, is composed of silt and clay and determines the cohesiveness of the sediment. Relatively small changes in composition of the sediment can cause the sediment matrix to change from a granular, sand-dominated matrix into one where the sand grains loose contact with each other and are captured in a mud-dominated matrix. The behaviour can then change abruptly from non-cohesive to cohesive or vice-versa (Winterwerp & van Kesteren 2004), changing the erosion threshold and erosive behaviour and its dynamics. Transport models suggest that changes in erosion/deposition parameters typically caused by benthic organisms, are sufficiently large to lead to significant effects at a larger spatial scale (Paarlberg *et al.* 2005), but these predictions are in need of direct experimental testing.

Ecosystem engineering, as coined by Jones *et al.* (1994), is a prime organism–environment interaction in intertidal areas (van de Koppel *et al.* 2001, 2005, Bouma *et al.* 2005, van Wesenbeeck *et al.* 2007) in which organisms influence each other via autogenic or allogenic modifications of their mutual habitat on different spatial and temporal scales (Woodin 1978, Woodin & Jackson 1979). The effects a benthic macrofaunal community can exert on its abiotic soft sediment environment are hard to distinguish, because of various naturally confounding factors. Each group of organisms uses specific traits to secure and maintain its niche within the system. Unravelling the reciprocal relationships between the dynamics of coastal sediments and their inhabitants, per species or functional group in the field, will shed light on the separate effects of each of these groups.

In this paper we present a concise set of observations on the interactions between biotic (i.e. MPB and benthic macrofauna) and abiotic (i.e. grain size distribution, bed level, erosion threshold) compartments of estuarine soft sediments obtained using a defaunation experiment in the field. Defaunation is a powerful and versatile *in situ* method for study of benthic macrofauna and its influences. It can be achieved by applying phylum-selective poisons (Underwood & Paterson 1993, de Deckere *et al.* 2001), or by inducing severe hypoxia (Beukema *et al.* 1999, Norkko *et al.* 2006).

Field experiments offer a number of advantages compared to laboratory experiments or (unmanipulated) field observations. Unmanipulated field observations are always to a certain degree cross-system comparisons, where interference of sediment type, elevation, tidal and wave exposure etc. may influence biotic–abiotic relationships. Experiments with a single species or with artificial species assemblages have limited applicability to the real world. Field experiments manipulating initial conditions offer the advantage that different stages of a community can be studied while it develops according to natural regulation. Covariation of different factors reflects this natural development. The disadvantage of the approach is that results may be scale-dependent (Norkko *et al.* 2006) and that analysis is complicated during the course of community recovery.

Here, we aim to address 3 issues: (1) by defaunating the experimental sediment, we were able to compare the sedimentological properties of naturally inhabited and azoic sediment during approximately the first month of the experiment, thus deriving an estimate of the influence of the total benthic community; (2) a high frequency sampling of control and defaunated sediments during the 8 mo following the defaunation further enabled us to follow the macrofauna recolonisation patterns and the corresponding developments in sedimentological properties; (3) the defaunation treatment provided us with information on how the temporal scale of macrofaunal response (i.e. recolonisation) is reflected in the temporal scale of sedimentological developments.

Materials & methods

Site, defaunation treatment and sampling

The site chosen for the experiment was Paulinapolder, a homogeneous intertidal flat, both in terms of macrofauna composition and sedimentology, situated on the southern shore of the Westerschelde estuary at 51°21′ 24″N, 3° 42′ 51″ E (Fig. 1). It is bordered by a sea defence (i.e.dike) at its back, running roughly from ENE to WSW. The prevailing winds are southwesterly and the experimental plots were shielded from them by the dike. Also, the site was largely protected from the forces of major flood currents and sea waves from the west by a breakwater barrier located ca. 50 m west of the plots. For further specifications of the estuary and/or the site see Herman *et al.* (2001), Widdows *et al.* (2004) and Ysebaert & Herman (2002).

For the experiment, 16 m^2 square plots were laid out at random, at least 5 m, but no more than 35 m, apart. We used 3 plots as controls and 3 were defaunated (i.e. treatment), by digging a 30 cm deep, 20 cm wide trench around each of them. The treated plots were covered and the trenches lined using waterproof 0.1 mm thick polyethylene (PE), and left to incubate. However, during the incubation, we observed small holes in the PE sheeting. We inserted an extra sheet (tarpaulin, 140 g m^{-2}) in the same manner as the first to cover the treated plots. Prior to the experiment, we tested how long the period of defaunation had to last to reach azoic conditions. After 1 mo almost all macrofauna were found dead, except for some *Heteromastus filiformis* individuals. Other polychaete worms were found dead at the sediment surface. At this time, dead bivalves already appeared as empty shell doublets. A 40 d period of defaunation resulted in an azoic sediment, without even the most stress-tolerant species such as *H. filiformis*. The dead polychaetes observed after 1 mo of defaunation were absent after 40 d, indicating decomposition of all soft organic matter within the plots. A time period of 40 d was thus adopted as the experimental defaunation time. At the start of the experiment, the sheets covering the plots were cut loose at the sediment surface and removed. The 30 cm deep lining of the trenches along the perimeter of the plots was left in place to prevent horizontal subsurface migration of infauna. By preventing this migration and relying solely on larval settlement, one is able to make general statements about larger areas affected by hypoxia and mass mortality of the macrofaunal community (i.e. upscaling). Whenever the lining emerged at the sediment surface, it was reinserted to minimise surface effects. To allow for a full recruitment and recolonisation potential of the experimental plots, the experiment was initiated (plots opened) on the 30 March 2005, which is prior to the recruitment peak of all important macrobenthic species in the area (Ysebaert 2000). After the plots were opened on Day 0, they were sampled consecutively at Days 0, 1, 2, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 175, 187, 203, 233 and Day 264 (19 December 2005), albeit not all variables could be sampled on all sampling days (see Table 1).

The outer rim of each plot (0.5 m wide) was not sampled, to minimise edge effects. The remaining inner area of 9 m2 was divided into 64 subquadrats of 37.5 × 37.5 cm. On each sampling day, 2 replicate subquadrats, never located next to each other, were randomly chosen beforehand. Depending on the parameter, 1 (macrofauna abundance and biomass, mud content and erosion threshold) or 2 (surface chl *a*, sediment water content and sediment bed level) subquadrats were then sampled in every plot. A portable bridge was used to sample in the centre of the plots so as not to disturb the sediment. All sampling was done from about 2 h before till 2 h after low tide. It is highly unlikely that time of sampling within the tidal cycle affected sediment parameters (e.g. water content), since the hydraulic conductivity of this muddy sediment was very low and the sediment remained fully water-saturated during the entire ebb period.

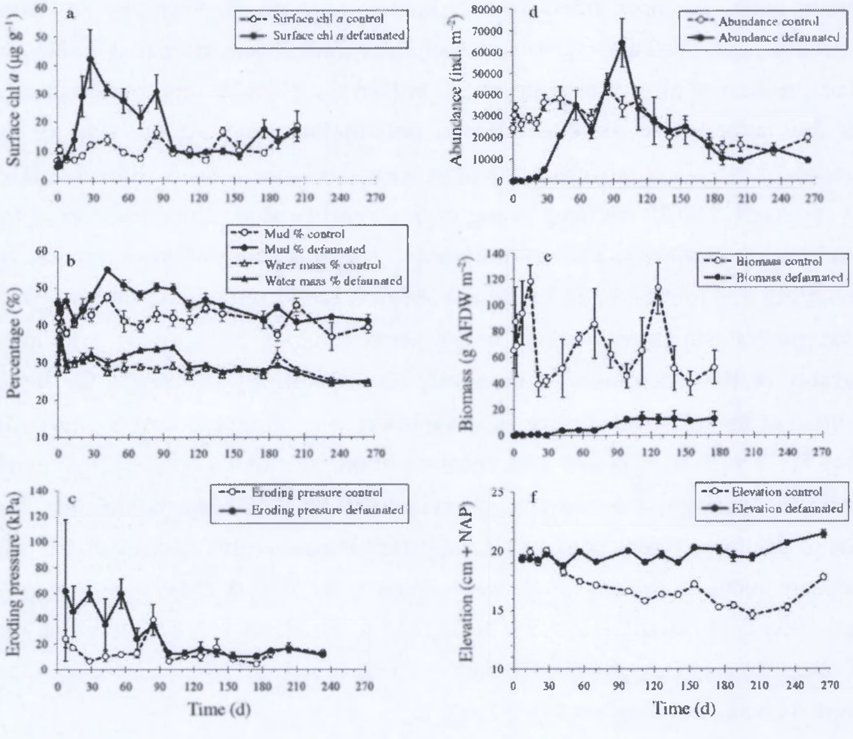


Fig. 2. Mean ± SEM temporal distribution of biotic and abiotic parameters. (a) Chl a concentration in the top 3 mm of the sediment, (b) mud percentages (circles) and the water mass percentages (triangles), (c) eroding pressure needed to make the transmission of light in the cohesive strength meter (CSM) sense head drop below 90%, (d) the total abundance (number of ind. per m^2), (e) the total biomass in grams ash-free dry weight (g AFDW) m^{-2} and (f) average bed elevation of plots normalised to the Dutch Ordnance Level (Normaal Amsterdams Peil, NAP). Note that in panels (b) and (f) the y-axis does not start at 0.

Macrofauna

The benthic macrofauna was sampled to a depth of 40 cm using a 125 mm inner diameter stainless steel corer. Samples were fixed with a buffered 8% formalin solution and taken to the lab in closed containers. The samples were sieved over a 0.5 mm mesh sieve and preserved using a neutralised 4% formalin solution with 0.01% Rose Bengal until they were processed. All macrofauna were sorted, counted and identified to the species level, except for nematodes and tubificid oligochaetes. Macrofaunal biomass was determined according to the methods described in Sistermans *et al.* (2005). These methods are ISO certified NEN 2000-9001. For further detailed information about the analysis of macrofauna in this experiment see Van Colen *et al.* (2008).

We assessed the bioturbatory actions of benthic macrofauna, rather than using a taxonomical classification, to study the effects on sedimentary properties. Gerino *et al.* (2003) used geochemical criteria for their classification. Here, we categorised the macrobenthos in relation to the erodability of the sediment surface layer, i.e. according to the most direct geomechanical effect for that group *sensu* Woodin & Jackson (1979). In this way we assessed the net effect that organisms have on the 'skin' of the sediment. Epi- or endofauna can have a net effect on the sediment surface by (1) diffusing a layer of surface sediment of a given depth, for example *Cerastoderma edule* (Flach 1996, Gerino *et al.* 2003), (2) leaving the sediment in a modified form with respect to grain size or critical shear stress after pelletisation of the surface layer, for example *Heteromastus filiformis* or *Hydrobia ulvae*, respectively (Cadee 1979, Orvain *et al.* 2004), (3) disrupting the surface by scraping it due to deposit feeding or grazing by for example *Corophium volutator* (de Deckere *et al.* 2001) and (4) modification of the benthic boundary layer and the shear exerted on the surface through tube building by, for example, *Pygospio elegans* (Bolam & Fernandes 2003). We, therefore, distinguished between these 4 major sediment surface effects: biodiffusors (BD), pelletisers (PE), surface disruptors (SD) and tube builders (TB). To illustrate the representation of certain geomechanical effects in both the defaunated and control sediment, we used indices of both biomass and abundance of the grouped organisms realising these effects. The index is calculated as follows: *Ti = (Di – Ci)/(Di + Ci)*, with *Ti* being the treatment index at Day *i* where *Di* and *Ci* are the parameter values (abundance or biomass) for an organism group in the defaunated and the control sediment, respectively. When the index is zero, the group is represented in equal densities or biomass in both treatments. When a particular group is overrepresented in the defaunated sediment, the index will approach 1. Conversely, when a group is overrepresented in the control sediment, the index will tend towards –1. In the control treatment, all groups were always present in substantial abundance and biomass, so that spuriously high values of the ratio statistic due to very low values of the denominator did not occur.

Photopigments, water content and grain size

Sediment material for quantification of photopigments, water content and grain size distribution was extracted from the experimental plots using perspex cores with an inner diameter of 36 mm. To reduce compaction of the sediment during extraction, the plunger of a 100 ml syringe was drawn up the core as it was pushed into the sediment. The cores were placed on dry ice (approx. –70°C) immediately after extraction to arrest enzymatic reactions and/or reworking by present infauna. Cores intended for photopigment analysis were kept in the dark in a closed PVC sleeve. Upon arrival in the lab, the photopigment cores were kept in the dark and stored at –80°C. The cores for sediment analysis were stored at –20°C until processing.

The cores were sliced at depths of 3, 10, 20, 30 and 50 mm. For photopigment analyses, only the top slice of 3 mm was used, based on the assumption that all living and active MPB is captured within this 3 mm layer (Kromkamp & Forster 2003).

Sediment was weighed wet, lyophilised and weighed again dry to yield water mass percentage or absolute water content (Flemming & Delafontaine 2000) for the 0 to 3, 3 to 10 and 10 to 20 mm depth slices pooled together. Sediment granulometric analysis was carried out on the upper 5 slices. Sediment grain size distribution and especially the percentage of the sediment fraction <63 μm (i.e. mud = silt + clay; Flemming & Delafontaine 2000, Winterwerp & van Kesteren 2004) was determined with a Malvern Mastersizer 2000, using laser diffraction.

Sediment for pigment analysis was lyophilised in the dark prior to analysis. Photopigments were extracted from the freeze-dried sediment by adding 10 ml 90% acetone, placing it in a ball mill for 20 s and centrifuging for 5 min at 1500 rpm (ca. 200 × *g*). The supernatant was analysed by HPLC following Jeffrey *et al.* (1997).

Bed level and sediment strength

The bed level of the plots was measured relative to fixed and known points in the vicinity, measuring sites of the Dutch National Institute for Coastal and Marine Management (RIKZ). Bed level measurements were performed using a rotating laser mounted on a tripod and a receiving unit on a measuring pole. Bed level values are expressed relative to NAP (Dutch Ordnance Level, similar to Mean Sea Level). Three replicate measurements were haphazardly taken per sampled subquadrat.

The sediment strength, or erosion threshold is taken as a measure of erodability of the sediment under a certain bottom shear, and was measured using a cohesive strength meter (CSM) (Tolhurst *et al.* 1999, Defew *et al.* 2002). The CSM uses a jet of water emitted perpendicularly to the sediment surface, disrupting the sediment matrix at the sediment–water interface. A series of tests with increasing pressure of the water jet is performed, according to the 'Mud 6' programme of the CSM Mk III (Sediment Services). The point of incipient erosion was determined as the pressure at which light transmission in the measuring cell decreased below 90%. Care should be taken not to confuse the reported pressure values, expressed in kPa, with bottom shear stress. Tolhurst *et al.* (1999) provide a conversion equation between eroding pressure measured by the CSM and horizontal shear stress.

Experimental design and statistical procedures

The results were analysed according to a complete randomised (CR) experimental design. The basic statistical design included Time and Treatment as separate categorical and fixed factors, and the Time × Treatment interaction factor. Due to logistical reasons, we were only able to sample macrofaunal abundance, macrofaunal biomass, percentage mud, absolute water content and surface erosion threshold once per plot. Variables that were sampled in duplicate within plots were surface chl *a* contents and sediment bed elevation. Depending on the response variable, there were additional levels of variance, namely Plot nested in Treatment and Time × Plot nested in Treatment (surface chl *a)* or Subquadrat nested in Plot nested in Treatment and Time × Subquadrat nested in Plot nested in Treatment (sediment bed elevation) where both Plot and/or Subquadrat were used as random categorical predictors. We were justified in using a classical ANOVA design analysis, even for the factor Time, because errors of individual plots (i.e. deviations of the Treatment–Time mean) were not significantly autocorrelated in time. Moreover, as several of our variables are known to be highly dependent on other factors (e.g. water content depends on mud content), we could introduce these variables as co-factors in the analysis. Using a repeated measurements (RM) design, which would have been the more conservative approach for this analysis, it would have been impossible to include these co-factors. For the sake of consistency, we used CR designs throughout. However, for the analyses where comparison with a RM was possible, we confirmed that there were no differences in model results or interpretation. Normal distribution of the data was ensured by visual inspection of Q-Q plots. Levene's test was used to check for homoscedasticity (homogeneity of variances; Underwood 1997). In cases where the data were heteroscedastic, transformation (either natural logarithm or arcsine square root) was conducted. The data shown in the graphs are untransformed data. Whenever significant effects became apparent, Student-Newman-Keuls (SNK) post hoc testing (Underwood 1997) was used to investigate how these differences manifested themselves. All univariate analyses were conducted using the STATISTICA data analysis software system, v7.1.

To assess single main effects and interaction effects of the biotic compartments of the system on its erodability, we regressed erosion threshold against chl *a* concentration, total macrofaunal abundance and total macrofaunal biomass (all variables natural-log transformed). In all cases we departed from a model which contained all explanatory variables and their interactions (i.e. overparameterised) and used a step-wise elimination of parameters, based on Akaike's Information Criterion (AIC; Crawley 2005), to reach a minimal adequate model (MAM). All multiple regressions were conducted in the open-source statistical environment R (R Development Core Team 2007, R Foundation for Statistical Computing, www.r-project.org).

Results

Biotic developments

At the onset of the experiment (removal of the PE sheeting; Day 0 = 30 March, 2005) the sediment in the defaunated plots was completely black and anoxic. Replicating Beukema *et al.* (1999), high numbers of empty shell doublets were observed on the sediment surface.

The most conspicuous development within the first month of the experiment was the growth of MPB (Fig. 2a). The control sediment was characterised by fairly constant surface chl *a* levels, varying between 6.72 ± 0.89 $\mu g\ g^{-1}$ dry sediment (mean ± SEM) at Day 0, 10.70 ± 1.61 $\mu g\ g^{-1}$ at Day 1 and 7.05 ± 0.78 $\mu g\ g^{-1}$ at Day 14. The MPB growth pattern in the defaunated sediment was quite different from that in the control sediment. Values started (Day 0) at 4.66 ± 0.71 $\mu g\ g^{-1}$ and increased exponentially to 45.78 ± 11.04 $\mu g\ g^{-1}$ at Day 28. After this initial exponential growth, chl *a* values decreased, but remained 2 to 3 times higher than control values until Day 84. After ca. 3 mo, at Day 98, chl *a* values in the defaunated sediment dropped drastically and then remained constant and low (around 10 μg g^{-1} sediment), similar to the control chl *a* levels. The factors Time, Treatment and Time × Treatment interaction had highly significant effects (Table 1). SNK post hoc tests revealed that the significant differences were for the period Day 21 to Day 84. Variances were a power function of the mean in both control and defaunation, and the relation between mean and variance was very similar between the 2 treatments. There was no sign that the defaunated plots were either more similar to each other than the control plots, or that they followed more diverse and independent time courses after the defaunation.

Regression analysis showed that the mean number of macrobenthic species counted in the control sediment was constant over time ($p = 0.32$, $r^2 = 0.05$), with an average of 13.5 ± 0.3 (SEM) species. The number of species in the defaunated sediment followed a saturation curve (exponential rise to maximum, $p < 0.0001$, $r^2 = 0.75$) reaching an average of 12.6 ± 0.6 after ca. 3 mo.

During the first 1.5 mo of recolonisation (until Day 42) the total macrofaunal abundances (total number of ind. m^{-2}) in the 2 treatments were significantly different, where the factors Time, Treatment and Time × Treatment interaction had highly significant effects (Table 1; Fig. 2d). The total abundance of macrofauna in the defaunated sediment started at zero, increasing to numbers similar to control plots in ca. 8 wk. From Day 42 onwards the abundance in the defaunated sediment was still lower, but did not differ significantly from that in the control sediment (SNK, $p = 0.43$). The abundances of both treatments did not significantly differ until Day 98 when the defaunated sediment reached a total macrofaunal abundance of about twice that of the control (SNK, $p = 0.0061$). This peak in abundance coincided with the collapse of the MPB concentration.

The conspicuous macrofaunal abundance peak in the defaunated sediment at Day 98 was made up mostly (60%) of the tube-dwelling spionid *Pygospio elegans* (Table 2) (Claparède 1863). Other major constituents of the macrobenthos were the polychaetes *Polydora ligni* (Webster 1879), *Heteromastus filiformis* (Claparède 1864), *Nereis diversicolor* (O.F. Müller 1776) and the tellinid bivalve *Macoma balthica* (Linnaeus 1758) (Van Colen *et al.* 2008). In terms of macrofaunal biomass, *P. elegans* accounted for 25.15% of the total macrofaunal biomass in the defaunated plots at Day 98. While less abundant in numbers, *N. diversicolor* contributed 29.45% and *M. balthica* another 25.97% to the total biomass. The abundance of cockles (*Cerastoderma edule*) was about 4 times lower in the defaunated plots, whereas their relative biomass was more than 200 times lower than in the control sediment (Table 2). Apart from *Hydrobia ulvae*, all species invaded the plots as small juveniles.

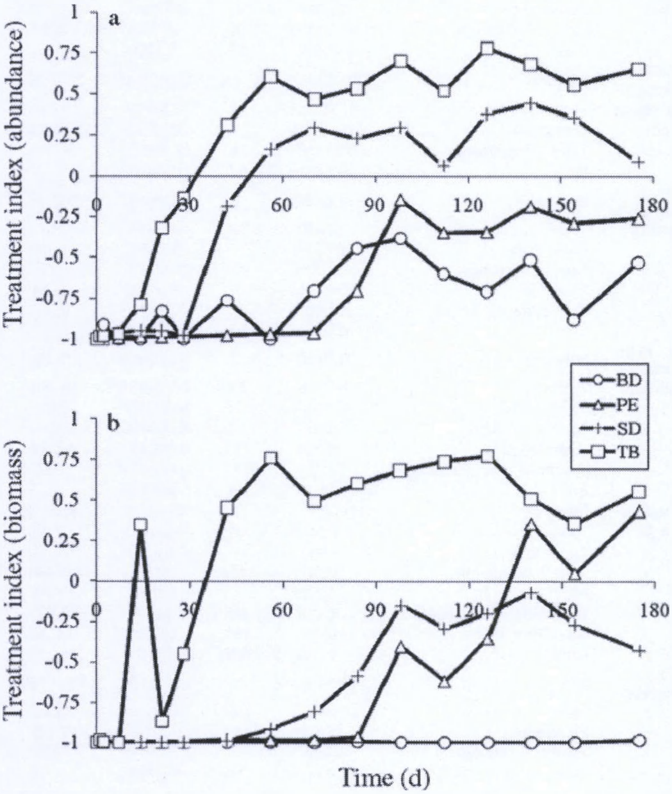


Fig. 3. Treatment index for the parameters (a) abundance and (b) biomass of the 4 geomechanical groups, where 0 = equal representation in both treatments, –1 = highly overrepresented in the control sediment and 1 = highly overrepresented in the defaunated sediment. BD = biodiffusor, PE = pelletiser, SD = surface disruptor, TB = tube builder

Time	Variable	Source of variation	SS	df	MS	*F*	p	Sign.
0–203	Surface chla (ln)	Intercept	1296.578	1	1296.578	2853.922	< 0.0001	
		Treatment	4.679	1	4.679	10.302	0.0326	*
		Plot(Treatment)	1.816	4	0.454	1.865	0.1261	
		Time	33.679	18	1.871	7.686	< 0.0001	!
		Time × Treatment	18.128	18	1.007	4.137	< 0.0001	!
		Time × Plot(Treatment)	17.285	71	0.243	1.404	0.0533	~
		Error	19.591	113	0.173			
0–175	Total abundance macrofauna (ln)	Intercept	8744.267	1	8744.267	87326.29	< 0.0001	
		Time	197.799	16	12.362	123.46	< 0.0001	!
		Treatment	87.829	1	87.829	877.12	< 0.0001	!
		Time × Treatment	185.111	16	11.569	115.54	< 0.0001	!
		Error	6.709	67	0.100			
0–175	Total biomass macrofauna (ln)	Intercept	601.6332	1	601.6332	1358.26	< 0.0001	
		Time	57.0282	16	3.5643	8.05	< 0.0001	!
		Treatment	270.2535	1	270.2535	610.13	< 0.0001	!
		Time × Treatment	69.1400	16	4.3212	9.76	< 0.0001	!
		Error	29.6771	67	0.4429			
0–203	Mud (< 63 μm) (arcsin)	Intercept	0.4545	1	0.4545	362.02	0	
		Time	0.0570	17	0.0034	2.67	0.0022	**
		Treatment	0.0054	1	0.0054	4.29	0.0420	*
		Time × Treatment	0.0376	17	0.0022	1.76	0.0526	~
		ln(chl a)	0.0131	1	0.0131	10.44	0.0019	**
		Treatment × ln(chl a)	0.0038	1	0.0038	2.99	0.0884	~
		Error	0.0854	68	0.0013			
0–203	Abs. water content (*arcsin*)	Intercept	0.0110	1	0.010958	13.99	0.0004	
		Time	*0.0838*	17	*0.004931*	6.30	< 0.0001	!
		Treatment	0.0005	1	0.000475	0.61	0.4389	
		Time × Treatment	0.0071	17	0.000420	0.54	0.9248	
		arcsin(mud)	0.0099	1	0.009871	12.60	0.0007	***
		Treatment × arcsin(mud)	0.0005	1	0.000517	0.66	0.4194	
		Error	0.0533	68	0.000783			
7–264	Elevation (ln)	Intercept	5083.800	1	5083.800	59324.43	< 0.0001	
		Time	1.397	16	0.087	13.26	< 0.0001	!
		Treatment	2.983	1	2.983	34.84	0.0041	**
		Time × Treatment	1.680	16	0.105	15.94	< 0.0001	!
		Plot(Treatment)	0.342	4	0.086	12.99	< 0.0001	!
		Time × Plot(Treatment)	0.415	63	0.007	0.65	0.9668	
		subq(Time × Treatment × Plot)	1.025	101	0.010	14.92	< 0.0001	!
		Error	0.273	402	0.001			
7–233	Erosion threshold (ln)	Intercept	539.9106	1	539.9106	1865.980	0	
		Time	15.8094	15	1.0540	3.643	0.0003	*
		Treatment	5.2738	1	5.2738	18.227	0.0001	!
		Time × Treatment	6.2272	15	0.4151	1.435	0.1682	
		Error	14.4672	50	0.2893			

Table 1. *Summary of the ANOVA of the measured environmental (biotic and abiotic) variables. Time: sampling days variables were measured. Variable: the analysed environmental variable with its appropriate transformation in parentheses. Source of variation: the factors taken into the explanatory model for each variable. ln = natural logarithm transformation; arcsin = arcsine square root transformation. Sign.: significance level; ~ = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ! = $p \leq 0.0001$*

Fig. 4. A defaunated plot, which was not sampled, ca. 2 mo after removal of the polyethylene cover. The sides of the square plot (corners designated with bamboo sticks) were 4 m in length. Note the different appearance of the plot compared to the surrounding ambient sediment. The sediment surface within the plot is very flat and devoid of faunal surface activity, unlike the surrounding sediment, which also displays sand ripples as a result of wave action.

Until Day 28, the total biomass (Fig. 2e) in the defaunated plots was 2, and sometimes even 3 (Day 14) orders of magnitude lower than in the control plots. Between Day 28 and Day 42 the total biomass in the defaunated plots increased with an order of magnitude, from 0.36 to 3.48 g AFDW m^{-2}, and remained stable for until Day 70. The total biomass then increased to 7.77 g AFDW m^{-2} at Day 84 and 12.03 g AFDW m^{-2} at Day 98, after which it remained stable.

Using our geomechanical classification of the abundant macrobenthic species, we calculated a treatment index *(Ti)* of the representation of the defined geomechanical traits in both treatments (Fig. 3), in terms of abundance and biomass. After 1.5 mo (Day 42), TB became more numerous in the defaunated sediment coinciding with stable, non-eroding sediment, while SD followed 2 wk later (Fig. 3a). The geomechanical traits biodiffusion and pelletisation were more numerous in the control sediment during the entire course of the experiment, although PE were closer to zero (i.e. equal representation over treatments) than

BD. In terms of biomass, only TB occurred in higher numbers in the defaunated sediment (Fig. 3b). Only in a later stage did PE become somewhat over-represented in the defaunated sediment. The BD biomass attained a *Ti* of ca. –1 throughout the entire experiment, which states its continuous high under-representation in the defaunated sediment. The surface disruptors tend more towards zero but remain over-represented in the control sediment.

Abiotic developments

After about 1.5 mo (Day 56) the defaunated plots were visibly more elevated than the surrounding sediment and remained this way throughout the course of the experiment (Table 1, Figs. 2f & 4). Also, the surface ripples normally seen on the control and on the surrounding ambient sediment were absent in the defaunated plots. This difference was not only caused by accretion of suspended sediment on the treated plots (F. Montserrat *et al.* unpubl. data), but mainly by erosion of the surrounding ambient sediment. Later in the experimental period (Day 203) there was overall deposition as the bed level increased in both treatments, but the defaunated plots remained significantly higher relative to NAP than the control plots.

Species	Feeding guild			Geomech. trait	Control				Defaunated			
					Abundance		Biomass		Abundance		Biomass	
	I	II	III		Actual	%	Actual	%	Actual	%	Actual	%
Cerastoderma edule	SF			BD	1793	5.1	25	53.7	801	1.2	0.03	0.2
Heteromastus filiformis	SSDF			PE	7497	21.4	2	4.2	5595	8.5	0.8	6.7
Hydrobia ulvae	G	SDF		PE	991	2.8	1	2.3	421	0.6	0.6	4.6
Macoma balthica	SDF	G	SF	SD	2526	7.2	8	18.2	3857	5.9	3.1	26
Nereis diversicolor	SDF	P	SF	SD	1698	4.9	0.7	1.5	3966	6	3.5	29.5
Polydora ligni	SDF	G	SF	TB	516	1.5	0.05	0.1	9874	15	0.7	5.9
Pygospio elegans	SDF	G	SF	TB	8257	23.6	0.6	1.4	39399	60	3	25.2
Total					23278	66.5	37.4	81.4	63913	97.4	11.7	97.9

Table 2. *The 7 most abundant species in both treatments at Day 98. For each species the feeding guild (in hierarchical order, if applicable) is listed from Gerino et al. (2003) and Ysebaert (2000). G = grazer, P = predator, SF = suspension feeder, SDF = surface deposit feeder, SSDF = subsurface deposit feeder, BD = biodiffusor, PE = pelletiser, SD = surface disruptor, TB = tube builder. Absolute abundances and biomasses are given as mean ± SEM.*

The sedimentology of the top 2 cm was also found to differ between the treatments (Fig. 2b). In the defaunated sediment, the percentage of mud was higher relative to the control sediment with significant effects for the factors Time and Treatment, but not for the Time × Treatment interaction factor (Table 1). During the initial weeks of the experimental period (Day 21 to Day 42) mud percentage in the control sediment also increased somewhat but remained lower than in the defaunated sediment. Depth distribution of mud in the top layers showed interesting patterns (Fig. 5). The top layer of the control sediment accumulated mud from Day 21 to Day 40, after which it decreased again slightly (Fig. 5a). As the surface eroded away and given the same coring depth, deeper layers containing more mud were reached. Thus, the mud profile obtained was a slightly concave one. The defaunated sediment, however, seemed to accumulate mud rather quickly in the first 6 wk, although the top layer always contained less mud than underlying layers (Fig. 5b). The exception was Day 40 where the depth-averaged mud percentage over the top 2 cm was around 55% against ca. 47% for the control sediment. As the bed level remained rather constant, so did the mud percentage of the deepest layers, yielding a convex mud profile. Around Day 120, when faunal abundance, and in particular abundance of deeper-burrowing species was restored (Van Colen *et al.* 2008), the pattern of mud depth-distribution returned to that of the controls.

The depth-averaged absolute water content (W_a; arcsine transformed) in the top 2 cm of sediment showed a significant effect of Time (Table 1, Fig. 2b), but without a significant effect for either Treatment or the Time × Treatment interaction terms. Instead, the factor mud content (arcsine transformed) did have a significant effect on the difference in W_a. Water content appeared to follow mud content closely, with some seasonal influence on the relationship.

One week after the removal of the plastic sheeting (Day 7) the defaunated sediment showed a high variability in erosion threshold. After 2 wk (Day 14), the defaunated sediment was characterised by a higher erosion threshold than the control sediments, and this lasted throughout the first 2 mo. The largest difference between the treatments coincided with the highest surface chl *a* values (MPB biomass) at Day 28. At Day 70 the threshold converged to the control sediment level. Both treatments experienced a last increase at Day 84, decreased again and remained at similar levels until the end of the experiment (Fig. 2c). There was a significant effect for both the factors Time and Treatment, but not for the Time × Treatment interaction factor with p = 0.1682 (Table 1).

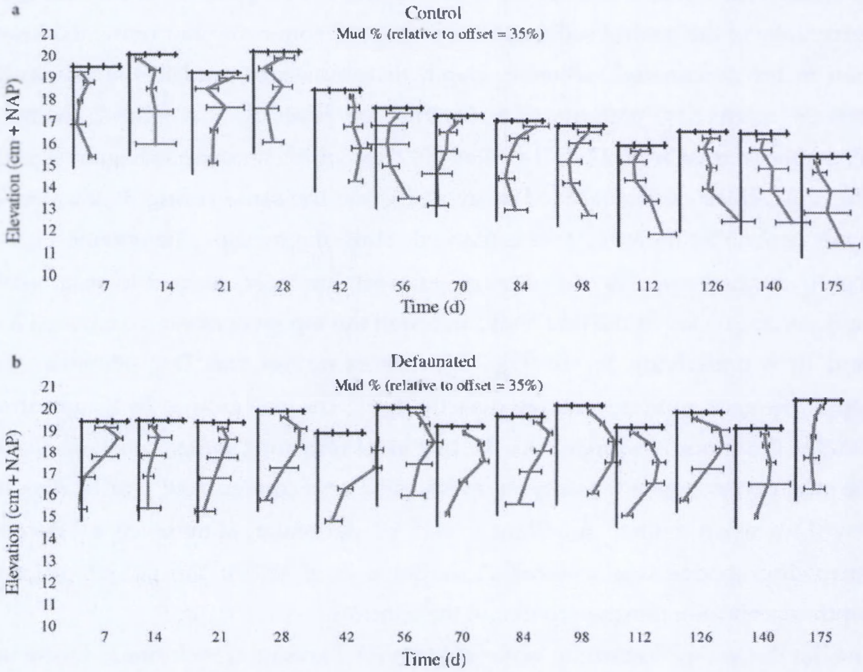


Fig. 5. The development of average mud percentage (% <63 µm) over time for the sediment layers 0–3, 3–10, 10–20, 20–30 and 30–50 mm in (a) control sediment and (b) defaunated sediment. The small sub-diagrams depict average percentage mud ± SEM, relative to an offset (base value) of 35%, for a given time in days after the start of the experiment and are positioned according to the sediment bed level in cm above NAP (Dutch Ordnance Level), which corresponds to the overall y-axis. The unit on the x-axes of each of the sub-diagrams is 5%.

We regressed erosion threshold against total macrofaunal abundance, total macrofaunal biomass and chl *a* (all variables natural log-transformed) for both treatments separately and for both treatments combined. In the cases of both the control plots and defaunated plots separately, no minimal adequate model

(MAM) was reached; i.e. no significant effects could be found for any of the variables or their interactions, either 2- or 3- way. However, when both treatments were combined, a MAM could be established, containing the parameters abundance, biomass, chl *a* and the interaction between abundance and biomass (Table 3). Consecutive simple regressions (both treatments combined) of erosion against abundance, biomass and chl *a* showed that these variables separately could account for 13% ($p = 0.0033$), 30% ($p \ll 0.0001$) and 32% ($p \ll 0.0001$) of the variance, respectively (Fig. 6). The fitted multiple regression model, containing the abovementioned 4 parameters (see also Table 3) had an overall significance level of $p \ll 0.0001$ with multiple $r^2 = 0.4833$.

	Estimate	**SE**	***t***	**p**
Intercept	3.00	0.90	3.34	0.0015
ln(abundance)	–0.23	0.11	-2.07	0.0429
ln(biomass)	–1.36	0.54	–2.53	0.0142
ln([chl *a*])	0.79	0.18	4.35	5.38×10^{-5}
ln(abundance) × ln(biomass)	0.13	0.05	2.40	0.0197

Table 3. *Parameters estimates and their respective SE for the multiple regression of erosion against total macrofaunal abundance, total macrofaunal biomass and [chl a].*

Discussion

Microphytobenthos regulation

Whereas the MPB reacted quickly and strongly and recolonised the defaunated plots in a matter of days, the macrofaunal abundance took several weeks to reach control levels. In the absence of grazing macrofauna, MPB experienced a period of exponential growth, whereas in the control sediment MPB was more constant as it was simultaneously being consumed, disturbed or buried by the intact macrofaunal community. Fig. 6a shows that, due to the presence of grazing or sediment-disturbing macrofauna, there is a narrower range of MPB biomass in the control plots than in the defaunated plots. The importance of macro-faunal checks on MPB has been reported in a number of studies (Blanchard *et al.* 2000, de Deckere *et al.* 2001).

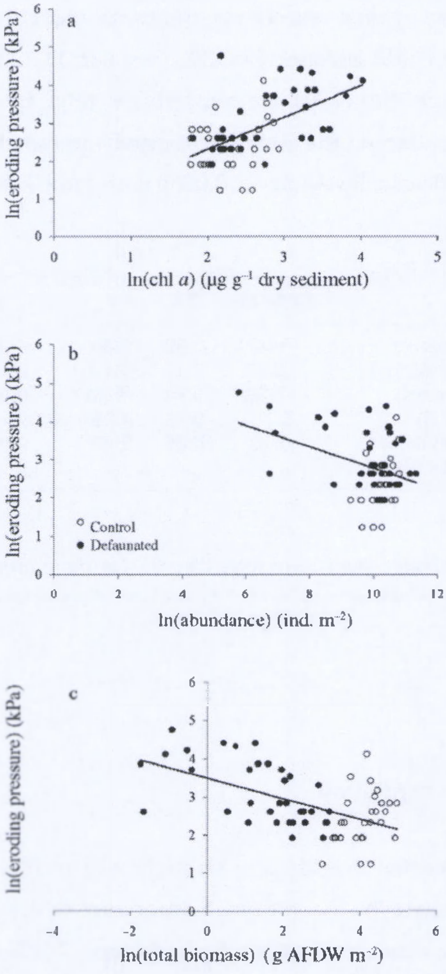


Fig. 6. Simple regression graphs of erosion threshold against (a) chl a, (b) total macrofaunal abundance, and (c) total macrofaunal biomass; s = control, D = defaunated. Grey solid lines: trendlines for both treatments combined. All variables are natural-log transformed.

The increased sediment strength during Day 14 to Day 56 of the experiment can be attributed to the increased abundance of benthic diatoms, which form a very strong, yet flexible layer on the sediment surface. This armouring acts as a first-order binding mechanism of the cohesive sediment (Holland *et al.* 1974, de Boer 1981, Paterson 1989, de Brouwer *et al.* 2003b) and causes the erosion threshold to be significantly higher than in the control plots. Mud content of the defaunated sediment also increased during the initial period of the experiment. However, this increase only started after some time, which was probably needed to develop a sufficiently large diatom mat capable of entraining mud from the water column. Water content of the sediment was not lower (on the contrary, even slightly higher) in the defaunated than in the control sediment, ruling out the possibility that increased sediment stability was caused by compaction only during the defaunation treatment.

Very recently, Larson & Sundbäck (2008) demonstrated the key role of diatoms in the recovery of ecosystem functions in shallow marine systems. In their experiments, diatoms appeared to possess an enormous resilience against hypoxic events and were able to reoxygenate the top layer of experimentally defaunated sediment in less than a week. MPB survival of low-oxygen conditions ensures that recolonisation by higher trophic levels is not limited by lack of primary production.

By providing ample food supply and by stabilising the sediment surface, MPB thus prepared favourable conditions for pioneer species. After recruitment and recolonisation started in the defaunated plots, the community developed towards a rather different composition from that in the control plots (Van Colen *et al.* 2008). Similar species were present in both treatments but when considering the geomechanical traits of those species their representation differed greatly. More specifically, TB attained both positive abundance (Fig. 3a) and biomass (Fig. 3b) indices from around 1 mo after the start of the experiment. The opportunist spionid and major constituent of the tube-building pioneers, *Pygospio elegans*, is a natural surface deposit feeder, which changes diet during its ontogeny (Hentschel 1998). While adults derive their nutrition from both MPB and phytodetritus, settled larvae and small juveniles forage mainly on benthic microalgae. The settled spionids foraged on the thick MPB layer, grew at a high rate and reached high densities (Van Colen *et al.* 2008) within the same period as the MPB was blooming on the defaunated plots.

Sediment characteristics

Although the sediment variables showed a clear relation with MPB in the first phase, we observed a decrease in the difference in erosion threshold and mud content approximately 1 mo before the MPB biomass collapsed. The decrease of the erosion threshold and the start of net loss of mud in the defaunated sediment coincided in time, suggesting that this was a consistent change in the sediment behaviour. To add to this consistency, the mud content showed the same pattern in both treatments. The much lower abundance and biomass of macrofauna combined with a well-developed diatom mat in the defaunated sediment caused the mud content to increase before it started to converge to constant control levels after Day 42. It appears that this moment of convergence coincides with the moment where macrofaunal abundance first reaches control levels (i.e. is not significantly different from that of the controls). The pioneer community then started to inhibit the armouring effect of the MPB by grazing and disturbance of the sediment surface.

When the macrofauna abundance experienced an overshoot (around Day 98), mud was lost faster in the defaunated than in the control plots (Figs. 2b & 5b). This is backed up well with the surface disrupting macrobenthos (SD) abundance attaining a positive *Ti* at that time. The over-representation of this group in the defaunated plots (Fig. 3a) induced a decrease in erosion threshold (Fig. 2c) and a faster loss of mud. Thus, macrofaunal influence seems to have overtaken the stabilising influence of MPB after about 2.5 mo.

Instead of being eroded after the disappearance of the protective effect of the diatom mat, the defaunated sediment remained more elevated than both the surrounding ambient sediment and the control plots. In fact, the sediment in the control plots was found to erode while the defaunated sediment remained at more or less the same level. Mud percentages did increase significantly in the defaunation treatment, but the increase was not extremely high and did not last for months. However, Mitchener & Torfs (1996) reported significant increases of the τ_{crit} (departing from a pure sand bed) at mud contents between 30 and 50%. Visual inspection of the sediment, as well as photographic documentation (Fig. 4) suggested that the increase measured in this experiment was sufficient to change the sediment behaviour from a slightly noncohesive, ripple-forming state in the controls, to a more cohesive, smooth surface in the defaunated treatment (Winterwerp & van Kesteren 2004). Independent measurements taken by RIKZ ca. 1 km east of our experimental plots in the same intertidal area show erosion in the order of 2 to 3 cm between May and November 2005 (G. Liek, RIKZ, pers. comm.). These bed elevation measurements are consistent with our own findings in the present study.

The subsurface lining of plastic sheeting hardly, if at all, affected the ripple migration within the defaunated plots. Not only does ripple migration occur mainly by sediment transport at the surface (van Rijn 1993), but also the period of the ripples in the ambient sediment was of a spatial scale (cm; Fig. 4) 2 orders of magnitude smaller than the spatial scale of the defaunated plots (m). If the subsurface lining had prevented ripple migration, this would have been observed primarily at the edges of the plots, and ripple formation would have been possible and visible in the middle part of the plots. This was not the case in our experiment.

Macrofaunal effects

The dependence of recolonisation on larval recruitment makes the succession dependent on the timing of opening the plots, because larvae of many species are only available during short periods. We timed our experiment to allow for recruitment of all important species (Ysebaert 2000). Some auxiliary plots were opened later in the year for other measurements (not discussed here) and faunal data of these plots showed that community species composition was very time-dependent (i.e. differed with availability of larval recruits). However, the sequence of faunal group composition was very similar, as was the sequence of sediment characteristics. Thus, we feel that our results are robust against these changes, provided that at least a minimum number of species is available for recruitment.

Tube building macrobenthos (TB) attained a positive *Ti* for both abundance and biomass after only 1 mo. Their very high numbers (>20 000 ind. m^{-2} at Day 42 to ca. 40 000 ind. m^{-2} at Day 98; Van Colen *et al.* 2008) coincided with the more elevated position of the defaunated plots as compared to the control plots and surrounding ambient sediment as described above in 'Abiotic developments'. In high densities this group effectively decreases erosion (Woodin & Jackson 1979, Jumars & Nowell 1984a, b, Murray *et al.* 2002). By creating milder hydrodynamics locally, they induced an increase in retention of mud at the sediment surface. Widdows *et al.* (2000) observed similar high densities of *Pygospio elegans* tubes protecting the sediment from erosion by increasing the height of the benthic boundary layer or even by replacing it to a higher position in the water column. Friedrichs *et al.* (2000) used the parameter roughness density (RD) to study flow attenuation in idealised tube 'meadows'. Assuming a tube diameter of 1 mm, the RD in the defaunated plots in our study would be around 0.03 at the time of the *P. elegans* abundance peak (Day 98). This RD value corresponds to RD values at and above which flow attenuation and net deposition of sediment took place in the work of Friedrichs *et al.* (2000). Bolam & Fernandes (2003) also found that in *P. elegans* patches in the Firth of Forth (SE Scotland) both silt/clay content and organic matter content were significantly higher. Both of these variables are known to contribute to a higher cohesiveness of the sediment (Winterwerp & van Kesteren 2004). Also, these tube meadows can better trap finer sediment fractions from the overlying water column. Rabaut *et al.* (2007) describe a similar phenomenon in aggregations of the tubeworm *Lanice conchilega* on the Belgian continental margin. Besides being associated with higher local species diversity, these *Lanice* 'reefs' alter hydrodynamics, retain sediment locally, and appear elevated above the surrounding sediment. In light of these very consistent findings, we postulate that the tubes formed by *P. elegans* in such high densities 'root down' the sediment, entrain mud particles and prevent it from eroding away.

The stabilising effect of the tube meadows was not directly reflected in the erosion threshold measurements. This can be partly attributed to the way the CSM applies water pressure to the sediment surface. The CSM uses a water jet acting perpendicularly on the sediment. A diatom mat is a more or less uniform layer on and integrated with the surface sediment, able to withstand (up to a certain point) the water pressure exerted by a CSM water jet. The spionid tubes can be seen to act as miniature vegetation *sensu* Scoffin (1970) and Woodin & Jackson (1979), where the meadow of tubes reduces the incident fluid momentum on the bed and retains (i.e. prevents resuspension of) the sediment but cannot counteract the perpendicular CSM water jet. Many other authors have used the CSM in different field and in lab experiments, all yielding consistent results. In our experiment there was not only a clear difference in erosion threshold between the treatments in the first 2 mo, but also little variability around the mean erosion threshold values in the control sediment, indicating that the resulting values do give a consistent measure of sediment surface strength. Even if the CSM does not give a strict quantitative measure of undrained shear strength of different sediments, it most certainly yields a qualitative difference regarding the erosion threshold of the 2 treatments. Widdows *et al.* (2007) demonstrated that the CSM is best in detecting the stabilising effect of MPB aggregates and suggest a field annular flume for measuring macrobenthic effects on erosion threshold and rate *in situ*. We could not deploy such a flume due to obvious experimental and logistical constraints. Sediment bed level did remain higher for several months after the erosion threshold, indicating the stabilising effect of the high-density tubeworm population.

Intricate interactions

Simple regression analysis of CSM eroding pressure results against the 3 biotic variables 'total macrofaunal abundance', 'total macrofaunal biomass' and 'sediment surface chl *a* content' all showed significant relations, but the relations were not particularly strong, not particularly useful for extrapolation and not particularly new for benthos–sediment interactions related research. Multiple regression analysis for both treatments separated did not yield very interpretable results, either. The range of values for both treatments separately was relatively small, and especially so for the control plots. When both treatments were combined, the range of values was larger and highly significant effects became apparent (Fig. 6). This is shown best in Fig. 6c, where the range of biomass values of the defaunated plots (black circles; starting at very low values) are in line with that of the control plots (open circles). The MAM, which could be established, contained all 3 variables (abundance, biomass and chl *a* as well as the interaction between abundance and biomass), and was able to explain slightly less than half of the variance (multiple $r^2 = 0.4833$).

It is possible that factors we did not measure in this experiment would have correlated better with the CSM measurements. In particular, direct measurements of EPS could have been more meaningful than chl *a* measurements, which are only a proxy of MPB biomass (Underwood & Paterson 1993) and not of MPB activity. However, total EPS measurement also neglects important chemical differentiation between different EPS classes, and is therefore not guaranteed to better reflect sediment strength than MPB biomass measures (de Brouwer *et al.* 2002, Wolfstein *et al.* 2002).

In light of the macrobenthic recolonisation recorded in our experiment (see also Van Colen *et al.* 2008), we attribute the interaction term Abundance × Biomass to a situation in which high densities of juvenile macrofauna (high density, low biomass) caused less erosion than a population of the same density which consists of adult individuals (high density, high biomass). The former would be the case in the defaunated plots after ca. 3 mo where high numbers of recruits had settled, the latter in the case of a natural situation.

Daborn *et al.* (1993), in their multidisciplinary field study in the Bay of Fundy, showed how seasonally regulated trophic cascades can lead to changing patterns in sediment dynamics. Here, we would like to add the feedback of the macrobenthos to the sediment.

An important pattern in the development of the macrofaunal community in the defaunated sediment was the highly negative treatment index *(Ti)* of biodiffusing (BD) macrobenthos. Biodiffusion (Gerino *et al.* 2003) is one of the processes which keeps the sediment surface loose and increases bottom roughness, rendering it more susceptible to erosion (Woodin & Jackson 1979). The BD group was made up by > 90% of the common cockle *Cerastoderma edule*. The highly negative *Ti* could be attributed to the absence of large, adult *C. edule* in the defaunated sediments. *C. edule* is a mobile, suspension feeding bivalve (Kamermans *et al.* 1992) which lives in the top 2 to 5 cm of the sediment. Ciutat *et al.* (2007) conducted a flume study in which suspended sediment concentrations (mg l^{-1}) and erosion rate (g m^{-2} s^{-1}) increased significantly as a function of increasing current speed and increasing cockle density. In addition, increased cockle density caused the critical erosion velocity (U_{crit}, the current velocity needed to erode 1 g m^{-2} of sediment) to decrease significantly. With geomechanical effects being highly density-dependent (Woodin & Jackson 1979), it should be noted that the maximum average cockle density recorded in the control plots of our study exceeded the maximum density used by Ciutat *et al.* (2007) by a factor of 10. By continuously mixing and disturbing the sediment, adult cockles can also cause a reduction of the presence of other pioneer species among which is *Pygospio elegans* (Woodin & Jackson 1979, Flach 1996). Bolam & Fernandes (2003) observed temporal mutual exclusion of *P. elegans* and *C. edule* in a Scottish intertidal area. Rhoads & Young (1970) identified this as 'trophic amensalism', with the cockles being the inhibitory group and the spionids the amensals. However, Snelgrove & Butman (1994) falsified most claims of the theory of trophic amensalism, mainly on the ground that, through modifications applied in several examples, it lacks generality. In terrestrial ecology, the term 'competitive displacement' is used to describe this type of organism–environment–organism interaction (Osakabe *et al.* 2006). Although not addressed in this study, the exceptionally high abundances of *P. elegans* also might have had an adverse effect on the larval settlement of other species, either directly through predation or indirectly (Cummings *et al.* 1996).

The drastic over-representation of tube building (TB) species in the defaunated plots caused a reduction in surface erosion and sediment resuspension. Furthermore, because of the severe under-representation of biodiffusing (BD) cockles, and the high absolute and relative biomass they represent in the control plots, the defaunated plots did not undergo the continuous sediment reworking, erosion and consequent decrease in sediment bed level as was the case in the control plots. We postulate that, preceded by the initially undisturbed and sediment-stabilising diatom mats, these factors combined (high TB density, low BD density and biomass) accounted for the largest part of the differences in sediment behaviour.

Biogeomorphological modelling

Paarlberg *et al.* (2005) modelled the effects of biological activity on a salt marsh-mudflat complex in the Paulinapolder intertidal area where our study was conducted. They modelled a situation devoid of biological activity (i.e. no macrobenthos), a situation where there is maximum stabilisation by microphytobenthos, and a situation where there is maximum destabilisation by macrobenthos. In the latter situation their model showed that the sediment was eroded. The model outcomes showed an increase in depth-averaged mud content in both the azoic and maximum stabilisation situations. Also, a bed level of about 10 cm lower than the azoic and maximum stabilisation scenarios was attained in the maximum destabilisation situation. A further situation was introduced where biological activity varied spatially in a 60 × 60 m chessboard pattern. In this situation the bed level showed the same difference of about 10 cm. Although the spatial scale in this model study differs somewhat from the present experiment, the model outcomes, with strong macrobenthos– sediment controls, are in the right order of magnitude, and qualitatively in accordance with our observations (Fig. 2). A logical next step in modelling is to add the dynamics of organism–sediment interactions, in such a way that the coupled physical-biological system can be used to explore time dynamics of the system. Such an approach has been useful in explaining salt marsh development (Temmerman *et al.* 2007). Studies by Le Hir *et al.* (2007) and by Wood & Widdows (2002) have included the temporal and seasonal dynamics of both MPB and macrofauna effects and arrive at similar qualitative and quantitative conclusions.

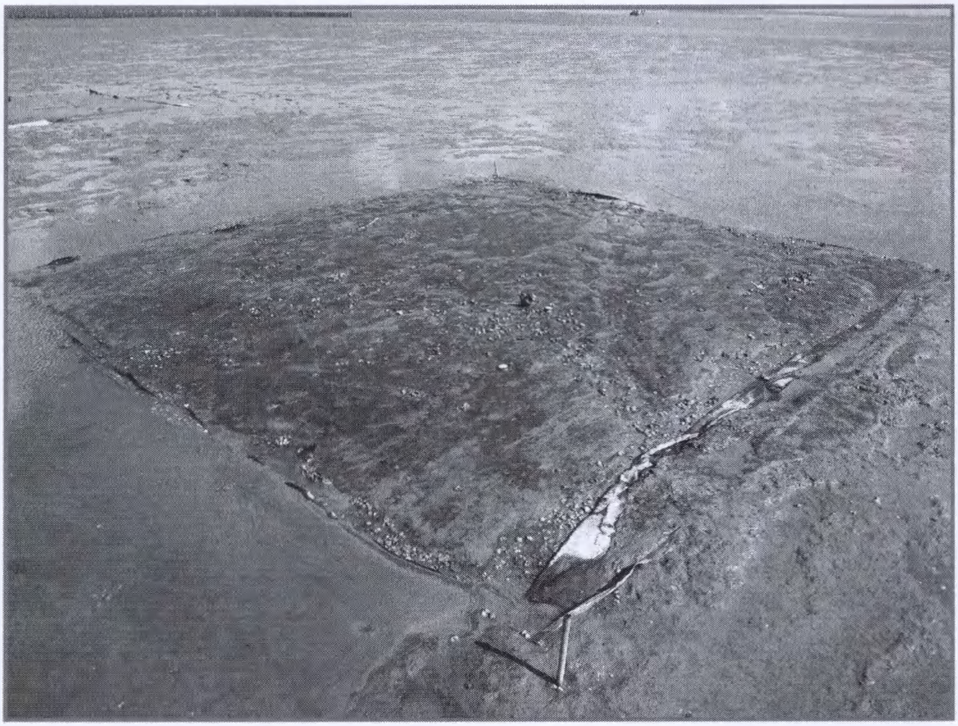
Both studies conclude that MPB effects are fairly well understood, relatively easy to model and overall stabilising. In both studies macrofauna effects are considered to be highly variable and difficult to unravel. Using the natural succession of benthic macrofaunal species our study has revealed separate effects of geomechanically functional groups in the field.

Conclusion

After an intense anthropogenic disturbance event, recolonisation of the site by the various functional groups of an intertidal community followed different dynamics. The groups of organisms affected each other through their influence exerted on the immediate environment. The time taken for the abiotic compartments to revert to what we perceive as a normal state is set by the speed of succession of the macrobenthic community as a whole. During a lag phase with low macrobenthic abundance, exponential growth of microphytobenthos and concomitant geomechanical changes in the sediment dominate the developments. This may facilitate recruitment of the macrobenthos, which, once sufficiently abundant, interacts intensely but in a complex way with microphytobenthos, sediment mud content (sediment cohesiveness) and sediment strength. The net effect of these interactions is slightly erosive, compared to a defaunated state. As demonstrated by this study, the dominant benthic macrofauna largely determines sediment behaviour.

Acknowledgements

We offer gracious thanks to J. van Soelen, B. Koutstaal, D. Peelaers and B. Beuselinck as well as the Masters students for their enormous efforts during the fieldwork. K. van den Meersche and F. Rossi are both kindly thanked for their patient advice on statistics. C. Kleppe, J. Sinke, Y. van der Maas, M. Houtekamer, H. Francke, D. van Gansbeke and D. Schram are thanked for their help in processing the sediment for pigment, granulometric and nutrient analyses. C. Woulds, J. Middelburg, H. Winterwerp and M.Vos proofread the manuscript in an earlier or later stage and provided valuable advice and comments. This research was supported by the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministry of Economic Affairs and the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen).



CHAPTER 5

BENTHOS-MEDIATED SEDIMENT DYNAMICS: IMPLICATIONS FOR GROWTH AND PRODUCTION OF JUVENILE RECRUITS

Van Colen C., Montserrat F., Vincx M., Herman P.M.J., Ysebaert T., Degraer S.

Abstract

Recruitment success of the bivalves *Macoma balthica* and *Cerastoderma edule* and the polychaetes *Nereis diversicolor* and *Pygospio elegans* was investigated in response to macrobenthos community-mediated differences in sediment characteristics. Population and growth dynamics were inferred from a recolonisation field study after experimentally induced hypoxia where differences in macrobenthos community structure mediated differences in food supply (i.e. benthic primary production) and susceptibility to physical stress (i.e. erosion).

Species feeding on microphytobenthos grew significantly faster in hypoxic patches (i.e. treatments) where fresh food supply was higher and physical stress was lower as compared to control sediments. Moreover, differences in growth rate were positively related to the consumer-specific microphytobenthos contribution to its diet and suggest that the faster growth in the treatments relates to a lower interference competition for food. In addition to the enhanced growth, lower post-settlement dispersal in the more stable treatments is suggested to contribute substantially to the significant higher cohort production of *M. balthica*, *N. diversicolor* and *P. elegans*.

Because recruitment is the foundation upon which all subsequent interactions within the community take place, our results illustrate that biotic-physical interactions that affect benthic primary production and sediment stability are of

fundamental importance to tidal flat macrobenthic community distribution and functioning.

Keywords: *Recruitment success, Growth, Production, Interference competition for food, Post-settlement resuspension*

Introduction

Events affecting recruitment of macrobenthos may control benthic species diversity, and hence ecosystem functioning (Naeem *et al.* 2002) since these events determine (1) the magnitude of propagule arrival to the community (i.e. net input of new individuals) and (2) the survival of settlers to the benthic stage. For species with pelagic larvae, recruitment has five major components: input of propagules into the water column, propagule dispersal, planktonic mortality, settlement and post-settlement growth and survival (Jenkins *et al.* 1999). Recruitment limitation involves both pre and post-settlement events determining recruitment success but the relative importance of primary (i.e. pre-settlement) and secondary (i.e. post-settlement) recruitment limitation differs between substrate type, scale and species (Fraschetti *et al.* 2003). Stoner (1990) suggested that pre-settlement events influence distribution of recruits because these events operate at a larger scale, whereas post-settlement events (survival, growth and secondary dispersal) affect juvenile density at a local scale. In general, pre-settlement mortality is thought to limit benthic populations because planktonic larvae are considered the most vulnerable stage in the life cycle of marine invertebrates (Thorson 1950). However, recent evidence illustrates that secondary recruitment limitation cannot be neglected since post-settlement mortality may exceed 90% of the larvae settled (Gosselin & Qian 1997). In addition to interactions between established adults and recruits (e.g. predation, interference by bioturbation, competition for food and space), exposure to physical and hydrodynamic disturbance and food limitation may affect post-settlement growth and survival (Fraschetti *et al.* 2003).

In this paper, we evaluated whether differences in community-mediated sediment characteristics affected the post-settlement growth and production of four macrobenthic species. Population and growth dynamics were inferred from a recolonisation field study after experimentally induced hypoxia where differences in biological activity of a control, 'mature' community and a recovering community mediated differences in food supply (i.e. benthic primary production) and susceptibility to physical stress (i.e. erosion) (Van Colen *et al.* 2008, Montserrat *et al.* 2008). The polychaetes *Nereis diversicolor* and *Pygospio elegans* and the bivalves *Macoma balthica* and *Cerastoderma edule* were chosen because these species (1) differed in microphytobenthos (MPB) contribution to their diet, (2) settled at high densities and (3) are common and important contributors to the macrobenthic community, and hence, to the functioning of NW European tidal flats (Bachelet & Dauvin 1993, Ysebaert & Herman 2002, Ysebaert *et al.* 2003, Volkenborn & Reise 2007, Van Colen *et al.* 2008).

Materials & methods

Data collection

Benthic recruits were collected with a 12.5 cm inner diameter corer from six replicated 4 x 4 m sediment patches at the Paulinapolder tidal flat (Westerschelde estuary, SW the Netherlands) during Spring and Summer 2005. Biweekly, from March 30th – August 3th, one sample was randomly taken from each patch, fixed with a neutralized 8 % formalin solution and subsequently sieved over a 500 μm mesh size. Three of these patches were disturbed by severe hypoxia for 40 days until March 30th (i.e. treatments) and three patches were left undisturbed (i.e. controls). Hypoxia was induced by covering the sediment surface by thick plastic sheets and resulted in a complete mortality of the macrobenthic community. Subsequently, macrobenthos recovery developed through different succession stages characterized by clear biotic and abiotic shifts (Van Colen *et al.* 2008). As a result of the low grazing pressure during the first month of recovery, benthic primary production – which was made up by > 80 % of diatoms (F. Montserrat, unpublished data) - strongly exceeded control values in the treatment patches during early recovery stages and returned to control levels after three months, along with an enhanced grazing and bioturbation impact, characterizing later succession stages (Fig. 1). Further, whereas the control patches constantly eroded during May and June, the bed level of treated patches remained more or less stable, most probably due to the combined effect of the dense diatom mat, the dense patches of tube-building polychaetes and the relatively low bioturbation disturbance during that period (Montserrat *et al.* 2008).

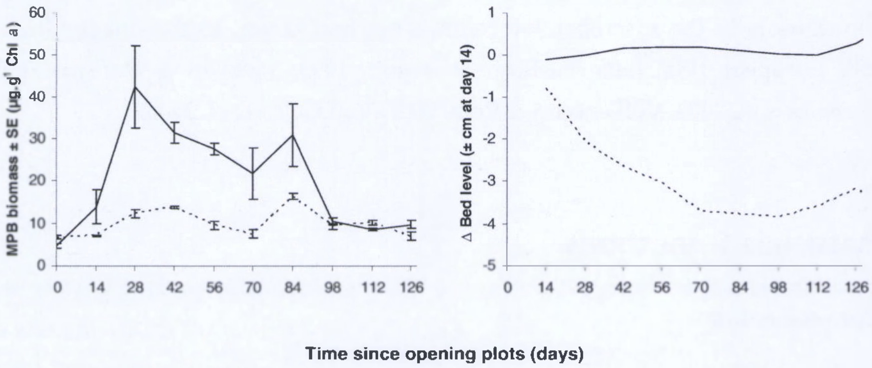


Fig.1. *Temporal variation in microphytobenthos biomass ± SE (left panel) and difference in bed level height (right panel), as compared to the first measurement (i.e. day 14) in control (dashed line) and treated patches (solid line). Curve fits for Δ bed level height are running averages (period = 2 sampling occasions).*

In similar treatment plots, used in a parallel experiment, Rossi & Middelburg (in prep.) quantified the MPB contribution to the consumers' diet by comparing the consumers' $\Delta\delta^{13}C$ (i.e. the increment of $\delta^{13}C$ due to a PLFA biomarker uptake, as compared to natural background values) to the MPB $\Delta\delta^{13}C$: $\Delta\delta^{13}C_{consumer}$ / $\Delta\delta^{13}C_{resource}$ (i.e. Δ-ratio). This approach revealed that *M. balthica* juvenile recruits relied primarily on MPB carbon, reaching a Δ-ratio close to 100 %, whereas benthic microalgae contributed less to the diet of *P. elegans* and *N. diversicolor* (Δ-ratio ~ 30 %). No label uptake was found in juvenile *C. edule* which supports the well-known filter feeding behavior on phytoplankton of this species (Herman *et al.* 2000, Rueda & Smaal 2002). For more details on MPB isotope tracking, information on the study site, experimental set-up and sample processing, the reader is referred to Van Colen *et al.* (2008), Montserrat *et al.* (2008) and Rossi *et al.* (2008).

Growth and production estimates and analysis

Species size-frequency analyses were performed for all sampling occasions for each replicate plot (n = all individuals in a replicate sample with a maximum of n = 50 individuals for *P. elegans*). Size classes were 1 mm shell width for *M. balthica* and *C. edule* and 0.05 mm and 0.2 mm 5th setiger width for *P. elegans* and *N. diversicolor*, respectively. These analyses revealed the occurrence of one clear juvenile cohort for *M. balthica* and *C. edule* throughout the sampling period, whereas no unimodal pattern for *P. elegans* and *N. diversicolor* was apparent. Consequently, size, standard deviation and density of juvenile cohorts of the latter two species were determined according to Bhattacharya (1967) using the Fisat II software (Gayanilo *et al.* 1996). This approach revealed the occurrence of several juvenile cohorts throughout the monitoring period for both of these species. Only data of the first appearing cohort of both species was used in this study.

Cohort growth rate and production – which takes both survival and growth into account - were used as indicators of recruitment success. Average daily cohort growth was estimated for each replicate treatment (n = 3) as the difference in mean size of the individuals of an identified cohort between the last and first appearance in the samples, normalized per unit time (size increment day^{-1}). A power function enabling conversions from body size (S, mm), i.e. 5th setiger width for polychaetes and shell width for bivalves, to biomass (W, g) was assessed from size and biomass measurements from the same complete individuals: $W_{N.\ diversicolor} = 0.001S^{2.6913}$, r = 0.96, n = 50; $W_{P.\ elegans} = 0.0004S^{1.9039}$, r = 0.76, n = 49; $W_{M.\ balthica} = 3.10^{-5}S^{1.8954}$, r = 0.91, n = 156; $W_{C.\ edule} = 2.10^{-5}S^{2.3193}$, r = 0.84, n = 153).

Bivalve biomass was obtained by determination of the ash free dry weight (4h combustion at 450°C of 24h, 60°C dried individuals) and polychaete biomasses were calculated by multiplying the organisms' blotted wet weight with a species-specific ISO certified wet weight-ash free dry weight conversion factor (Sistermans *et al.* 2007). Cohort production for each replicate treatment (n=3) was estimated using the weight increment method (Crisp 1971) (Table 4, a-d in Appendix 3). After data transformation in order to meet homogeneity of variances (Levene test) and normality (Shapiro-Wilks test) assumptions, Student t-tests were applied to analyze differences in initial settlement density and size, cohort growth rate and production between treatments and controls.

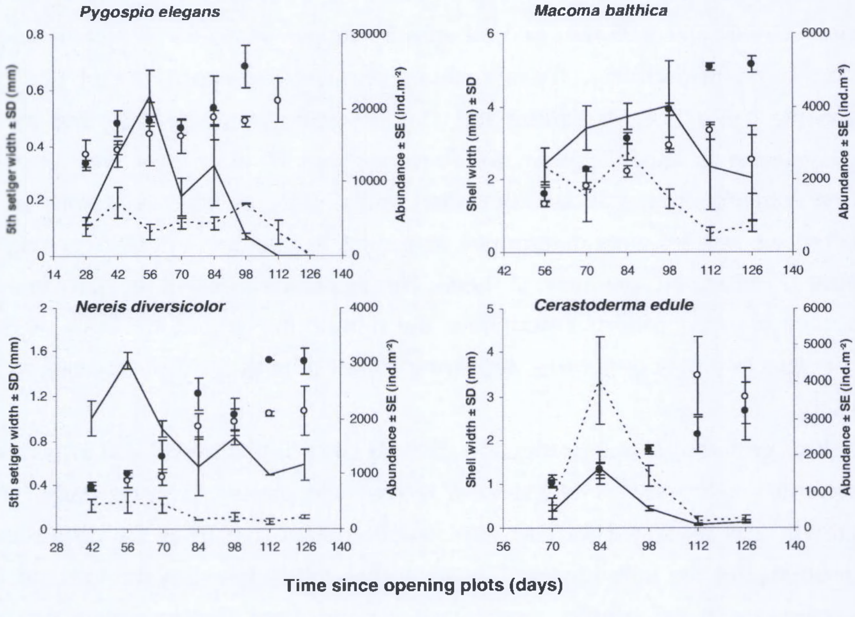


Fig. 2. *Temporal variation of mean size ± SD (circles, left y-axis) and density ± SE (lines, right y-axis) of the identified juvenile cohorts of* Pygospio elegans, Nereis diversicolor, Macoma balthica *and* Cerastoderma edule *in control (open circles, dashed line) and treatment patches (closed circles, solid line).*

Results & discussion

Temporal variation of mean cohort size and density are presented in Fig. 2. Settlement size (i.e. mean cohort body size at first appearance in samples) did not differ significantly between treatments and controls for all species (t-test, df = 4, p > 0.05). Juvenile *M. balthica, P. elegans* and *N. diversicolor* treatment cohorts grew significantly faster as compared to the controls (+ 31.0 µm day^{-1} shell width for *M. balthica,* + 2.2 µm day^{-1} 5th setiger width for *P. elegans* and + 5.4 µm day^{-1} 5th setiger width for *N. diversicolor*), whereas growth rates were not significantly different between control and treatment sediments for *C. edule* (Fig. 3a, Table 1). Furthermore, differences in growth rate between control and treatment patches were positively related to the microphytobenthos contribution to the consumers' diet: +102% for *M. balthica,* which almost completely feed on MPB; + 77 %, for *P.elegans* (feeding less on MPB, i.e. ~30%); + 67 % for *N. diversicolor* (feeding less on MPB, i.e. ~30%) and no significant difference for *C. edule,* which does not feed on MPB.

Taking into account the species-specific microphytobenthos contribution to the macrobenthos diet (Herman *et al.* 2000, Rossi & Middelburg in prep.) and assuming a net growth efficiency of 60 % and 75 % for macrobenthos and epistrate feeding nematodes, respectively (Van Oevelen *et al.* 2006), one can estimate that 17.3 % of the produced microalgal carbon during the 126-day experimental period is consumed by the macrobenthos and epistrate feeding nematodes in the control sediments, whereas grazing of these organisms only account for 3.5 % of the produced microalgal carbon in the treatments. One can expect that an organism feeds more efficiently, and thus spends less energy, in a more stable environment where there is less interference with other organisms. Hence, the faster growth in the treatment cohorts presumably relates to a lower interference competition for food (i.e. microalgal carbon). Furthermore, differences in grazing pressure and bioturbation intensity have been shown to alter benthic diatom community composition (e.g. Hagerthey *et al.* 2002). Therefore, alterations in diatom species-specific nutritional value or cell wall characteristics, affecting the benthos' digestion efficiency (e.g. Kawamura *et al.*

1995), may have affected differences in growth rate between controls and treatments. Differences in body size became clearly more pronounced at the end of the study period. In addition to their deposit feeding behaviour, large *M. balthica* also filterfeed and this filterfeeding behaviour is known to increase with body size (Herman *et al.* 2000, Rossi *et al.* 2004). As a result of their enhanced initial growth, the threshold body size which enables proper filterfeeding (i.e. 3 mm, Herman *et al.* 2000) was more rapidly reached by *M. balthica* juveniles in the treatments as compared to the controls. Consequently, additional food intake via the filterfeeding pathway may have contributed to the large difference in *M. balthica* body size between controls and treatments at the end of the experiment.

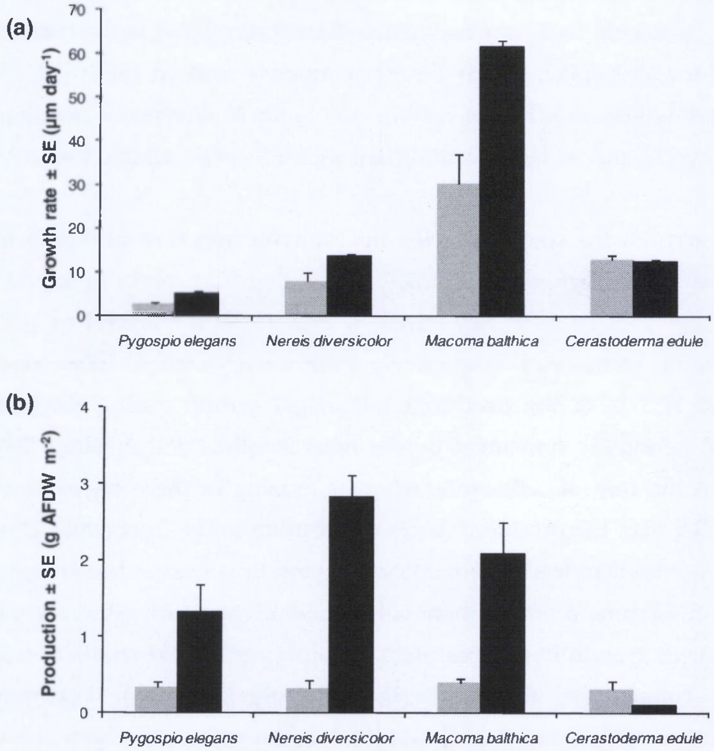


Fig. 3. (a) Mean growth rate ± SE and (b) production ± SE of Pygospio elegans, Nereis diversicolor, Macoma balthica *and* Cerastoderma edule *in control (grey bar) and treatment patches (black bar).*

In addition to their faster growth in the treatment plots, distinctly higher post-settlement densities of *P. elegans, N. diversicolor* and *M. balthica* in the treatments (Fig. 2) resulted in a significantly higher production of these species in the treatments (Fig. 3b, Table 1). In contrast to the eroding control sediments, treatment sediments were found to be more stable, presumably due to the stabilizing effect of a dense diatom mat and the dense aggregations of polychaete tubes (Montserrat *et al.* 2008). Consequently, enhanced accumulation due to a lower resuspension of settled recruits is hypothesized to primarily cause the higher abundances of *P. elegans, N. diversicolor* and *M. balthica* in the treatments.

	t-statistic	**Df**	**p**	**Transformation**
Growth rate				
Pygospio elegans	-2.983	4	**0.041**	Log (x+1)
Nereis diversicolor	-2.891	4	**0.045**	Log (x+1)
Macoma balthica	-4.640	4	**0.010**	Arcsine (x)
Cerastoderma edule	0.763	4	0.488	Log (x+1)
Production				
Pygospio elegans	-2.811	4	**0.048**	Log (x+1)
Nereis diversicolor	-8.720	4	**0.001**	Log (x+1)
Macoma balthica	-5.279	4	**0.006**	Log (x+1)
Cerastoderma edule	1.843	4	0.139	Log (x+1)

Table 1. *Student t-test results for difference in growth rate and production of the identified cohorts between control and treatments.*

Conclusion

In summary, the present study shows that the growth rate and production of microphytobenthos feeding macrobenthos juveniles is enhanced in a community with low bioturbation intensity. The available data suggest that this enhanced recruitment success resulted both from a lower post-settlement resuspension and lower interference competition for food due to an initially higher primary production. Consequently, because recruitment is the foundation upon which all subsequent interactions within the community take place (Woodin *et al.* 1995), biotic-physical interactions that affect primary production and sediment stability are of fundamental importance to tidal flat macrobenthic community structure and functioning.

Acknowledgements

We thank D. Van Gansbeke and M. Houtekamer for their assistance during the processing of the samples, as well as B. Beuselinck, D. Peelaers, J. van Soelen and B. Koutstaal for their help during sampling. This research was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen), the FWO Vlaanderen (FWO-KISS 3G048005), the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministry of Economic Affairs. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446).

148084

CHAPTER 6

TIDAL FLAT NEMATODE RESPONSES TO HYPOXIA AND SUBSEQUENT MACROFAUNA-MEDIATED ALTERED SEDIMENT PROPERTIES

Adapted from:

Van Colen C., Montserrat F., Verbist K., Vincx M., Steyaert M., Vanaverbeke J., Herman P.M.J., Degraer S., Ysebaert T. (in press)

Tidal flat nematode responses to hypoxia and subsequent macrofauna-mediated altered sediment properties
Marine Ecology Progress Series

Abstract

To assess the role of macrofauna-mediated sediment changes on nematode community recovery, the temporal development of macrobenthos, nematode communities and sediment properties following hypoxia were examined in 16 m^2 replicated plots over a 6-months period.

Hypoxia drastically changed nematode community composition (i.e. reduced diversity and abundances of all dominant nematodes, except *Odontophora spp.*) but complete mortality, as was the case for the macrobenthos, did not occur. Macrofauna diversity recovered slowly but community composition approached that of control communities after several months. In contrast to this, nematode diversity recovered to control values within one month but, subsequently, decreased again and hence no clear convergence towards the control community composition was apparent. This diversity decline and lack of community recovery was mainly attributed to abundance overshoots of the epistrate feeding nematodes *Chromadora spp.*, *Daptonema spp.* and *Ptycholaimellus ponticus* in the treatments, which dominated the treatment community after two months.

Nematode community reassembling was strongly related to the coupled macrobenthos-environmental temporal development. The dynamics of two sediment characteristics, which were both mediated by the colonizing macrobenthos, are presented as possible determinant factors for this relationship: (1) low nematode post-settlement resuspension resulting from stable sediments at early macrofauna recovery stages and (2) enhanced nematode reproduction and settlement success in a dense microphytobenthos mat in relation to the temporal variation in macrobenthos grazing pressure and bioturbation. In conclusion, the strong relationships between macrobenthos, environmental and nematode community development after hypoxia highlight the importance of macrobenthos-sediment interactions in the recovery and structuring of nematode communities.

Keywords: *Benthic community recovery, Macrofauna – meiofauna interactions, Sediment dynamics, Hypoxia, Intertidal mudflat, Westerschelde estuary*

Introduction

Benthic communities of estuarine and coastal ecosystems are increasingly affected by disturbances. Besides physical disturbances, such as fishing, dredging and dredge disposal which have adversely impacted benthic communities over the last decades (Newell *et al.* 1998, Thrush & Dayton 2002), shallow estuarine and coastal sediments are worldwide exposed to enhanced anthropogenic nutrient inputs often causing permanent or seasonally depleted dissolved bottom oxygen (DO) concentrations characterized by a high benthic mortality (Cloern 2001, Diaz 2001). While hypoxic (DO < 2 mg l^{-1}) and anoxic (DO = 0 mg l^{-1}) environments have existed through geological time, their occurrence in shallow coastal and estuarine areas appears to be increasing worldwide, most likely accelerated by human activities, e.g. agricultural runoff and industrial sewage (Wolanski 2007).

Research on the restoration of benthic communities from prolonged and/or seasonal periods of hypoxia has mainly been targeted to macrobenthos (e.g. Gamenick *et al.* 1996, Beukema *et al.* 1999, Van Colen *et al.* 2008), whereas recovery of meiobenthic organisms has been examined to a much lesser extent. Nematodes often constitute up to 90 % of the meiobenthic community in intertidal flats (Soetaert *et al.* 1994) and are predators of meio –and microfauna and serve as a food source for macrofauna and juvenile fish (Heip *et al.* 1985, Coull 1990). In tidal flats, nematodes reach extremely high abundances in the upper centimeters (> 10^6 ind. m^{-2})(e.g. Steyaert *et al.* 2003) and their distribution is determined by sediment composition, salinity, organic content and oxygen (e.g. Soetaert *et al.* 1994, Steyaert *et al.* 2003). Furthermore, macrobenthic organisms have the potential to affect nematode communities since macrobenthos can drastically change the physical and chemical characteristics of the sediment (i.e. bio-engineering capacity) (e.g. Reise 1983, Olafsson 2003 and references therein). Especially after disturbances causing severe benthic mortality (e.g. prolonged hypoxia), macrobenthos may be expected to influence nematode community structure considerably since recolonizing macrobenthos is known to change physical and chemical sediment properties, often in a successional sequence (e.g. Pearson & Rosenberg 1978, Montserrat *et al.* 2008). Since nematodes have relatively short generation times, they may be expected to rapidly respond to such macrobenthos-mediated changing environments. A few studies have examined macrobenthos and nematode community responses to disturbed tidal flat sediments simultaneously (e.g. Dittmann *et al.* 1999, Bolam *et al.* 2006) but the pattern and the importance of macrobenthos-nematode interactions during the recovery processes remains poorly understood.

In order to investigate how macrofauna-mediated changes in sediment properties may affect nematode community recovery we conducted a field experiment in which we have simultaneously examined the temporal development of sediment properties, the macrobenthic and nematode community following hypoxia, over a 6-month period. We specifically addressed the following questions:

- How do nematodes respond to, and develop after, hypoxia in terms of diversity, abundance and community structure?
- Are the temporal patterns of nematode recovery related to the temporal patterns in the coupled macrobenthos-environmental development?

Materials & methods

Experimental set up, sampling and laboratory treatment

The experiment was conducted at Paulinapolder, a tidal flat located along the southern shore of the polyhaline part of the Westerschelde estuary, the Netherlands (51°21'24'' N, 3°42'51'' W). Nematodes constitute 81 – 98 % of the meiobenthos abundance and occur predominantly (~ 65 %) in the upper cm layer at the study site (C. Van Colen, unpublished data). Triple replicated 4 x 4 m treatment and control (i.e. undisturbed sediment) plots were randomly positioned within a 25 x 25 m homogeneous study site, at least 5 metres from each other. Hypoxic conditions were created by covering the treatment plot sediments by a water-proof polyethylene sheet (0.1 mm thick) and a tarpaulin sheet (140 g m^{-2}) and digging in the edges till 30 cm, following Beukema *et al.* (1999) and Dittman *et al.* (1999). After 40 days (30 March 2005), the plots were opened and biotic and abiotic samples were collected at low tide at a randomly selected subplot (37.5 x 37.5 cm) immediately after opening of the plots (day 0) and subsequently with a maximum interval of two weeks, during six months. Depending on the response variable, one (macrofauna, nematodes, organic matter, mud content and sediment stability) or two (bed level elevation, surface Chlorophyll *a*, oxygen concentration, water content, nutrient pore water concentration) subplots were sampled in each plot. Nematode samples were collected from the upper centimetre using a perspex corer (inner ø 3.6 cm, i.e. 10 cm^2) and subsequently fixed in a neutral 4 % formaldehyde tap-water solution. Treatment plots were characterized by different macrofauna communities and sediment properties at day 0, 28, 56, 98, 112 and 175 (Van Colen *et al.* 2008).

Nematode samples from these sampling occasions were further processed in order to understand how nematode communities respond to and recover from hypoxia and how macrofauna-mediated environmental changes may affect this process. Nematodes retained on a 38 µm sieve were extracted from the sediment by centrifugation with Ludox (Heip *et al.* 1985). After staining with Rose Bengal, 120 nematodes were randomly selected, transferred to glycerine and mounted on slides for identification to species or genus level using the NEMYS database (Steyaert *et al.* 2005). For further information on the study site, experimental set up and abiotic and biotic sample processing, the reader is referred to Van Colen *et al.* (2008), Montserrat *et al.* (2008) and Rossi *et al.* (2008).

Statistical analyses

Two-way analysis of variance was used to test for significant effects of the factors Time and Treatment (i.e. hypoxia vs. control) on univariate community characteristics (total nematode abundance, number of genera, Hill's N_1 diversity index and the abundances of the genera contributing most to the dissimilarity between control and treatment assemblages at each sampling occasion, as revealed by SIMPER analysis). Prior to the analyses, Bartlett's and Cochran's tests were used to verify for homogeneity of variances and data not meeting these criteria were appropriately transformed. Additionally, to assess recovery status, treatment effects within a sampling occasion were explored by one-way analysis of variance. In the context of the present study we defined recovery of the impacted plots as having occurred when the impacted plots have attained a state that is no longer significantly different from the control plots.

A correlation based principal component analysis (PCA) using normalised Euclidean distance was performed to visualize treatment and temporal differences in the environmental variables. The effect of Treatment (averaged over time groups) and Time (averaged over treatment groups) on nematode community structure was examined using two-way crossed analysis of similarity (ANOSIM) on square root transformed abundance data and visualized using mulitidimensional scaling (MDS). Furthermore, dissimilarities between control and treatment nematode communities at each sampling occasion and the contribution of those genera responsible for the dissimilarity were determined using the similarities of percentage procedure (SIMPER). Finally, the RELATE routine was applied to examine whether the environmental, macrofauna and nematode community development followed similar directional changes over time (i.e. seriation) and the BEST routine was applied in order to assess relations between the environmental, macrofauna and nematode multivariate patterns.

Univariate statistical analyses were performed using Statistica 7.0 (Statsoft, Inc. 1984-2004), multivariate analyses were performed using the Plymouth Routines In Multivariate Ecological Research (PRIMER) package, version 6 β (Clarke & Gorley 2006). For a detailed analysis of the environmental and macrobenthos recovery pattern, the reader is referred to Van Colen *et al.* (2008) and Montserrat *et al.* (2008).

Results

Nematode response to and recovery from hypoxia

Total nematode abundance, total number of genera and Hill's N_1 diversity index, were significantly reduced in the treatment plots at day 0 (Fig. 1). According to the SIMPER analysis, differences between the control and treatment communities at day 0 mainly resulted from significantly reduced abundances of *Oncholaimellus sp.1* (-61 %) and species belonging to the genera *Chromadorita* (-87 %), *Viscosia* (-70 %), *Daptonema* (-80 %) and *Neochromadora* (-100 %). Next to *Neochromadora,* only less abundant genera (i.e. < 18 ind. $10cm^{-2}$ in control plots at day 0) were absent at day 0 in the treatment plots. Large but non-significant reductions in abundances of other species and genera in the treatment plots were found for *Ptycholaimellus ponticus* (-59 %), *Anoplostoma viviparum* (-37 %), *Sphaerolaimus spp.*(-58 %), *Sabatieria spp.* (-38 %) and *Paramonhystera spp.* (-24 %). The only abundant genus, not reduced after hypoxia, was *Odontophora* (28 ± 14 ind. $10cm^{-2}$ ± SE; + 3 %). Highest abundances in the treatment plots were reached for *Anoplostoma* *viviparum, Oncholaimellus sp.1* and *Paramonhystera spp.* (58 ± 23; 49 ± 22 and 41 ± 17 ind. 10cm^{-2} ± SE, respectively). However, these species were more abundant in the control plots.

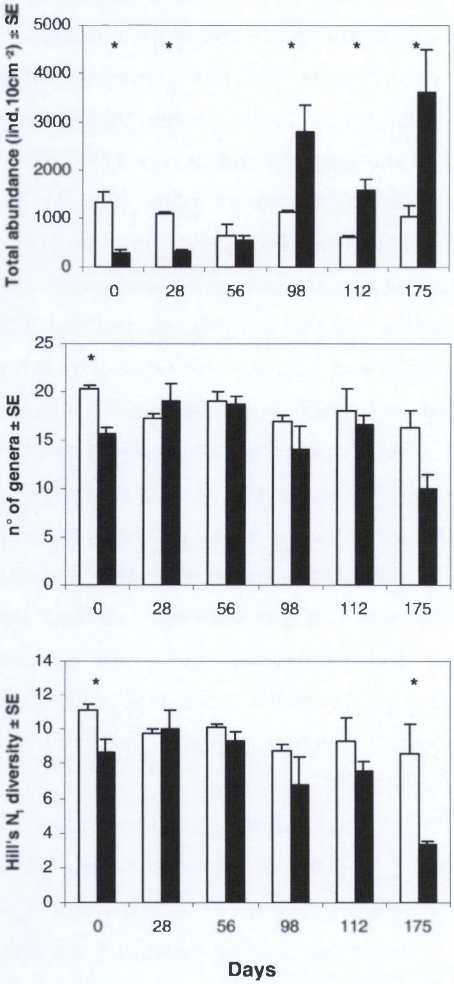


Fig. 1. *Temporal variation of general univariate community characteristics in control (white) and treatment (black) plots: Total abundance, total number of genera and Hill's* N_1 *diversity. * significant treatment effects for sampling occasions, deduced from one-way analysis of variance.*

Two-way analysis of variance indicated a significant Time and significant Treatment effect for the total number of genera and Hill's N_1 diversity index, whereas only a significant Time effect was found for the total nematode abundance (Table 1). Total nematode abundance recovered at day 56, followed by a significant overshoot in the treatment plots, whereas the total number of genera and Hill's N_1 diversity index already recovered at day 28. However, the latter variables were consistently lower in the treatment plots from day 56 onwards as compared to the controls and at day 175, Hill's N_1 diversity index was significantly lower in the treatment plots (Fig. 1). Results of two-way analysis of variance revealed a significant Time and Treatment effect for *Chromadora spp.* and *Ptycholaimellus ponticus,* whereas only significant Treatment effects were found for *Oncholaimellus sp.1, Viscosia spp.* and *Anoplostoma viviparum* and a significant Time effect was apparent for *Daptonema spp.* (Table 1). All these species and genera had recovered or showed an abundance overshoot in the treatment plots at day 175. Abundance overshoots in the treatment plots were noticed for *Daptonema spp., Ptycholaimellus ponticus* and *Chromadora spp.* from day 56 onwards (Fig. 2). The contribution of the juveniles to the total abundance of *Daptonema spp.* remained quite stable in the treatment plots during the course of the experiment, whereas this variable showed a distinct temporal pattern for *Ptycholaimellus ponticus* and *Chromadora spp.* An increase in the relative abundance of *Chromadora spp.* juveniles occurred during the second month after hypoxia, while the highest relative juvenile abundances of *Ptycholaimellus ponticus* were present from day 98 onwards.

Two way crossed ANOSIM revealed significant treatment (R = 0.748, p = 0.01) and temporal (R = 0.6, p = 0.01) differences on the nematode community structure as is also shown by the clear separation and temporal variation of both communities in the non-metric multidimensional scaling (MDS) (Fig. 3). Temporal variation in the treatments was greater as compared to the controls but no apparent convergence of the treated community towards the control community occurred throughout the experiment. Consequently, at day 175 both communities were still clearly different (ANOSIM R = 0.593, averaged dissimilarity = 41.8 %, Table 2). Species contributing most to the community dissimilarity changed throughout the experiment (Table 2). Until day 56, dissimilarities between both communities were mainly attributed to the lower abundances of *Oncholaimellus sp.1*, *Viscosia spp.* and *Anoplostoma viviparum* in the treatment plots, while abundance overshoots in the treatments of *Daptonema spp.*, *Ptycholaimellus ponticus* and *Chromadora spp.* contributed relatively most to the dissimilarity from day 98 onwards.

	Treatment		Time	
	F	*p*	*F*	*p*
Total abundance †	0.29	0.592	4.06	**0.006**
Total number of genera ‡	7.56	**0.010**	4.25	**0.005**
Hill's N_1 diversity ‡	12.34	**0.001**	5.93	**0.001**
Daptonema spp.‡	1.06	0.312	6.00	**0.001**
Oncholaimellus sp.1 ‡	22.80	**< 0.001**	1.15	0.359
Chromadora spp.‡	60.30	**< 0.001**	15.67	**< 0.001**
Viscosia spp.‡	24.14	**< 0.001**	1.07	0.396
Anoplostoma viviparum †	21.19	**< 0.001**	2.35	0.066
Ptycholaimellus ponticus ‡	6.60	**0.016**	9.32	**< 0.001**

Table 1. *Two-way analysis of variance tests for univariate nematode community characteristics and dominant nematode genera (df = 1, 5; respectively for treatment and time). Values in bold are significant terms (p < 0.05). Assumptions for homogeneity of variances were met for all variables (Bartlett-Cochran test; p > 0.05). Analysis performed on square root (†) and log(x+1) (‡) transformed data.*

Day	Anosim R	% D	Species contribution to the dissimilarity	%	Species contribution to the dissimilarity	%	Species contribution to the dissimilarity	%
0	0.63	46.8	*Oncholaimellus sp.1*	10.3	*Chromadorita*	8.8	*Viscosia spp.*	8.7
28	1	52.4	*Viscosia spp.*	12.2	*A. Viviparum*	11.7	*Oncholaimellus sp.1*	10.9
56	0.74	37.5	*P.ponticus*	*10.3*	*Oncholaimellus sp.1*	9.0	*Viscosia spp.*	8.7
98	0.74	36.5	*Chromadora spp.*	*19.1*	*P.ponticus*	*14.7*	*Daptonema spp.*	*13.2*
112	1	40.5	*Chromadora spp.*	*16.3*	*Daptonema spp.*	*14.1*	*P.ponticus*	*8.8*
175	0.59	41.8	*Chromadora spp.*	*29.1*	*P.ponticus*	*6.2*	*Paramonhystera*	5.6

Table 2. *Results of ANOSIM and SIMPER analysis based on square root transformed nematode genera abundances indicating the strength of separation between the communities (Anosim R) and the total dissimilarity (% D) between control and treated communities at each sampling occasion and the contribution of the most discriminating genera to this dissimilarity. Italicized values indicate higher genera abundances in the treatment plots.*

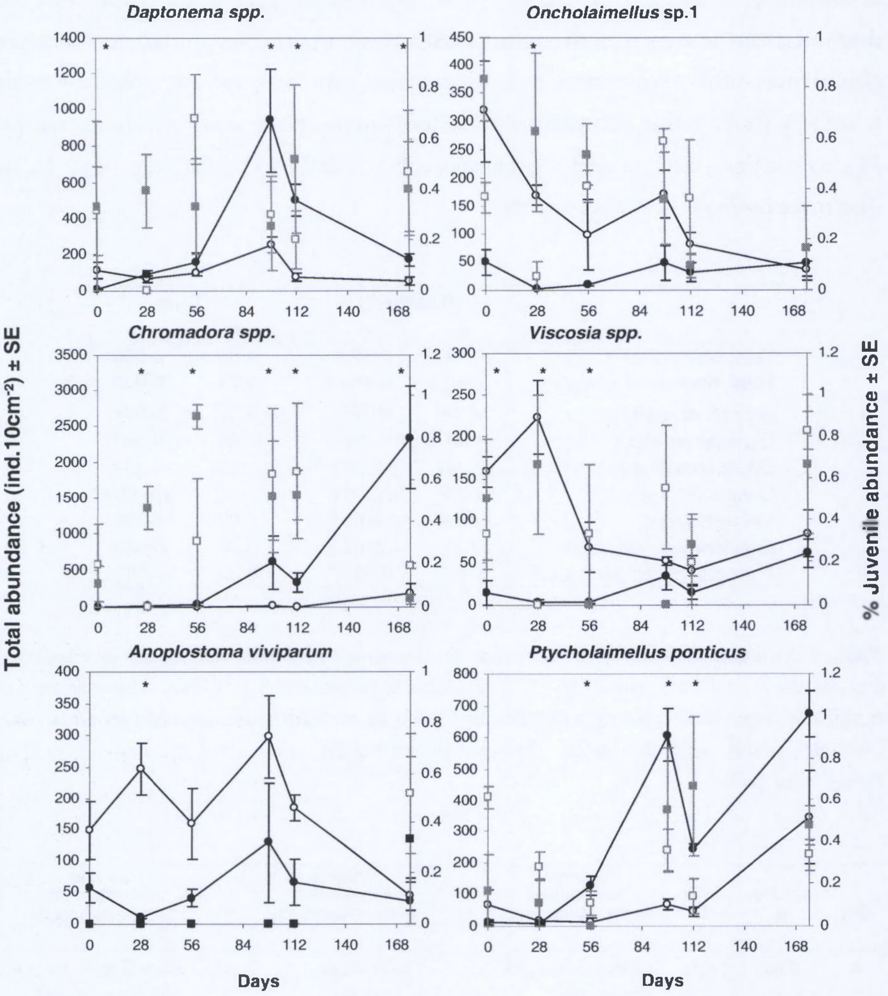
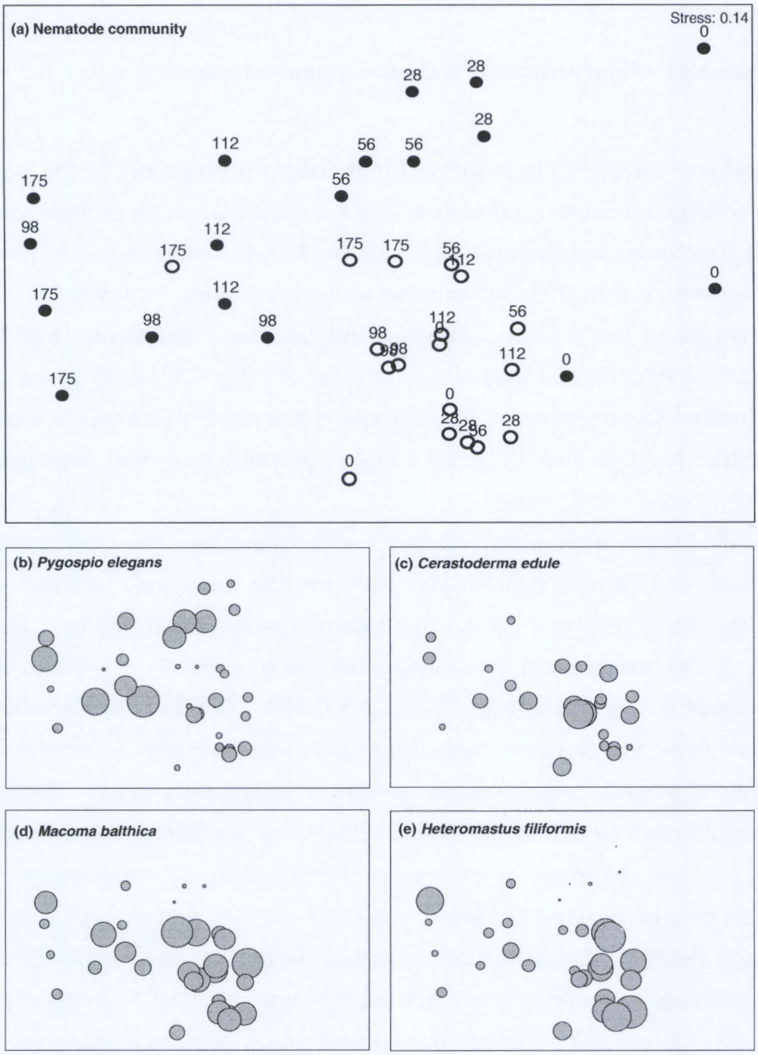


Fig. 2. Temporal variation of the total abundance of the dominant nematodes Daptonema spp., Chromadora spp., Anoplostoma viviparum, Oncholaimellus sp.1, Viscosia spp. *and* Ptycholaimellus ponticus *in control (open circles) and treated (filled circles) plots. The contribution of juveniles to the total abundances is given on the secondary y-axis (control = open grey squares, treatment = filled grey squares). Asterisks indicate significant treatment effects for the total abundance per sampling occasion, deduced from one-way analysis of variance.*

Relationships with macrofauna and environmental recovery

Principal component analysis showed that sediment properties in the treatment plots converged towards control conditions during the course of the experiment (Fig. 4). Treatment sediments at day 0, 28 and 56 were more reduced (lower free oxygen penetration and higher ammonium concentration, i.e. lower PC1 values) as compared to the control sediments and treatment sediments from day 98 onwards. Furthermore, treated sediments at day 28 and 56 could be distinguished from later sampling occasions and control sediments since they had higher elevation and contained a higher proportion of mud, organic carbon and Chl*a* (i.e. lower PC1 values).

From day 28 onwards, RELATE test within the treatment plots revealed a significant and strong relationship between the temporal variation of the nematode community and (1) the macrofauna species biomasses (ρ = 0.830; p = 0.043), (2) the macrofauna species abundances (ρ = 0.782; p = 0.020) and (3) environmental development (ρ = 0.572; p = 0.035). BEST analysis revealed that maximal matching between nematode and macrobenthic assemblages was explained by four macrobenthic species: *Cerastoderma edule, Heteromastus filiformis, Macoma balthica* and *Pygospio elegans* (ρ = 0.879, ρ = 0.952; for their abundance and biomass, respectively). Furthermore, a combination of the variables oxygen penetration depth, sediment stability and organic carbon best explained the multivariate nematode pattern in the treatment plots (BEST, ρ = 0.964). Adding chlorophyll a to this combination resulted in a slightly lower correlation (ρ = 0.952). Within the control plots, temporal variation of the nematode community was only, but to a lesser extent, related to the temporal variation in environmental characteristics (ρ = 0.572, p = 0.035). BEST revealed that a combination of water content, bed level and sediment stability best explained the multivariate nematode pattern in the control plots (ρ = 0.879).



(a) Nematode community

(b) *Pygospio elegans*

(c) *Cerastoderma edule*

(d) *Macoma balthica*

(e) *Heteromastus filiformis*

Fig. 3. *(a) MDS ordination diagram based on square root transformed data of nematode genera abundances in treatment (filled circles) and control plots (open circles) at the sampling occasions. To visualize the relationship with the macrobenthos recovery and resemblance to control plots, abundances of* P. elegans *(b) and* C. edule *(c) and biomasses of* M. balthica *(d) and* H. filiformis *(e) are superimposed on the same ordination, using circles which sizes reflect the magnitude of these variables.*

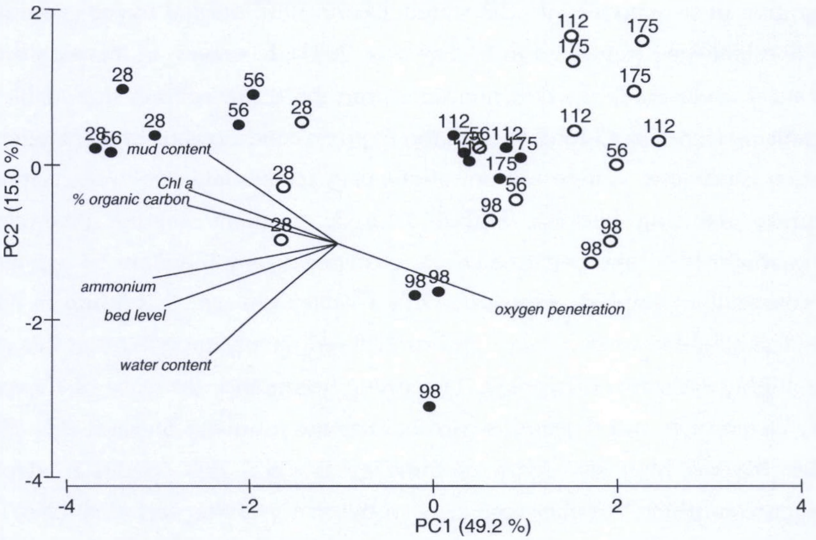


Fig. 4. Principal component analysis ordination (PCA) based on normalised environmental data, showing the temporal changes in the control (open circles) and treatment plots (filled circles). Vectors presenting the environmental variables relative to PC1 and PC2 are superimposed. Note that sampling occasion day 0 (extreme high ammonium concentrations in the treatments) is omitted to enhance visualisation.

Discussion

Nematode response to hypoxia

The induced hypoxic conditions did not result in complete nematode mortality. Such severe mortality was however observed for the macrobenthos (see Van Colen *et al.* 2008), which, consistently with Josefson & Widbom (1988) and Mödig & Olafsson (1998), indicates a higher resistance of nematodes to hypoxic conditions as compared to the macrobenthos. Nonetheless, hypoxia adversely impacted the nematode community, i.e. reduced number of genera and abundances causing a less diverse community, in general. Such effects have commonly been noticed after hypoxic events and have been attributed to migration of nematodes into the water column until normal oxygen conditions are re-established (reviewed in Wetzel *et al.* 2001). However, in this experiment, the sheet cover made upward migration into the water column impossible and organisms were forced to cope with the hypoxic conditions or to die. Under low oxygen conditions, sulfide concentrations may accumulate due to the activity of sulphate reducing bacteria. Sulfide is toxic to many marine invertebrates (Baggarinao 1992) and negative effects of hypoxia may therefore be aggravated by concomitant sulphide exposure. Only *Odontophora spp.* was found in almost identical abundances in hypoxic and control sediments, implying that this genus was highly resistant to hypoxia. The strong abundance decrease of *Daptonema spp.*, *Viscosia spp.* and *P. ponticus* corroborates the results of Steyaert *et al.* (2007), indicating the high sensitivity of these genera and this species to depleted oxygen conditions. Another similarity to the study of Steyaert *et al.* (2007) was that *Sabatieria* abundances were moderately reduced although species belonging to this genus have often been shown to resist hypoxic events (e.g. Bouwman *et al.* 1984, Wetzel *et al.* 2002). Further, no significantly higher abundances were noticed for any genus, indicating that, in hypoxic conditions, no nematode species belonging to a certain genus could take advantage of the reduced abundances of others.

Nematode recovery and relation with the coupled macrofauna - environmental recovery

Nematode diversity recovery occurred fast (i.e. within one month) in comparison with macrofauna diversity recovery, indicating the higher resilience of nematodes as compared to the macrofauna. Macrobenthos diversity recovery took six months and was strongly dependant on successful colonization of juvenile macrobenthos (Van Colen *et al.* 2008). In contrast, both juvenile and adult recruitment were important nematode recovery mechanisms in this study (Fig. 2). Fast nematode recovery has often been found in tidal flats (e.g. Savidge & Taghon 1988, Atilla & Fleeger 2000) and is probably mainly attributable to strong currents in these areas. Commito and Tita (2002) found that suspended nematodes in the water column of an intertidal flat are in particular epistrate feeders, most probably because they are more susceptible to erosion induced sediment transport. In this experiment, the strong abundance increase of the epistrate feeders *Ptycholaimellus ponticus* and *Chromadora spp.* and the facultative epistrate feeder *Daptonema spp.* in the treatments occurred concomitantly with the sediment erosion in the control plots between day 28 and day 98, which suggests that recolonisation via the resuspension pathway is an important recovery mechanism in the present study. Moreover, the strong abundance overshoots of these epistrate feeding nematodes in the treatments (1) resulted in a diversity decrease in the treatment community from two months after opening of the plots onwards and (2) largely contributed to the lack in community recovery (i.e. lack of directional nematode reassembling towards the controls).

Nematode reassembly was strongly related to the development of both macrofauna and environmental characteristics following hypoxia, whereas the temporal variation in the control plots was only related to environmental temporal variation. Moreover, a subset of species (*P. elegans, M. balthica, C. edule* and *H. filiformis*), all identified to mediate sediment properties significantly in this experiment (Van Colen *et al.* 2008, Montserrat *et al.* 2008), best 'matched' the nematode development following hypoxia. Macrobenthos-mediated physical-biological interactions have been shown to influence nematode communities (Olafsson 2003 and references therein) and two types of such interactions are hypothesized as structuring factors of the nematode community recovery in this experiment: (1) sediment bio(de)stabilization and post-settlement resuspension and (2) the development and decomposition of a dense microphytobenthos mat due to temporal variation in macrobenthic grazing and bioturbation.

Interaction 1: Macrofaunal bioturbation and post-settlement resuspension

In comparison with the controls, treatment sediments remained at the same intertidal height, were more stable and consisted of a higher proportion of mud during the second and third month after hypoxia (Montserrat *et al.* 2008). This presumably resulted from the combined effect of the dense aggregations of opportunistic biostabilizing tube-building polychaetes (*P. elegans, Polydora cornuta*) and the low biomass of biodestabilizing species (*M. balthica, C. edule* and *H. filiformis*) during early macrobenthic recovery stages (Fig. 3). These conditions may reduce the boundary shear stress, so that fine particles (e.g. nematodes) are less easily suspended after settlement (Eckman 1983). Hence, lower resuspension in the treatments may explain the enhanced accumulation of mud particles and nematodes as compared to eroding control sediments. These findings corroborate Thrush *et al.* (1997) who found increased sediment instability after the experimental removal of the tube-building polychaete *Boccardia syrtis* from a New Zealand sandflat.

Interaction 2: Macrobenthos grazing, bioturbation and microphytobenthos

The development of a dense microphytobenthos bloom, resulting from the low grazing pressure by macrofauna during the first month, might have favoured nematodes with the reproductive potential to take advantage of an organic enrichment, such as *Ptycholaimellus ponticus* and *Chromadora spp.* (Gee & Warwick 1985, Schratzberger & Warwick 1998, Schratzberger *et al.* 2004). Juvenile abundance contributes largely to the total abundance overshoots of the diatom feeding nematodes *P. ponticus* and *Chromadora spp.* Moreover, for *P. ponticus* it was particularly clear that initial colonization predominantly occurred through adult recruitment while the proportion of juveniles of this species became larger at a later stage. Further, significantly higher abundances in the treatment plots were also found for the non-selective deposit feeder *Daptonema spp.* Based on observations from microcosm feeding experiments Moens & Vincx (1997) concluded that species belonging to this genus also feed on diatoms. High abundances for *Daptonema spp., P. ponticus* and other chromadorid nematodes have been reported from different recolonisation studies (Wetzel *et al.* 2002, Schratzberger *et al.* 2004) indicating the opportunistic behaviour of these nematodes.

In addition to a lower post-settlement mortality and enhanced reproduction due to the favourable conditions during the second and third month, enhanced settlement may have contributed to the significant abundance overshoot in the treatment plots from day 98 onwards. Evidence for such active settlement in a diatom biofilm was found by Ullberg and Olafsson (2003). However, it should be noted that the results from the referred study are deduced from a still-water laboratory experiment while active habitat choice becomes presumably less important at higher hydrodynamic conditions (i.e. field conditions), as suggested by Commito and Tita (2002). As far as we know, evidence for active habitat choice of nematodes from field experiments is still lacking.

The dominance of *Ptycholaimellus ponticus* and *Chromadora spp.* in the treatment community was also maintained after the decline of tube-building populations and microphytobenthos biomass resulting in the recovery of the sediment stability at day 98 – 112 (Montserrat *et al.* 2008). These changes, characterizing the shift between intermediate and later macrobenthos succession stages, were related to the enhanced grazing pressure of surface deposit feeding macrofauna populations and enhanced sediment disturbance resulting from the colonization of *H. filiformis* and growth of *M. balthica* and *Nereis diversicolor* (Van Colen *et al.* 2008). However, macrobenthos grazing pressure and disturbance due to bioturbation remained lower as compared to the controls since macrobenthos biomass had not yet recovered after six months. Further, some epistrate feeding nematodes can also feed on mineralized material (i.e. throphic plasiticity)(Moens & Vincx 1997, Danovaro & Gambi 2002). Therefore, in addition to a lower competition with macrofauna, *P. ponticus* and *Chromadora spp.* probably also benefit from the subsequent decomposition of the microphytobenthos bloom by bacteria.

Conclusion

This study shows that, in comparison with the macrobenthos, nematode communities are much more resistant to hypoxia. Following severe hypoxia, nematode community reassembly was strongly related to the recovery of the macrobenthos community and the concomitant changes in sediment properties. The dynamics of two sediment characteristics, which were both mediated by the colonizing macrobenthos, were suggested as determinant factors for these relationships: (1) low post-settlement resuspension resulting from low macrobenthic bioturbation impact at early macrofauna recovery stages and (2) the development and subsequent decomposition of a dense microphytobenthos mat in relation to the temporal variation of macrobenthos grazing pressure and bioturbation.

Acknowledgements

This research is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen), the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministery of Economic Affairs. The authors want to thank D. Van Gansbeke, D. Schram, M. Houtekamer, C. Kleppe, H. Francke and Y. van der Maas for their assistance during the processing of the samples, as well as B. Beuselinck, D. Peelaers, J. van Soelen and B. Koutstaal for their help during sampling. S. Van Gaever and B. Mercx are acknowledged for their assistance and providing information on nematode identifications. T. Moens and three anonymous referees are acknowledged for their valuable comments and advice on this paper. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446, contribution number 9003).

CHAPTER 7

A LONG-TERM STUDY OF THE MACROBENTHOS RECOVERY FOLLOWING HYPOXIA-INDUCED MASS MORTALITY

Van Colen C., Montserrat F., Vincx M., Herman P.M.J., Ysebaert T., Degraer S.

Abstract

Macrobenthos recovery after hypoxia-induced mass mortality was assessed during 3 years in replicated 16 m^2 plots located at an estuarine tidal mudflat (Paulinapolder, Westerschelde estuary, SW the Netherlands).

During the first 2 years, a Pearson-Rosenberg type of community recovery towards the control sediments took place along with the improving bottom water oxygen conditions. After 3 months, the spionid polychaetes *Pygospio elegans* and *Polydora cornuta* became superabundant (i.e. opportunistic peak), followed rapidly by a steep decline (i.e. ecotone point). Subsequently, a moderate increase in species richness and a steep increase in biomass, related to the growth of the long-lived species *Scrobicularia plana* and *Macoma balthica* occurred (i.e. transition region). Beyond this transition region, however, the recovering community diverged again from the ambient sediments. This divergence mainly resulted from the higher biomasses of *M. balthica* and *S. plana* in the recovering plots towards the end of our experiment. Enhanced recruitment success and subsequent growth of both species in absence of high interference from bioturbation during early recovery stages is hypothesized as the causal pattern for this biomass overshoot in the recovering community at the long-term. In particular, biomass of *Cerastoderma edule*, an important long-lived bioturbator at the study site, remained low in the recovering plots in comparison with the

ambient sediments due to a general, successive recruitment failure in 2006 and 2007.
The present study indicates that, despite community recovery may follow a more or less predictable pathway at early recovery stages (i.e. Pearson-Rosenberg recovery pattern), divergence may occur at the long term because biotic interactions at early recovery stages may become important at later recovery stages whenever the influence of the affected species becomes greatest. Consequently, such lagged effects may have important consequences for macrobenthos distribution, especially in areas which are frequently subjected to disturbances.

Keywords: *Macrobenthos, Recovery, Succession, Hypoxia, Tidal mudflat*

Introduction

Worldwide, about 85 % of the coastline (Airoldi & Beck 2007) is threatened by a wide range of anthropogenic disturbances (Halpern *et al.* 2007, 2008). For instance, enhanced anthropogenic nutrient inputs originating from increasing agricultural runoff and industrial sewage have resulted in depleted dissolved bottom water oxygen concentrations (DO) in shallow estuarine and coastal sediments. When DO declines below 0.5 mg l^{-1}, mass mortality of the macrobenthos may occur (Diaz & Rosenberg 1995). Soft-sediment macrobenthic communities provide important ecological goods and services. For example, estuarine tidal flat macrobenthos recycle nutrients (e.g. Lohrer *et al.* 2004), considerably alter sediment transport processes (e.g. Meysman *et al.* 2006, Solan *et al.* 2008) and represent an important trophic linkage, being a food source for epibenthic crustaceans, fish and birds (e.g. Hampel et al 2004) and by feeding on benthic algae and bacteria (e.g. Herman *et al.* 2000, Van Oevelen *et al.* 2006). Hence, depletion of the benthos can have dramatic consequences on estuarine ecosystem functioning. For environmental impact assessment and management it is therefore important to gather knowledge on macrobenthic recovery dynamics after severe disturbance events (e.g. hypoxia).

The Pearson-Rosenberg (PR) model is a generally applied method for examining macrobenthos community response to disturbance, based on the development of the community species richness, abundance and biomass (SAB) (Pearson & Rosenberg 1978). The model originally exemplified the changes in faunal community structure in a stable, subtidal muddy habitat along a gradient from excessive organic enrichment to 'normal' conditions but has been shown to hold for physical disturbance (Rhoads & Germano 1986) and recovery from oxygen deficiency (Rosenberg *et al.* 2002) as well. According to the PR model (Fig. 1), species richness, abundance and biomass are low at highest organic enrichment. A peak in abundance represents the point along the organic enrichment gradient were small-sized opportunists become superabundant and cause a secondary biomass maximum. This 'opportunistic peak' is followed by a steep decrease in abundance and a rapid increase in species richness, defined as the 'ecotone point'. Beyond this ecotone, a 'transition region' occurs where the long-lived fauna dominate the community and cause a primary peak in biomass due to moderate organic enrichment. Afterwards, the benthos declines to its background values. Furthermore, Pearson & Rosenberg (1987) stressed the availability of food as a structuring factor of their PR model and Nilsson & Rosenberg (1997) related the successional community changes along the disturbance gradient to a benthic habitat quality index, which takes the presence of (sub)surface biotic structures and the redox potential discontinuity (RPD) layer into account. The main advantage of the PR model is that the use of different simple univariate community characteristics allows for the definition of a general stress relationship, which can be compared across different ecosystems (Rakocinski *et al.* 2000). Moreover, the ratios between the different metrics in the model, have stimulated the development of different biotic indices (see Pinto *et al.* 2009 for a review).

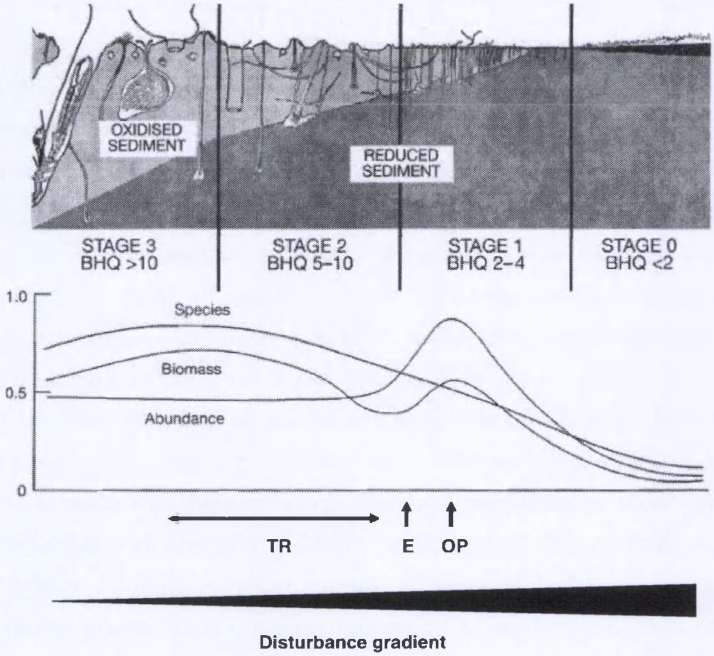


Fig. 1. *Graphical model of infaunal succession stage distribution along a gradient of increasing environmental disturbance (after Pearson & Rosenberg 1978), and the associated benthic habitat quality (BHQ) index (after Nilsson & Rosenberg 1997). OP = peak of opportunists, E = ecotone point and TR = the transition region.*

Despite the general applicability of the PR model, recovery studies in shallow water and tidal flat sediments have yielded variable results, not always in full accordance to a typical PR recovery pathway (Thrush & Whitlatch 2001). This may be ascribed to the limited temporal and spatial scale at which studies have been conducted: studies with a limited time frame may have been too short to identify full recovery, passing through all successional stages whereas small-scale studies will not identify different recovery stages due to the fast immigration of ambient organisms (Günther 1992). In accordance to the PR model, the two large-scale recolonisation studies in North-Western European tidal flats which have lasted > 1 year (Beukema *et al.* 1999, Dittmann *et al.* 1999) indicate an abundance overshoot of typical opportunistic species and early colonizers. However, both studies are not consistent with the total model because not all metrics have been measured, e.g. biomass (Dittmann *et al.* 1999) or the total abundance remains high after an opportunistic peak due to very high abundances of later colonists (Beukema *et al.* 1999).

We assessed the recovery of a macrobenthic tidal mudflat community in large plots after complete mortality due to experimentally induced hypoxia. Previously, we described the macrobenthic early succession during the first recruitment season (i.e. 6 months, April – September) (Van Colen *et al.* 2008). In the present paper, we addressed whether the macrobenthic community reassembled to control conditions at the long term (i.e. three years) or not and whether this recovery occurred in accordance with to PR model, or not.

Materials & methods

Study site and sampling

Temporal variation within the macrobenthos after hypoxia-induced mass mortality was assessed in replicated 16 m^2 plots, located at the Paulinapolder tidal mudflat (median particle size = 65 μm, mud content = 51 %). This tidal flat is located in the polyhaline part of the Westerschelde estuary (SW the Netherlands), has a semi-diurnal local tidal regime with a mean tidal range of 3.9 m and salinity ranges from 16 PSU in winter to 27 PSU in summer (Ysebaert 2000). Hypoxic conditions were created by covering the sediment for 40 days with a water-proof polyethylene sheet (0.1 mm thick) and a tarpaulin sheet (140 g m^{-2}) following Thrush *et al.* (1996), Beukema *et al.* (1999) and Dittman *et al.* (1999) which resulted in a total mortality of the macrobenthos community. At the opening of the plots, the sediment was hypoxic, characterized by a black surface and contained significantly higher NH_4^+ pore water concentrations in the upper

10 cm as compared to the controls (i.e. > 30000 μg l^{-1} vs. ~ 4000 μg l^{-1}). After the opening of the plots (i.e. 30 March 2005), macrobenthos and environmental temporal development was monitored 21 times during the first 9 months (i.e. until 19 December 2005), and subsequently at every end of March and September until 26 March 2008. Furthermore, to assess recovery status of the reassembling macrobenthos community, samples were also collected from control (i.e. undisturbed) plots. For every sampling occasion, we randomly sampled in three replicated treatment and control plots. In March 2006, however, extra control samples taken in the immediate vicinity of the control and treatment plots indicated large differences from the 'original' control samples (i.e. average community dissimilarity = 30 % with significant lower abundances for *Pygospio elegans* (787 vs. 5174 ind. m^{-2}) and *Aphelochaeta marioni* (1521 vs. 2445 ind. m^{-2}) in the 'original' control samples). To enable a further, proper investigation of the colonization and recovery status, unbiased by effects due to the repeated sampling, we removed the March 2006 sampling point from the dataset and continued sampling in three 'new', control and treatment plots. The 'new' treatment plots were opened at the same time as the 'original' ones but were generally undisturbed. Macrobenthos was sampled with a core (inner ø 12.5 cm) to a depth of 40 cm and fixed with a neutralised 8% formalin solution. In the laboratory, the samples were sieved through a 0.5 mm mesh and the residual was fixed and preserved until processing using a neutralised 4 % formalin solution with 0.01 % Rose Bengal. All macrofauna was sorted, counted and identified to the species level, except for tubificid oligochaetes. Bivalve biomasses were obtained by determination of the ash free dry weight (4h combustion at 450°C of 24h, 60°C dried individuals) and the biomass of other macrobenthos was calculated by multiplying the organisms' blotted wet weight with a species-specific ISO certified wet weight-ash free dry weight conversion factor (Sistermans *et al.* 2007). For further details on the study site, experimental set up and abiotic and biotic sample processing, the reader is referred to Van Colen *et al.* (2008), Montserrat *et al.* (2008) and Rossi *et al.* (2008).

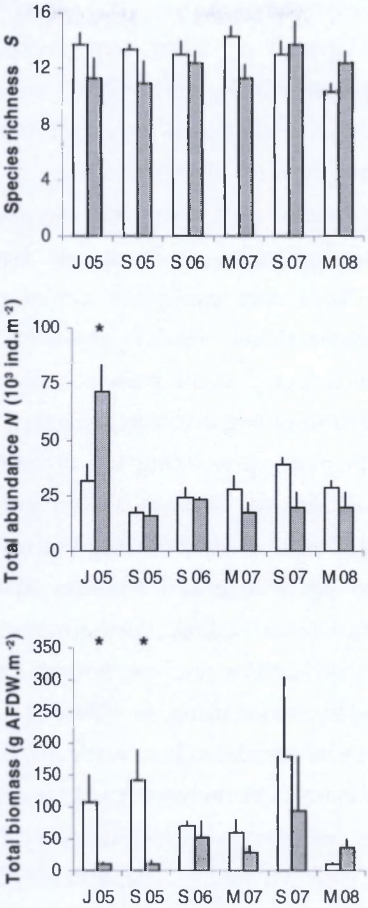


Fig. 2. Mean + SEM temporal distribution of species richness (upper panel), total abundance (middle panel) and total biomass (lower panel) in control (white bars) and treatment sediments (grey bars). J, S and M refer to June, September and March, respectively. Asterisks denote significant differences between treatments according to Mann-Whitney-U testing.

Statistical analysis

In order to analyze macrobenthos recovery patterns at the long term, community composition data representing different succession stages inferred from our detailed short-term recovery monitoring (i.e. March 2005, June 2005 and September 2005; Van Colen *et al.* 2008) were completed with community composition data of September 2006, March 2007, September 2007 and March 2008. The temporal variation of species richness, total abundance, total biomass and the abundance of the four most abundant species at each sampling occasion in the treatment plots, was examined using one-way analysis of variance, after data were log(x+1) transformed in order to met homogeneity of variance (Bartlett-Cochran test). To detect significant differences between sampling occasions, Student-Neuman-Keuls (SNK) post-hoc tests were applied. Furthermore, we defined recovery as the point at which Mann-Whitney U-tests (MWU), performed at each sampling occasion separately, detected no significant differences between both plot types. Using the PRIMER v6 software package (Clarke & Gorley 2006), differences between control and treatment communities at each sampling occasion were analyzed using the one-way ANOSIM test on square root transformed abundance and biomass data and differences were visualized by multi-dimensional scaling. Furthermore, dissimilarities between treatment and control communities and the species that contributed most to these dissimilarities were identified using the SIMPER test. Finally, the Warwick statistic (W, Clarke 1990) was calculated to investigate changes in the community biomass/abundance relationship. W increases to +1 for communities with an even abundance across species but biomass is dominated by a single species, whereas W decreases to – 1 in the converse case (Clarke & Gorley 2006).

Results

Univariate analyses

Table 1 presents the temporal variation of NH_4^+ pore water concentrations in the upper 10 cm, Chl a concentration in the upper 3 mm and mud content in the upper first cm sediment layer of control and treatment plots. After recovering sediments initially developed higher Chl a and mud content values as compared to the controls, these variables returned to ambient levels in September 2005 (Table 1)(Montserrat et al. 2008, Van Colen et al. 2008). The NH_4^+ pore water concentrations in the upper sediment layers (0-1-2-3 cm) returned to ambient levels in September 2005, whereas such recovery in the deeper sediment layers took longer (i.e. March 2007).

In the treatments, highest species richness and total biomass was found in September 2007 (i.e. 2.5 yr after opening of the plots), whereas the total abundance peaked in June 2005. These general community characteristics all exhibited a significant temporal variation but recovery to control values was variable-specific (Fig. 2). Mann-Whitney-U tests (MWU) performed for each sampling occasion separately depicted (1) no significant differences in species richness from June 2005 onwards, (2) a significantly higher abundance in June 2005 and (3) no biomass recovery before September 2006.

The temporal variation in abundance of the four most dominant species at each sampling occasion, is presented in Fig. 3. These species comprised > 70 % of the total macrobenthic abundance at each sampling occasion and all, except *Hydrobia ulvae,* displayed a significant temporal variation ($p < 0.05$; one-way analysis of variance). Dominant species in June 2005 were the bivalve *Macoma balthica* and the polychaetes *Nereis diversicolor, Heteromastus filiformis, Polydora cornuta* and *P. elegans,* comprising together > 95 % of the total abundance; the spionid polychaetes *P. elegans* and *P. cornuta* showed a significant abundance overshoot (MWU $p < 0.05$) and the other species were no longer significantly different from the controls. Further, the abundance of all dominant species in June 2005 was significantly reduced at all later sampling occasions (SNK post-hoc test $p < 0.05$) but, nonetheless, still dominated the community in September 2005 (i.e. > 80 % of the total abundance). Contrary, significantly higher abundances as compared to June 2005 were found for tubificid oligochaetes from September 2006 onwards and for *A. marioni* from March 2007 onwards (SNK post-hoc test $p < 0.05$). Nevertheless, in comparison with the controls, a significantly lower abundance of the tubificid oligochaetes still remained in March 2008 (MWU $p < 0.05$).

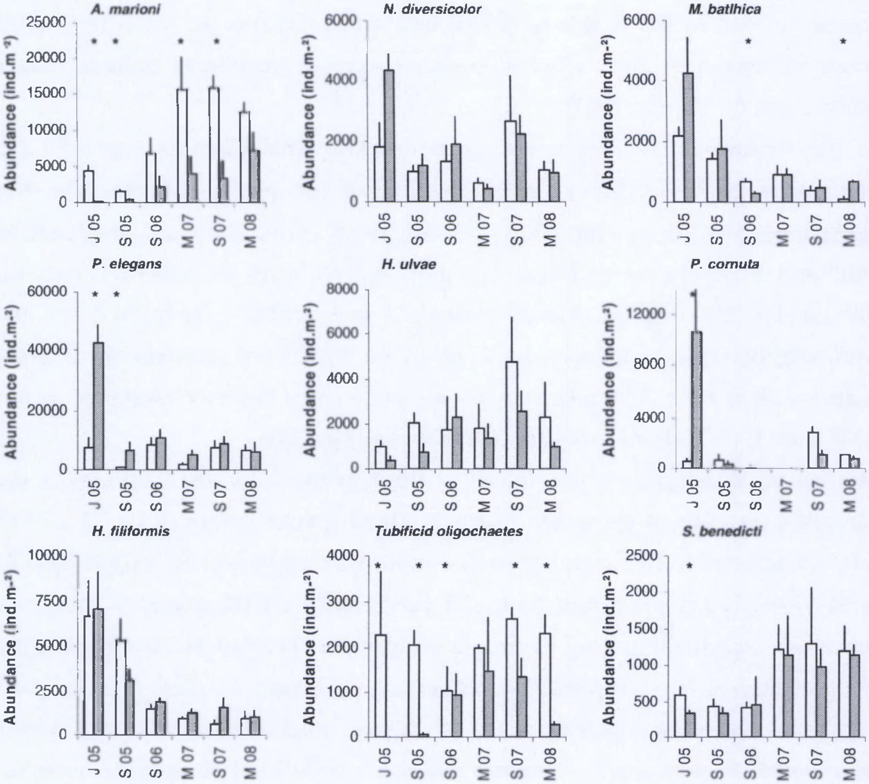


Fig. 3. Mean + SEM temporal distribution of the abundance of Aphelochaeta marioni, Pygospio elegans, Heteromastus filiformis, Nereis diversicolor, Hydrobia ulvae, tubificid oligochaets, Macoma balthica, Polydora cornuta *and* Streblospio benedicti *in control (white bars) and treatment sediments (grey bars). J, S and M refer to June, September and March, respectively. Asterisks denote significant differences between treatments according to Mann-Whitney-U testing.*

	M05	J05	S05	S06	M07	S07	M08
Control							
NH_4+ 0-1cm	3643 ± 198	7787 ± 892	4386 ± 2552	10048 ± 2394	1548 ± 180	5306 ± 576	2725 ± 261
NH_4+ 1-2 cm	4205 ± 586	12996 ± 693	6436 ± 2507	9730 ± 3073	3093 ± 487	4774 ± 106	3473 ± 857
NH_4+ 2-3 cm	3521 ± 1169	16846 ± 3874	4641 ± 696	6783 ± 589	2520 ± 376	2568 ± 369	2436 ± 926
NH_4+ 3-5 cm	4328 ± 1424	16259 ± 1197	3731 ± 2215	6603 ± 2158	1853 ± 320	3118 ± 840	1931 ± 344
NH_4+ 5-10 cm	4124 ± 176	6597 ± 268	2871 ± 714	3904 ± 707	1852 ± 187	3757 ± 542	3424 ± 1079
Chl a 0-3 mm	6.7 ± 0.4	9.3 ± 0.7	9.4 ± 1.2	16.8 ± 4.1	3.8 ± 0.7	15.2 ± 2.2	3.5 ± 1.5
% Mud 0-1 cm	40.7 ± 1.8	36.8 ± 2.8	42.3 ± 5.2	35.6 ± 1.3	29.9 ± 4.3	38.8 ± 1.6	39.6 ± 2.6
Treatment							
NH_4+ 0-1cm	51338 ± 20684	7829 ± 2295	4960 ± 1702	8309 ± 1387	1826 ± 289	3864 ± 205	2562 ± 64
NH_4+ 1-2 cm	46477 ± 20196	5039 ± 182	6566 ± 1611	10554 ± 3253	2208 ± 539	3191 ± 441	2130 ± 465
NH_4+ 2-3 cm	40772 ± 18479	4463 ± 593	6798 ± 1947	8040 ± 1461	2345 ± 737	3500 ± 1179	1612 ± 220
NH_4+ 3-5 cm	35089 ± 15214	5292 ± 2209	6638 ± 1993	3022 ± 51	2899 ± 660	2516 ± 478	1202 ± 52
NH_4+ 5-10 cm	26683 ± 9838	9816 ± 4506	4382 ± 2648	3877 ± 750	1826 ± 196	2117 ± 290	2611 ± 455
Chl a 0-3 mm	4.7 ± 0.5	10.5 ± 0.8	17.5 ± 4.1	16.0 ± 4.5	3.8 ± 0.8	18.5 ± 5.4	6.0 ± 4.0
% Mud 0-1 cm	44.7 ± 2.2	47.9 ± 0.7	40.9 ± 1.0	37.9 ± 2.8	24.9 ± 6.9	41.6 ± 2.0	36.9 ± 0.8

Table 1. *Averaged ± SE ($n = 3$) NH_4^+ pore water concentrations in the upper 10 cm, Chl a concentration in the upper 3 mm and mud content in the upper first cm sediment layer of control and treatment plots. J, S and M refer to June, September and March, respectively.*

Multivariate biotic analyses

Based on both species biomasses and abundances, the temporal variation of the change in the dissimilarity between treatment and control communities illustrated a clear community recovery until September 2006-March 2007 (Table 2). Overall, a faster convergence during this recovery period was found for analyses based on species abundances than for analyses based on species biomasses. According to their *R*-statistic, control and treatment communities were no longer clearly separable in September 2006 and March 2007 ($R < 0.25$), which is further illustrated by their overlap in de MDS ordination diagram (Fig. 4a, c). Later on, the averaged community dissimilarity based on biomass increased again, indicating a divergence between control and treatment communities (Table 2, Fig. 4c, d). Species that contributed most to the community dissimilarity before September 2006 were the spionid polychaetes *P. elegans* and *P. cornuta* for analyses based on abundances and the bivalves *Cerastoderma edule* and *M. balthica* for analyses based on species biomasses (Table 2). From September 2006 onwards, differences in abundances were mainly due to *A. marioni* and Oligochaeta which only partly recovered. For biomass, *C. edule* remained low in the treatment plots throughout the study period, whereas distinct, however not significantly, higher biomasses were observed in the treatments for *Srobicularia plana* in September 2007 and March 2008 and for *M. balthica* in March 2008 (Fig. 5). In general, despite a small drop in March 2007, an increase of the Warwick-statistic was apparent throughout the course of the experiment (Fig. 6). This indicates a shift from a community dominated by a few small-sized short-lived species in June 2005 (e.g. *P. elegans* and *P. cornuta*) towards a community where the biomass is dominated by a few large, long-lived species, represented by rather few individuals in March 2008 (e.g. *S. plana* and *M. balthica*) (Table 2, Fig. 6).

Discussion

Along with improving bottom water oxygen conditions, as indicated by the decrease in ammonium pore water concentrations, macrobenthos species richness, abundance and biomass (SAB) recovery performed a clear nonlinear pattern, as is summarized in Fig. 6. In June 2005, a few opportunistic species became superabundant, causing a distinct abundance peak (Van Colen *et al.* 2008, this study). Subsequently, a moderate increase in species richness and a steep increase in biomass, related to the growth of the long-lived species *M. balthica* and *S. plana,* occurred from September 2005 to September 2006. Until September 2006-March 2007, the macrobenthos thus, in general, reassembled in concordance with the Pearson-Rosenberg species-abundance-biomass curves (Pearson & Rosenberg 1978) and the associated successive zones in benthic habitat quality

(BHQ) (Nilsson & Rosenberg 1997) (Fig. 1, 6). Yet, a significant increase in species richness and a clear drop in the total biomass at the ecotone point, where later colonists replace the dominance of early colonizing opportunists was lacking. At the study site, the majority of the species spawn in spring and subsequently pass through a pelagic larval stage and therefore were able to settle rapidly into the treatment plots in late spring or summer (i.e. within 3 months after hypoxia)(Van Colen *et al.* 2008). Recovery of the few species without a pelagic larval phase (*A. marioni* and tubificoid oligochaetes) took longer and resulted in a modest extra increase in species richness after June 2005. This finding corroborates with the general idea that species colonization at a large scale, and thus recovery time, is mainly dependent on postlarval settlement by pelagic recruits because these stages can disperse over large areas (e.g. Günther 1992, Whitlatch *et al.* 1998). Further, the absence of a significant biomass drop is presumably related to the combined effect of (1) the very low body mass of the small-sized opportunists *P. elegans* and *P. cornuta* (Anger *et al.* 1996, Zajac 1991) and (2) the growth of the long-lived species *M. balthica, H. filiformis* and *N. diversicolor*, which had already settled at high abundances before the ecotone point. In concordance with the PR model, biomass recovery was slow which corroborates Beukema *et al.* (1999) who found no biomass recovery during their 4 year monitoring period because > 4 year old *Mya arenaria* were present in the control sediments. Furthermore, beyond biomass recovery at the end of the transition period (i.e. September 2006), we observed a marked temporal variation in total community biomass with a maximum biomass in September 2007, flanked by steep drops in March 2007 and March 2008 (Fig. 2, 6). These dips corresponded with the biomass decreases of the large bivalves *C. edule* and *S. plana* (Fig. 5). In contrast to *M. balthica, C. edule* and *S. plana* are extremely vulnerable to low (winter) temperatures (Guerreiro 1998, Strasser *et al.* 2001). Therefore, besides biomass increase during the growing season (i.e. spring-summer), the temporal variation in biomass seems also related species-specific winter mortality. In addition to the seasonal peak in availability of dispersive benthic settlers, this temporal variation in biomass further highlights the importance of seasonality in the interpretation of recovery patterns, in general.

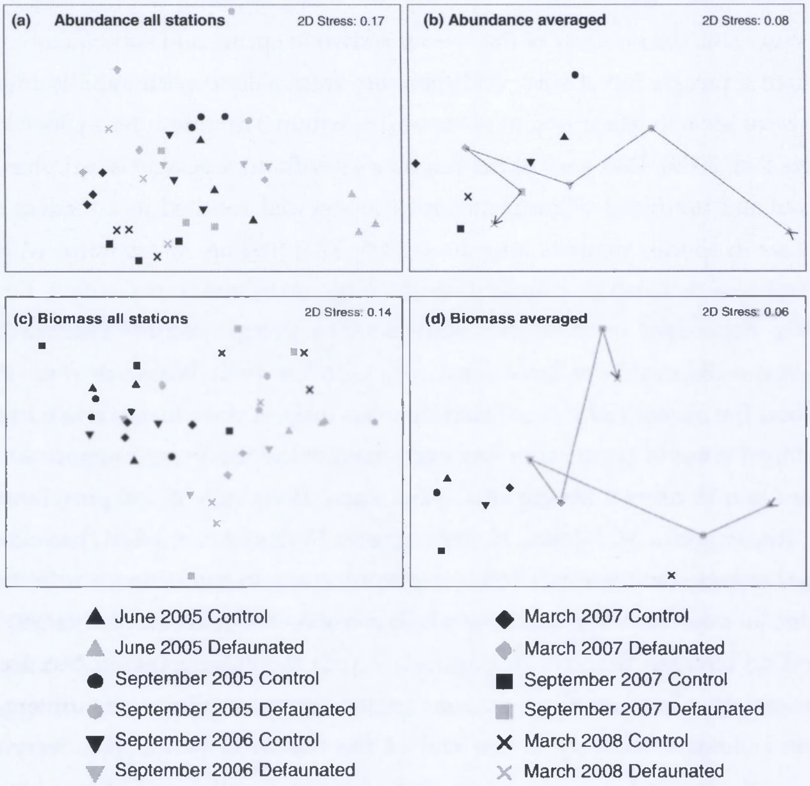


Fig. 4. Multi-dimensional scaling showing the changes in benthic community structure from June 2005 to March 2008, based on squareroot transformed abundance (a, b) and biomass (c, d) data. The line in b and d indicates the averaged recovery trajectory.

A further remarkable fact that appears from this study is that, with respect for biomass community (dis)similarities, control and treatment communities diverged again beyond the transition period. This pattern mainly resulted from the higher biomasses of *S. plana* in September 2007 and March 2008 and of *M. balthica* in March 2008 in the treatments due to a significant higher recruitment in the treatments of *M. balthica* in 2005 and of *S. plana* in 2006 and 2007 (Fig. 5, Van Colen *et al.* 2008). As most probably, the surface deposit feeding juveniles of these two species has benefited from the low bioturbation of *C. edule* in the treatments throughout the course of the experiment. After the moderate recruitment in 2005, *C. edule* abundances declined in both control and treatments which corresponded with its observed recruitment failure in 2006 and 2007 in the whole Westerschelde estuary (Kesteloo *et al.* 2007, 2008). *C. edule* is a suspension feeding bivalve which crawls through the superficial sediment layers, thereby affecting both the abiotic and biotic parts of its environment (Ciutat *et al.* 2007, Flach 1996) and is considered an important bioturbator at our study site (Chapter 8). Interference by bioturbation is recognised as an important process affecting benthic recruitment success (Fraschetti *et al.* 2003). Moreover the inhibitory impact of *C. edule* on benthic recruitment success has been demonstrated by Flach (1996) and lowered macrobenthic post-settlement growth has been observed in areas with higher *C. edule* biomasses (Chapter 5). This community divergence indicated that macrobenthic community recovery should not necessarily be convergent and deterministic at the long term because biotic interactions at early recovery stages may give rise to community divergence at later stages whenever the impact of the affected species becomes greatest. Hence, in addition to changes in environmental variables, community divergence resulting from a lagged effect of biotic interactions during early recovery may explain macrobenthic patchiness within one tidal flat which is subjected to relatively small-scale disturbances (e.g. hypoxic conditions resulting from algal bloom decomposition; Bolam & Fernandez 2002, Norkko & Bonsdorff 1996, Reise 1985) and/or differences in community structure between disturbed and non-disturbed sites at a larger scale.

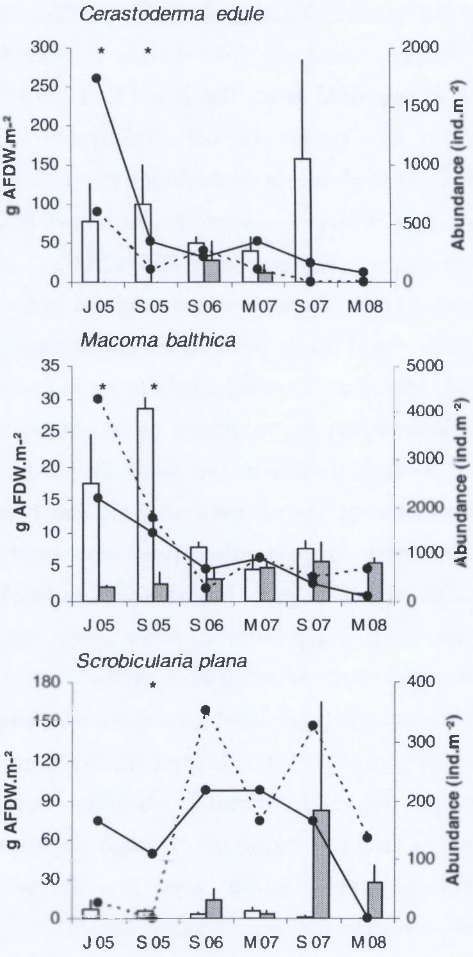


Fig. 5. Mean + SEM temporal distribution of the biomass of Cerastoderma edule *(upper panel),* Macoma balthica *(middle panel) and* Scrobicularia plana *(lower panel) in control (white bars) and treatment sediments (grey bars). J, S and M refer to June, September and March, respectively. Averaged abundance in controls (solid line) and treatments (dashed line) is presented on the right y-axis. Asterisks denote significant biomass differences between treatments according to Mann-Whitney-U testing.*

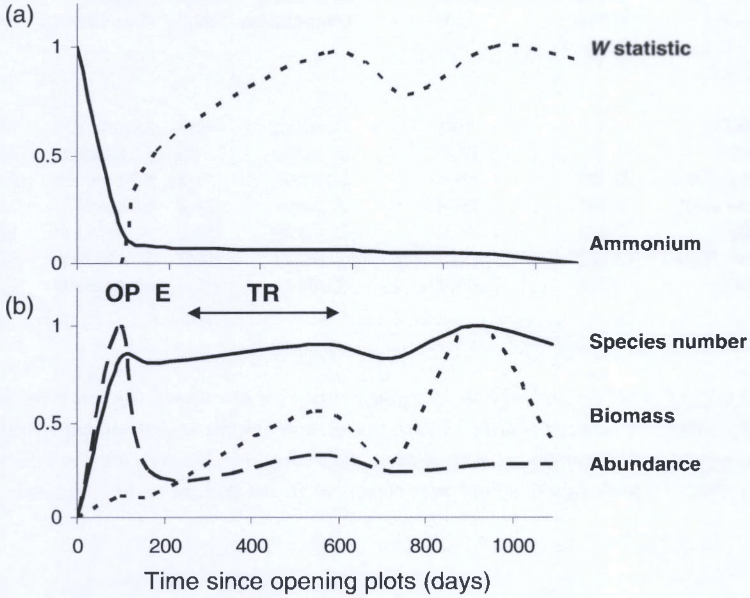


Fig. 6. Temporal variation of (a) the ammonium pore water concentration at the 3-5 cm sediment layer and the Warwick (W) statistic, and (b) species richness, total abundance and total biomass. Data points are normalized and OP, E and TR refer to the peak of opportunists, the ecotone point and the transition region of the Pearson-Rosenberg model (1978).

	R-value	Dissimilarity (%)	Species	D%	Species	D%
(a) Abundance						
March 2005	1	100	*H. filiformis*	25.5	*A. marioni*	13.6
June 2005	1	36.3	*P. elegans*	26.2	*P. ligni*	17.6
September 2005	0.63	27.4	*P. elegans*	23.6	Oligochaeta	17.2
September 2006	-0.037	11.3	*A. marioni*	32.4	*P. elegans*	11.5
March 2007	0.185	17.3	*A. marioni*	39.2	*P. elegans*	16.6
September 2007	0.519	20.5	*A. marioni*	31.4	*P. ligni*	9.6
March 2008	0.519	17.6	Oligochaeta	20.3	*A. marioni*	17.9
(b) Biomass						
March 2005	1	100	*C. edule*	39	*M. balthica*	13.6
June 2005	1	61.4	*C. edule*	45	*M. balthica*	14.5
September 2005	0.926	56.4	*C. edule*	53	*M. balthica*	20.6
September 2006	0.185	20.3	*S. plana*	24.2	*C. edule*	23.6
March 2007	0.074	16.6	*C. edule*	54.2	*A. marioni*	10.2
September 2007	0.222	56.3	*C. edule*	50.9	*S. plana*	32.6
March 2008	0.519	41.4	*S. plana*	54.3	*M. balthica*	13.9

Table 2. *Results of SIMPER and ANOSIM analysis based on abundance (a) and biomass (b), indicating the difference between control and treatment communities at each sampling occasion and the two species which contributed most to these differences. Underlined contributions to the dissimilarity (%D) indicate higher abundances/biomasses in the treatments as compared to the controls.*

Acknowledgements

We thank D. Van Gansbeke, G. De Smet and I. Pauwels for their assistance during the processing of the samples, as well as B. Beuselinck, D. Peelaers, J. van Soelen and B. Koutstaal for their help during sampling. This research was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen), FWO Vlaanderen (FWO-Kiss 3G048005), the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministry of Economic

Affairs. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446).

CHAPTER 8

SEDIMENT SEGREGATION BY BIODIFFUSING BIVALVES

Submitted as:

Montserrat F., Van Colen C., Provoost P., Ponti M., Vanden Meersche K., Ysebaert T., Herman P.M.J.
Sediment segregation by biodiffusing bivalves
Estuarine, Coastal and Shelf Science

Abstract

The selective processing of sediment fractions (sand and mud; > 63 μm and ≤ 63 μm median grain size) by macrofauna was assessed using two size classes of inert, UV-fluorescent sediment fraction tracers (luminophores). The luminophores were applied to the sediment surface in 16 m^2 replicated plots, defaunated and control, and left to be reworked by infauna for 32 days. As the macrofaunal assemblage in the ambient sediment and the control plots was dominated by the common cockle *Cerastoderma edule*, this species was used in an additional mesocosm experiment. The diversity, abundance and biomass of the macrobenthic assemblage did not return to control values within the experimental period. Both erosion threshold and bed elevation increased in the defaunated plots as a response to the absence of macrofauna and an increase in microphytobenthos growth. In the absence of macrobenthos, we observed an accretion of 7 mm sediment, containing ca. 60 % mud. Image analysis of the vertical distribution of the different luminophore size classes showed that the cockles preferentially mobilised fine material from the sediment, thereby rendering it less muddy and effectively increasing the sand : mud ratio. Luminophore profiles and budgets of the mesocosm experiment under "no waves-no current" conditions support the field data very well.

Keywords: *Intertidal cohesive sediment, Sand, Mud, Erosion, Deposition, Luminophores, Image analysis, Bioturbation, Ecosystem engineering*

Introduction

Intertidal soft sediments are governed in their dynamics (e.g. erosion, deposition, transport) by both physical and biological processes (Borsje *et al.* 2008, Daborn *et al.* 1993, Nowell *et al.* 1981, Rhoads 1974). Intertidal sediments consist of a mixture of gravel, sand and mud. Mud (< 63 μm), in turn, consists of a mixture of silt (quartz powder) and clay where per particular estuarine system, the ratio between silt and clay is constant (Flemming 2000). A sediment matrix built up of coarse sediment particles (sand and gravel) in contact with each other displays a granular behaviour. Gradually increasing the mud content of such a matrix will cause the interstitial spaces to be filled up and the sand particles to lose contact with each other. Once the matrix is dominated by mud instead of by sand particles, the sediment behaviour will change from non-cohesive to cohesive; the sediment will behave less granular and more like a firm gel (Van Ledden 2003, Winterwerp & Van Kesteren 2004 and references therein).

Besides being ca. two orders of magnitude smaller than sand, clay particles are not spherical but consist of layered platelets. Their shape and size give them a high specific surface area and an electrical charge which interacts with ambient (water and organic) molecules and other particles (Winterwerp & Van Kesteren 2004). The sediment mud content is therefore positively correlated with organic matter (OM) content (Hedges & Keil 1995) and respiration and mineralisation rates (Aller 1994), but also with other biogeochemical characteristics such as the capacity to bind heavy metals and other pollutants (Bouezmarni & Wollast 2005). In sediment beds dominated by mud, cohesive forces between the particles are dominant as sand and silt particles are captured in a clay-water matrix. Therefore, as mud (clay) strongly influences mechanical properties like the cohesiveness of the sediment and thus erosive behaviour (Dade *et al.* 1992, Mitchener & Torfs 1996, Van Ledden *et al.* 2004), coastal and estuarine sediment transport and morphology can differ with different mud contents.

In coastal lagoons and tidal basins, the main physical drivers for sediment mud content are hydrodynamic energy gradients (Oost 1995, Van Ledden 2003 and references therein). Classical hydrodynamic models which treat sand-mud segregation in coastal areas do not incorporate biological processes (Van Ledden 2003). Even the most recent state-of-the-art morphodynamic models which recognise that small-scale benthic biologically-mediated processes reflect in large-scale sediment dynamics, have a grid size in the order of km and a sub-grid level in the order of 200-800 m (Borsje *et al.* 2008). Unless averaged over the grid cells, these models cannot account for small- to mid-scale biological processes which can cause sharp boundaries between sandy and muddy areas in nature and create a patchy intertidal landscape.

Benthic animals can change the sediments' erodability autogenically (i.e by their own physical structures; Luckenbach 1986, Montserrat *et al.* 2008, Van Duren *et al.* 2006) or allogenically (i.e. bioturbation; Andersen *et al.* 2002, Rhoads & Young 1970, Willows *et al.* 1998). Also, by modifying the composition (i.e. the sand : mud ratio), organisms can alter the sediments' geomechanical properties like cohesiveness and erosion threshold (Le Hir *et al.* 2007 and references therein; Winterwerp & Van Kesteren 2004). For example, biodeposition is a process in which suspension-feeding fauna capture fine sediment fractions from the water column and deposit them nearby as (pseudo)faeces (Haven & Morales-Alamo 1966, Oost 1995). If this material is not immediately resuspended, the surrounding sediment will become muddier and more cohesive. Benthic invertebrates are also known to cause incorporation or expulsion of fine sediment fractions through bioturbation (Volkenborn *et al.* 2007a) resp. bioventilation or bioresuspension (De Deckere *et al.* 2000). Differential processing of sediment fractions through bioturbation is a process that can change the sediment composition and have profound consequences for its biogeochemistry (Volkenborn *et al.* 2007b).

In order to study bioturbation processes numerous tracer techniques have been developed over the last three decades (Maire *et al.* 2008), including the use of phaeopigment concentration profiles (Gerino *et al.* 1998), radionuclides (Andersen *et al.* 2000, Mulsow *et al.* 2002, Sandnes *et al.* 2000; Wheatcroft & Martin 1996), inert fluorescent sediment tracers called luminophores (Gerino 1990, Mahaut & Graf 1987) and even silver and gold (Wheatcroft *et al.* 1994). Especially the use of luminophores has taken a flight in bioturbation studies to model and quantify vertical particle transport and to estimate biodiffusion coefficients (D_b) of both single species (Duport *et al.* 2006, Maire *et al.* 2007) and of multi-species communities (Duport *et al.* 2007, Gilbert *et al.* 2007). The luminophores have been applied in frozen "cakes" or as a layer either at the sediment surface, at a certain depth or both (Caradec *et al.* 2004) to distinguish between transport directions. Solan *et al.* (2004) combined the use of luminophores with an *in situ* time-lapse sediment profile camera, obtaining a series of cross-sectional images of the sediment in which both the reworking of tracer and natural benthic activity were captured.

In all abovementioned cases only one size class of luminophore was used. To our knowledge and as mentioned in a review by Maire *et al.* (2008), there have been no studies in which two or more size classes of luminophores were deployed to study particular bioturbation processes. Here, we did use two size classes of luminophores, each as a tracer for the sediment fractions mud (< 63 µm) and sand (> 63 µm and < 2000 µm), to determine selectivity between sediment fractions by bioturbating macrofauna.

To detect possible differential processing of sediment fraction tracers and resulting possible changes in sand : mud ratio in this experiment, we used defaunation to exclude the effect of macrobenthos *in situ* following exactly the same method and spatial scale as in Van Colen *et al.* (2008) and Montserrat *et al.* (2008). In this light, the focus of this study is to assess the temporal and spatial scale of bioturbation of a natural community and one that has been removed, using two size classes of luminophores, each with a different colour, as conservative sediment fraction (mud and sand) tracers. Using the same sediment fraction tracers, we also conducted a mesocosm experiment under controlled conditions, with the most important macrobenthos found in the field experiment. Our hypothesis is that the net effect of the macrofaunal assemblage is to remove the fine sediment fraction, to yield less muddy sediment, with a consequent lower erosion threshold.

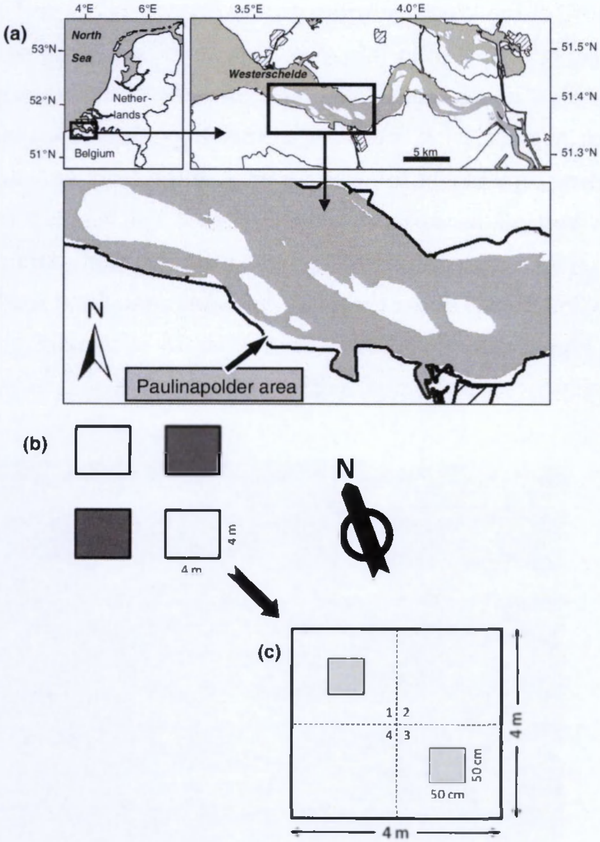


Fig. 1. (a) The geographic position of the research site within the polyhaline part of the Westerschelde estuary, SW Netherlands. (b) Schematic representation of the defaunated (dark) and control (light) replicated plots. Distance between plots is not to scale. (c) Schematic representation of the placement of the luminophore tiles in (grey) 50 x 50 cm squares.

Materials & methods

Site, defaunation treatment and luminophore application

The experiment was conducted in an intertidal flat area (Paulinapolder) on the southern shore of the Westerschelde estuary (51° 21′ 24″ N and 3° 42′ 51″ E; SW Netherlands; Fig. 1a). Within this area, four 16 m² square plots were randomly chosen to be used in this study (Fig. 1b). Two plots were defaunated by covering the sediment in plastic foil, inducing severe hypoxia, for a period of 94 days. After this period, the plastic foil was cut open at the sediment surface, referred to as day 0. A vertical subsurface lining of plastic foil was left intact to prevent horizontal subsurface immigration of macrobenthic infauna. For more information and details about the exact defaunation method see Montserrat *et al.* (2008) and Van Colen *et al.* (2008). Two other 16 m² square plots consisted of untreated ambient sediment and were used as controls.

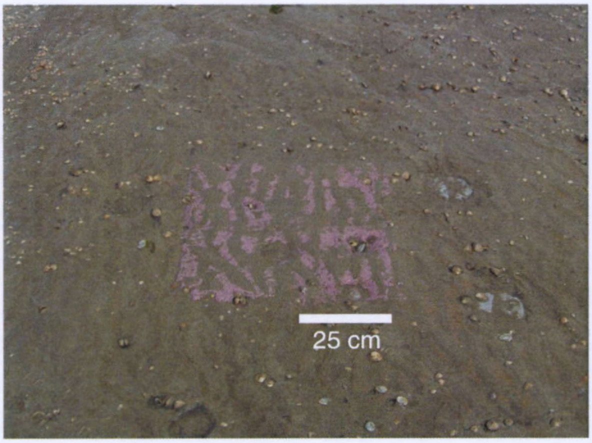


Fig. 2. *A square of 2 x 2 luminophore tiles after 2 days in the field experiment.*

The used fluorescent sediment tracers (luminophores, Environmental Tracing Systems, UK; http://www.environmentaltracing.com/) were coded "Magenta" and "UV Blue Mostyn" with a median grainsize (D_{50}) of 41 μm and 129 μm, respectively. Overlap between the two fraction tracers existed in that the D_{90} of "Magenta" was 86 µm and the D_{10} of "UV Blue Mostyn" was 72 µm. Luminophores were volumetrically mixed in the ambient sand : mud ratio of the sediment and in turn volumetrically mixed 1:1 with sieved (1 mm) ambient sediment. The sediment-tracer mix was poured in plaster moulds of 25 x 25 x 0.5 cm and frozen at -20° C to make sediment 'tiles'. At t = 0, the frozen tiles were then placed in a square of 2 x 2 tiles covering 0.25 m^2, both in the north-west and in the south-east corners, at ca. 1 m from the edges of the experimental plots (Fig. 1c).

Sampling

The experiment started on the 23rd of May 2005 by opening the plastic sheeting (referred to as t = 0, see above). The plots were sampled at t = 1 hour, 1 tide, 3 days, 7 days, 14 days and 32 days. The collection consisted of samples taken for luminophore analysis, macrobenthos abundance, macrobenthos biomass, erosion threshold and bed elevation. Afterwards, measurements of the latter two variables was continued and done approximately every month for a total period of 154 days (until 24 October 2005) to monitor abiotic sedimentological responses over a longer period. Samples were taken in replicates per plot (i.e. quadrant 1 and 3; Fig. 1c). For luminophore sampling, cores made out of 30 cm long PVC pipes with an inner diameter of 36 mm and a wall thickness of 2 mm were used. To minimize compaction of the sediment while pushing down the core, a plunger of a 100 ml syringe was drawn upwards. Luminophore cores were stored on dry ice (approx. – 70° C) in the field to arrest macrofaunal reworking, taken to the lab and further stored at -20° C until processing. Macrofauna was sampled to a depth of 30 cm, using an 11 cm inner diameter stainless steel corer. Macrofauna samples were taken to the lab, sieved over a 1 mm sieve and preserved in a neutralised 4% formaldehyde solution with 0.01 % Bengal Rose until processing. Macrofaunal abundance is given as individuals per square meter (ind. m^{-2}). Macrofaunal biomass was determined according to Sistermans *et al.* (2007) and given in grams ash-free dry weight per square meter (g AFDW

m^{-2}). The sediment strength, or erosion threshold is taken as a measure of erodability of the sediment under a certain bottom shear, and was measured using a cohesive strength meter Mk III (CSM, Sediment Services, UK; Tolhurst *et al.* 1999). The measurements were performed according to the 'Mud 6' programme of the CSM Mk III. The sediment bed level elevation was measured relative to NAP (= Normaal Amsterdams Peil; Amsterdam Ordnance Datum, similar to Mean Sea Level). Between five and eight replicate measurements were haphazardly taken per sampled quadrant. Bed level measurements were performed using a rotating laser mounted on a tripod and a receiving unit on a measuring pole. There was evidence of baseline variability in the bed elevation measurements; to correct for this variability, the bed elevation values are given as the difference in elevation ± standard deviation (SD) between the defaunated and the control plots. Assuming there is no covariance between the treatments, the SD for the difference in bed elevation is calculated according to: $\sigma^2_{\mathrm{Defaunated\text{-}Control}} = \sigma^2_{\mathrm{Defaunated}} + \sigma^2_{\mathrm{Control}}$

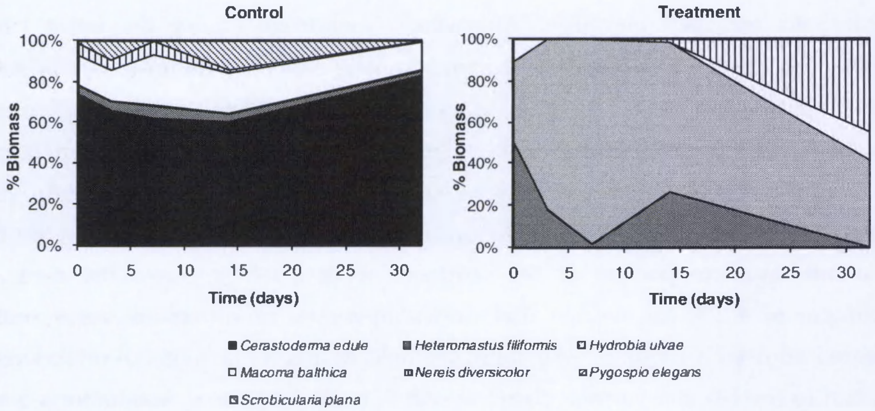


Fig. 3. The relative contribution to the total biomass in both treatments of the seven most abundant species. In the right hand panel (defaunated) there is a minor contribution of C. edule *at the end of the experimental period and no contribution of* S. plana.

The luminophore cores were taken from within the 0.25 m^2 tracer area (Fig. 1c). All other samples were taken from the adjacent sediment within the experimental plots, max. 30 cm away from the luminophore tiles. To prevent disturbance of the sediment profile, all holes left in the sediment after extraction of the respective cores were filled up with PVC tubes of the same diameter and length, which were closed at one side with duct tape.

Mesocosm experiment

In addition to the field experiment, a mesocosm experiment was done to assess the bioturbatory effects of the most important species in terms of biomass in the ambient sediment: the common cockle *Cerastoderma edule* Linnaeus (1758) (Van Colen *et al.* 2008). In this mesocosm experiment, PVC cores (66 mm inner diameter, 2 mm wall thickness, 85 mm height) were closed on one side with duct tape and filled with a column of 1 mm sieved ambient sediment. Half of the cores each received one live cockle haphazardly drawn from a population with shell length 29.47 ± 2.35 mm (mean ± SD) and wet weight 9.64 ± 2.18 g (mean ± SD). In each of these cores, the animal was gently pushed into the sediment until flush with the sediment surface. Then, a 3 mm thick disc of frozen luminophore / sediment mix with a diameter of 66 mm was placed on top of the sediment in each experimental core. We replicated 9 times to yield 18 cores in total. One-third, i.e. 3 "Cockle - No Cockle" (C –NC) pairs, was immediately frozen at -20^0 C to serve as a t = 0 where no sediment reworking could have taken place. The remaining 6 replicates (12 cores in total) were placed in C - NC pairs within each of the six mesocosm units of ca. 8 dm^3. All mesocosm units within the setup were simultaneously subjected to a light : dark cycle of 12 : 12 hours, a temperature of 15^o C – 18^o C and a diurnal ebb-flood cycle of 4 x 6 hours. The ebbing and flooding of the units was accomplished with peristaltic pumps which administered the water in low speed and directly to the bottom of the units so wave and/or current action generated by this very gentle flow was regarded as negligible. Experimental units were checked daily, and whenever an animal was found dead, it was replaced by one not more than 6 % different in length and not more than 26 % different in wet weight. After 21 days, the mesocosm experiment was terminated by removing all cores from the setup and placing them at -80^0 C for 1.5 hours, after which they were placed at -20^0 C until processed.

Luminophore image analysis

Both the PVC cores used to extract material from the tracer-enriched sediment surface from the field and the PVC cores used in the mesocosm experiment were, while still frozen, longitudinally cross-sectioned using a band saw. The frozen smear left by the band saw was chafed away using a razor. Every half core was photographed in the light and in the dark under blacklight, using a digital mirror-reflex camera (Canon EOS 350D with 18-55 mm EFS objective) mounted on a stand. The images measured 2304 by 3456 pixels, and were saved using JPEG compression. The area photographed measured 43 by 67 mm, resulting in a resolution of 18.66 μm x 19.39 μm per pixel. The images were analyzed using custom-made Matlab scripts. First, the images were corrected for the uneven sediment surface by drawing a line on the image taken under normal light, and vertically translating the pixel columns of the image taken under UV light accordingly. Subsequently, discriminant analysis was used to classify all pixels into one of the classes "red luminophores" (i.e. mud fraction mimic), "blue luminophores" (i.e. sand fraction mimic) or "background" (i.e. ambient sediment), based on their brightness values in the red, green and blue bands (each between 0 and 255). A training set was composed by manually selecting pixels pertaining to each of the three classes. The classification outcome was verified by comparing false-colour and original images. Finally, the number of pixels belonging to the different classes was determined for each pixel row.

Data analysis

All field results were analysed according to a Complete Randomised (CR) experimental design. We performed analysis of variance using general linear modelling (GLM-ANOVA) to test for differences between time (i.e. sampling occasions), treatments, plots and, in the case of bed level measurements, quadrants. To ensure normal distribution of the residuals, visual inspection of Normal P-Plots was performed. Macrofauna data were $(x+1)^{0.25}$ transformed in the case of abundance and $\ln(x+1)$ transformed in the case of biomass (Quinn & Keough 2002).

The GLM for macrofauna (both for abundance and biomass) included Time and Treatment as separate fixed factors and Plot nested in Treatment with Plot as a random factor, plus the interaction terms Time x Treatment and Time x Plot nested in Treatment. The GLM for erosion threshold included Time, Treatment, Plot nested in Treatment and the interaction term Time x Treatment. Containing an extra source of variation, the GLM for bed level elevation included Time, Treatment, Quadrant nested in Plot nested in Treatment and the interactions terms Time x Treatment and Time x Quadrant nested in Plot nested in Treatment. Analyses on luminophore budgets from the field experiment were done using analysis of covariance (ANCOVA), with treatment as a covariate. Analyses of the sediment budgets from the lab experiment were done using a full-factorial ANOVA with Time, Treatment and Fraction, including all interaction terms as factors. All data analyses have been performed using R: A Language and Environment for Statistical computing (R Development Core Team, 2007; Vienna, Austria; http://www.R-project.org).

Results

Field experiment - General observations

In similarly defaunated plots, used in a parallel experiment, the sediment was rendered azoic after 40 days of hypoxia (Montserrat *et al.* 2008, Van Colen *et al.* 2008). Therefore, in this experiment it was assumed that after 94 days of induced hypoxia all macrobenthos was dead and decomposed in the defaunation-treated plots. The tracer tiles were placed in quadrant 1 and 3 (Fig. 1c) and all samplings were done after 1 hour. Because of the warm weather, the tiles thawed very quickly and shortly after placement on the control plots, various benthic animals and particularly cockles would emerge from underneath the tiles. After the sampling, when the tide returned, we observed the applied tracer tiles for several minutes and no significant amount of tracer was transported away from the plots. Also, when we returned to do the sampling after one tide, the tracer tiles were still very well visible. After two days, mini-ripples present on the ambient sediment (and thus also the control plots) had migrated over the tracer tiles (Fig. 2). These observations indicated that, after thawing, the tracer tiles became integrated with the ambient sediment matrix and did not cause or display any anomalous sediment behaviour.

Field experiment – Benthic assemblage

Throughout the experimental period, both the total abundance and total biomass in the defaunated plots were at least an order of magnitude lower than in the control plots. Total macrofaunal abundance in the control plots ranged between 13 511 ± 624 ind. m^{-2} (mean ± SEM) at day 0 and 8159 ± 3084 ind. m^{-2} (mean ± SEM) at day 32 , whereas that in the defaunated plots started at 245 ± 39 ind. m^{-2} (mean ± SEM) and increased to 1579 ± 538 ind. m^{-2} (mean ± SEM) at day 32. Total abundance was found to be different with significant effects of Time (Table 1, GLM-ANOVA, $p = 0.0305$), Treatment ($p = 0.0091$) and Time x Treatment interaction ($p = 0.0009$). The total macrofaunal biomass in the control plots ranged between 110.82 ± 27 g AFDW m^{-2} (mean ± SEM) at day 0 and 78.24 ± 38 g AFDW m^{-2} (mean ± SEM) at day 32, whereas that in the defaunated plots started at 0.04 ± 0.02 g AFDW m^{-2} (mean ± SEM) at day 0 and increased one order of magnitude to 0.51 ± 0.3 g AFDW m^{-2} (mean ± SEM) at day 32. Total macrofaunal biomass was found to be different with significant effects of Time (GLM-ANOVA, $p = 0.0044$), Treatment ($p = 0.0176$), Plot nested in Treatment ($p = 0.0219$) and Time x Treatment interaction ($p = 0.0021$).

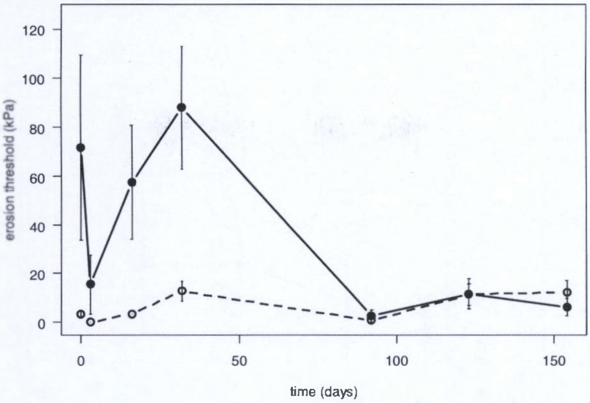


Fig. 4. The mean ± SEM eroding pressure (measured with CSM Mk IV) as a function of time in control (dashed line) and treatment (solid line) sediments.

There were seven species that accounted for 75 to 99 % of the total macrobenthic biomass in both treatments (Fig. 3). A conspicuous difference between the treatments was that in the control plots the bulk of the biomass was made up by adult *Cerastoderma edule*. Its biomass varied between 61 % at day 0 and 83 % at day 32 (Fig. 3), while in the defaunated plots adult *C. edule* did not occur within the 32 day experimental period. Within the first week, the common Mudsnail, *Hydrobia ulvae* (Pennant 1777) was -with 94 % of the biomass at day 7- the dominant species of the highly impoverished assemblage in the defaunated plots, whereas it never constituted more than 2.7 % of the natural assemblage in the control plots. The contribution of *H. ulvae* to the total biomass in the defaunated plots decreased to ca. 40 % towards day 32. Simultaneously, *Nereis* (= *Hediste*) *diversicolor* (Müller 1776) started to recolonise the defaunated plots and contributed about 40 % to the assemblage in the defaunated plots. Only at the end of the experimental period, freshly recruited, juvenile *Macoma balthica* (L.) contributed almost 14 % to the total macrobenthic biomass in the defaunated plots, while in the control plots it remained stable around 15 % throughout the entire period.

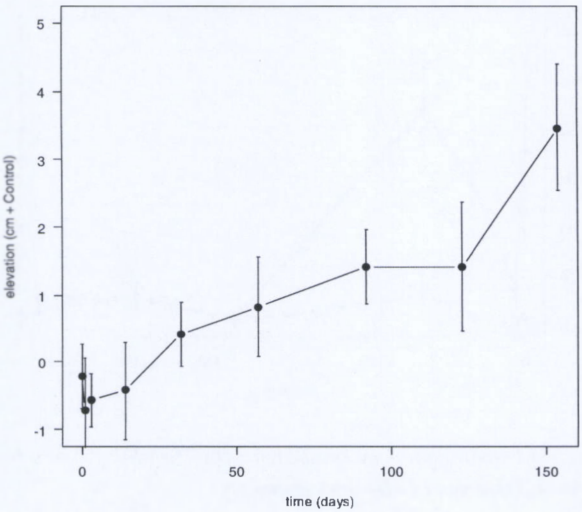


Fig. 5. *The mean ± SD elevation difference of the defaunated plots, compared to the controls, in cm.*

Field experiment - Sediment characteristics

The sediment erosion threshold displayed different patterns between the treatments for the first four samplings (up till day 32). The defaunated sediment had a higher erosion threshold than the control plots, albeit with a very high variability around the mean (Fig. 4). In the control plots both the mean erosion threshold and variance were low. The greatest difference between the two treatments occurred at day 32. This great difference in erosion threshold between the treatments coincided with a visible bloom of microphytobenthos in the defaunated plots (chl *a* data not shown here), as the surface sediment turned a dark brown as observed in Montserrat *et al.* (2008). Upon inspection of the Normal P-Plot of the erosion data, violation of the assumption of homoscedasticity was suspected. To correct for this, Quinn & Keough (2002) suggest square root + 1 transformation for data with right skew. It is obvious from Fig. 4 that there was no difference between the treatments for the long-term erosion threshold measurements (day 92 and onward). Therefore, only the first four measurements (day 0, day 3, day 14 and day 32) were addressed in the statistical analysis. GLM-ANOVA found only a significant effect of Treatment (p = 0.0006; Table 1).

Variable	Source of variation	SS	Df	MS	F	p
total abundance macrofauna	time	47.337	9	5.260	2.788	0.0305
fourth root(x+1)	treat	412.625	1	412.625	108.590	0.0091
	plot(treat)	7.603	2	3.801	2.026	0.1602
	time*plot(treat)	34.000	18	1.889	1.565	0.1226
	time*treat	95.521	9	10.613	5.626	0.0009
	Error	44.670	37	1.207		
total biomass macrofauna	time	74.865	9	8.318	4.2316	0.0044
ln(x+1)	treat	512.682	1	512.682	55.3433	0.0176
	plot(treat)	18.536	2	9.268	4.7312	0.0219
	time*plot(treat)	35.410	18	1.967	1.2961	0.2458
	time*treat	86.174	9	9.575	4.8709	0.0021
	Error	56.158	37	1.518		
elevation *ln(x+1)*	time	125.09	8	15.64	2.4579	0.0267
	treat	38.86	1	38.86	0.0318	0.8644
	quad(treat*plot)	7314.51	6	1219.08	191.6367	<< 0.0001
	time*quad(treat*plot)	286.26	45	6.36	16.0367	<< 0.0001
	time*treat	85.20	8	10.65	1.6742	0.1312
	Error	54.74	138	0.40		
erosion threshold	time	25.77	1	25.77	3.50	0.0808
square root(x+1)	treat	137.01	1	137.01	18.63	0.0006
	plot(treat)	9.18	1	9.18	1.25	0.3153
	time*treat	2.20	2	1.10	0.15	0.7041
	time*plot(treat)	1.34	2	0.67	0.09	0.9131
	Error	110.40	15	7.36		

Table 1. *Summary of the analysis of variance of the measured environmental (biotic and abiotic) variables. The first column (variable) states the analyzed variable with its appropriate transformation italicized. In the second column (source of variation), the factors taken into the explanatory model for each variable can be found. The last column (p) states the p-value of the effect.*

The sediment bed level elevation relative to NAP of the defaunated plots started somewhat lower than the control plots. From day 7 onwards, the bed level of the defaunated plots started to rise (ANCOVA: $p = 0.0072$), while at the same time a decrease in the bed level of the control plots was observed (ANCOVA: $p = 0.0179$; Multiple $r^2 = 0.4174$).

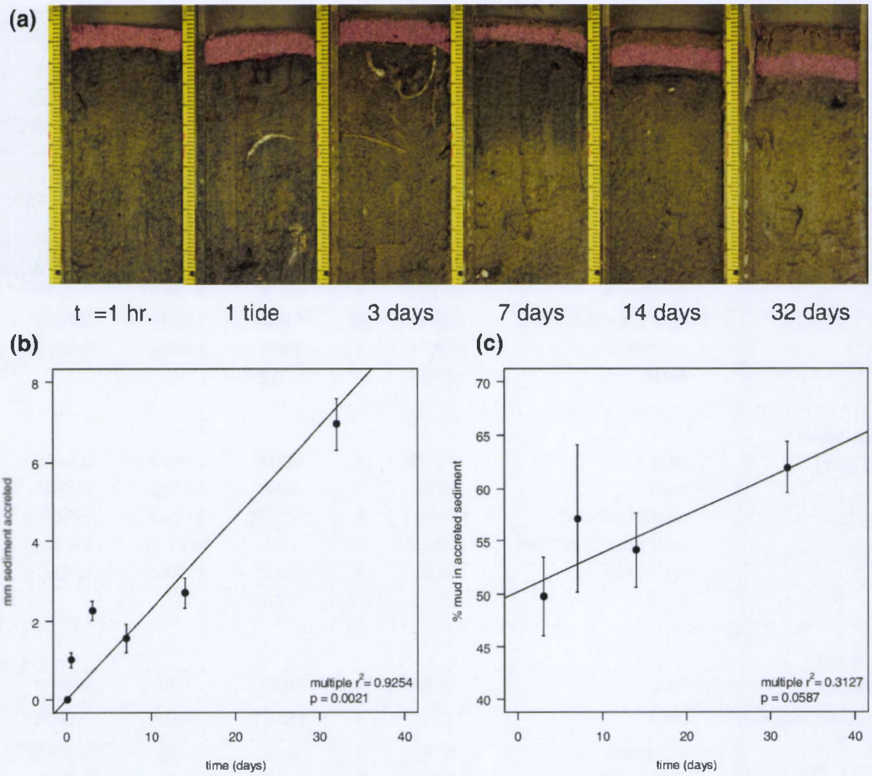


Fig. 6. (a) Longitudinally cross-sectioned cores containing sediment extracted from the defaunated plots, with a pink layer of sediment-luminophore mix. Each frame is representative of the cross sections of the cores during the mentioned sampling occasion (from left to right: t = 0, 1 tide, 3 days, 7 days, 14 days and 32 days, resp.). (b) The mean ± SEM amount of accreted material on top of the defaunated plots, in mm. The accreted material was measured from the underside of the luminophore layer. (c) The mean ± SEM percentage mud (sediment fraction ≤ 63 μm) in the accreted material on the corresponding sampling occasions, in %.

The difference in elevation of the defaunated plots increased to 4.2 mm above the control plots on day 32 and even up to 34.7 mm on day 154 (Fig. 5). As the Normal P-Plot of the elevation data did not show any evidence of heteroscedasticity, the data were not transformed. There were significant effects (Table 1) for the factors Time ($p = 0.0267$), Quadrant nested in Treatment nested in Plot ($p \ll 0.0001$) and Time x Quadrant nested in Treatment nested in Plot interaction ($p \ll 0.0001$), but not for Treatment ($p = 0.8644$) or for Time x Treatment interaction ($p = 0.1321$).

On day 7, ca. 2 mm of material had accreted on top of the sediment in the defaunated plots (Fig. 6a & 6b). The accretion occurred in a linear fashion (linear regression: slope = 0.19 mm day^{-1}, $p = 0.0021$, Multiple $r^2 = 0.9254$), reaching approximately 7 mm at day 32. The percentage of mud in the accreted material (Fig. 6c) also increased linearly, reaching around 60 % at day 32, although this was not statistically significant (linear regression: slope = 0.36 % day^{-1}, $p = 0.0587$, Multiple $r^2 = 0.3127$). At the same time in a parallel experiment, the top 1 cm of the ambient sediment contained 45 % mud and similarly defaunated sediment contained ca. 50 % mud (Montserrat *et al.* 2008).

Field experiment – Luminophore profiles

The vertical profiles of the luminophores are shown in Fig. 7a-d. In the case of both the coarse and the fine tracer in the defaunated plots, we observed a subsidence of the layer of luminophores which is in effect the burial of the layer by accreted sediment (see above). The peak of the pixel count over the vertical can be seen to be gradually moving downwards (Fig. 7a & 7b). For both fractions of tracers within the defaunated plots, we clearly observed a loss of material over time.

In one of the two control plots, a small amount of the coarse tracer (Fig. 7c) was already found at 1 - 1.5 cm depth after one hour (0 days, first panel). At day 3, a slight diffusion of the luminophore layer to approx. 1 cm can be seen, but by day 7 and onwards, a rapid loss of material had occurred. Apart from the advection-diffusion transport processes, we observed some non-local vertical transport to depths up to ca. 1.5 - 2 cm (Fig. 7c, 7 days and 32 days). The fine tracer displayed a similar profile over time as it was quickly mixed to about 1 cm depth in one of the control plots, but was not found below that depth at subsequent samplings. At day 14 and day 32, only a small fraction of the initial amount of fine tracer was detected in the control plots.

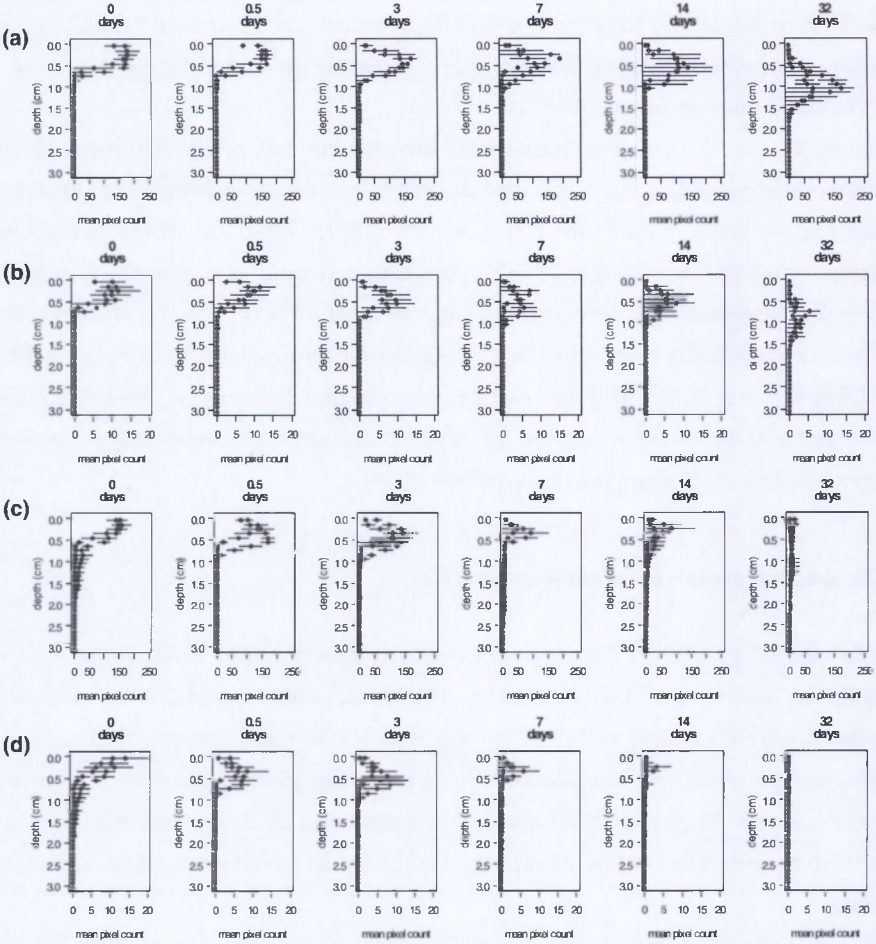


Fig. 7. Luminophore profiles as a function of sediment depth in the field defaunation experiment. The solid circles are the mean values per plot (n = 2) with the error bars representing the SEM (subquadrants per plot, n = 2). (a) Defaunated plots, coarse luminophores. (b) Defaunated plots, fine luminophores. (c) Control plots, coarse luminophores. (d) Control plots, fine luminophores.

Field experiment – Sediment budgets

Depth-integrated pixel counts decreased for both tracer fractions in both treatments (Fig. 8). In all cases there was a rapid decrease of material within the first four samplings (0 days – 7 days), followed by a steady but much slower loss of material. In the control plots, almost 100 % of the fine luminophores were lost by the end of the experiment. We regressed the natural log-transformed depth-integrated mean pixel count for both fraction tracers against Time with Treatment as a covariate. Between tracer fraction, the fine tracer was lost faster than the coarse tracer (ANCOVA: $p \ll 0.0001$). Starting from a full factorial (overparametrised) model containing Time, Treatment and Fraction, and all interactions as factors, the factors Treatment ($p = 0.1133$) and Treatment x Fraction did not have an effect ($p = 0.963$). Removal of both of these factors yielded a minimal adequate model (MAM): ln(pixel) = Time + Fraction + Time x Treatment + Time x Fraction + Time x Fraction x Treatment, of which the parameter estimates are given in Table 2. The loss rates are different between sampling occasions (Time) and between fractions and the fraction-specific loss rate differs between treatments.

Integrated over the top 3 cm of sediment, the tracer ratio of coarse : fine increased over time for both treatments. However, the coarse : fine ratio increased more rapidly for the control than for the defaunated plots (ANCOVA: $p \ll 0.0001$) during the experimental period (Fig. 9). The coarse : fine luminophore ratio in the control plots increased 7.3 times (15.09 to 113.47), against an increase of 2.35 times in the defaunated plots (17.87 to 42.01). The ratio (natural log transformed) of coarse : fine luminophores was significantly different with strong effects of the factors Time (ANOVA, $p \ll 0.0001$), Treatment (ANOVA, $p = 0.0008$) and the Time x Treatment interaction factor (ANOVA, $p \ll 0.0001$).

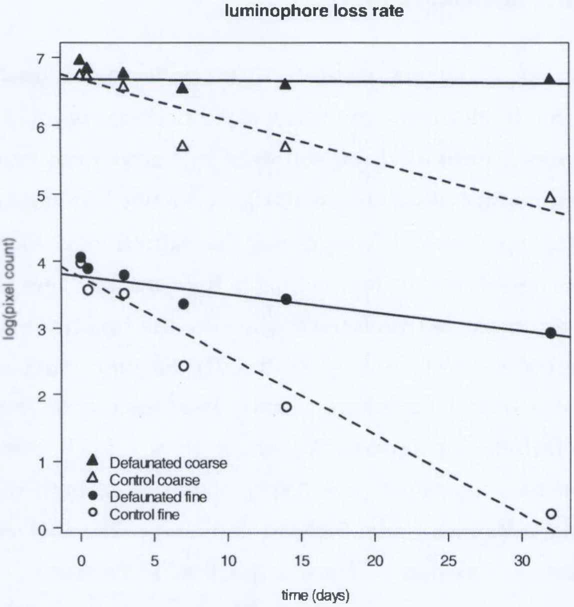


Fig. 8. The luminophore loss rate as a function of time. The y-axis denotes the natural log-transformed pixel count for the coarse luminophores (triangles) and for the fine luminophores (circles). The open symbols and the dashed lines represent the values and linear regression for the control plots, the solid black symbols and solid black lines represent the values and linear regression for the defaunated plots.

Parameter	Estimate	Std. Error	p - value
Intercept	3.767	0.096	<< 0.0001
Time	-0.119	0.008	<< 0.0001
Fraction	2.903	0.136	<< 0.0001
Time x Treatment	0.091	0.01	<< 0.0001
Time x Fraction	0.058	0.012	<< 0.0001
Time x Treatment x Fraction	-0.033	0.014	0.0311

Table 2. *Parameter estimates and their respective standard errors for the minimal adequate model obtained by analysis of covariance of the natural logarithm-transformed luminophore fraction-specific pixel count for both treatments.*

Mesocosm experiment – profiles and budgets

The luminophore profiles of the experimental cores in the mesocosm experiment were similar to those extracted in the field experiment, but with obvious differences like the absence of accretion of sediment in the "No Cockle" (NC - comparable to "Defaunated") cores, and the consequent subsidence of the luminophore layer. Large parts of the luminophore layer on top of some of the NC cores were lost during processing. These cores were therefore omitted from the analysis. The cockles in the "Cockle" (C - comparable to "Control") cores were recorded moving, burrowing and 'shaking' (*sensu* Flach *et al.* 1996 and references therein) or 'coughing' (rapid valve adductions), thereby expelling visible amounts of tracer which accumulated around the base of the cores during the experiment. In both the C and the NC cores which were immediately frozen (the "0 days" cores) there had been no sediment reworking: both treatments displayed the same profiles for both tracer fractions (data not shown).

In the "21 days" cores, luminophore material in the layer which constituted the initial tracer disc (i.e. 0 - 3 mm) had disappeared from this layer in both the C and the NC cores. However, the tracer profiles of the NC cores were very similar to any of the "0 days" cores, in the sense that the initial luminophore layer was largely intact. The loss from the initial layer in the NC cores was around 30 % of the coarse tracer and 5 % of the fine tracer (Fig. 10a-b). Compared to the C cores, the loss was 86 % for the coarse and 89 % for the fine tracer. Besides expelling sediment, the cockles' movements also transported the tracer material downward into the sediment to form a three-layer system (Fig. 10c-d). Starting from the sediment surface the first layers (0 – 5.5 mm depth), being the remnants of the luminophore disc, consisted of 25 % of the initial values for the coarse tracer and 17 % of the initial values for the fine tracer (i.e. the luminophore disc at 0 days). A second, uniformly mixed layer started at 5.5 mm deep and continued to around 23.5 mm deep. This uniform layer consisted of 32 % of the starting values for the coarse tracer and 9 % of the initial values for the fine tracer. Below this uniform layer, the amount of tracer decreased exponentially as a function of sediment depth for both fractions. The percentage of initial coarse tracer found in the deeper layers was 7 %, against 1 % of initial fine tracer.

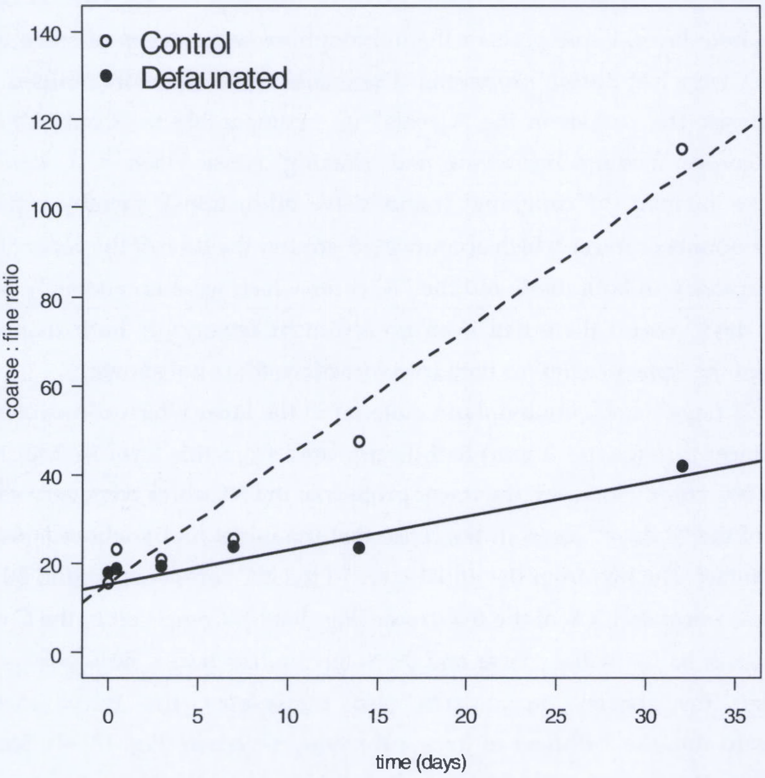


Fig. 9. The ratio of coarse : fine luminophores as a function of time. The open symbols and the dashed line represent the values and linear regression for the control plots, the solid black symbols and solid black line represent the values and linear regression for the defaunated plots.

In both the C and the NC cores we observed a decrease of tracer material from the sediment column as a whole. In the NC cores there was a decrease of 29 % of coarse tracer and a 4 % decrease of fine tracer in the 21 days experimental period (Table 3). Within the same period, we recorded a 37 % decrease of coarse tracer and a 73 % decrease of fine tracer material in the C cores (Table 3). ANOVA (Time, Treatment and Fraction as factors, full factorial design) showed significant effects of Time ($p \ll 0.0001$), Treatment ($p \ll 0.0001$) and Time x Treatment interaction ($p = 0.0007$), but no significant effect of either the factor Fraction ($p = 0.0915$) or the Time x Fraction interaction ($p = 0.6791$). The Time x Treatment interaction and the Time x Treatment x Fraction interaction both had a significant effect ($p = 0.0002$ and $p = 0.0018$, resp.). The resultant coarse : fine ratio decreased slightly from 0.94 to 0.7 between 0 and 21 days in the NC cores (Fig. 11). In the C cores, the coarse : fine ratio started at 1.05 at 0 days and increased almost two and a half times to 2.48 at 21 days.

	Coarse 0 days	**Coarse 21 days**	**Fine 0 days**	**Fine 21 days**
No Cockle (NC)	561 ± 6	400 ± 4	599 ± 6	575 ± 6
Cockle (C)	504 ± 5	320 ± 1	479 ± 5	129 ± 1

Table 3. *Depth-integrated luminophore fraction-specific mean ± SEM pixel counts for both treatments (NC and C) on both t = 0 days and t = 21 days.*

Discussion

Luminophore use in bioturbation experiments

The two different luminophore size classes have proven to be very good visual tracers for bioturbation processes in the sediment. Both the direct profile-imaging as well as the budgeting of both fractions in the sediment column have yielded valuable results and have shed light on how fauna can induce differential processing of sediment fractions, and its net effect on local vertical grainsize distribution. Also, the luminophore layer in the defaunated plots enabled us to observe both gross and net effects of the absence of bioturbation; the actual amount and composition of accreted material on top of the layer could be measured directly.

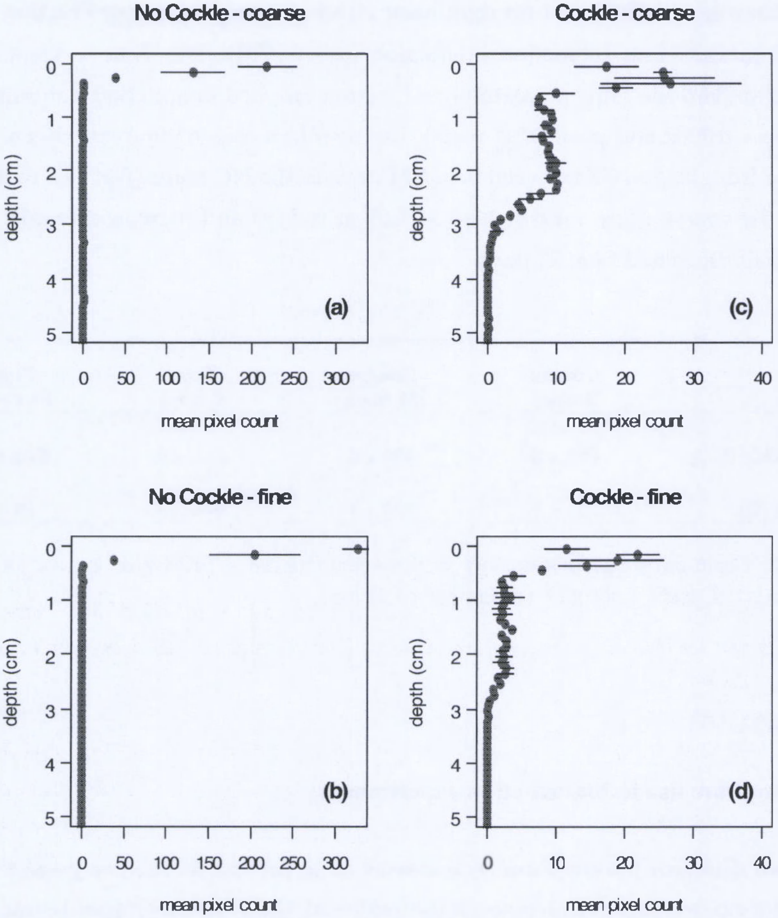


Fig. 10. The luminophore profiles, as a function of depth, of the cores used in the mesocosm experiment at t = 21 days. (a) No Cockle cores, coarse luminophores. (b) No Cockle cores, fine luminophores. (c) Cockle cores, coarse luminophores. (d) Cockle cores, fine luminophores.

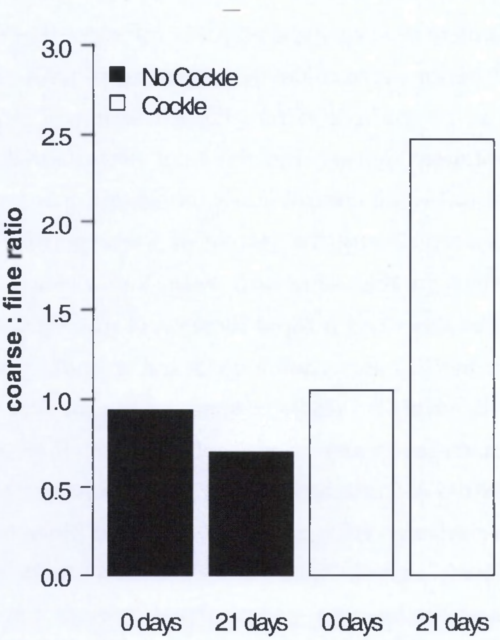


Fig. 11. The depth-integrated coarse : fine luminophore ratio for the cores used in the mesocosm experiment for the No Cockle (black bars) and for the Cockle (white bars) treatments on t = 0 days and t = 21 days.

The reported overlap in size distributions between the two luminophore types can be avoided by sieving them before application. We have not sieved the fractions due to logistical constraints, but in this case the overlap most probably reflected the natural situations where smaller particles can coagulate into larger flocs. Nevertheless, the use of two sizes of luminophores has been very successful in determining selectivity between sediment size fractions.

Macrobenthic assemblage

The defaunation treatment was very successful in the sense that we succeeded to create two very different macrofaunal assemblages with their respective bioturbatory effects to be studied. Within the experimental period of 32 days both the total macrofaunal density and the total macrofaunal biomass in the defaunated plots did not reach control levels, indicating that recolonisation has not reached its end-stage during the course of the experiment. In a parallel defaunation experiment in the same area both Van Colen *et al.* (2008) and Montserrat *et al.* (2008) described a rapid increase of microphytobenthos (MPB; chl *a* data not shown here) biomass and a quick but temporary recolonisation of mobile species, like the mudsnail *Hydrobia ulvae,* within the first month, whereas deeper burrowing macrofauna arrived only after two to three months. Besides the mudsnail, the tellinid *Macoma balthica* and the polychaetes *Nereis diversicolor* and *Heteromastus filiformis* also colonised the defaunated plots, but as these were all juvenile individuals -given their low biomass- their contribution to bioturbation processes within the experimental period can be considered marginal. *H. ulvae* is a highly mobile species, able to cover large distances (Andersen *et al.* 2002) while crawling over the sediment surface and grazing on MPB. The recolonisation patterns observed in the first month by Van Colen *et al.* (2008) are therefore very similar to our recordings within the 32 days experimental period.

The macrofaunal assemblage in the control plots was dominated by *Cerastoderma edule* in terms of biomass and also, considering its shape, in terms of biovolume (Hillebrand *et al.* 1999 cited in Gilbert *et al.* 2007). *C. edule* is a motile, suspension feeding bivalve crawling through the top 3-5 cm of the sediment, able to affect both the biotic and abiotic parts of its environment (Ciutat *et al.* 2007, Flach 1996, Montserrat *et al.* 2008). The effect of *C. edule* on the benthic boundary layer (BBL) is twofold: (1) the physical structure of its shell protruding from the sediment surface increases bottom roughness and (2) its movements cause biodestabilisation of the sediment surface. Both mechanisms translate into a significant decrease of the U_{crit} (the critical erosion velocity – the velocity needed to erode 1 g of sediment m^{-2}) and a significantly higher sediment transport with an increase of animal density (Ciutat *et al.* 2007). Following the classification used by Montserrat *et al.* (2008) to describe bioturbating fauna by their primary geomechanical effect, the macrofaunal assemblage at the Paulinapolder site - and therefore that in the control plots- was dominated by biodiffusors (*C. edule*), with some contribution of gallery burrowers (*N. diversicolor*) and surface disruptors (*M. balthica*).

Tracer profiles and budgets

The apparent subsidence of the luminophore layer (Fig. 6a) was a very conspicuous effect of the absence of the natural assemblage and the concurrent lack of vertical sediment dynamics in the defaunated plots. In the absence of bioturbation, accretion of muddy sediment occurred in a linear fashion over time, causing downward advective transport of the luminophore layer. Once the luminophores in the defaunated plots were covered with a sufficiently thick layer of accreted sediment, they were not a part of the sediment-water interface anymore and thus unavailable for erosion. The luminophore layer itself remained relatively unchanged during the first samplings but had clearly been affected by advection/diffusion processes by the end of the experiment (Fig. 7a-b and 6a). Despite the diffusive processes, mainly mediated by recolonised juvenile fauna, the total amount of luminophores in the defaunated plots did not change dramatically. The coarse luminophores virtually ceased to disappear from the sediment column after a layer of almost 2 mm was deposited on day 7. Three weeks later, on day 32, between 65 and 70 % of the coarse tracer was still present in the sediment column, under a layer of 7 mm accreted sediment which consisted of ca. 60 % mud. In contrast, the fine tracer was continuously removed from the sediment, albeit at a slow rate. However, both tracer fractions were overall more conserved in the defaunated sediment than in the non-treated controls.

In the control plots, the natural macrofaunal assemblage was quick to rework the tracers, relocating a portion to deeper sediment layers. There was some variation between the two plots with respect to vertical distribution of tracers (Fig. 7c: 0 days [= 1 hr.], 7 days and 32 days) which can be attributed to species known to be responsible for non-local transport, mainly *M. balthica* (Gingras *et al.* 2008, Michaud *et al.* 2006) and *N. diversicolor* (Duport *et al.* 2006, Fernandes *et al.* 2006). Lateral heterogeneity in bioturbation, which occurs typically over short time scales (Maire *et al.* 2007), may explain why the variation is found only in one plot or only during one sampling occasion.

Differential tracer processing

In the absence of macrofaunal bioturbators in the field experiment, there was accretion of mud, stabilisation of the accreted material and the sediment surface (Montserrat *et al.* 2008) and consequent subduction of the sediment tracers. In the control sediment we observed differential incorporation of both tracer fractions. These luminophore profiles could be used for estimating a biodiffusion coefficient (D_b) as a measure of sediment reworking rate on a community level. However useful, the use of biodiffusion models to estimate D_b should only be applied on larger spatial scales and, more important, longer temporal scales (Meysman *et al.* 2008a, Meysman *et al.* 2008b). The period of 32 days of the field experiment was simply too short.

The presence of benthic animals in both the field and the mesocosm experiments caused differential incorporation of luminophores into the sediment but, more importantly, also a qualitatively high rate of resuspension. This resulted in the loss of a significant amount of the luminophores, and then in particular within the first week. The net effect of the cockle-dominated macrofaunal assemblage in the control plots was to mobilise the sediment, which resulted in a higher treatment-specific loss rate of both tracer fractions. Between treatments, there was preferential remobilisation of the fine luminophores, which had a higher loss rate than the coarse luminophores. Even in the defaunated plots, there was loss of fine luminophores, but at a much lower rate than in the presence of a natural assemblage. These results are well supported by the data from the mesocosm experiment. There was a significant higher loss of fine luminophores from the cores, while still a high percentage of the coarse luminophores were retained, resulting in an increased coarse : fine ratio. We have shown here that the main effect of both the cockle-dominated natural assemblage and the cockles in the mesocosm experiment is selective removal of fine material from the sediment surface layers.

The selective removal of fines can be accomplished by the characteristic movement through the surface sediment layers or by the sudden adduction of the valves (resp. 'ploughing' and 'shaking') (Flach 1996). Although *C. edule* tends to have its natural abundance peak in fine to medium sands (Ysebaert *et al.* 2002), it does not directly utilise the bottom sediment and is therefore not dependent on its composition. However, cockles are negatively influenced by higher suspended sediment concentrations (SSC) to which they respond with a higher shaking frequency to get rid of fine material that clogs their gills (Ciutat *et al.* 2007).

We observed the shaking in the mesocosm experiment when the cockles were burrowing into the sediment as the water level receded. First, the agile foot was inserted into the sediment in a manner as described for crack propagation by burrowing marine polychaetes (Dorgan *et al.* 2005). The cockle then slowly opened its shell valves and adducted them quickly. In doing so, it forced the water from its mantle cavity and created a small body of quicksand beneath its shell and, at almost the same moment, pulled itself down into the sediment with its foot. This was repeated until the cockle is burrowed beneath the sediment surface and only its siphons are visible. In nature, the burrowing behaviour of cockles causes sediment to become resuspended (more than is already caused by waves or valve adductions); the coarse fractions settle relatively fast and the fine fractions will be transported away by the overlying water.

Ecosystem engineering effects

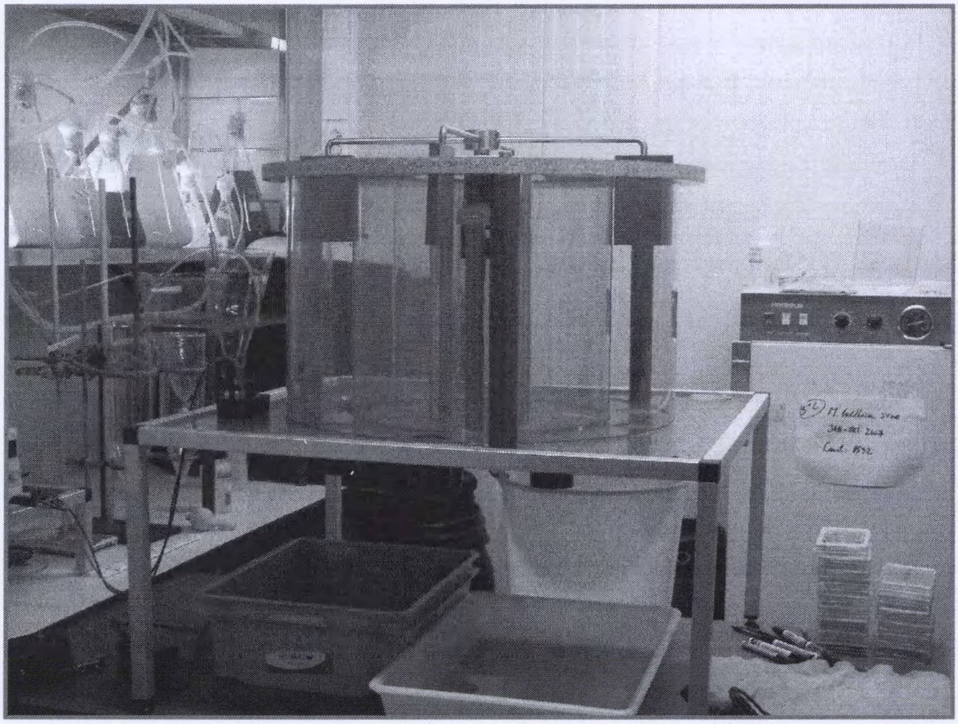
Using the luminophore coarse : fine ratio as a proxy for the sediment sand : mud ratio, the cockle-dominated benthic assemblage's main effect was to render the sediment less muddy. The control sediment was characterised by a significantly higher erodability, compared to the situation without benthic fauna, in which net accretion of mud occurred. An increase in mud content, and the fact that it was not homogeneously mixed into the sediment by infauna as in a natural situation, can induce a local change in ecosystem functioning of the sediment through known biogeochemical cascades. First of all, a higher mud content entails a higher organic matter (OM) content with concomitant higher respiration rates (Aller & Aller 1998). Secondly, the increased mud content decreases the permeability of the sediment and flow of pore water, decreasing the oxygen penetration depth (Winterwerp & Van Kesteren 2004). Thirdly, a higher mud content will cause benthic primary production and standing stocks to increase (Van De Koppel *et al.* 2001). These profound changes would in turn reflect on the entire benthic community (micro-, meio- and macro-) and could have possibly set back the system to an earlier successional state (Connell & Slatyer 1977, Pearson & Rosenberg 1978, Van Colen *et al.* 2008).

Thrush *et al.* (2006) demonstrated the important structuring role of large surface-dwelling, suspension-feeding bivalves in the biogeochemistry and makeup of the macrobenthic assemblage in intertidal systems. Volkenborn *et al.* (2007b) attribute similar mechanisms to the lugworm *Arenicola marina* in the German Wadden Sea. *A. marina* is a model ecosystem engineer and has all the characteristics of a climax species: a long-lived, slow-growing, deep-burrowing, sediment-regenerating bioturbator (Riisgård & Banta 1998), able to inhibit the settlement of other (pioneer) species (Flach 1992) and even plants (Van Wesenbeeck *et al.* 2007). The physical restructuring of the bottom by its bioturbation increases the bottom roughness and resuspension of fine material, keeping the intertidal areas it inhabits more sandy (Volkenborn *et al.* 2007a). The influence *A. marina* exerts on the entire spectrum within the intertidal (grain size distribution - pore water flow - bacterial community - micro-/meio-/macrobenthos) enables it to maintain its habitat in a preferred state, which can abruptly change when it is excluded (Van De Koppel *et al.* 2001, Volkenborn *et al.* 2007a). The cockles in our experiment appear to fulfil a similar role in this particular system, including the inhibition of opportunist pioneer species and physical ecosystem engineering, through mechanical disturbance of the sediment.

The absence of the cockle-dominated assemblage made it clear that these animals and their behaviour can cause large differences in sediment behaviour (Montserrat *et al.* 2008). As demonstrated in this study, the sediment erosion threshold during the first month was much higher in the defaunated plots than in the control plots. Also, the sediment bed level of the defaunated plots increased due to accumulation of muddy material. The lower abundance of grazing and mechanically disturbing fauna in the defaunated plots enabled MPB to form dense mats (Montserrat *et al.* 2008) which armour the sediment and can entrain mud. Constituting an important part of the macrobenthic assemblage, and backed up by the mesocosm experiment results, *C. edule* was responsible for a large part of the bioturbatory effects in this system. This study has demonstrated that the burrowing and ploughing behaviour of these biodiffusing bivalves is keeping the sediment matrix in a less muddy state -and therefore less organically enriched and less cohesive- by removing fine material from the sediment through an interaction of organism traits and hydrodynamic forcing.

Acknowledgements

The authors would like thank Jos van Soelen, Bas Koutstaal and the masters' students who helped during the fieldwork and the processing of the samples for their efforts. Jon Marsh and several members of the team at Environmental Tracing Services helped us with advice on how to apply and process the luminophores, for which they have our thanks. This research is supported by the Dutch Technology Foundation STW, applied sciences division of NWO and the Technology Program of the Ministry of Economic Affairs.



~148092

CHAPTER 9

SETTLEMENT OF *MACOMA BALTHICA* LARVAE IN RESPONSE TO BENTHIC DIATOM FILMS

Accepted with minor revision as:

Van Colen C., Lenoir J., De Backer A., Vanelslander B., Vincx M., Degraer S., Ysebaert T.
Settlement of *Macoma balthica* larvae in response to benthic diatom films
Marine Biology

Abstract

We investigated the role of multi-species benthic diatom films (BDF) on the settlement of *Macoma balthica* larvae in mesocosm still-water experiments and flume experiments. Observations during five minutes revealed that in a still-water environment, the larval settlement response was lower and the average burrowing time (i.e. penetration into the BDF) was slower in older BDF as compared to control and younger BDF. The different settlement responses to different ages of BDF were related to the concentration of chlorophyll *a* and extracellular polymeric substances of the BDF, suggesting that a higher physical resistance during penetration into a dense matrix of diatoms and its associated sugar and protein compounds results in a lower settlement response in dense BDF at the very short term. In a hydrodynamic environment, *M. balthica* larvae settled significantly more in BDF as compared to control sediments. Comparison with the settlement of polystyrene mimics and freeze-killed larvae revealed that, active selection, active secondary dispersal and, at low flow velocities (5 cm s^{-1}), also passive adhesion are important mechanisms that determine the settlement success of *M. balthica* larvae in estuarine biofilms.

Our findings suggest that benthic diatoms may significantly affect *M. balthica* settlement behaviour and recruitment in estuarine tidal flats.

Keywords: *Benthic diatom film, Larval settlement, Hydrodynamics, Settlement preferences, Macoma balthica*

Introduction

One of the most important challenges in estuarine benthic ecology is to understand the spatial and temporal variability in soft-sediment communities. Recruitment is of fundamental importance to macrobenthic community structure because it is the foundation upon which all subsequent interactions within the community take place (Woodin *et al.* 1995).

The majority of marine macrobenthic invertebrates display a life cycle with a dispersive (i.e. pelagic) larval phase during which they distribute and settle down into new habitats and develop to the benthic stage. Settlement of marine benthic invertebrates is mediated by a wide set of factors, e.g. flow characteristics (Crimaldi *et al.* 2002), organic content of the sediment (Grassle *et al.* 1992), sediment disturbance (Woodin *et al.* 1998, Marinelli & Woodin 2002, Marinelli & Woodin 2004), sediment grain size (Pinedo *et al.* 2000), nutrient pore water concentrations (Engstrom & Marinelli 2005), presence of conspecific juveniles or adults (Snelgrove *et al.* 2001), metabolites of sympatric organisms (Woodin *et al.* 1993, Esser *et al.* 2008) and the presence of bacteria (Dobretsjov & Qian 2006, Sebesvari *et al.* 2006). Furthermore, during recent years, there is growing evidence that also marine biofilms are instrumental to habitat selection and the onset of settlement events for many benthic organisms (reviewed in Qian *et al.* 2007).

Marine biofilms are highly variable in time and composition, forming complex aggregates composed of diatoms, bacteria, protozoa and fungi (Decho 2000), all enmeshed in a matrix of extracellular polymeric substances (EPS). Both facilitative and inhibitive effects of marine biofilms on larval settlement have been reported, which are often attributed to waterborne bacterial EPS depending on origin, surface chemistry, micro-topography and metabolic activity of the biofilm (reviewed in Qian *et al.* 2007). The proportion of benthic diatoms in biofilms of estuarine tidal mudflats can be significantly high (Sabbe & Vyverman 1991, MacIntyre *et al.* 1996). Lam *et al.* (2003) showed that relative space occupation of diatoms can mediate larval settlement of the polychaete *Hydroides elegans*. Hence, next to the bacterial compound of a marine biofilm, also the specific role of diatoms in the settlement of tidal flat invertebrate larvae requires specific interest. Moreover, postlarvae of herbivore benthic invertebrates often feed on diatoms. Thus, recruitment success of these larvae may depend on differences in diatom community composition because of their post-larval dietary requirements.

Marine biofilms have intensively been investigated with respect to their role in larval settlement of barnacles, ascidians, bryozoans, sea urchins, gastropods and polychaetes (e.g. Keough & Raimondi 1995, Olivier *et al.* 2000, Harder *et al.* 2002, Lam et al 2003, 2005, Dahms *et al.* 2004, Sebesvari *et al.* 2006, Chiu *et al.* 2006, Dworjanyn & Pirozzi 2008) but far less is known about diatom film mediation on bivalve settlement, especially in soft-sediments. The baltic tellin *M. balthica* is an infaunal surface deposit-feeding and facultative suspension feeding bivalve (Rossi *et al.* 2004) which displays a pelagic larval stage (Caddy 1967). This species occurs from the Gironde estuary in Southwest France to the polar region in Greenland and Siberia (Meehan 1985). In north-western European tidal flats, *M. balthica* is one of the most common bivalves that reaches densities ranging from tens to hundreds of individuals m^{-2} (Beukema 1976, Van Colen *et al.* 2006, 2008) and is an important food source for wading birds, benthic and epibenthic organisms (Hulscher 1982, Zwarts & Blomert 1992, Hiddink *et al.* 2002a, b). Further, this species influences the geochemistry of the sediment, and thus tidal flat energy cycling in general, by their bio-engineering impact due to burrowing and feeding actions (e.g. Marinelli & Williams 2003). Hence, successful recruitment of *M. balthica*, and bivalves in general, is of crucial importance to maintain tidal flat ecosystem functioning.

In general, the final recruitment success is determined by two major classes of processes: (1) primary dispersal and initial settlement and (2) post-settlement processes (i.e. mortality and secondary dispersal and settlement) (Armonies 1992). In this paper, we report and discuss the results of larval response tests of the bivalve *Macoma balthica* to multi-species benthic diatom films (BDF), using still-water assays and flume experiments. Multiple choice flume experiments enable the determination of settlement preferences because bivalve larvae can select a preferred settlement site in a hydrodynamic environment (e.g. Grassle et al 1992, Snelgrove *et al.* 1998, Engstrom & Marinelli, 2005). In addition, observations from still-water assays provide valuable information on some specific conditions which influence successful establishment within a given habitat (Marinelli & Woodin 2004).

Specifically, the following null hypotheses concerning *M. balthica* larval settlement in response to BDF were tested:

H_{01}: Settlement response (i.e. rejection/acceptance) does not differ between different ages of BDF in a still-water environment (Experiment 1).

H_{02}: Settlement choice is not influenced by BDF in a hydrodynamic environment (Experiment 2).

H_{02a}: Settlement choice is not influenced by flow velocity.

H_{02b}: Settlement choice does not differ from deposition of dead larvae and polystyrene mimics, thus settlement is a passive, depositional, process.

H_{03}: In a hydrodynamic environment, the settlement response after primary settlement is not determined by BDF (Experiment 3).

Materials & methods

Collection, production and cultivation of larvae

Collection

Adult *M. balthica* were repeatedly collected from Paulinaschor (The Netherlands, 51°21'24" N, 3°42'51" W) during low tide in February-March 2008 and stored at 5 °C in aerated basins (40×33×14 cm), prefilled with sieved sediment (1 mm) and 2 µm filtered seawater (FSW) with a salinity of 27 ± 1 practical salinity units (PSU). Each basin contained ~ 150 individuals which were fed 3 times wk^{-1} with a mixture of concentrated algae (*Isochrysis galbana* and *Tetraselmis* sp.; Reed Mariculture).

Larval production

Individual *M. balthica* were induced to spawn, following the procedure of Honkoop *et al.* (1999) and Bos (2005). Therefore, the adults were exposed to the selective serotonin re-uptake inhibitor (SSRI) fluoxetine, preceded by a Δ 10 °C temperature shock. SSRI's prevent the deterioration of neurotransmitters, so nerves are stimulated longer and more intensely than usual (Honkoop *et al.* 1999). On average, 35 % of the adults could be induced to spawn. Fertilisation was carried out by pipetting eggs of several females into a beaker and adding 1 to 3 ml of sperm suspension derived from at least 5 males. The resultant mixture was left undisturbed for 4 hours at 15 °C. Fertilised eggs (ø ~100 µm) were then separated from all other matter by rinsing them over stacked sieves of 125 and 32 µm. Subsequently, they were transferred into 2 L glass bottles (further referred to as batches), containing 15° C UV-irradiated filtered 27 ± 1 PSU seawater (UV FSW) dosed with 1.5×10^{-5} g l^{-1} Penicillin G potassium salt and 2.5×10^{-5} g l^{-1} streptomycin sulphate. The bottles were placed on a roller-table (3 rpm) to avoid sinking of larvae.

Cultivation & maintenance

At day 4, all larvae had reached the D-stage, named after its resemblance to the letter D, and from this moment on, live *Isochrysis galbana* (10^5 cells ml^{-1}) was added to the UV FSW which was refreshed every other day. Subsamples were taken to measure larval mortality. During the cultivation, we observed a mortality of on average 36 % of the brood stock at day 20, which is a mortality rate of about 0.02 d^{-1}. 21 to 24 days after fertilization, the larvae metamorphosed to the benthic stage as indicated by the development of a foot (i.e. pediveliger stage). 25 day old larvae (270 μm ± 4 SE μm), actively moving their feet, were used in all experiments and are further referred to as *M. balthica* larvae or alive larvae (experiment 2).

Settlement response in still-water (Experiment 1)

Sediment processing

Sediment was collected from Paulinaschor at low tide. Collection was confined to the top 2 cm and sieved over a 1 mm mesh sized sieve in the laboratory to remove macrobenthic organisms and larger debris. Subsequently the sieved sediment was heated at 180° C during 4 hours. This sediment has a median grain size of 89.6 ± 1.07 SE μm and the mud content is 30.8 ± 0.52 SE % (Malvern Mastersizer 2000 laser diffraction) and is further referred to as control sediment. This sediment was preferred above muffled sediment as a control since pilot tests revealed an inhibitory impact of muffling on settlement responses which was not related to changes in organic content and or grain size. The inhibitory influence of muffled sediment therefore presumably relates to the dissolution of material from the muffled sediment into the water column.

For the assays, 2.5 g of control sediment was transferred into each well of a sterile 12-well microplate (3.8 cm^2 well surface area, TPP, Switzerland) resulting in a 7 mm sediment layer. To develop a benthic diatom film (BDF), the control sediments were inoculated with 3 ml of axenic diatom cultures and incubated at 18° C, 14/10 hours day/night light regime (145 µmol photons $m^{-2} s^{-1}$). The diatoms used in this experiment were *Navicula phyllepta, N. gregaria, N. arenaria* and *Cylindrotheca closterium.* These species were isolated from the tidal mudflat at Paulinaschor and were dominant components of the microphytobenthos at that site (Sabbe & Vyverman 1991, Forster *et al.* 2006). Cells for inoculations were harvested from monoclonal, exponentially growing cultures at 19°C ± 1°C and illuminated at a rate of 90 µmol photons m^{-2} s^{-1} with a light/dark cycle of 14/10 hours. The experimental microcosms were inoculated with a fixed total biovolume of 1 10^8 μm^3 (biovolume of *N. phyllepta, N. gregaria, N. arenaria* = 3 10^7 μm^3; biovolume of *C. closterium* = 1 10^7 μm^3). To obtain different BDF, sediments were incubated for 0, 4, 11 and 21 days respectively for the control, "low", "medium" and "high" treatment. Every day, 1.2 ml of the F/2 medium (Guillard 1975) of all treatments was refreshed in a flow bench without disturbing the sediment. Control sediments were maintained under the same incubation conditions. This resulted in an averaged *C. closterium* – *N. phyllepta* – *N. arenaria* – *N. gregaria* relative biovolume of 14% – 26% – 32% – 31%, 22% – 17% – 34% - 26% and 27% – 16% - 31% - 25%, for the "low", "medium" and "high" treatment, respectively. Experimental sediments were further characterized by their Chl*a* and EPS concentration. Chl*a* concentration was determined by HPLC analysis of the supernatant, extracted from the lyophilized sediment by adding 10 ml 90% acetone. The EPS concentration was measured spectrophotometrically using the phenol-sulphuric acid assay (Dubois *et al.* 1956) on the colloidal carbohydrate fraction of the supernatant extracted after lyophilization (De Brouwer & Stal 2001).

Experimental protocol

In order to observe settlement responses (i.e. acceptance/rejection) to different ages of BDF, *M. balthica* larvae were labelled with fluorescent microparticles (Radglo, Radiant Color, N.V., Houthalen, Belgium) to obtain a contrast with the bioassay sediment. These microparticles are non-toxic and have a spherical diameter of 2 to 10 µm. Feeding larvae ingest these particles resulting in a gut region filled with fluorescent pigment (Lindegarth & Jonsson 1991, Jonsson *et al.* 1991), which become visible by illumination of the larvae with UV-light (365 nm). To assure uptake by the larvae, fluorescent pigment particles were supplied to feeding larvae (10^5 particles ml^{-1}) 24 hours prior to the experiments. Since the particles are insoluble in water, one droplet of detergent was added to facilitate suspension of these particles. Preliminary test showed that mortality rate was not affected as a result of fluorescent labelling. Prior to the still-water bioassays, 2 ml F/2 medium of each well was pipetted out and 2 ml of 27 PSU sterile UV FSW was added to the wells without disturbing the sediment. *Macoma balthica* larvae were picked out from two independent batches, using a stereomicroscope and UV-light to check their viability and dyeing. For each bioassay ($n = 6$ $batch^{-1}$), fifteen larvae were gently added to a well with a glass pipette and timing started when the pipette was empty. All pipettes were checked for remaining larvae, i.e. larvae that were not added to the well. During 5 minutes the burrowing larvae were counted and their disappearance in the sediment was timed. After this time period, larvae that were still on the sediment surface were interpreted as not settled.

To quantify bacterial contamination of the BDF due to experimental handling procedures, bacteria were extracted from the biofilm, stained with Acridine Orange and bacterial cell densities were enumerated on 0.2 µm black polycarbonate filters under blue-green light excitation (480 – 195 nm). Recorded bacterial densities were marginal, varying between 160 – 630 cells mm^{-2} and did not differ significantly between treatments (t-test; $p > 0.05$).

Statistical analysis

Burrowing time and percentage of larval settlement (n° of settled larvae/n° of total added larvae) after 60, 120, 180, 240 and 300 seconds were used as response variables to identify settlement responses of *M. balthica* larvae to the different biofilms. Burrowing time data were root transformed and percentage of larval settlement data were arcsine transformed to gain normality (Shapiro-Wilks' tests) and homogeneity of variances (Cochran & Bartlett tests). The effect on burrowing time was investigated using two-factor analysis of variance with Batch as random factor and Treatment as fixed factor, followed by Tukey's multiple comparison post-hoc tests. Larval settlement data were analyzed using a repeated measures design with Batch as random factor and Treatment and Time as fixed factors. Tukey's multiple comparison tests were performed to investigate significant differences between treatments, whenever the Treatment within Batch factor was significant. Since the sphericity assumption for repeated measurements was violated by our data, adjusted F tests using the Greenhouse-Geiser correction were calculated, resulting in more conservative p-levels (Quinn and Keough 2002). Further, regression analysis was performed to investigate relationships between the percentage of larval settlement, averaged burrowing time and the BDF characteristics (Chl *a* and EPS).

Annular flume experiments (Experiment 2 & 3)

Annular flume characteristics

According to the Plymouth Marine Laboratory annular flume (Widdows *et al.* 1998), a flume was constructed of polystyrene material, forming a circular channel 10 cm wide (inner ø 44 cm, outer ø 64 cm), 35 cm deep and with a maximum volume of 60 L. The channel flow was driven by contact on the water surface with 4 pvc paddles (9x14 cm), which were attached to a rigid support system driven by a variable speed DC motor. On the bottom of the tank, pvc pots (inner ø 5 cm) can be attached, flush with the flume bottom and O-rings sealed the pots to prevent water loss. The annular flume is a good compromise in terms of portability and the spatial coverage (0.17 m^2) and allowed simultaneous testing of treatments in a realistic fully developed benthic boundary layer where larvae and sediment treatments could easily be removed and recovered after each trial. The disadvantage of annular flumes in general is the effect of secondary circulation. However, secondary flows are kept to an acceptable minimum (~ 3% of tangential flow) with the 10 cm channel width of the flume in the current study (J. Widdows, pers. comments). To characterize the fluid dynamic environment, velocity profiles were measured at 8 cm above the bottom with a SonTek Micro ADV (Acoustic Doppler Velocimeter), mounted through the bottom of the flume. A linear relation between free stream velocity and rounds per minute was found (free stream velocity = 1.7785 x RPM – 0.5672 (r^2 = 0.998)).

Sediment processing

The same control sediment as for the still-water bioassays was used. To yield the BDFs, the pvc-pots, prefilled with control sediment, were inoculated with a mixture of diatoms (total biovolume = 4.68 10^8 μm^3; relative biovolume = 30-30-30-10 %, respectively for *N. phyllepta, N. gregaria, N. arenaria* and *C. closterium*). Control and BDF sediments were incubated for 11 days at 18° C, 14/10 hours day/night light regime (145 µmol photons m^{-2} s^{-1}) and 10 ml of the F/2 medium was refreshed every day. Chl*a* and EPS concentrations of the upper 5 mm were determined according to the abovementioned methods (Experiment 1).

Settlement choice in a hydrodynamic environment (Experiment 2): protocol

The proportional distribution of alive larvae, freeze-killed larvae (further referred to as dead *Macoma*) and spherical polystyrene (PS) mimics (ø 250-400 µm) between BDF and control sediments was tested in a first set of experiments to examine processes affecting settlement of *M. balthica* larvae (i.e. active habitat selection vs. passive deposition). Therefore two BDF and two control sediments were screwed into the bottom of the flume (flume bottom surface occupied = 4.6 %; intersect between pots = 37.4 cm) for each experimental trial (n = 4) and the flume was filled with 50 L of FSW (15° C, 27 PSU). Subsequently, 500 juvenile *Macoma* and ~ 5000 PS mimics were randomly added to the flume and flow was initiated and maintained for 3 hours at 5 cm s^{-1} or 15 cm s^{-1}. In addition, two trials at 5 cm.s^{-1} and two trials at 15 cm s^{-1} were conducted with 500 dead larvae. Sinking velocities of the three types of 'settlers' in 15° C, 27 PSU still FSW were 2.8 ± 0.5 SE mm s^{-1}, 2.6 ± 0.2 SE mm s^{-1} and 1.6 ± 0.2 mm s^{-1}, respectively for alive larvae, dead larvae and PS mimics. Furthermore, no resuspension of the sediment was observed at 5 cm s^{-1} and 15 cm s^{-1} during pilot tests performed with neutral red dyed sediment. Hence, secondary dispersal after primary settlement is expected due to active choice, rather than occurring passively by sediment resuspension. After 3 hours, the experimental sediments were closed with inox plates, the flume was drained and the top 2 cm of the sediments was preserved in a 4 % buffered formalin – tap water solution, stained with Rose Bengal and the settled juveniles were sorted out under a stereomicroscope.

Settlement response after primary settlement (Experiment 3): protocol

Thirty *M. balthica* larvae were added to the control and BDF sediments and left to settle for 30 minutes. Subsequently the above standing F/2 medium was removed from each pvc pot and checked for unsettled juveniles. For each experimental trial, two control and two BDF sediments were screwed into the flume, flush with the flume bottom. Then, the flume was filled with 50 L of FSW (15° C, 27 PSU) and the flow was initiated at 5 cm s^{-1}. After 10 minutes, the flow was stopped and the experimental sediments were closed with inox plates, the flume was drained and the top 2 cm of the sediments was preserved in a 4 % buffered formalin – tap water solution, stained with Rose Bengal and the settled juveniles were sorted out under a stereomicroscope.

Statistical analysis

For Experiment 2, replicated G-tests for goodness of fit (Sokal & Rohlf 1995) were conducted to determine significant deviations from the 1/1 (i.e. even) distribution, the averaged distribution of the PS mimics, dead larvae and the averaged distribution of alive larvae, dead larvae and PS mimics at 15 cm s^{-1}. The two BDF and the two control sediments per experimental trial were pooled and only the juvenile % inside sampling pots was retained for statistical analysis. All results have been expressed as relative % recovered from BDF and control sediments and the percentages were adjusted to give composition, i.e. their cumulative abundance equals 100%. As such, the weight of all replicates in a replicated statistical test is equal (Moens *et al.* 1999). Measurement of the pooled G statistic (G_P) enabled interpretation of the significance of the overall deviation from the tested distribution over all replicates. G_P was calculated at a critical probability of $\alpha' = \alpha/k$, with k equal to the number of multiple pairwise tests (i.e. Bonferroni approach). As such, G-tests for PS mimics and alive larvae were performed at $\alpha = 0.008$ (i.e. 0.05/6). Experiment 3 was analyzed using a mixed model analysis of variance with Batch and Trial as random effects and Treatment as fixed effect. The proportion remaining to the sediments was arcsine-square root transformed to meet assumptions of normality (Shapiro-Wilks' tests) and homogeneity of variances (Cochran & Bartlett tests).

Results

Benthic Diatom Film characteristics

Manipulation of the incubation time successfully resulted in different BDFs. Chlorophyl *a* and EPS concentration of these BDFs (Table 1) were significantly different between treatments for each experiment (t-test, $p < 0.05$). Initiation of the flow slightly reduced the Chl*a* content of the BDF (-14 %, -12% and -29 %; respectively for 10 min at 5 cm s^{-1}, 3 hours at 5 cm s^{-1} and 3 hours at 15 cm s^{-1}) due to biofilm erosion during the first minute after initiation of the flow. However, differences between control and BDF sediments remained large and significant (t-test, $p < 0.05$).

	Chl*a* **(μg.g^{-1} dry sediment)**	**EPS** **(g glucose.g^{-1} dry sediment)**
Control sediment	$0.01 \pm 2.0\ 10^{-4}$	$1.1\ 10^{-4} \pm 7.9\ 10^{-6}$
Experiment 1		
4 day old	3.13 ± 0.81	$1.6\ 10^{-4} \pm 5.5\ 10^{-5}$
11 day old	8.46 ± 0.59	$1.8\ 10^{-4} \pm 5.9\ 10^{-5}$
21 day old	15.35 ± 3.6	$2.3\ 10^{-4} \pm 7.8\ 10^{-5}$
Experiment 2 & 3		
11 day old	7.04 ± 1.17	$1.7\ 10^{-4} \pm 2.9\ 10^{-7}$

Table 1. *Chla and EPS concentration ± SE of the benthic diatom film and control sediments in all experiments. Determination of BDF characteristics is based on the upper 7 mm of the sediment for Experiment 1 and the upper 5 mm of the sediment for Experiment 2 and 3.*

Settlement response in still-water (Experiment 1)

All *Macoma* larvae started to burrow within the first minute after their addition to the wells. The percentage of larval settlement significantly differed between treatments and times. Consequently, H_{01} was rejected, i.e. the settlement response differed between different ages of BDF in a still-water environment. No significant differences between the two batches were found and the interaction between Time and Treatment nested in Batch was not significant (Table 2). In general, the settlement response to controls and 4 day old BDF was higher than in 11 day old and 21 day old BDF. The percentage of larval settlement increased with time for all treatments and, in Batch 1, significant differences remained between 11 day old BDF and control sediments, even after 300 seconds (Tukey's test, $p < 0.05$) (Fig 1a). Consistently, the average burrowing time was significantly different between treatments with highest burrowing times in 11 day old and 21 day old BDF for both batches (Table 3, Fig1b). The percentage of larval settlement was significantly negatively related to the Chl*a* concentration and the colloidal EPS fraction of the BDFs (r^2 = 0.68 and r^2 = 0.52; respectively). No significant relations were found between the averaged burrowing time per treatment and BDF characteristics.

Settlement choice (Experiment 2)

Mean recovery rate of alive and dead larvae was 98% at both flow velocities, indicating that loss of larvae due to stickiness to the walls and paddles is marginal. On average, 5.8 ± 1.5 SE % of the live larvae and 6.0 ± 1.5 SE % of the dead larvae were recovered in the control and BDF sediments at 5 cm s^{-1}. At 15 cm s^{-1}, the total percentages of settlement in control and BDF sediments were 4.6 ± 1.5 SE % and 4.4 ± 1.4 SE %, respectively for alive larvae and dead larvae. Significantly more live larvae settled in BDF than in control sediments at 5 cm s^{-1} (Gp = 36.6, $p < 0.001$) and 15 cm s^{-1} (Gp = 59.2, $p < 0.001$) and the distribution of alive larvae did not differ between both flow velocities (Gp = 2.9, p = 0.087). Consequently, H_{02} is rejected while H_{02a} cannot be rejected, i.e. settlement is influenced by BDF but the settlement preference for BDF is independent of flow velocity.

The distribution of PS mimics did not differ significantly from the even distribution at both flow velocities (Gp = 6.8, p = 0.009; Gp = 0.3, p = 0.56, respectively for 5 cm s^{-1} and 15 cm s^{-1}). Consistently, the distribution of alive larvae significantly differed from the passive deposition of PS mimics at both flow velocities (Gp = 71.3, $p < 0.001$; Gp = 72.0, $p < 0.001$, respectively for 5 cm s^{-1} and 15 cm s^{-1}). Hence, H_{02b} is rejected, i.e. habitat selection for BDF is not a passive, depositional process. However, deposition of dead larvae was significantly higher in BDF at 5 cm s^{-1} (66 %; Gp = 20.8, $p < 0.001$), whereas the distribution of dead larvae did not differ significantly from the even distribution at 15 cm s^{-1} (Gp = 1.0, p = 0.32). Hence, based on comparison between distribution of dead and alive larvae, H_{02b} could only be rejected at a flow velocity of 15 cm s^{-1}.

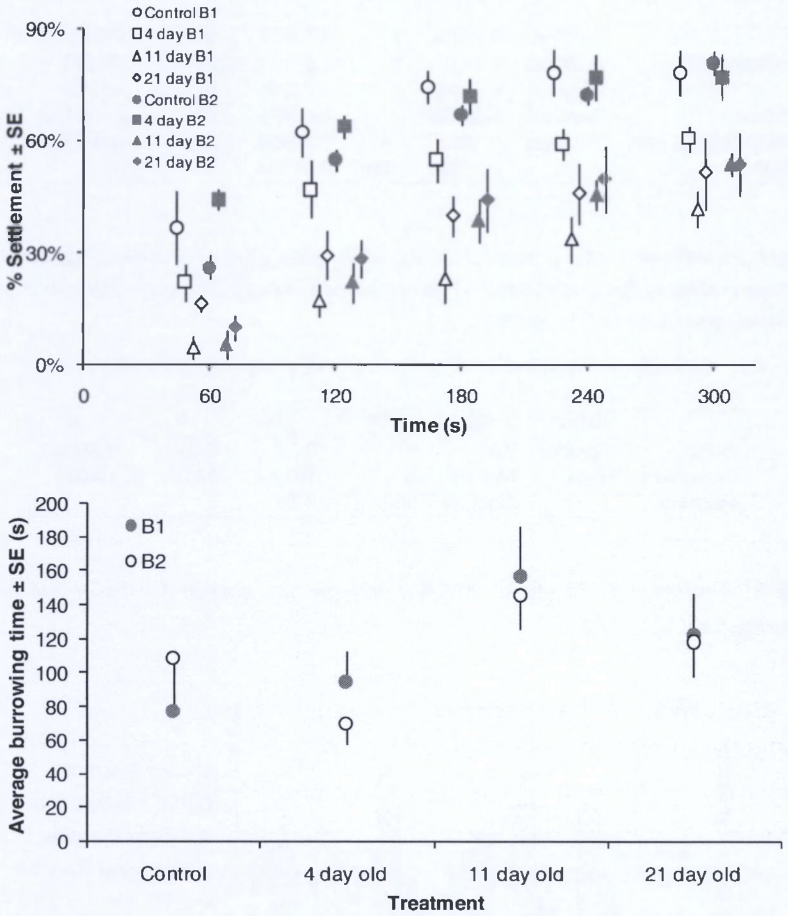


Fig. 1. Experiment 1. Upper panel: the percentage of settlement in the different treatments in relation with the time after addition of the larvae. Data plotted are means of six replicates per batch. Lower case letters indicate significant differences between treatments at each Time. Lower panel: averaged burrowing time ± SE of the larvae in the different treatments. B1 = batch 1, B2 = batch 2. Lower case letters indicate significant differences between treatments.

	Effect	SS	Df	MS	F	p	G-G adjusted p
Batch	Random	0.13432	1	0.13432	0.2012	0.669487	
Treatment(batch)	Random	4.02861	6	0.67144	96.7442	**<0.001**	
Time	Fixed	4.01163	4	1.00291	138.3899	<0.001	**<0.001**
Batch*Time	Random	0.02899	4	0.00725	1.0377	0.407856	0.99304
Treatment(Batch)*Time	Random	0.16657	24	0.00694	0.4967	0.977122	0.91774
Residual		2.51526	180	0.01397			

Table 2. *Experiment 1. Mixed model ANOVA table for the effect of Treatment, Batch and Time on the percentage of larval settlement. Adjusted p-levels are calculated for Time effects based on the Greenhouse-Geiser (GG) correction.*

	Effect	SS	Df	MS	F	p
Batch	Random	0.33	1	0.33	0.04	0.850811
Treatment	Fixed	544.40	3	181.47	19.35	**<0.001**
Residual		3282.39	350	9.38		

Table 3. *Experiment 1. Two-factor ANOVA table for the effect of Treatment and Batch on burrowing time.*

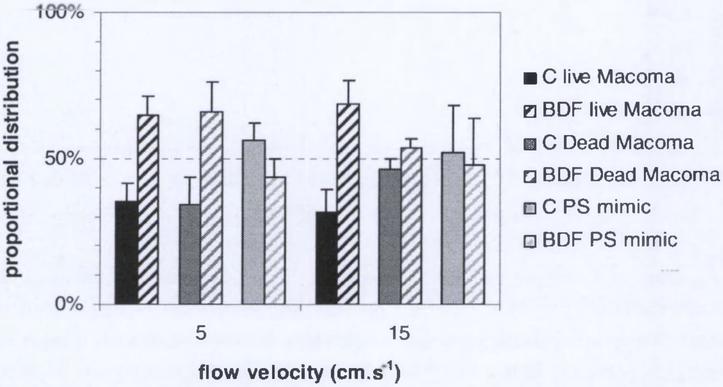


Fig. 2. *Experiment 2. Proportional distribution ± SE of the recruited alive Macoma, dead Macoma and PS mimics and dead pediveliger mimics in control (c) and benthic diatom film (bdf) sediments at 5 cm* s^{-1} *and 15 cm* s^{-1}*.*

Settlement response after primary settlement (Experiment 3)

Analysis of the above standing medium showed a larval addition efficiency of 100 % in both control and BDF sediments. Retention rates of *M. balthica* larvae were significantly higher in BDF (58 %) as compared to controls (40%)(Fig. 3, Table 4). Despite the lower larval retention rate to BDF in Trial B of Batch 2, no Batch nor a Trial effect was found indicating that, overall, the strength of response did not significantly vary over replicates. Consequently, H_{03} was rejected, i.e. secondary dispersal after primary settlement is influenced by BDF.

	Effect	SS	Df	MS	F	p
Treatment	Fixed	0.034331	1	0.034331	10.799	**0.021805**
Batch	Random	0.002726	1	0.002726	2.066	0.224013
Trial (Batch)	Random	0.005279	4	0.001320	0.415	0.792597
Residual		0.015895	5	0.003179		

Table 4. *Experiment 3. Mixed model ANOVA table for the effect of Treatment and Trial on the percentage of remaining larvae.*

Discussion & conclusions

In this study we investigated the role of multi-species benthic diatom films on the settlement of *Macoma balthica* larvae. Successful settlement is a crucial element in the recruitment of invertebrate larvae and thus in determining macrobenthic community structure. Settlement of invertebrate larvae is known to be mediated by marine biofilms and both biofilm induced facilitative and inhibitive effects on settlement have been demonstrated (reviewed in Pawlik 1992, Wieczorek *et al.* 1995). Our results show that the settlement of *M. balthica* larvae is also influenced by benthic diatoms and the outcome of the different experiments allows the suggestion of the underlying mechanisms.

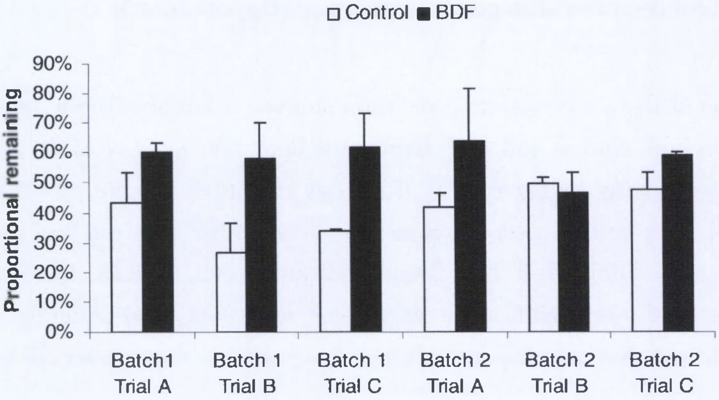


Fig. 3. *Experiment 3. Retention percentages ± SE of primary settled larvae in response to BDF (black bars) and control sediments (white bars).*

In a still-water environment, the average burrowing time depends on the age of BDF. During the first five minutes after inoculation, the settlement response was higher and the average burrowing time was faster in controls and younger BDF than in older BDF. Although the role of diatom-derived chemical cues cannot be ruled out, our results suggest that a physically mediated process is probably responsible for this difference in settlement response. First, in all treatments, larvae started to burrow directly after their inoculation and no "rejection behavior" was observed. Second, no significant differences were found between controls and 4 day old BDF, neither for % of settlement, nor for burrowing time. Third, complete settlement in dense BDF sediments was observed in still water after 30 minutes, preceding addition to the flume in Experiment 3. A higher resistance during penetration into a dense matrix of diatoms and their associated sugar and protein compounds therefore probably resulted in a reduced settlement in old, dense BDF at the short-term. This hypothesis is supported by the negative relationship between the % of larval settlement and the Chl *a* and colloidal EPS concentration. At the very short-term, settling *M. balthica* larvae in dense BDF are therefore, more susceptible to epibenthic predation in comparison with larvae that burrow in less dense BDF. However, at the medium and longer term, a beneficial effect may be expected in dense BDF due to better growing conditions as a result of the high food supply, making the bivalves also less susceptible to epibenthic predation (Hiddink *et al.* 2002, Hiddink 2003).

Macoma balthica larvae settled significantly more in BDF as compared to the controls and this distribution was not significantly different between flow velocities of 5 cm s^{-1} and 15 cm s^{-1}. However, the underlying mechanism of habitat selection seems to differ between both flow velocities. At 15 cm s^{-1}, significantly higher proportions of *M. balthica* larvae settled in BDF as compared to PS mimics and dead larvae, which both displayed a not significantly different recruitment pattern from the even distribution (i.e. no preference). At 5 cm s^{-1}, however, dead larvae performed a similar preference for BDF as alive *M. balthica* larvae, suggesting passive deposition of larvae to the BDF as a settlement mechanism at this flow velocity. The difference between inert, spherical PS mimics and dead, flatter larvae suggests that flow-dependent adhesion to the biofilm is an important settlement mechanism at lower flow velocities. Adhesion to biofilms is a complex process which remains poorly understood, but biochemical (e.g. production of viscoelastic substances, wettability of the surface), behavioral or physical (e.g. surface energy of the substratum) mechanisms may all be involved (Zardus *et al.* 2008). At higher flow velocities, substratum shear stress may be too high, inhibiting passive adhesion of dead larvae to the biofilm and the enhanced settlement of *M. balthica* larvae in BDF is due to active selection. Furthermore, the results obtained from experiment 3 highlight the importance of post-settlement dispersal in the final habitat selection. Thus, next to passive adhesion to the biofilm, also active behavior (i.e. rejection of the initial settlement site) plays a role at low flow velocities. Whenever no suitable settlement site is encountered, *M. balthica* larvae can actively re-enter the water column after initial settlement by migration to the surface and secreting a byssus thread, which allows resuspension along with currents (i.e. byssus drifting, Beukema & de Vlas 1989).

Higher recruitment success into dense biofilms has been noticed in the field for *Macoma balthica* (Van Colen *et al.* 2008) and for benthic invertebrates in general (e.g. Keough & Raimundi 1995). Furthermore, within the whole distribution area of *M. balthica,* primary settlement of postlarvae occurs predominantly on high tidal flats and offshore secondary dispersal occurs from late summer on towards the lower tidal flats (Reading 1979, Martini & Morrison 1987, Beukema & de Vlas 1989, Van der Meer *et al.* 2003). Beukema & de Vlas (1989) and Hiddink (2003) attribute this preference for primary settlement at high tidal flats to the lower predation pressure of epifaunal organisms and the lower disturbance by wave action at these sites. Furthermore, as a result of lower sediment resuspension, biofilms tend to develop more stable and are more productive in the more sheltered, upshore tidal flats (de Jong & de Jonge 1995). Hence, taken our results into account, enhanced primary settlement of *M. balthica* pediveligers in the upper tidal flats may, next to the above mentioned theories, also result from habitat selection for biofilms. However, the nature of the diatom-derived settlement cue for *M. balthica* larvae remains unknown. Such settlement cues have extensively been studied in relation to the bacterial compound of the biofilm (e.g. Bao *et al.* 2007), whereas the specific cues derived from diatoms have been investigated to a much lesser extent. Based on manipulation of the different components of biofilms, Lam *et al.* (2003) reported that the settlement of the serpulid polychaete *Hydroides elegans* is induced due to the presence of capsular surface EPS, produced by specific diatoms. Such diatom-derived sugar compounds have also been identified as settlement and metamorphosis cues for barnacles, limpets and bryozoans (Dahms *et al.* 2004, Patil & Anil 2005, Jouuchi *et al.* 2007). Further experiments, in which the chemical compounds derived from the different diatom species (e.g. EPS) are manipulated, are needed to elucidate these diatom-derived cues regarding the settlement of *M. balthica* larvae.

Acknowledgments

This research is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen). We would like to thank John Widdows and Peter Herman for their comments on the flume and experimental design, Tjeerd Bouma for his help with the ADV measurements, Jurgen Verstraeten and Yves Israël for the construction of the annular flume and Annick Verween, Annick Van Kenhove, Danielle Schram, Annelien Rigaux and Dirk Van Gansbeke for their assistance during processing of the samples and maintenance of the larval cultivation. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446).

CHAPTER 10

GENERAL DISCUSSION: WEAVING ECOLOGICAL THEORY WITH TIDAL FLAT RESTORATION

The previous chapters of this thesis have presented and discussed the results from an integrative field experiment and specific mesocosm experiments related to the settlement, recolonisation and succession of a macrobenthic community or particular species and its impact on its environment, i.e. a tidal mudflat. These findings provide information for ecosystem-based management and basic fundamental research. In what follows, I address how ecological theory can be used to enhance our knowledge in restoration ecology. First, I discuss the recovery patterns of different ecosystem functions and processes and how these relate to community recovery (i.e. biodiversity – ecosystem function relationships). Secondly, I propose a conceptual model of benthic tidal flat restoration based on results derived from this thesis and literature. At the end, I present some future research topics which are necessary to bridge the gap between tidal flat restoration ecology and ecological theory.

On the recovery of functionality: lessons learned from an integrative field experiment

The term "recovery" implies that a system will return to a previous condition after having been in a degraded one, which is often interpreted as being in poor ecological quality status. Recovery will occur once the causal stressors have been abated or removed and may occur naturally (i.e. passive recovery) or may be accelerated through management actions (i.e. active recovery or restoration)

(Elliott *et al.* 2007). Restoration success is often set to the systems' original structural and functional state (National Research Council 1992, Bradshaw 1997) and, hence, implies that this original state is known. Except for recovery from recent short-term disturbances, this seems unrealistic because, as summarized by Choi (2004), it is rarely possible to determine what the 'historic' ecosystem looked like, ecological damage is often irreversible for certain sites and economic and social costs may be really prohibitive. Restoration goals should thus be set realistically in an ecologically, economically and socially acceptable framework (Hobbs & Norton 1996). Moreover, the recovered ecosystem should be able to function appropriately now and in the future, not necessarily with the past environment. The achievement of such healthy ecosystem will depend on the ability of ecosystem functions and important properties to return to the point where the system keeps 'working' (i.e. resilience). Because physical and biological temporal variability are part of nature, the appropriate functional recovery/restoration endpoint should therefore be set to a state where the system will persist despite natural temporal fluctuations (i.e. dynamic equilibrium).

The discussion about which diversity is needed to maintain sustainable ecological functioning, or vice versa, has now become an emergent theme in marine benthic ecological research. Recent meta-analysis depicts that marine biodiversity often has a positive effect on ecosystem functions (e.g. Stachowicz *et al.* 2007). However, empirical evidence has also been found for idiosyncratic as well as for the lack of any relationships between marine biodiversity and ecosystem functioning (e.g. Bolam *et al.* 2002, Ieno *et al.* 2006). Moreover, the magnitude of the relation seems to depend on the ecological redundancy within the community and the environmental context (e.g. Naeem *et al.* 2002, Waldbusser & Marinelli 2006, Rossi *et al.* 2008). However, both aspects vary considerably in a successional pathway during recovery depending on dispersal constraints (see further) and abiotic-biotic interactions (e.g. Chapters 3, 4, 7). Consequently it is poorly understood to what extent diversity and ecosystem functioning is related to each other during recovery from disturbance (Solan *et al.* 2004, Ives & Carpenter 2007).

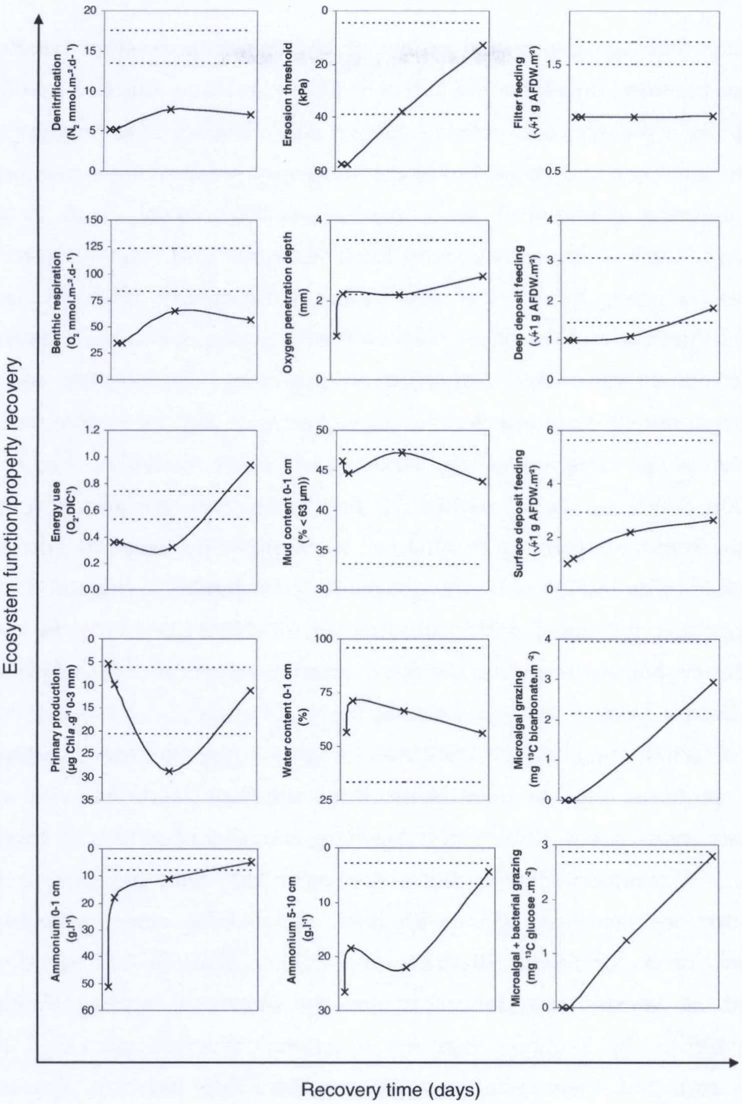


Fig. 1. *Recovery of ecosystem functions and sediment properties following hypoxia as deduced from the field experiment presented in Chapter 3, 4, 7, Andersson (2007) and Rossi et al. (in press). Data points indicate averaged values of the data available for the different succession stages (azoic, stage 1, 2 and 3) during the first six months of recovery. Dashed horizontal lines depict variance due to natural temporal variation in the controls (i.e. averaged minimum and maximum value throughout the course of the experiment).*

Concordant with the conceptual model of changes in ecosystem health with changes in pressure, developed by Tett *et al.* (2007) and later adjusted by Elliott *et al.* (2007), Fig. 1 presents the recovery pattern and resilience of several important ecosystem functions and properties in our field survey following experimentally induced hypoxia (Chapter 3, 4, 6, Andersson 2007, Rossi *et al.* in press). According to their ability to withstand the disturbance and their lag in recovery (cf. hysteresis types, Elliott *et al.* 2007, Diaz & Rosenberg 2008), as assessed during the first six months of recovery, different patterns became apparent for several functional parameters and sediment properties. Mud content and water content were not severely affected, i.e. these variables did not evolve out of the natural temporal variation range, whereas all other variables did. Oxygen penetration depth recovered within the first week, whereas ammonium pore water concentrations, primary production, microalgal and bacterial grazing and particle mixing, as indicated by the temporal development in erosion threshold and energy use, recovered within the first six months of recovery. In contrast, denitrification, benthic respiration and secondary production of filter feeders and surface deposit feeders showed a strong lag in recovery (i.e. hysteresis type II, Elliott *et al.* 2007). The observed difference between ecosystem function/process – recovery patterns (Fig. 1) may result from different relations with several community metrics, e.g. (functional) diversity, biomass of particular functional groups, ... Assignment of colonizing species in our field experiment to the combination of their trophic group, life habit and mobility category enabled the calculation of a functional diversity index (i.e. Shannon-Wiener diversity calculated on species abundances pooled by functional group) (Micheli & Halpern 2005). Fig. 2 shows that the functional diversity increased during recovery and that there was a positive relationship between species and functional diversity. This relationship was significant and explained to the same extent by a linear model ($R^2 = 0.82$, slope = 0.62) and a log linear model ($R^2 = 0.81$, slope = 0.62). Despite this low functional redundancy, Pearson – product moment correlations did not detect significant correlations between the ecosystem functions or sediment properties and functional diversity of the different community maturity stages (Table 1). Significant strong correlations were, however, present between the community biomass and the ecosystem functions and properties which strongly depend on bioturbation and bio-irrigation activities: denitrification, benthic respiration, energy use, erosion threshold, oxygen penetration depth. Correlations between NH_4^+ pore water concentrations, mud content and community biomass were significant at the $\alpha = 0.1$ level (Table 1). In addition, Chapter 3 depicted that primary production is related to both the grazing pressure of surface deposit feeding populations and bioturbation activities. Consequently, our results show that the recovery of ecosystem functions and properties may be expected to be controlled by the recovery of the community biomass. Hence, yearly successful recruitment of ecosystem engineers such as large, long-lived bio-diffusing and bio-irrigating species and benthic grazers are crucial in recovering ecosystem health after severe hypoxia.

Function/property	**Macrobenthic functional diversity**		**Macrobenthic biomass**	
	r	p	r	p
Denitrification	0.77	0.413	**0.93**	0.007
Benthic respiration	0.59	0.216	**0.86**	0.027
Energy use	0.38	0.457	**0.89**	0.015
Primary production	0.34	0.507	-0.40	0.428
Erosion threshold	-0.69	0.131	**-0.85**	0.029
Mud content 0-1 cm	-0.12	0.820	-0.80	0.054
Water content 0-1 cm	-0.65	0.163	-0.25	0.637
Oxygen penetration depth	0.47	0.343	**0.85**	0.031
Ammonium 0-1 cm	0.01	0.988	0.80	0.057
Ammonium 5-10 cm	0.01	0.982	0.78	0.070

Table 1. *Correlation coefficients and their significance of macrobenthos functional diversity and biomass with sediment properties and ecosystem functions. Relations are based on Pearson-product moment correlation of log (x+1) transformed averaged data of succession stage 1, 2 and 3 and control data from the corresponding periods (total n = 6). Correlation coefficients in bold are significant at p < 0.05.*

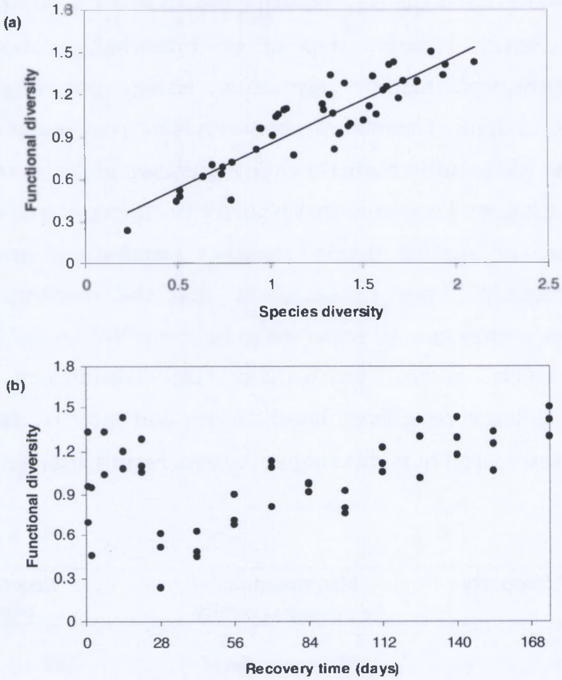


Fig. 2. (a) Relationship between Shannon-Wiener species diversity and functional diversity (i.e. Shannon-Wiener diversity calculated on species abundances pooled by functional group. (b) Temporal variation of the functional diversity since the opening of the plots. Each data point is the diversity measurement of the recovery monitoring over the first six months in a treatment plot.

Towards a conceptual model of tidal flat macrobenthos recovery

Historically, ecological succession has been viewed as a process of progressive community change over time passing through a series of distinct successional stages resulting from (1) the ability of species to recruit and (2) interactions between early and later colonists (Clements 1916, Connell & Slatyer 1978). Hence, when these processes occur consistently in a particular way this would enable a relatively predictable recovery pathway (i.e. canalization, Berlow 1997). However, evidence is accumulating that many aspects of benthic communities with relevance to succession theory are variable in time and space (e.g. recruitment limitation, Fraschetti *et al.* 2003). Consequently, succession pathways may be relatively stochastic and unpredictable. Given that both deterministic and stochastic processes are important in community recovery following disturbance, what are the implications for restoration ecology?

The conceptual model, presented in Fig. 3, attempts to incorporate both contrasting views and is further exemplified with results from this thesis and information from the literature. Before a species from a regional species pool can colonize a disturbed site, adequate dispersal into this site is needed and, subsequently, the environmental conditions at the site and interactions with the local assemblage have to allow survival. These processes can be interpreted as filters with the mesh sizes as a function of the strength of dispersal, abiotic and biotic constraints that need to be overcome by the colonizing species (Lake *et al.* 2007) and will vary according to the intertidal position on the tidal flat (Chapter 2). In addition, these mesh sizes may also depend on stochastic events. For instance, a mismatch between the phytoplankton bloom and bivalve spawning may cause a recruitment failure of pelagic recruits (e.g. Bos *et al.* 2006) (i.e. dispersal constraint), increased primary production resulting from the accumulation of organic matter may enhance microbial activity which, in turn, may cause seasonal hypoxia (Diaz & Rosenberg 2008) (i.e. abiotic constraint) or recruitment failure of the cockle, *Cerastoderma edule* (Kesteloo *et al.* 2007, 2008) may enhance recruitment success of surface deposit feeders due to a lowered interference from bioturbation (Flach 1996) (i.e. lowered biotic constraint). Our results indicate that dispersal constraints are related to the mobility, the seasonal recruitment peak and development mode of the different species. Species with a mobile adult life habit are able to colonize rapidly when conditions are optimal (species A in Fig. 3, e.g. *Hydrobia ulvae*) or performed a somewhat slower recovery when the initial prevailing abiotic and biotic conditions are only suboptimal (species B, e.g. *Nereis diversicolor*)(Chapter 3). Colonization of species with a pelagic larval stage are dependent on the seasonal availability of their dispersive larvae (species C, D) whereas species without a pelagic dispersal larval stage only recover at a late recovery stage (species E, e.g. oligochaetes and *Aphelochaeta marioni*)(Chapter 3, 7). Dispersal constraints may be expected to decrease with recovery time because the chance that (1) the regional species pool has produced dispersive larval stages and (2) colonization of species without a dispersive stage by erosion-induced colonization via the water column increases with time. Further, both biotic and abiotic sediment characteristics may affect species colonization, directly or indirectly via coupled biotic-abiotic effects. The importance of such biotic-abiotic interactions and the strength of the different aspects of these processes may be expected to change in a successional sequence towards the achievement of a 'normal' diverse community that is characterized by strong biological-physical interactions and which functions properly. For instance, the recovery of the oxygenation status (i.e. ammonium pore water concentrations) was strongly related to increase of macrobenthic biomass (Table 1). The recovery of macrobenthos biomass resulted in the enhancement of sediment particle mixing (i.e. bioturbation) as indicated by the increase in O_2/DIC ratio (Fig. 1, Table 1) and the decrease in the erosion threshold (Chapter 4). Consequently, constraints resulting from interference by bioturbation increase throughout the course of recovery whereas constraints due to sediment oxygenation status (e.g. Marinelli & Woodin 2002, Engstrom & Marinelli 2005) decrease. Moreover, at early recovery stages, the algal bloom and dense patches of tube-dwellers (species C in Fig. 3, *Pygospio elegans* and *Polydora cornuta*) presumably stabilized the sediment, thereby counteracting the inhibitory effects of strong bioturbating infauna (species D, e.g. *Macoma balthica, C. edule, Scrobicularia plana, Heteromastus filiformis*) at early recovery stages. Furthermore, hampering of colonization due to limited food availability is probably not a big issue in the eutrophic Westerschelde estuary. However, Chapters 5 and 9 indicate that the settlement and recruitment success of larvae can be enhanced by the development of an algal bloom in the absence of a high grazing pressure at early recovery stages (Fig. 1). Finally, direct biotic interactions may increasingly hamper recruitment success with recovery time due to ingestion of pelagic larvae (e.g. André & Rosenberg 1991, Troost *et al.* 2008) or predation of juvenile settlers (e.g. Cummings *et al.* 1996, Hiddink *et al.* 2002).

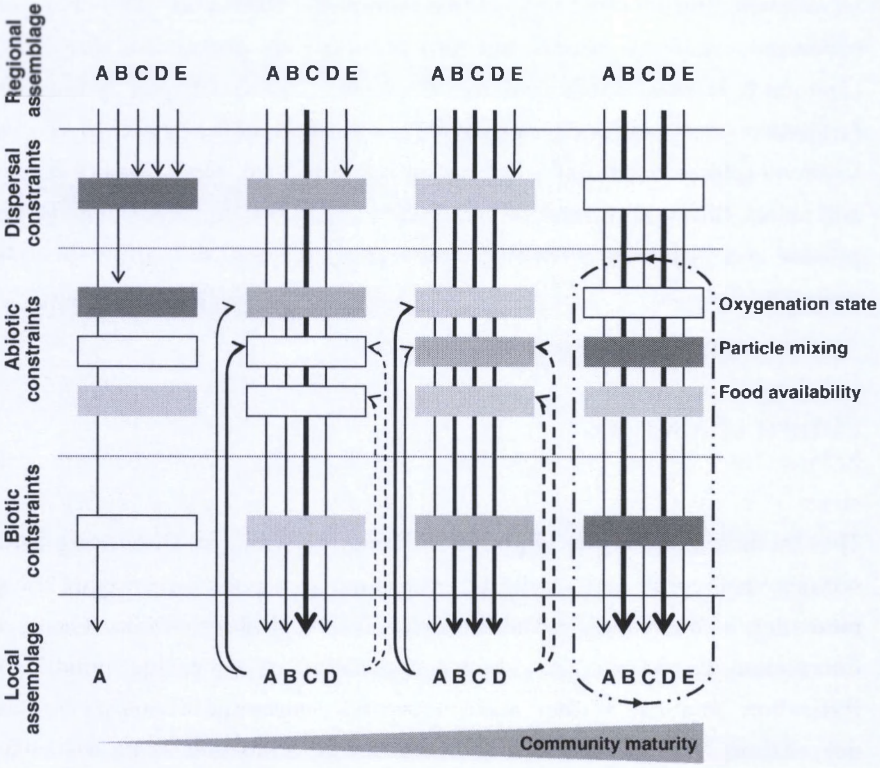


Fig. 3. *A conceptual model of macrobenthos recovery/succession. Following hypoxia-induced mass mortality, macrobenthic community recovery is hampered by dispersal, abiotic and biotic factors. The transition from dark grey towards white boxes indicates a declining severity of these constraints and can be interpreted as the mesh size of the different constraints that colonizing species have to pass through. The boldness of the bracket arrows is positively related to the importance of facilitative (solid lines) and inhibitory (dashed lines) biotic-abiotic interactions. Bolder straight arrows depict increasing importance of such interactions by the particular species in the local assemblage. A, B = motile species, C = tube-building species with pelagic larval stage, D = strong bioturbative species with pelagic larval stage, E= species without pelagic larval stage. See text for specific examples deduced from this thesis.*

In conclusion, the prevalence of structuring biotic-abiotic interactions emphasizes the importance of the successful recruitment of keystone species for tidal flat functional and macrobenthic recovery. For instance, the failure of cockle recruitment during the consecutive summers 2006 and 2007 hampered community recovery directly but also indirectly by giving rise to a shift in community biomass dominance from *C. edule* to *S. plana* (Chapter 7). In order to facilitate recovery, restoration managers can think about the introduction of such keystone species if 'natural' colonization is constrained. Mesh sizes of dispersal and abiotic filters can further be enlarged by (1) enhancing connectivity between pristine and disturbed or created sites and (2) the use of appropriate soils at proper tidal elevations.

Future challenges

This thesis highlights the importance of macrobenthos in structuring benthic ecology, in general, and should be considered as a basis for bridging the gap between fundamental ecological research and ecological restoration. Ideally, this information would enable a better application of conceptual models into restoration projects. Within such improved conceptual framework, habitat degradation, management and recovery can be addressed using a variety of indicators and outcomes of restoration efforts would then be partly predictable and economic, ecological and social promises could be made to policy makers and the public at large. For this, the following aspects appear crucial to me:

- Further expanding our insights in ecosystem function – biodiversity relationships and how both facets evolve following different types, intensities and scales of disturbance

- Providing empirical evidence about how much disturbance the tidal flat ecosystem can cope with before ecosystem functions deteriorate or evolve to an alternative stable state
- Investigating the role of sediment properties and biotic interactions as settlement cues and structuring factors for macrobenthos post-settlement recruitment success.

These goals can be achieved by means of manipulation of species interactions, sediment and hydrodynamic properties in both mesocosm and field experiments. Moreover, restoration projects provide an ideal setting to test hypotheses regarding the abovementioned current shortcomings in our knowledge.

APPENDIX 1

MACROBENTHIC COMMUNITY STRUCTURE AND DISTRIBUTION IN THE ZWIN NATURE RESERVE (BELGIUM AND THE NETHERLANDS)

Accepted for publication as:

Van Colen C., Snoeck F., Struyf K., Vincx M., Degraer S. (in press)
Macrobenthic community structure and distribution in the Zwin nature reserve
(Belgium and the Netherlands)
Journal of the Marine Biological Association of the United Kingdom

---

Abstract

Distribution and structure of intertidal macrobenthic communities in the Zwin nature reserve, a lagoonal inlet consisting of marsh and tidal flat habitats, was investigated using univariate and multivariate analyses. Macrobenthos community structure was related to environmental characteristics and discussed in the framework of the implemented extension of the nature reserve. Based on explorative multivariate techniques, five different sample groups (SGs) were distinguished, which were, in general, located in different habitats of the Zwin nature reserve. The ecologically most important SGs, consisting of the highest macrobenthic density, diversity and highest densities of *Nereis diversicolor* and *Scrobicularia plana*, important prey species for wading birds and fish occurred in the deep tidal inlet channels. This habitat was characterized by fine to medium sand sediment and strong tidal currents, guaranteeing water renewal at each high tide. Other SGs were found in less and erratically submersed and thus stressed areas (i.e. tidal pond, salt pans and shallow flats). These assemblages were characterized by typical r-strategists (i.e. *Capitella capitata* and *Polydora cornuta*) and typical supralittoral, mobile species (i.e. *Orchestia gammarellus* and *Collembola spp.*). Being ecologically most important, the extension of wide, tidal creeks should be a prime target within the future development and management of the nature reserve.

Keywords: *Macrobenthic community structure and habitat preferences, Zwin nature reserve, Intertidal habitat*

Introduction

Estuaries and their adjacent intertidal habitats fulfil several important ecosystem functions (e.g. high productivity, nursery and feeding habitats for epibenthic fishes, crustaceans and birds) and services (e.g. pollution filter, counteracting coastline erosion) (McLusky & Elliott 2004). Consequently many of these habitats are incorporated into nature reserves and designated as Special Areas of Conservation (SACs) according to the EU Habitats Directive (Anon. 1992). However, during the last 4 decades, coastal and estuarine ecosystems have been exposed to enhanced anthropogenic nutrient inputs and have been heavily exploited (e.g. dredging for building harbours or aggregate extraction, fishing, tourism) resulting in a general decrease in quality of these ecosystems (Bachelet *et al.* 2000, Diaz 2001, Wolanksi 2007). For instance, 2500 hectares of tidal flat and marsh habitats have been lost in the Schelde estuary (the Netherlands) since 1900, mainly due to land reclamation, deepening of the shipping channel and reinforcement of dykes (Eertman et al. 2002). To comply with the Water Framework Directive (WFD, Anon. 2000), the Flemish and Dutch governments have proposed to extend the Zwin nature reserve with 120 to 240 hectares of marshes and tidal flats, to be taken from the adjacent, formerly reclaimed polders. The Zwin nature reserve is a lagoonal inlet (i.e. seawater enters inlets on each tide), that has achieved an international reputation because its function as important breeding and wintering habitat for birds, especially waders (Struyf & Degraer 2003).

Since macrofauna is essential for tidal flat ecosystem functioning as food resource (e.g. Cramp & Simmons, 1977: (wading) birds; Hampel *et al.* 2005: fish) and nutrient cycling (McLusky & Elliott 2004, Wolanski 2007), knowledge on the structure and distribution of the macrobenthic community in the present Zwin nature reserve is essential as a baseline for the appropriate design and evaluation of the implemented restoration project. However, at present, knowledge on macrobenthic community structure and distribution patterns is lacking. Benthic research in the Zwin nature reserve has formerly focused solely on nekton communities of the tidal creeks (Hampel *et al.* 2004, 2005) and the effects of emersion on macrobenthos in one selected tidal creek (Van Colen *et al.* 2006). The aims of this study were therefore to describe the macrobenthic spatial structure in terms of diversity, abundance, and their relation with the environmental characteristics in the Zwin nature reserve.

Materials & methods

Study area, sampling and laboratory treatment

The Zwin nature reserve (51°21′ N, 3°22′ E) extends 2.3 km along the North Sea coastline and is situated along the southern shore of the Schelde estuary mouth at the Belgian-Dutch border. It has a total surface area of 158 ha and comprises of dunes, salt marshes, salt pans, a constructed tidal pond and two large inlet channels with adjacent tidal flat -and creek systems (Fig. 1).

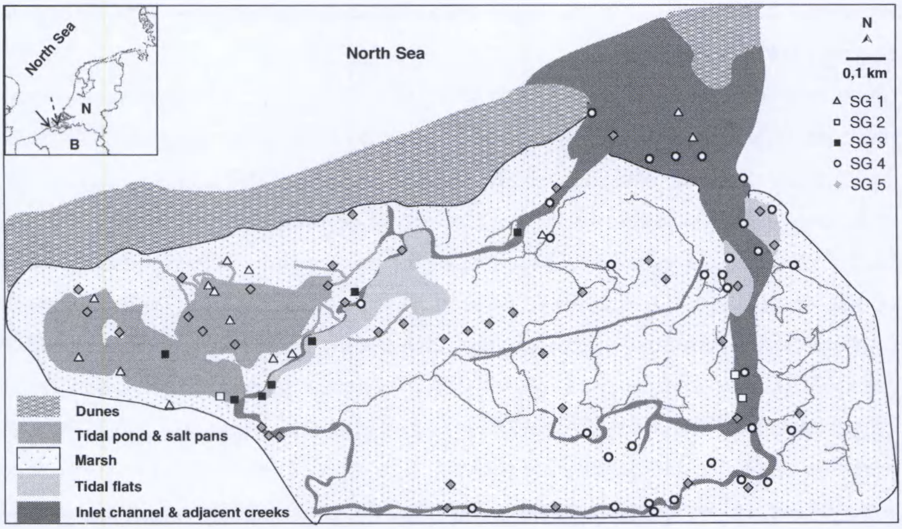


Fig. 1. Location of the Zwin nature reserve (solid arrow), at the mouth of the Schelde estuary (dashed arrow), along the southern shore (Belgian (B) – Dutch (N) border). Sampling stations are indicated by their sample groups (SG 1 – 5), distinguished by the multivariate analyses.

In Autumn 2003, at low tide, unreplicated macrobenthos samples were collected with a stainless corer (ø 12.5 cm; penetration depth: 40 cm) from 104 randomly selected intertidal locations. Samples were sieved over a 500 μm mesh sized sieve and the residual was fixed and preserved with a 8% formaldehyde-seawater solution. All macrofauna was sorted, identified to the species level and counted except for nematodes. Oligochaetes were only partly identified to species level: a set of 730 oligochaetes was randomly selected from the samples for species identification. This set allowed to identify the oligochaete species list of the Zwin nature reserve, but oligochaetes were truncated for all further analyses. Species were classified into functional groups according to their feeding guilds (surface deposit feeders, subsurface deposit feeders, suspension feeders, omnivores and scavengers), based on available literature (Fauchauld & Jumars 1979, Ysebaert *et al.* 2003, Volkenborn & Reise 2007). Species belonging to more than one feeding guild were assigned to their most common feeding mechanism.

At each location, (1) sediment characteristics and (2) intertidal elevation relative to mean high water tide level (MHWS, i.e. marsh border)) were measured. The distance below MHWS only gives a relative indication of the tidal position of the sampling location within the Zwin nature reserve (e.g. deep inlet channel, steep vs. shallow creeks, flats). Given the highly diverse geomorphology of the Zwin nature reserve, the relative intertidal elevation does not allow to calculate – ecologically more relevant – submersion and emersion times. Samples for grain size distribution of the top 10 cm were collected using a 5 cm inner diameter perspex corer and analysed for median grain size and mud content (volume percentage < 63 µm) with a LS Coulter particle size analyser.

Data analysis

Typical hyperbenthic and epibenthic species were excluded from the analysis. Furthermore, samples containing maximal one individual were not taken into account for the multivariate analyses (n=95 samples). Descriptive multivariate techniques on fourth root transformed data were used to analyse the community structure: group-averaging cluster analysis based on Bray-Curtis similarity followed by a similarity profile test (SIMPROF, Clarke & Gorley 2006) and multidimensional scaling (MDS). The main species contributing to the dissimilarity between the significantly ($p < 0.05$) separated sample groups, resulting from SIMPROF, were identified using the similarity percentage routine (SIMPER, Clarke & Gorley 2006) and indicator species for each sample group, were identified by Indicator Species Analysis (INDVAL) (Dufrêne & Legendre 1997). Sample groups were characterized by means of their averaged abundance, species richness, Shannon-Wiener diversity index, taxon composition, feeding group composition and physical habitat characteristics (median grain size, mud content and relative intertidal height). The relationships between community characteristics, community structure and environmental variables were investigated using the BIO-ENV procedure (Clarke & Gorley 2006) and Spearman rank correlation (Sokal & Rohlf 1981). Environmental variables and species densities were superimposed on MDS ordination diagrams using correlation vectors in order to allow a better visualization of the relation between sample groups, species and environment. Multivariate analyses were performed using the Plymouth Routines In Multivariate Ecological Research (PRIMER) package, version 6 (Clarke & Gorley 2006).

Classis	Species	Abbreviation	Feeding guild
Bivalvia	*Cerastoderme edule*	Cer edu	SF
Bivalvia	*Macoma balthica*	Mac bal	SDF
Bivalvia	*Scrobicularia plana*	Scr pla	SDF
Collembola	*Collembola sp.*	Coll sp	SDF
Crustacea	*Orchestia gammarellus*	Orc gam	SDF
Crustacea	*Sphaeroma rugicauda*	Sph rug	SDF
Gastropoda	*Hydrobia ulvae*	Hyd ulv	SDF
Nematoda	*Nematoda sp.*	Nema sp.	O
Oligochaeta	*Enchytraeidae sp.*	Olig sp	SSDF
Oligochaeta	*Tubificidae sp.*	Olig sp	SSDF
Oligochaeta	*Tubificoides benedeni*	Olig sp	SSDF
Oligochaeta	*Tubifex costatus*	Olig sp	SSDF
Polychaeta	*Aphelochaeta marioni*	Aph mar	SDF
Polychaeta	*Capitella capitata*	Cap cap	SSDF
Polychaeta	*Eteone longa*	Ete lon	P
Polychaeta	*Nereis diversicolor*	Ner div	O
Polychaeta	*Heteromastus filiformis*	Het fil	SSDF
Polychaeta	*Malacoceros tetracerus*	Mal tet	SDF
Polychaeta	*Polydora cornuta*	Pol cor	SDF
Polychaeta	*Pygospio elegans*	Pyg ele	SDF
Polychaeta	*Scolelepis squamata*	Sco squ	SDF
Polychaeta	*Streblospio benedicti*	Str ben	SDF

Table 1. *List of macrobenthic species recorded in the Zwin nature reserve during this study. For each species the feeding type is given. SDF; surface deposit feeder, SSDF; subsurface deposit feeder, SF; suspension feeder, O; omnivore, P; predator. Species abbreviations, used in Table 2 and Table 3, are given.*

Results

General characterization of the macrobenthos

A total of 23 macrobenthic species were encountered (Table 1). Macrofaunal species richness varied between 0 and 11 spp.sample^{-1}, with an average of 3.4 ± 0.21 SE species . The total macrobenthic density ranged from 0 to 71376 ind.m^{-2}, with an average of 7260 ± 1099 SE ind.m^{-2}. The most widely distributed species were *Nereis diversicolor* (Müller 1776)(recorded in 87 % of all samples) and *Oligochaeta spp.* (75%). Other species occurred in less than 20 % of the samples.

Total macrobenthic density was dominated by oligochaetes (60%) and polychaetes (38%), while bivalves, arthropods, gastropods and nematodes all contributed to <1% of the total density. Based on a feeding guild approach, subsurface deposit feeders dominated the macrobenthos (71% of the total macrobenthic density), followed by omnivores/scavengers (20 %), surface deposit feeders (9%) and suspension feeders (<1%). Five species contributed 96% of the total macrobenthic density: *Oligochaeta spp.* (60%), *N. diversicolor* (19%), *Heteromastus filiformis* (Claparède 1864)(9%), *Aphelochaeta marioni* (Saint-Joseph 1894)(6%) and *Capitella capitata* (Fabricius 1780)(2%).

Community structure of the macrobenthos

Five sample groups (SGs) were significantly separated based on SIMPROF (Fig. 2). Nevertheless, the MDS ordination displayed an overlap between SGs (except for SG 2) and a rather high stress (0.18) which means that not too much reliance should be placed on the details of the plot (Clarke & Warwick 2001) (Fig. 3). Sample groups 2, 3 and 4 were clearly more diverse as compared to SG 1 and 5 (Table 2).

Diverse sample groups (SG 2, 3 and 4)

Sample group 2 displayed the highest Shannon-Wiener diversity (1.21 ± 0.13 SE), followed by SG 3 and 4 (respectively, 1.05 ± 0.19 SE and 1.00 ± 0.06 SE). Further, in SG 2, averaged species richness was relatively high (7.6 ± 1.6 SE spp.sample^{-1}) in comparison with other SGs and three species had a significant indicator value (IV) > 40: *Malacoceros tetracerus* (Schmarda 1861), *Pygospio elegans* (Claparède 1863) and *A. marioni*. These three surface deposit feeding species reached their highest densities in SG 2 (respectively, 841 ± 476 SE ind.m^{-2}, 163 ± 47 SE ind.m^{-2} and 5622 ± 4754 SE ind.m^{-2}) and can be considered highly indicative for this SG. SG 3 and SG 4 were numerically dominated by Oligochaeta spp., while A. marioni was far less abundant in these SGs (Table 2). Consequently, *Oligochaeta spp.* and *A. marioni* contributed largely to the dissimilarity between SG 3, 4 and SG 2 (Table 3). The dissimilarity between SG 3 and 4 was mainly determined by the subsurface deposit feeder *C. capitata* (C% = 20.7; see Table 3). This species reached its highest density in SG 3 (1688 ± 877 SE ind.m^{-2}, i.e. 21 % of the total density), while it was absent in SG 4. INDVAL analysis revealed significant IVs > 40 for *C. capitata* and *Polydora cornuta* (Bosc 1802) in SG 3, while *Scrobicularia plana* (da Costa 1778) and *Oligochaeta spp.*, were highly indicative for SG 4 (IV = 44 and 35, respectively).

Less diverse sample groups (SG 1 and 5)

SG 1 and 5 were characterized by a low macrobenthic diversity (species richness = 1.6 ± 0.2 SE spp.sample^{-1} and 2.6 ± 0.2 SE spp.sample^{-1}, respectively for SG 1 and SG 5; Shannon-Wiener diversity = 0.25 ± 0.09 SE and 0.53 ± 0.06 SE, respectively for SG 1 and SG 5). Furthermore, the lowest macrobenthic density (716 ± 108 SE ind.m^{-2}) was found in SG 1 (Table 2). Oligochaeta spp. numerically dominated SG 5 (6878 ± 2304 SE ind.m^{-2}, i.e. 90 % of the total density), while oligochaetes were absent in SG 1. Consequently, *Oligochaeta spp.* contributed largely (i.e. 53%, SIMPER, Table 3) to the dissimilarity between both SGs. SG 5 was further characterized by the highest densities of the mobile species *Hydrobia ulvae* (Pennant 1977), *Orchestia gammarellus* (Pallas 1766) and *Collembola spp*. The latter two species occurred exclusively in SG 5.

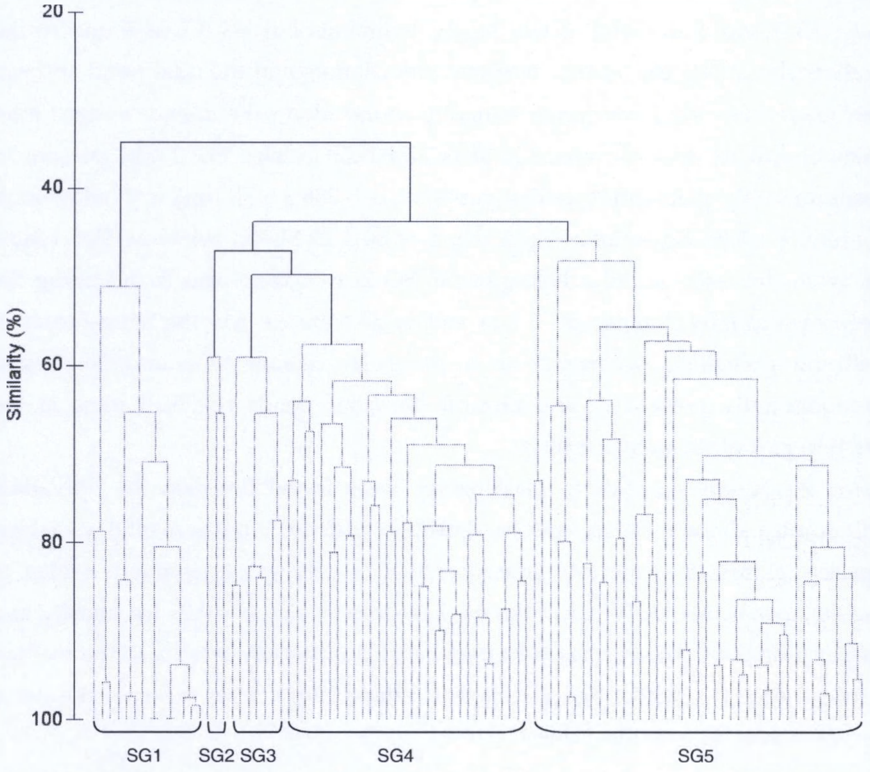


Fig. 2. Dendrogram representing the group average linking cluster analysis based on fourth root transformed macrobenthos density data. The five sample groups (SGs), identified by SIMPROF are indicated by the solid lines and brackets.

Distribution and relations with the abiotic environment

BIO-ENV revealed a weak correlation between the macrobenthic community structure and the environmental variables: the combination of median particle size and relative intertidal height only explained 0.1 % of the biotic structure in the multidimensional space. Nevertheless, in general, distinct distribution patterns, often corresponding with different habitat types (i.e. large tidal inlets, small to medium sized creeks, flats, tidal pond or salt pans) were found for the SGs.

Both SG 3 and 4 occurred in fine sandy sediments but SG 3 was found in the shallow flats along the smaller western inlet channel and the tidal pond and salt pan area while SG 4 was predominantly found along the deeper eastern inlet channel and its adjacent muddier flats and tidal creeks. SG 2 was present in medium sandy sediments (median particle size = 286 ± 5 SE μm) with a low mud content (4 ± 2 SE %), at an average depth of 50 ± 29 SE cm below MHMS (Table 2). From the three samples belonging to SG 2, two were also found along the deep eastern inlet channel. SG 5 was widely distributed over the nature reserve, with no prevailing occurrence to a particular habitat type and SG 1 was predominantly present in and around the tidal ponds and salt pans in the western part of the nature reserve.

Some weak, but significant, relationships were found between the univariate community characteristics, species densities and the environmental variables. Species richness and the total macrobenthos density were positively related to the distance below MHWS and the median particle size ($r = 0.21$ for density and species richness). The species richness was also positively related to the median particle size ($r = 0.29$). Species specific relationships with the environmental variables are presented in Table 4.

Discussion

Macrobenthos community structure, species richness, density and species densities were found to be related to the mud content, median particle size and relative intertidal elevation of the samples. This is consistent with literature, indicating the importance of sediment characteristics and hydrodynamic conditions (e.g. submersion time, current velocities) in the distribution of macrobenthic communities in estuarine intertidal habitats (e.g. Warwick *et al.*

1991, Ysebaert et al 2003). However, correlation coefficients were relatively low. This may partly result from the unreplicated sampling design, given the high small-scale patchiness within the macrobenthos in intertidal areas. Further, other variables may explain the distribution of the distinguished species assemblages as well. Based on a review of > 50 studies, Snelgrove & Butman (1994) pointed out that although grain size is usually correlated with the benthic distribution patterns, this correlation is not solely due to grain size alone but also to other variables, related to grain size, such as organic matter content. In addition, salinity conditions and ecological interactions (e.g. predation pressure) can explain macrobenthic distribution patterns in tidal flat habitats (Ysebaert *et al.* 2003, Williams *et al.* 2004, Beukema & Dekker 2005).

Sample group 2 and especially SG 4 were here considered ecologically most important because their high species richness and density of larger macrobenthic species (e.g. 2860 ind. *N. diversicolor* m^{-2}, 126 ind. *S. plana* m^{-2} and 24 ind. *M. balthica* m^{-2}), which may be expected to contribute most to the waders' diet (Cramp & Simmons 1977). Other typical species for SG 2 and 4 were *A. marioni*, *M. tetracerus*, *S. plana* and *P.elegans*. In comparison with the polyhaline tidal flat areas further upstream the Schelde estuary, species composition, total density and diversity is quite similar (Ysebaert *et al.* 2003, C. Van Colen unpublished data) and similar species assemblages have commonly been observed in similar estuarine environments (e.g. Beukema 1976, 1981, Dörjes *et al.* 1986). Sample groups 2 and 4 were mainly found within the entrance channel and adjacent wider creeks at the eastern part of the Zwin nature reserve. Contrary to the habitats of the other SGs, this system is characterized by relatively strong tidal currents guaranteeing water renewal at each high tide. This creates a consequent relatively stable highly productive environment in which biomass can accumulate in larger organisms (i.e. k-strategists) (Gamito 2006), compared to the lesser (shallow flats, i.e. SG 3) and erratically submersed and thus stressed areas (SG 1 and SG 5, i.e. tidal pond and salt pan). Given its high macrobenthic value, the extension of wide, tidal creeks should be a prime target within the future development and management of the nature reserve.

	SG 1		SG 2		SG 3		SG 4		SG 5	
Environment										
Median particle size	245 (23)		286 (5)		227 (29)		158 (16)		135 (16)	
Mud content	16 (6)		4 (2)		17 (7)		28 (4)		42 (4)	
Relative intertidal height	32 (9)		50 (29)		7 (7)		53 (10)		20 (4)	
Macrobenthos										
S (total)	6		13		8		15		14	
S (mean)	1.6 (0.2)		7.6 (1.6)		5.1 (0.6)		5.1 (0.2)		2.6 (0.2)	
N	716 (108)		10718 (4894)		8160 (1529)		11275 (1743)		7649 (2285)	
H' (e)	0.25 (0.09)		1.21 (0.13)		1.05 (0.19)		1.00 (0.06)		0.53 (0.06)	
Top 10 density										
	Ner div	646	Aph mar	5622	Oli spp.	2828	Oli spp.	5175	Oli spp.	6878
	Het fil	35	Ner div	2743	Ner div	2386	Ner div	2860	Ner div	556
	Cap cap	17	Het fil	1059	Cap cap	1688	Het fil	1932	Hyd ulv	98
	Aph mar	6	Mal tet	841	Het fil	838	Aph mar	194	Pol cor	29
	Pol cor	6	Cap cap	190	Str ben	210	Scr pla	126	Orc gam°	22
	Pyg ele	6	Pyg ele	162	Pol cor	105	Sph rug	111	Cap cap	16
			Pol cor	135	Aph mar	58	Pyg ele	79	Sph rug	14
			Cer edu	27	Mal tet	47	Nem spp.	63	Nem spp.	10
			Ete lon	27			Ete lon	50	Col spp.°	8
			Hyd ulv	27			Mac bal°	24	Het fil	6
Relative feeding guild density										
Surface deposit feeders	2 (1)		49 (15)		6 (3)		11 (3)		8 (2)	
Subsurface deposit feeders	11 (5)		13 (3)		65 (7)		54 (4)		65 (5)	
Omnivores/scavengers	87 (6)		38 (14		29 (7)		35 (5)		27 (7)	
Indicator species			Mal tet	88	Cap cap	62	Scr pla	44		
			Pyg ele	72	Pol cor	44	Oligo	35		
			Aph mar	41						

Table 2. *Environmental and macrobenthic characterization of the sample groups. Averaged values of all sampling stations per SG are given for median grain size (µm), mud content (%), and relative intertidal height (cm below MHWS), total species richness (N total), mean species richness (N mean), total density and density of the 10 most abundant species (ind.m^{-2}), Shannon-Wiener diversity index H'(e)(nits), feeding guild composition (%). Standard errors are given in parentheses. Species with a significant Indicator Value > 40 for a particular SG are presented as well. °: unique species for this SG. Species abbreviations are listed in Table 1.*

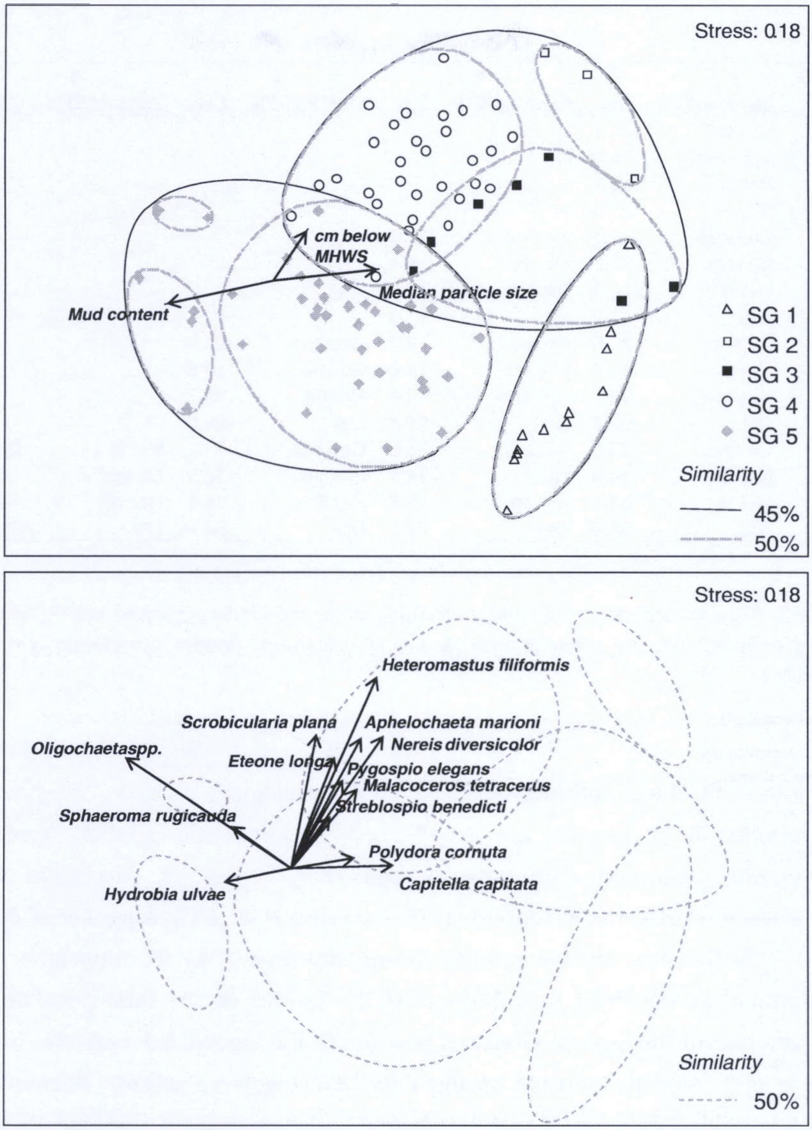


Fig. 3. *(a) Multidimensional scaling (MDS) ordination plot of the macrobenthos community structure, based on bray-curtis similarity of fourth root transformed species densities. The significantly separated sample groups by SIMPROF, are indicated by different symbols. Similarity clusters (45% and 50%) and correlation vectors of the mud content, median particle size and relative intertidal height are superimposed in (a), 50% similarity clusters and species correlations > 0.25 are superimposed in (b).*

SG	1		2		3		4	
	Species/D%	C%	Species/D%	C%	Species/D%	C%	Species/D%	C%
2	Mal tet	17.4						
	Aph mar	16.8						
	Het fil	16.2						
	D%	70.0						
3	Cap cap	25.0	Aph mar	15.0				
	Oli spp.	23.6	Oli spp.	14.4				
	Het fil	16.4	Mal tet	13.9				
	D%	63.0	D%	53.6				
4	Oli spp.	30.6	Oli spp.	18.7	Cap cap	20.7		
	Het fil	20.0	Mal tet	14.8	Oli spp.	14.8		
	Ner div	9.6	Aph mar	14.4	Ner div	10.7		
	D%	67.8	D%	52.8	D%	50.2		
5	Oli spp.	53.0	Oli spp.	15.6	Cap cap	24.2	Het fil	23.2
	Ner div	14.8	Het fil	14.1	Oli spp.	18.9	Oli spp.	14.6
	Het fil	6.6	Mal tet	13.6	Het fil	16.1	Ner div	14.3
	D%	63.4	D%	77.5	D%	58.4	D%	53.6

Table 3. *Total dissimilarity (D%) and contribution of the three most important taxa (C%) to the dissimilarity between any combination of the five sample groups. Species abbreviations are listed in Table 1.*

In contrast, SG 3 was characterized by typical r-strategists, such as *C. capitata* and *P. cornuta*. Both species are small-sized opportunistic species, typically dominating disturbed environments and early stages of succession after disturbance (Pearson & Rosenberg 1978, Carvalho *et al.* 2005, Magni *et al.* 2005). SG 1 was the less diverse sample group, dominated by *N. diversicolor* and predominantly occurred in sediments at the border of the tidal pond in the western part of the lagoon. Given its position in the lagoon, the seawater in this area is only refreshed during spring tides, causing large salinity fluctuations. Ysebaert *et al.* (2003) found that *Nereis diversicolor* dominated the macrobenthic community of the meso/oligohaline transition zone of the Schelde estuary. This zone is subject to large, seasonal salinity fluctuations. Hence, *N. diversicolor* can be considered as a species capable to cope with this variability.

Species	Median particle size	Mud content	Relative intertidal height
Aphelochaeta marioni	0,10	**-0,32**	0,01
Capitella capitata	0,09	**-0,37**	-0,01
Cerastoderma edule	**-0,26**	-0,17	-0,05
Collembola spp.	**-0,26**	-0,02	-0,16
Eteone longa	-0,02	**-0,22**	**0,23**
Hediste diversicolor	0,03	-0,08	0,16
Hydrobia ulvae	**-0,32**	0,05	-0,09
Macoma balthica	**-0,21**	**-0,23**	0,08
Malacoceros tetracerus	0,03	**-0,34**	0,01
Nematoda spp.	**-0,27**	-0,05	0,02
Nereis diversicolor	0,13	**-0,31**	**0,22**
Oligochaeta spp.	**-0,30**	0,23	-0,01
Orchestia gammarellus	**-0,28**	0,01	-0,16
Polydora cornuta	0,05	**-0,31**	0,00
Pygospio elegans	0,00	**-0,31**	**0,31**
Scolelepis squamata	**-0,25**	-0,17	0,05
Scrobicularia plana	-0,13	-0,06	0,14
Sphaeroma rugicauda	**-0,27**	-0,01	0,05
Streblospio benedicti	-0,14	**-0,21**	-0,10

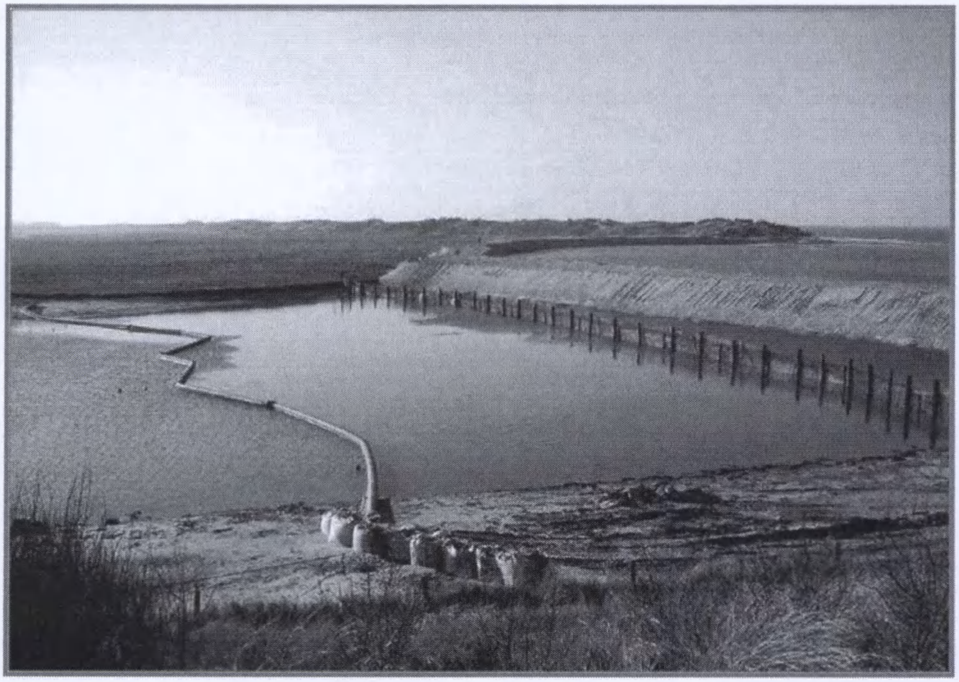
Table 4. *Spearman rank correlations between species densities and environmental variables. Significant correlations are presented in bold.*

Conclusion

The Zwin nature reserve hosts a rich (max. 71376 ind.m^{-2}) and species diverse (max. 11 spp.sample^{-1}) macrobenthic life. Based on multivariate analyses, five significantly different sample groups were detected. Macrobenthic density, species richness and species densities were correlated to sediment median grain size, mud content, elevation, as well as larger landscape features. The wide, tidal inlet channel was identified as ecologically most important because it contained the highest species richness and densities of larger macrobenthic species. The extension of wide, tidal creeks will result in added values in terms of both benthos and birds and should therefore be a prime target within the future development and management of the nature reserve.

Acknowledgments

The first author is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT Vlaanderen). The authors want to thank Jan Soors (INBO) for the help with the determination of oligochaetes, Bart Beuselinck for assisting in sampling and Danielle Schram for processing the granulometric samples. This paper contributes to the Ghent University BBSea Project (GOA 01600705) and the EU Network of Excellence Marbef (GOCE-CT-2003-505446; contribution number 8052).



148096

APPENDIX 2

DOES MEDIUM-TERM EMERSION CAUSE A MASS EXTINCTION OF TIDAL FLAT MACROBENTHOS? - THE CASE OF THE TRICOLOR OIL POLLUTION PREVENTION IN THE ZWIN NATURE RESERVE (BELGIUM AND THE NETHERLANDS)

Published as:

Van Colen C., Vincx M., Degraer S. (2006)
Does medium-term emersion cause a mass extinction of tidal flat macrobenthos? - The case of the Tricolor oil pollution prevention in the Zwin nature reserve (Belgium and The Netherlands)
Estuarine, Coastal and Shelf Science 68: 343-347

Abstract

As a result of the *Tricolor* oil pollution in the Southern Bight of the North Sea (winter 2003) the Zwin nature reserve, consisting of tidal flats and salt marshes, was blocked from the North Sea by use of a sand barrier. Hence, macrobenthic tidal flat organisms, by nature strongly dependent on the cyclic incoming seawater, were emersed during a period of 27 days. Because the effect of medium-term emersion on the ecologically important benthic life could not be assessed beforehand, the damming was taken as an opportunity to examine these effects. This study demonstrated that (1) no species vanished due to emersion, (2) although the emersion might have caused some mortality, a mass mortality within the macrobenthos did not occur, and (3) the supra-littoral amphipods *Talitrus saltator* and *Orchestia gammarellus* performed a strong, though ephemeral immigration into the intertidal zone during the period of emersion. In view of both its minor impacts on the macrobenthos and its effectiveness in preventing oil pollution in the Zwin nature reserve, damming as a measure against oil pollution may be considered effective protection, especially during winter.

Keywords: *Tidal flat macrobenthos, Tricolor oil pollution, Zwin nature reserve, Emersion, Mortality, Immigration*

Introduction

On the 14th December 2002, the car carrier "Tricolor" collided with the container ship "Kariba" in the English Channel and sank in French waters near the Belgian border (Fig.1). Five weeks later, on the 22nd January 2003, approximately 170 tons of fuel leaked from the wreck of the Tricolor during salvage operations. Due to the meteorological conditions, with strong onshore winds, the oil washed ashore on French, Belgian and Dutch beaches and threatened some coastal nature reserves, including the salt marshes and tidal flats of the Zwin nature reserve.

Oil spills do have significant, though short-term negative impacts on coastal areas, whereas the effects of oil pollution in salt marshes may be measurable for years or even decades after the event (Hester & Mendelssohn 1999). The impact of oil pollution on salt marsh vegetations is dramatic, e.g. mortality of the marsh vegetation and reduced subsequent growth (de la Cruz *et al.* 1981, Hester & Mendelssohn 1999). Oil pollution in salt marshes may further reduce faunal densities by its acute, short-term toxicity (e.g. Sanders *et al.* 1980, McDonald *et al.* 1991, Widbom & Oviatt 1994) and cause avoidance by mobile organisms (Moles *et al.* 1994). It was therefore proposed to protect the lagoonal Zwin salt marsh by means of a sand barrier.

Unfortunately, such a measure also excludes the tidal regime from the area, causing a permanent exposure to the air of tidal areas. Closure of intertidal areas from the natural tidal cycle can result in minor to irreversible effects on organisms and the ecosystem, depending on the time scale of the impact and the sensitivity of the organisms themselves. The macrobenthos, largely depending on the incoming water, was believed to be particularly vulnerable to an emersion

event (Fortuin *et al.* 1989). Though, the mortality rate in such cases is known to depend on temperature, the duration of emersion, condition of the organisms, species identity and population structure (Hummel *et al.* 1994); for some species a mortality rate up to 50% per day has been found after two days of emersion.
The construction of a sand barrier as a method of protection against oil pollution therefore led to much debate in the case of the "Tricolor" calamity: the choice being between keeping out chronic oil pollution versus the risk of high mortality of the macrobenthos due to emersion. In the end, blocking both inlets by use of a sand barrier was chosen as a protective measure (Fig. 1). The entrance on the Dutch part of the nature reserve was closed for 27 days during winter (i.e. from 7th February 2003 till 6th March 2003).

The damming-up was taken as an opportunity to study the *in situ* tolerance of north-western European tidal flat macrobenthos to medium-term emersion during wintertime, with a view to making better estimates in the future whenever this measure of protection could be needed again. Two questions were therefore raised:

(1) Do common and abundant species vanish as a consequence of emersion?
(2) if not, do species vary in their responses to the disturbance of medium-term emersion?

Materials & methods

The Zwin nature reserve (51°21′ N, 3°22′ E) extends for 2.3 km along the North Sea coastline and is situated at the mouth of the Western Scheldt estuary at the Belgian-Dutch border (Fig.1). It has a total surface area of 158 ha consisting for the greatest part of salt marshes, tidal flats and tidal creek systems. This nature reserve has achieved international recognition, mainly because of its significance as important bird area (Struyf & Degraer pers. comm.).

Data on the distribution and diversity of the macrobenthic species and communities in the Zwin nature reserve do not exist. To be able to investigate the emersion effect on a maximum number of species, a wide variety of tidal flat habitats along a diverse creek was thus sampled. At eight sampling stations, each situated in a separate habitat, two replicate samples were collected (sampling design: 8 sites x 2 replicates x 4 sampling occasions; Fig. 1; Table 1).

Macrobenthic samples were collected with a hand core (ø 10 cm) to a depth of 15 cm and sieved alive over a 500 μm mesh sized sieve. The residual was fixed and preserved with an 8% formaldehyde-seawater solution. All macrofauna was sorted, counted and identified to the species level, except for nematodes and oligochaetes. In order to avoid biased results due to sampling methodology, species that were considered to be sampled non-representatively (max. < 1 ind.sample^{-1} at the initial phase) were not taken into account for further analyses. The results presented here only take into account the common and abundant species.

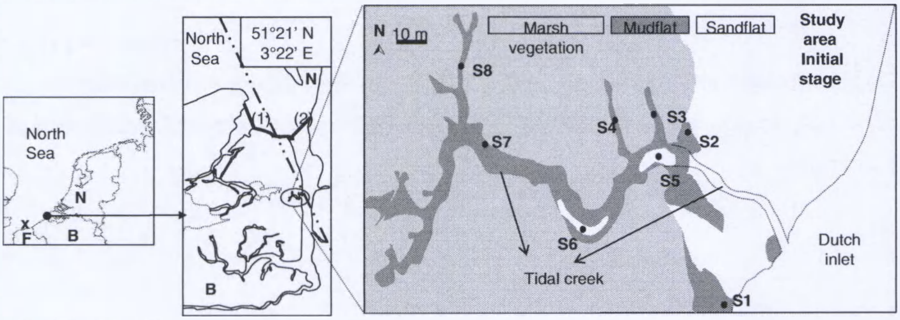


Fig. 1. Study area with indication of the sampling stations (S1eS8) and the main habitat characteristics. (1) Sand barrier in the Belgian entrance creek, (2) sand barrier in the Dutch entrance creek. The Belgian (B, Belgium)eDutch (N, the Netherlands) border is marked with a dotted line. X position of the "Tricolor" wreck on 14 December 2002 off the French (F) coastline.

Physical parameter	**Sampling station**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Mud content (%)	59	51	56	77	0	12	78	51
Median particle size (µm)	41	66	40	30	270	245	24	57
% time of submersion	33	4	17	16	15	24	17	12

Table 1. *Physical parameters (median particle size (mm), mud content (%), time of tida submersion (%) measured at mean-tide) per sampling station.*

To detect possible effects due to the emersion period, macrobenthic samples were collected three days before (B), two times during (after 12 and 21 days of emersion, E12 and E21, respectively), and 26 days after the emersion period (A), which should allow study of the subsequent mortality appearing in the first month after restoration of the natural tidal cycle (Hummel *et al.* 1986).

Results

In total, 14 species (7 annelids, 3 molluscs, 3 crustaceans and unidentified nematodes) were recorded in the area (Table 2). All species present before emersion, were encountered afterwards. No uniform clear trend was found in the density pattern during the emersion period in the study area. Total macrobenthic density increased in some stations and decreased in others (Fig. 2). The most abundant polychaetes were, in order of overall total abundance *Nereis diversicolor, Heteromastus filiformis, Pygospio elegans, Streblospio benedicti,* and *Aphelochaeta marioni*. The density of *N. diversicolor, H. filiformis* and *S. benedicti* generally declined strongly (minimum 23 %) during the emersion period wherever those species were present abundantly (>1500 ind.m^{-2}) before the construction of the sand barrier (e.g. station 4, Fig. 3). *Orchestia gammarellus* and *Talitrus saltator* were not present before and 12 days after the construction of the sand barrier. After three weeks of emersion the abundance of these organisms appeared at high levels (2101 ± 1210 ind.m^{-2} of *O. gammarellus* and 3501 ± 2483 ind.m^{-2} of *T. saltator*) at two stations near the high-water mark (S4 and S8). After the removal of the sand barrier both species were no longer recordered (Fig. 4).

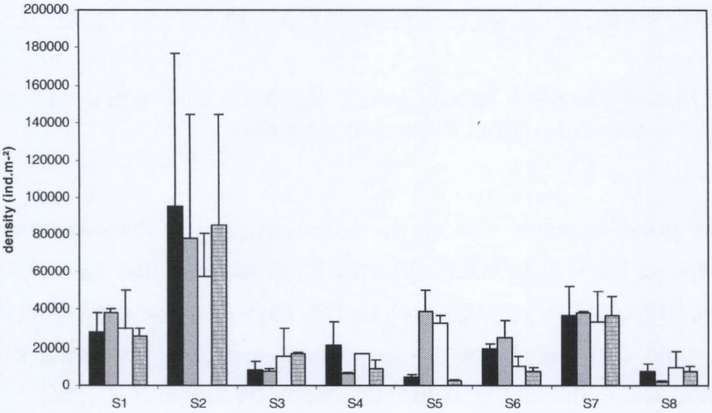


Fig. 2. Total macrobenthic density. Density values (± SE) before (black bar), after 12 days of emersion (grey bar), after 21 days of emersion (white bar) and 26 days after (dark grey bar) the removal of the sand barrier, per station are given.

Discussion

Do common and abundant species vanish as a consequence of emersion?

All species that were present in the study area before emersion were still present after emersion: no species vanished as a consequence of the emersion. The absence of *M. aestuarina* and *L. rugicauda* during the emersion is however of interest. Both species were rather rare at the initial stage (max. 1 ind.sample^{-1}), therefore with a low chance of collecting these species on each sampling occasion. We were therefore unable to determine whether their absence was a real response by both species or must be considered a sampling artefact.

Species	B	E 12	E 21	A
Annelida				
Aphelochaeta marioni	+	+	+	+
Heteromastus filiformis	+	+	+	+
Manayunckia aestuarina	+	-	-	+
Nereis diversicolor	+	+	+	+
Oligochaeta spp.	+	+	+	+
Pygospio elegans	+	+	+	+
Streblospio benedicti	+	+	+	+
Crustacea				
Lekanesphaera rugicauda	+	-	-	+
Orchestia gammarellus	-	-	+	-
Talitrus saltator	-	-	+	-
Mollusca				
Hydrobia ulvae	+	+	+	+
Macoma balthica	+	+	+	+
Scrobicularia plana	+	+	+	+
Nematoda	+	+	-	+

Table 2. *List of observed species. Presence/absence before (B), during (E12 and E21) and after (A) the emersion period are given: present (+), absent (-).*

Do species vary in their responses to the disturbance of medium-term emersion?

In general, neither the macrobenthic density nor the density of the representatively sampled species was seriously affected by the medium term emersion. In other words, a mass mortality due to the medium-term emersion was not observed.

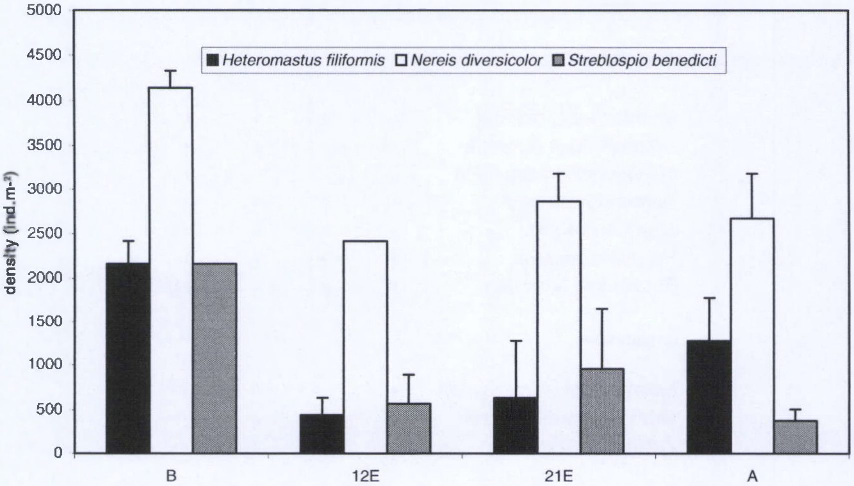


Fig. 3. Density of Nereis diversicolor, Heteromastus filiformis *and* Streblospio benedicti. *Density values (±SE) (B) before, (E12) after 12 days of emersion, (E21) after 21 days of emersion, and (A) 26 days after the removal of the sand barrier, in station 4 are given.*

A consistent decrease of species-specific densities (sampling occasions E21 versus B) however was detected in those stations where the species were abundant. This may point towards mortality in the macrobenthos during the period of emersion; trends, observed in stations, where species are abundantly present, should be considered as being most representative. Unfortunately, within this study it is impossible to attribute those decreases in species-specific density to the medium-term emersion. Because the whole study area was emersed, no control samples are available and thus the alternative hypothesis of natural winter mortality (Ysebaert 2000) cannot be rejected. Both processes might even have been acting in conjunction: in the absence of the buffering capacity of the seawater during emersion, causing major drops in bottom temperature during winter (pers. obs.), the effect of natural winter mortality might have been emphasised. Low bottom temperatures, often occurring during severe winters, are known to impact drastically on tidal flat macrobenthos, such as *Cerastoderma edule* (e.g. Beukema 1979, Dörjes 1986).

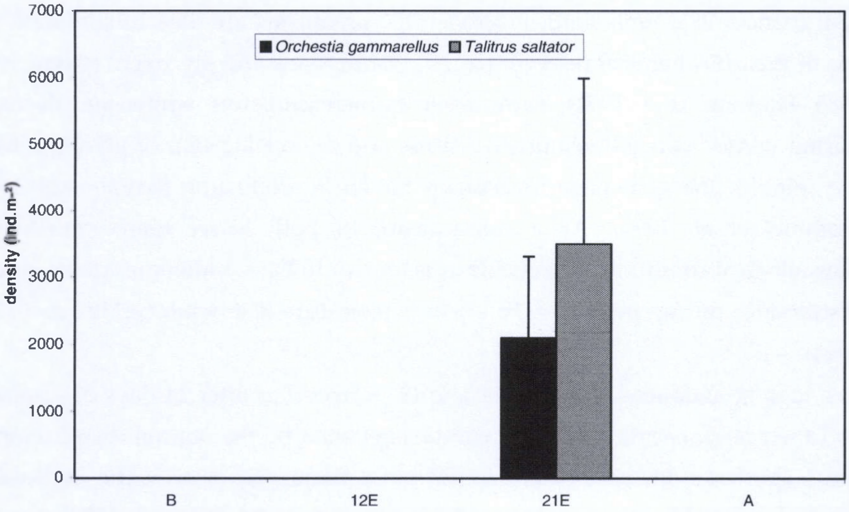


Fig. 4. *Density of* Talitrus saltator *and* Orchestia gammarellus. *Total density values (±SE) (B) before, (E12) after 12 days of emersion, (E21) after 21 days of emersion and (A) 26 days after the removal of the sand barrier are given.*

The observed high resistance of the tidal flat macrobenthos to the emersion is presumably linked to the fact that this study was performed in winter. Strong seasonal differences in the sensitivity of the macrobenthos to prolonged emersion have been found (Hummel & Bogaards 1989, Fortuyn *et al.* 1989). Ambient temperature is considered to be the most important factor, regulating this seasonality (Hummel *et al.* 1994). Hummel *et al.* (1988) showed that macrobenthos mortality during emersion increased progressively, with increasing temperature. As a consequence of lower temperature stress in addition to the stress from emersion, lower temperatures in winter during emersion might result in a lower mortality of the macrobenthic organisms.

The absence of the incoming water during flood and the associated lack of food and oxygen supply during a period of emersion also imposes physiologically stressful conditions on the macrobenthos (Hummel *et al.* 1994). However, during disturbances (e.g. emersion), macrobenthic organisms are able to stimulate the use of their biochemical reserves (e.g. lipids, proteins and glycogen) (Bayne *et al.* 1985, Hummel *et al.* 1994). These reserves increase before winter and decrease during winter as result of nutritive stress and the reallocation of glycogen from the soma to the gonads as investment for the reproduction (Bayne *et al.* 1985, Hummel *et al.* 1994). As a consequence of both lower temperatures and physiological condition macrobenthos is known to have a lower mortality due to disturbance during periods with lower temperature (e.g. winter) (Hummel *et al.* 1994).

The high abundances of *T. saltator* and *O. gammarellus* after 21 days of emersion and their absence after emersion can be explained by the natural distribution of these species. Both species are found most frequently nearby the high-water mark in supra-littoral, semi-terrestrial habitats (Jones & Wigham 1993). During the period of emersion, the tidal flats, which used to be inundated twice a day, now become a suitable for both species: an ephemeral immigration of both species into the intertidal zone during the third week of emersion from these habitats therefore seems likely.

In view of its minor impact on the macrobenthos and its effectiveness in preventing oil pollution in semi-enclosed salt marshes, such as the Zwin nature reserve, damming as a measure against oil pollution may be considered effective. Furthermore, given the long-lasting and severe impact of oil pollution in salt marshes (Hester & Mendelssohn 1999) in contrast to the minor impact of medium-term emersion, as demonstrated here for macrobenthos during wintertime, this strategy of pollution prevention should be considered a better alternative to tolerating an oil slick, followed by a subsequent clean-up.

Acknowledgments

The authors want to thank everybody who assisted in sampling and analysing the macrobenthic samples (Jan Wittoeck, Danny Peelaers, Bart Beuselinck, Gert Van Hoey, Sofie Vandendriessche, Annelies Goffin and Jeroen Speybroeck) and the sedimentological samples (Dannielle Schram). Valuable comments on the manuscript were provided by Tom Ysebaert (NIOO-KNAW) and three anonymous referees. We also express special thanks to Kris Struyf, conservator of the Zwin nature reserve, for his logistic support of this study.

APPENDIX 3
SUPPLEMENTARY MATERIAL

Supplementary material to Chapter 3

Table 1. (a) Abundance (ind. m^{-2}) and biomass (g AFDW m^{-2}) of the taxa present in the control macrobenthic assemblage, averaged over the duration of the experiment (March-September 2005) with indication of their functional group(s). (b) Environmental characterization of the study site, based on averaged values of the monitored environmental variables over the duration of the experiment (March-September 2005) in the control plots.

Table 2. Results of the SIMPER analyses between the species assemblage in the defaunated and control plots on each sampling occasion, based on standardized squareroot transformed abundance and biomass data. Species that contribute most to the observed dissimilarities and their percentage of contribution are given. Underlined percentages of dissimilarity indicate higher abundance/biomass in the control plots for the considered species.

Table 3. Relative abundance and biomass of the species in the defaunated plots. The tree most dominant species are given, whenever they contribute to > 5 % of the total abundance and biomass.

Fig. 1. Temporal variation in relative abundance and biomass of functional groups in the defaunated and control plots.

Supplementary material to Chapter 5

Table 4a-d. Production estimation following Crisp (1971) for the juvenile cohorts of *Pygospio elegans* (a), *Nereis diversicolor* (b), *Macoma balthica* (c) and *Cerastoderma edule* (d) in the tri-replicated control and treatment plots.

Table 1

(a) Biotic characterization

Species	Abundance	Biomass	Functional group
Abra tenuis	56.7	< 0.01	biodestablizing, discretely mobile surface deposit feeder
Gammarus spp.	24.0	0.46	mobile surface deposit feeder
Aphelochaeta marioni	3710.9	0.52	biodestablizing, discretely mobile surface deposit feeder
Capitella capitata	8.0	< 0.01	biodestabilizing, discretely mobile subsurface deposit feeder
Cerastoderma edule	876.4	43.76	biodestabilizing, discretely mobile suspension feeder
Eteone longa	909.9	0.19	mobile, biodestabilizing omnivore/scavenger
Heteromastus filiformis	11454.6	4.68	biodestabilizing, discretely mobile subsurface deposit feeder
Hydrobia ulvae	869.2	1.21	mobile surface deposit feeder
Macoma balthica	1505.9	7.92	biodestablizing, discretely mobile surface deposit feeder
Malacocerus tetracerus	1001.8	0.10	biodestablizing, discretely mobile surface deposit feeder
Nemertinae spp.	32.8	0.03	mobile, biodestabilizing omnivore/scavenger
Nereis diversicolor	856.4	3.53	mobile, biodestabilizing omnivore/scavenger
Tubificid oligochaeta	3511.1	0.27	biodestabilizing, discretely mobile subsurface deposit feeder
POLYDORA CORNUTA	183.7	0.02	tube-building surface deposit feeder
Pygospio elegans	4207.0	0.28	tube-building surface deposit feeder
Retusa obtusa	51.1	< 0.01	mobile, biodestabilizing omnivore/scavenger
Scrobicularia plana	76.7	5.23	biodestablizing, discretely mobile surface deposit feeder
Streblospio benedicti	568.8	0.14	biodestablizing, discretely mobile surface deposit feeder

(b) Abiotic characterization

Chlorofyll a (µg.g^{-1})	11.00
Sediment stability (Kpa)	12.85
oxygen penetration (µm)	2618.87
Ammonium (µg.l^{-1})	5084.93
Total organic carbon (%)	0.78
Total nitrogen (%)	0.07
Mud content (%)	41.54
Median particle size (µm)	74.5
% very fine sand	39.45
% fine sand	17.76
% medium sand	1.23
Water content (%)	62.13
Total organic matter (%)	4.17
Bed level (cm + N.A.P.)	17.9
Emersion time at mean tide (h)	5 – 5.5

Table 2

Days (d)	Dissimilarity Abundance (%)	Species contribute most to the abundance dissimilarity			Dissimilarity Biomass (%)	Species contribute most to the biomass dissimilarity		
0	-	-	-	-	-	-	-	-
		-	-	-		-	-	-
1	54.63	*H.ulvae*	*H.filiformis*	*A.marioni*	83.06	*H.ulvae*	*C.edule*	*S.plana*
		19.52	14.87	13.38		26.19	23.77	11.29
2	60.32	*H.ulvae*	*H.filiformis*	*M.balthica*	81.85	*H.ulvae*	*C.edule*	*M.balthica*
		29.16	25.58	7.53		25.17	22.02	11.67
7	51.82	*H.ulvae*	*Oligochaeta*	*H.filiformis*	82.83	*H.ulvae*	*C.edule*	*S.plana*
		19.64	15.37	10.48		33.13	28.05	10.9
14	50.37	*H.ulvae*	*Oligochaeta*	*H.filiformis*	84.78	*H.ulvae*	*C.edule*	*S.plana*
		19.11	15.99	14		30.03	27.34	13.98
21	47.56	*H.ulvae*	*H.filiformis*	*P.elegans*	84.59	*H.ulvae*	*C.edule*	*P.elegans*
		21.48	19.66	13.66		22.62	18.7	16.7
28	54.76	*P.elegans*	*H.filiformis*	*A.marioni*	64.7	*H.ulvae*	*C.edule*	*M.balthica*
		21.62	20.32	16.65		22.62	17.92	15.65
42	48.07	*H.filiformis*	*P.elegans*	*Oligochaeta*	74.53	*C.edule*	*H.ulvae*	*P.elegans*
		20.06	19.11	11.48		25.66	19.69	16.13
56	42.96	*H.filiformis*	*P.elegans*	*A.marioni*	71.86	*C.edule*	*H.ulvae*	*P.elegans*
		26.34	20.71	11.15		28.09	22.24	19.38
70	43.59	*H.filiformis*	*P.elegans*	*Oligochaeta*	61.81	*C.edule*	*P.elegans*	*S.plana*
		20.02	14.61	12.61		28.41	25.72	10.26
84	34.94	*P.elegans*	*H.filiformis*	*A.marioni*	39.14	*C.edule*	*H.ulvae*	*P.elegans*
		15.66	14.79	13.4		36.77	15.51	15.47
98	35.97	*A.marioni*	*P.elegans*	*Oligochaeta*	48.71	*C.edule*	*P.elegans*	*S.plana*
		15.51	13.52	12.76		23	17.96	15.15
112	31.41	*A.marioni*	*Oligochaeta*	*P.cornuta*	46.44	*C.edule*	*P.elegans*	*N.diversicolor*
		15.24	14.63	13.94		32.74	18.02	10.46
126	35.02	*P.elegans*	*P.cornuta*	*A.marioni*	58.06	*C.edule*	*P.cornuta*	*A.marioni*
		18.96	12.47	11.86		28.98	13.19	10.19
140	30.5	*P.ligni*	*P.elegans*	*Oligochaeta*	48.24	*C.edule*	*N.diversicolor*	*S.plana*
		14.53	14.52	12.02		31.85	17.66	8.92
154	30.07	*P.elegans*	*Oligochaeta*	*A.marioni*	41.39	*C.edule*	*H.filiformis*	*N.diversicolor*
		17	16.18	11.52		30.74	18.17	16.75
175	26.97	*P.elegans*	*Oligochaeta*	*M.tetracerus*	48.72	*C.edule*	*S.plana*	*S.benedicti*
		25.47	16.84	10.59		27.55	12.76	12.43

Table 3

Days (d)	Relative abundance (%)			Relative biomass (%)		
0	-	-	-	-	-	-
1	*H.ulvae*	*Oligochaeta*	*M.tetracerus*	*H.ulvae*	-	-
	40	20	13.3	87.6		
2	*H.ulvae*	-	-	*H.ulvae*	-	-
	80			98.6		
7	*H.ulvae*	*H.filiformis*	Nemertinae	*H.ulvae*	-	-
	40	20	10	95.5		
14	*H.ulvae*	*P.elegans*	*H.filiformis*	*H.ulvae*	*P.elegans*	-
	39.8	34.2	7.8	60.3	37.5	
21	*H.ulvae*	*P.elegans*	-	*H.ulvae*	-	-
	43.4			92.1		
28	*P.elegans*	-	-	*H.ulvae*	*P.elegans*	-
	89.1			55.1	37.3	
42	*P.elegans*	*N.diversicolor*	-	*H.ulvae*	*P.elegans*	-
	86.4	8.2		53.6	41.5	
56	*P.elegans*	*N.diversicolor*	*M.balthica*	*P.elegans*	*H.ulvae*	*N.diversicolor*
	77.4	9.5	6.9	65	15.5	11
70	*P.elegans*	*M.balthica*	*N.diversicolor*	*H.filiformis*	*H.ulvae*	*P.elegans*
	62.4	19.9	19.5	33.7	18.7	17.6
84	*P.elegans*	*N.diversicolor*	*M.balthica*	*P.elegans*	*M.balthica*	*M.balthica*
	61.3	9.2	8.3	34.5	23	21.2
98	*P.elegans*	*P.cornuta*	*N.diversicolor*	*N.diversicolor*	*M.balthica*	*P.elegans*
	59.7	14.9	6.1	29.5	26	25.1
112	*P.elegans*	*H.filiformis*	*P.cornuta*	*N.diversicolor*	*M.balthica*	*P.cornuta*
	45.3	21.5	9.2	25.4	23.4	16.2
126	*P.elegans*	*H.filiformis*	*P.cornuta*	*N.diversicolor*	*M.balthica*	*H.filiformis*
	38.6	22.6	14.1	39.6	26.5	16.4
140	*P.elegans*	*H.filiformis*	*P.cornuta*	*H.filiformis*	*N.diversicolor*	*M.balthica*
	34.1	21	12.2	33.5	31.1	25.2
154	*P.elegans*	*H.filiformis*	*M.balthica*	*M.balthica*	*N.diversicolor*	*H.filiformis*
	45.9	15.1	10	28.8	26.2	14.8
175	*P.elegans*	*H.filiformis*	*M.balthica*	*M.balthica*	*H.ulvae*	*H.filiformis*
	41.2	19.8	11.2	29.8	20.8	29.4

Fig. 1

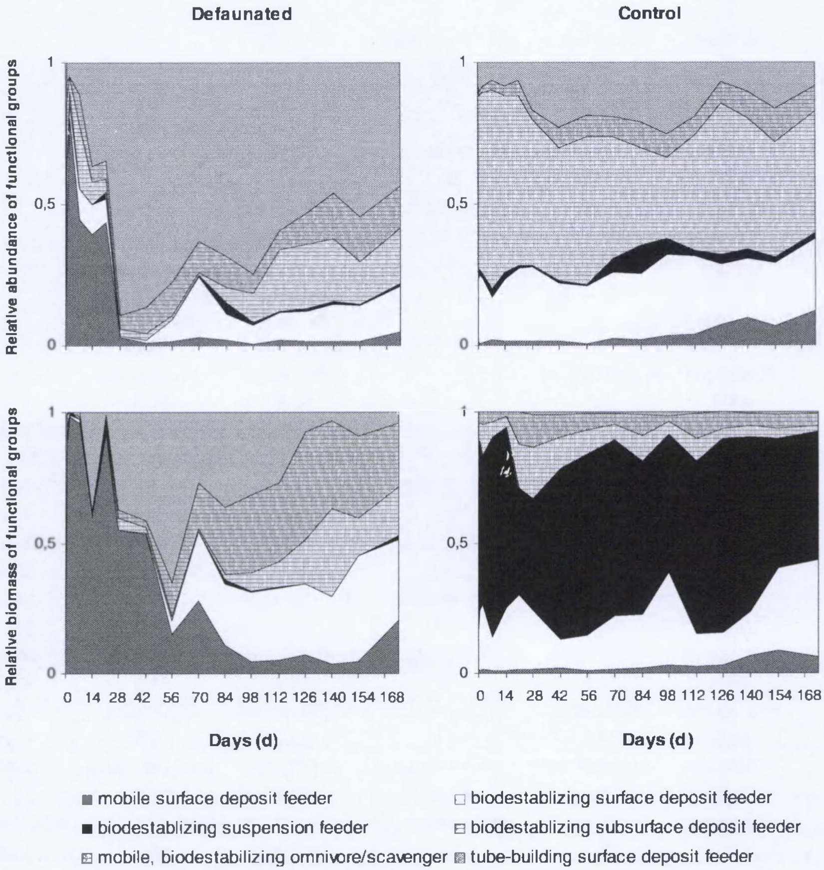

Defaunated
Control
Relative abundance of functional groups
Relative biomass of functional groups
1
0,5
0
0 14 28 42 56 70 84 98 112 126 140 154 168
Days (d)
▨ mobile surface deposit feeder
☐ biodestablizing surface deposit feeder
■ biodestablizing suspension feeder
▤ biodestablizing subsurface deposit feeder
▨ mobile, biodestabilizing omnivore/scavenger
▨ tube-building surface deposit feeder

Table 4a

	Time since opening plots						
	28	**42**	**56**	**70**	**84**	**98**	**112**
Abundance (ind.m^{-2})							
Control 1	3324	5297	5052	3015	3379	7136	3809
Control 2	2077	4646	3103	5361	6073	6802	5542
Control 3	3389.333	7093.333	3207.333	4375	4291.333	6969	3200
Treatment 1	4481	12029	25235	12344	16401	3017	
Treatment 2	3667	17602	14872	8177	19061	1767	
Treatment 3	5630	12037	24971	3374	1314	3077	
Mean biomass per Individual (g AFDW)							
Control 1	6.18E-05	6.21E-05	7.99E-05	6.66E-05	9.62E-05	9.73E-05	0.000123
Control 2	7.6E-05	7.22E-05	0.000135	0.00011	0.000117	0	0.000176
Control 3	4.25E-05	6.18E-05	4.93E-05	7.22E-05	0.000111	0.000108	0.000108
Treatment 1	5.16E-05	0.000117	0.000107	8.78E-05	0.000151	0.000231	0
Treatment 2	4.54E-05	8.2E-05	0.000106	8.75E-05	7.12E-05	0.000203	0
Treatment 3	5.24E-05	0.000102	9.62E-05	0.000107	0.000151	0.000151	0
Δ Biomass							
Control 1		3.14E-07	1.77E-05	-1.3E-05	2.96E-05	1.16E-06	2.56E-05
Control 2		-3.8E-06	6.27E-05	-2.5E-05	6.7E-06	-0.00012	0.000176
Control 3		1.93E-05	-1.3E-05	2.29E-05	3.83E-05	-2.9E-06	0
Treatment 1		6.57E-05	-1E-05	-1.9E-05	6.34E-05	8.01E-05	0
Treatment 2		3.66E-05	2.41E-05	-1.9E-05	-1.6E-05	0.000132	0
Treatment 3		4.92E-05	-5.5E-06	1.07E-05	4.44E-05	0	0
Mean abundance (ind.m^{-2})							
Control 1		4310.5	5174.5	4033.5	3197	5257.5	5472.5
Control 2		3361.5	3874.5	4232	5717	6437.5	6172
Control 3		5241.333	5150.333	3791.167	4333.167	5630.167	5084.5
Treatment 1		8255	18632	18789.5	14372.5	9709	1508.5
Treatment 2		10634.5	16237	11524.5	13619	10414	883.5
Treatment 3		8833.5	18504	14172.5	2344	2195.5	1538.5
Production (g AFDW m^{-2})							
Control 1		0.001354	0.091742	-0.05345	0.09452	0.006123	0.139835
Control 2		-0.01265	0.242751	-0.10463	0.038305	-0.75234	1.087155
Control 3		0.101249	-0.06443	0.086965	0.166145	-0.0162	0
Treatment 1		0.542469	-0.18627	-0.36573	0.911427	0.77732	0
Treatment 2		0.388852	0.391018	-0.21453	-0.22107	1.370554	0
Treatment 3		0.434723	-0.10162	0.151937	0.103974	0	0

Table 4b

	Time since opening plots						
	42	**56**	**70**	**84**	**98**	**112**	**126**
Abundance (ind.m^{-2})							
Control 1	407.4367	162.9747	162.9747	0	325.9493	81.48733	244.462
Control 2	651.8986	814.8733	570.4113	162.9747	244.462	244.462	244.462
Control 3	244.462	488.924	570.4113	162.9747	81.48733	81.48733	162.9747
Treatment 1	2444.62	2770.569	1629.747	570.4113	1792.721	977.848	1711.234
Treatment 2	2200.158	3259.493	1711.234	651.8986	1792.721	0	733.386
Treatment 3	1385.285	3096.519	2118.671	2200.158	1385.285	0	1059.335
Mean biomass per Individual (g AFDW)							
Control 1	0.000103	0.000177	0.0002	0	0.000672	0.001069	0.000577
Control 2	7.8E-05	8.78E-05	0.000108	0.001034	0.000856	0.00119	0.001727
Control 3	5.93E-05	8.73E-05	9.77E-05	0.000646	0.001292	0.00114	0.0015
Treatment 1	6.04E-05	0.000134	0.000153	0.002261	0.000768	0.003056	0.002404
Treatment 2	7.77E-05	0.000138	0.000494	0.001794	0.001633	0	0.003609
Treatment 3	7.52E-05	0.000191	0.000419	0.001204	0.001029	0	0.003113
Δ Biomass							
Control 1		7.34E-05	2.35E-05	-0.0002	0.000672	0.000397	-0.00049
Control 2		9.84E-06	2.03E-05	0.000926	-0.00018	0.000334	0.000537
Control 3		2.8E-05	1.04E-05	0.000548	0.000647	-0.00015	0.000359
Treatment 1		7.39E-05	1.84E-05	0.002109	-0.00149	0.002288	-0.00065
Treatment 2		6E-05	0.000356	0.0013	-0.00016	-0.00163	0.003609
Treatment 3		0.000115	0.000229	0.000784	-0.00017	-0.00103	0.003113
Mean abundance (ind.m^{-2})							
Control 1		285.2057	162.9747	81.48733	162.9747	203.7183	162.9747
Control 2		733.386	692.6423	366.693	203.7183	244.462	244.462
Control 3		366.693	529.6676	366.693	122.231	81.48733	122.231
Treatment 1		2607.595	2200.158	1100.079	1181.566	1385.285	1344.541
Treatment 2		2729.826	2485.364	1181.566	1222.31	896.3606	366.693
Treatment 3		2240.902	2607.595	2159.414	1792.721	692.6423	529.6676
Production (g AFDW m^{-2})							
Control 1		0.020928	0.003838	-0.01631	0.109453	0.0809	-0.0802
Control 2		0.007218	0.014043	0.339526	-0.03632	0.081642	0.131263
Control 3		0.010282	0.00551	0.200945	0.079045	-0.01239	0.043922
Treatment 1		0.192616	0.040592	2.319635	-1.76425	3.169922	-0.87774
Treatment 2		0.163866	0.884716	1.536164	-0.19605	-1.46414	1.323547
Treatment 3		0.258527	0.59654	1.693602	-0.3129	-0.7128	1.649064

Table 4c

	Time since opening plots					
	56	70	84	98	112	126
Abundance (ind.m^{-2})						
Control 1	3015.031	407.4367	3178.006	977.848	244.462	407.4367
Control 2	2770.569	1385.285	2770.569	1466.772	407.4367	896.3606
Control 3	1303.797	2770.569	2363.133	1874.209	814.8733	814.8733
Treatment 1	2933.544	2892.8	3585.443	6274.524	1140.823	1222.31
Treatment 2	2852.057	2770.569	3259.493	2281.645	1140.823	488.924
Treatment 3	1466.772	4644.778	4400.316	3585.443	4726.265	4400.316
Mean biomass per individual (g AFDW)						
Control 1	4.39E-05	8.2E-05	0.000116	0.000227	0.000325	0.000106
Control 2	4.92E-05	9.33E-05	0.000153	0.00027	0.000255	0.000118
Control 3	5.3E-05	0.000102	0.000136	0.000211	0.000312	0.000347
Treatment 1	5.9E-05	0.000154	0.000249	0.000435	0.000629	0.00062
Treatment 2	9E-05	0.000141	0.000294	0.000373	0.000652	0.000665
Treatment 3	6.63E-05	0.000137	0.000248	0.000398	0.000675	0.00073
Δ Biomass						
Control 1		3.81E-05	3.43E-05	0.00011	9.82E-05	-0.00022
Control 2		4.41E-05	5.99E-05	0.000117	-1.5E-05	-0.00014
Control 3		4.94E-05	3.31E-05	7.53E-05	0.000102	3.45E-05
Treatment 1		9.45E-05	9.57E-05	0.000185	0.000194	-8.9E-06
Treatment 2		5.1E-05	0.000153	7.99E-05	0.000278	1.33E-05
Treatment 3		7.1E-05	0.000111	0.00015	0.000277	5.56E-05
Mean abundance (ind.m^{-2})						
Control 1		1711.234	1792.721	2077.927	611.155	325.9493
Control 2		2077.927	2077.927	2118.671	937.1043	651.8986
Control 3		2037.183	2566.851	2118.671	1344.541	814.8733
Treatment 1		2913.172	3239.121	4929.983	3707.674	1181.566
Treatment 2		2811.313	3015.031	2770.569	1711.234	814.8733
Treatment 3		3055.775	4522.547	3992.879	4155.854	4563.291
Production (g AFDW m^{-2})						
Control 1		0.065239	0.061518	0.228948	0.060004	-0.07131
Control 2		0.091659	0.124393	0.248302	-0.01426	-0.08926
Control 3		0.100551	0.085053	0.159446	0.136612	0.028097
Treatment 1		0.275392	0.310115	0.914133	0.719104	-0.0105
Treatment 2		0.1434	0.459914	0.221494	0.476057	0.01086
Treatment 3		0.216815	0.500511	0.598	1.150973	0.253942

Table 4d

	Time since opening plots				
	70	84	98	112	126
Abundance (ind.m^{-2})					
Control 1	325.9493	3015.031	1874.209	407.4367	81.48733
Control 2	814.8733	2689.082	896.3606	0	407.4367
Control 3	814.8733	6356.012	1548.259	162.9747	244.462
Treatment 1	162.9747	1140.823	651.8986	162.9747	81.48733
Treatment 2	814.8733	1303.797	407.4367	81.48733	162.9747
Treatment 3	325.9493	2526.107	570.4113	81.48733	244.462
Mean biomass per					
Individual (g AFDW)	1.9E-05	3.08E-05	8.97E-05	0.000228	0.000318
Control 1	2.24E-05	3.08E-05	7.29E-05	0	0.000135
Control 2	3.3E-05	5.34E-05	6.93E-05	0.000522	0.000342
Control 3	1.71E-05	3.9E-05	8.54E-05	0.000175	0.000342
Treatment 1	2.08E-05	5.02E-05	7.88E-05	7.32E-05	0.000126
Treatment 2	2.52E-05	3.3E-05	7.31E-05	0.000115	0.000149
Treatment 3					
Δ Biomass					
Control 1		1.18E-05	5.89E-05	0.000138	9.04E-05
Control 2		8.41E-06	4.2E-05	-7.3E-05	0.000135
Control 3		2.04E-05	1.6E-05	0.000452	-0.00018
Treatment 1		2.19E-05	4.64E-05	8.99E-05	0.000166
Treatment 2		2.94E-05	2.86E-05	-5.6E-06	5.26E-05
Treatment 3		7.79E-06	4.01E-05	4.18E-05	3.45E-05
Mean abundance (ind.m^{-2})		1670.49	2444.62	1140.823	244.462
Control 1		1751.978	1792.721	448.1803	203.7183
Control 2		3585.443	3952.136	855.617	203.7183
Control 3		651.8986	896.3606	407.4367	122.231
Treatment 1		1059.335	855.617	244.462	122.231
Treatment 2		1426.028	1548.259	325.9493	162.9747
Treatment 3					
Production (g AFDW m^{-2})		0.019713	0.144074	0.157848	0.022088
Control 1		0.014731	0.075351	-0.03266	0.027416
Control 2		0.072968	0.063073	0.386983	-0.03664
Control 3		0.014275	0.041591	0.036647	0.020337
Treatment 1		0.03116	0.024448	-0.00136	0.006429
Treatment 2		0.011107	0.062077	0.013622	0.005629
Treatment 3		0.500511	0.598	1.150973	0.253942

CITED LITERATURE

Albert RC (1988) The Historical Context of Water-Quality Management for the Delaware Estuary. Estuaries 11: 99-107

Alden RW (1997) Dynamics of an estuarine ecosystem - The Chesapeake Bay experience: Statistical approaches and water quality patterns. Oceanologica Acta 20: 55-69

Aller RC (1994) Bioturbation and Remineralization of Sedimentary Organic-Matter - Effects of Redox Oscillation. Chemical Geology 114: 331-345

Aller RC, JY Aller (1998) The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. Journal of Marine Research 56: 905-936

Andersen TJ, Jensen KT, Lund-Hansen L, Mouritsen KN, Pejrup M (2002) Enhanced erodibility of fine-grained marine sediments by Hydrobia ulvae. Journal of Sea Research 48: 51–58

Andersen TJ, Mikkelsen OA, Moller AL, Pejrup M (2000) Deposition and mixing depths on some European intertidal mudflats based on Pb-210 and Cs-137 activities. Continental Shelf Research 20: 1569-1591

Andersson MGI (2007) Nitrogen cycling in a turbid tidal estuary. PhD thesis Utrecht University.

Andre C, Rosenberg R (1991) Adult-Larval Interactions in the Suspension Feeding Bivalves Cerastoderma-Edule and Mya-Arenaria. Marine Ecology Progress Series 71: 227-234

Anger K, V Anger, Hagmeier E (1986) Laboratory studies on the larval growth of Polydora ligni, Polydora ciliata and Pygospio elegans (Polychaeta, Spionidae). Helgoländer Meeresuntersuchungen 40: 377-395

Anon. (1992) Council\Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official journal of the European Community L206 35: 7-50

Anon. (2000) Council\Directive 2000/60/EC of 23 October 2000 on the establishing a framework for community action in the field of water policy. Official journal of the European Community L327: 1-72

Armonies W (1992) Migratory Rhythms of Drifting Juvenile Mollusks in Tidal Waters of the Wadden Sea. Marine Ecology Progress Series 83: 197-206

Armonies W, Reise K (2000) Faunal diversity across a sandy shore. Marine Ecology Progress Series 196: 49-57

Aronson RB, Precht WF (1995) Landscape Patterns of Reef Coral Diversity - a Test of the Intermediate Disturbance Hypothesis. Journal of Experimental Marine Biology and Ecology 192: 1-14

Austen MC, Widdicombe S (1998) Experimental evidence of effects of the heart urchin Brissopsis lyrifera on associated subtidal meiobenthic nematode communities. Journal of Experimental Marine Biology and Ecology 222: 219-238

Bachelet G, Dauvin JC (1993) The Quantitative Distribution of the Benthic Macrofauna in Intertidal Sands of Arcachon Bay. Oceanologica Acta 16: 83-97

Bachelet G, de Montaudouin X, Auby I and Labourg PJ (2000) Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. Ices Journal of Marine Science 57: 1495–1506

Banta GT, Holmer M, Jensen MH, Kristensen E (1999) Effects of two polychaete worms, Nereis diversicolor and Arenicola marina, on aerobic and anaerobic decomposition in a sandy marine sediment. Aquatic Microbial Ecology 19: 189–204

Bao WY, Satuito CG, Yank JL, Kitamura (2007) Larval settlement and metamorphosis of the mussel Mytilus galloprovincialis in reponse to biofilms. Marine Biology 150: 565-574

Barille L, Cognie B (2000) Revival capacity of diatoms in bivalve pseudofaeces and faeces. Diatom Research 15: 11–17

Barnes RD (1982) Invertebrate Zoology, 4th edition. Holt-Saunders, Tokio.

Barnes RSK (1998) The effects of movement on population density estimates of mudflat epifauna. Journal of the Marine Biological Association of the United Kingdom 78: 377-385

Bayne BL, Brown DA, Burns K, Dixon DR, Ivanovici A, Livingstone DR, Lowe DM, Moore MN, Stebbing ARD, Widdows J (1985) The effects of stress and pollution on marine animals. Praeger, New York, 384 pp.

Benedetti-Cecchi, L. 2000. Predicting direct and indirect interactions during succession in a mid-littoral rocky shore assemblage. Ecological Monographs 70: 45-72

Berlow EL (1997) From canalization to contingency: Historical effects in a successional rocky intertidal community. Ecological Monographs 67: 435-460

Bertness MD (2007) Atlantic shorelines. Natural history and ecology. Princeton University Press, Princeton, New York

Beukema JJ (1976) Biomass and species richness of the macrobenthic animals living on tidal flats of the Dutch Wadden-sea. Netherlands Journal of Sea Research 10: 236-261

Beukema JJ (1979) Biomass and species richness of the macrobenthic animals living on a tidal flat area in the Dutch Wadden Sea: effects of a severe winter. Netherlands Journal of Sea Research 13: 203 – 223

Beukema JJ (1981) Quantitative data on the benthos in the Wadden Sea proper. In Dankers N., Kühl H. and Wolff Q.J. (eds) Invertebrates of the Wadden Sea. Balkema, Rotterdam, pp. 134 – 142

Beukema JJ, Cadeé GC (1996) Consequences of the sudden removal of nearly all mussels and cockles from the Dutch Wadden Sea. Marine Ecology – Pubblicazioni Della Stazione Zoologica Di Napoli I 17: 279–289

Beukema JJ, Dekker R (2005) Decline of recruitment success in cockles and other bivalves in the Wadden Sea: possible role of climate change, predation on postlarvae and fisheries. Marine Ecology Progress Series 287: 149-167

Beukema JJ, Devlas J (1989) Tidal-Current Transport of Thread-Drifting Postlarval Juveniles of the Bivalve Macoma-Balthica from the Wadden Sea to the North-Sea. Marine Ecology Progress Series 52: 193-200

Beukema JJ, Flach EC, Dekker R, Starink M (1999) A long term study of the recovery of the macrozoobenthos on large defaunated plots on a tidal flat in the Wadden Sea. Journal of Sea Research 42: 235-254

Beukema JJ, Flach EC, Dekker R, Starink M (1999) a longterm study of the recovery of the macrozoobenthos on large defaunated plots on a tidal flat in the Wadden Sea. Journal of Sea Research 42: 235–254

Bhattacharya CG (1967) A simple method of resolution of distribution of Gaussian components. Biometrics 23: 115-135

Blanchard GF, Guarini JM, Provot L, Richard P, Sauriau PG (2000) Measurement of ingestion rate of Hydrobia ulvae (Pennant) on intertidal epipelic microalgae: the effect of mud snail density. Journal of Experimental Marine Biology and Ecology 255: 247–260

Bolam SG, Fernandes TF (2002) Dense aggregations of tubebuilding polychaetes: response to small-scale disturbances. Journal of Experimental Marine Biology and Ecology 269: 197–222

Bolam SG, Fernandes TF (2003) Dense aggregations of Pygospio elegans (Claparede) effect on macrofaunal community structure and sediments. Journal of Sea Research 49: 171–185

Bolam SG, Fernandes TF, Huxham M (2002) Diversity, biomass, and ecosystem processes in the marine benthos. Ecological Monographs 72: 599-615

Bolam SG, Schratzberger M, Whomersley P (2006) Macro- and meiofaunal recolonisation of dredged material used for habitat enhancement: temporal patterns in community development. Marine Pollution Bulletin 52: 1746-1755

Bolam SG, Whomersley P (2005) Development of macrofaunal communities on dredged material used for mudflat enhancement: a comparison of three beneficial use schemes after one year. Marine Pollution Bulletin 50: 40-47

Bolam SG, Whomersley P, Schratzberger M (2004) Macrofaunal recolonization on intertidal mudflats: effect of sediment organic and sand content. Journal of Experimental Marine Biology and Ecology 306: 157-180

Borja A, Dauer DM, Diaz R, Llanso RJ, Muxika I, Rodriguez JG, Schaffner L (2008) Assessing estuarine benthic quality conditions in Chesapeake Bay: A comparison of three indices. Ecological Indicators 8: 395-403

Borsje BW, De Vries MB, Huscher S, De Boer GJ (2008) Modeling large-scale cohesive sediment transport affected by small-scale biological activity. Estuarine Coastal and Shelf Science 78: 468-480

Bos OG (2005) Recruitment variation of Macoma balthica (L.) is there a role for larval food limitation? PhD thesis Groningen University

Bos OG, Philippart CJM, Cadee GC, van der Meer J (2006) Recruitment variation in Macoma balthica: a laboratory examination of the match/mismatch hypothesis. Marine Ecology Progress Series 320: 207-214

Bouezmarni M, Wollast R (2005) Geochemical composition of sediments in the Scheldt estuary with emphasis on trace metals. Hydrobiologia 540: 155-168

Bouma TJ, De Vries MB, Low E, Peralta G, Tanczos IC, Van de Koppel J, Herman PMJ (2005) Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. Ecology 86: 2187–219

Bradshaw AD (1997) What do we mean by restoration? In: Urbanska KM, Webb NR , Edwards PF (eds) Restoration Ecology and Sustainable Development, Cambridge University Press, Cambridge UK

Bremner J, Rogers SI, Frid CLJ (2003) Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. Marine Ecology Progress Series 254: 11-25

Brey T (1991) Interactions in soft bottom benthic communities: quantitative aspects of behaviour in the surface deposit feeders Pygospio elegans (Polychaeta) and Macoma balthica (Bivalvia). Helgoländer Meeresuntersuchungen 45: 301-316

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18: 119-125

Caddy JF (1967) Maturation of Gametes and Spawning in Macoma Balthica (L). Canadian Journal of Zoology 45: 955-965

Cadee GC (1979) Sediment reworking by the polychaete Heteromastus filiformis on a tidal flat in the Dutch Wadden Sea. Netherlands Journal of Sea Research 13: 441–456

Caradec S, Grossi V, Hulth S, Stora G, Gilbert F (2004) Macrofaunal reworking activities and hydrocarbon redistribution in an experimental sediment system. Journal of Sea Research 52: 199-210

Carvalho S, Moura A, Gaspar MB, Pereira P, da Fonseca LC, Falcão M, Drago T, Lietão F, Regala J (2005) Spatial and inter-annual variability of the macrobenthic communities within a coastal lagoon (Óbidos lagoon) and its relationship with environmental parameters. Acta Oecologia 27: 143-159

Choi YD (2004) Theories for ecological restoration in changing environment: toward 'futuristic' restoration. Restoration Ecology 19: 75-81

Ciutat A, Widdows J, Pope ND (2007) Effect of Cerastoderma edule density on near-bed hydrodynamics and stability of cohesive muddy sediments. Journal of Experimental Marine Biology and Ecology 346: 114-126

Clarcke KR (1993) Non-parametric multivariate analysis of changes in community structure; Australian Journal of Ecology 18: 117-143

Clarcke KR, Gorley RN (2001) Primer v5: User manual/tutorial. Primer-E, Plymouth Marine Laboratory, Plymouth, U.K.

Clarke KR, Gorley RN (2006) Primer v6: User manual/tutorial. Primer-E, Plymouth Marine Laboratory, Plymouth, U.K.

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed. PRIMER-E: Plymouth, U.K.

Clements FE (1916) Plant succession: an analysis of the development of vegetation. Washington: Carnegie Institution of Washington.

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210: 223-253

Cohen JE (1997) Population, economics, environment and culture: an introduction to human carrying capacity. Journal of applied ecology 34: 1325-1333

Commito JA, Tita G (2002) Differential dispersal rates in an intertidal meiofauna assemblage. Journal of Experimental Marine Biology and Ecology 268: 237-256

Connell JH (1978) Diversity in Tropical Rain Forests and Coral Reefs - High Diversity of Trees and Corals Is Maintained Only in a Non-Equilibrium State. Science 199: 1302-1310

Connell JH, Hughes TE, Wallace CC, Tanner JE, Harms KE, Kerr AM (2004) A long-term study of competition and diversity of corals. Ecological Monographs 74: 179-210

Connell JH, Slatyer RB (1977) Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist 111: 1119-1144

Costanza R, dArge R, deGroot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, ONeill RV, Paruelo J, Raskin RG, Sutton P, vandenBelt M (1997) The value of the world's ecosystem services and natural capital. Nature 387: 253-260

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11: 1304-1315

Cramp S. & Simmons K.E.L. (eds) (1977) The birds of the western Palearctic. Oxford University Press, Oxford.

Crawley MJ (2005) Statistics: an introduction using R. John Wiley & Sons, Chichester

Crimaldi JP, Thompson JK, Rosman JH, Lowe RJ, Koseff JR (2002) Hydrodynamics of larval settlement: The influence of turbulent stress events at potential recruitment sites. Limnology and Oceanography 47: 1137-1151

Crisp DJ (1971) Energy Flow measurements. In: Holme NA, McIntyre AD (eds) Methods for the study of marine benthos. Blackwell, Oxford, pp. 197-279

Cummings VJ, Pridmore RD, Thrush SF, Hewitt HE (1996) Effect of the spionid polychaete Boccardia syrtis on the distribution and survival of juvenile Macomona liliana (Bivalvia: Tellinacea). Marine Biology 126: 91–98

Daborn GR, Amos CL, Brylinski H, Drapeau G, Faas RW, Grant J, Long B, Paterson DM, Perillo GME, Piccolo MC (1993) An ecological cascade effect: migratory birds affect stability of intertidal sediments. Limnology and Oceanography 38: 225-231

Dade WB, Nowell ARM, Jumars PA (1992) Predicting Erosion Resistance of Muds. Marine Geology 105: 285-297

Dahms HU, Dobretsov S, Qian PY (2004) The effect of bacterial and diatom biofilms on the settlement of the bryozoan Bugula neritina. Journal of Experimental Marine Biology and Ecology 313: 191-209

Danovaro R, Gambi C (2002) Biodiversity and trophic structure of nematode assemblages in seagrass systems: evidence for a coupling with changes in food quality. Marine Biology 141: 667-677

de Boer PL (1981) Mechanical effect of micro-organisms on intertidal bedform migration. Sedimentology 28: 129–132

de Brouwer JFC, Bjelic S, de Deckere EMGT, Stal LJ (2000) Interplay between biology and sedimentology in a mudflat (Biezelingse Ham, Westerschelde, The Netherlands). Continental Shelf Research 20: 1159–1177 de Brouwer JFC, de Deckere EMGT, Stal LJ (2003a) Distribution of extracellular carbohydrates in three intertidal mudflats in Western Europe. Estuarine Coastal and Shelf Science 56: 313–324

de Brouwer JFC, Neu TR, Stal LJ (2003b) On the function of secretion of extracellular polymeric substances by benthic diatoms and their role in intertidal mudflats: a review of recent insights and views. In: Kromkamp JC, De Brouwer JFC, Blanchard GF, Forster RM, Creach V (eds) Microphytobenthos in estuaries. Royal Netherlands Academy of Arts & Sciences (KNAW), Amsterdam, pp 45–61

de Brouwer JFC, Ruddy GK, Jones TER, Stal LJ (2002) Sorption of EPS to sediment particles and the effect on the rheology of sediment slurries. Biogeochemistry 61: 57–71

de Brouwer JFC, Stal LJ (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Marine Ecology Progress Series 218: 33-44

de Deckere EMGT, Tolhurst TJ, de Brouwer JFC (2001) Destabilization of cohesive intertidal sediments by infauna. Estuarine Coastal and Shelf Science 53: 665–669

de Deckere EMGT, Van De Koppel J, Heip CHR (2000) The influence of Corophium volutator abundance on resuspension. Hydrobiologia 426: 37-42

de la Cruz AA, Kackney CT, Rajanna B (1981) Some effects of crude oil on a Juncus tidal marsh. Journal of the Elisha Mitchell Society 97: 14-28

Decho AW (2000) Microbial biofilms in intertidal systems: an overview. Continental Shelf Research 20: 1257-1273

Defew EC, Tolhurst TJ, Paterson DM (2002) Site-specific features influence sediment stability of intertidal flats. Hydrology and Earth System Sciences 6: 971–981

Degraer S, Mouton I, De Neve L, Vincx M (1999) Community structure and intertidal zonation of the macrobenthos on a macrotidal, ultra-dissipative sandy beach: Summer-winter comparison. Estuaries 22: 742-752

Dejong DJ, Dejonge VN (1995) Dynamics and Distribution of Microphytobenthic Chlorophyll-a in the Western Scheldt Estuary (Sw Netherlands). Hydrobiologia 311: 21-30

Dekker R, Beukema JJ (1999) Relations of summer and winter temperatures with dynamics and growth of two bivalves, Tellina tenuis and Abra tenuis, on the northern edge of their intertidal distribution. Journal of Sea Research 42: 207-220

Diaz RJ (2001) Overview of hypoxia around the world. Journal of environmental quality 30: 275-281

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology: an annual review 33: 245-303

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321: 926-929

Dittmann S, Günther C-P, Schleier U (1999) Recolonization of tidal flats after disturbance. In: Dittmann S. (ed) The Wadden Sea Ecosystem: Stability, Problems and Mechanisms. Springer, Berlin, pp 175-192

Dobretsov S, Qian PY (2006) Facilitation and inhibition of larval attachment of the bryozoan Bugula neritina in association with mono-species and multi-species biofilms. Journal of Experimental Marine Biology and Ecology 333: 263-274

Dorgan KM, Jumars PA, Johnson B, Boudreau BP, Landis E (2005) Burrow extension by crack propagation. Nature 433: 475-475

Dörjes J, Michaelis H, Rhode B (1986) Long-term studies of macrozoobenthos in intertidal and shallow subtidal hatitats near the island of Norderney (East Frisian coast, germany). Hydrobiologia 142: 217-232

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs 67: 345-366

Duport E, Gilbert F, Poggiale JC, Dedieu K, Rabouille C, Stora G (2007) Benthic macrofauna and sediment reworking quantification in contrasted environments in the Thau Lagoon. Estuarine Coastal and Shelf Science 72: 522-533

Duport E, Stora G, Tremblay P, Gilbert F (2006) Effects of population density on the sediment mixing induced by the gallery-diffusor Hediste (Nereis) diversicolor O.F. Muller, 1776. Journal of Experimental Marine Biology and Ecology 336: 33-41

Dworjanyn SA, Pirozzi I (2008) Induction of settlement in the sea urchin Tripneustes gratilla by macroalgae, biofilms and conspecifics: A role for bacteria? Aquaculture 274: 268-274

Easterling DR, Karl TR, Gallo KP, Robinson DA, Trenberth KE, Dai A (2000) Observed climate variability and change of relevance to the biosphere. Journal of Geophysical Research-Atmospheres 105: 20101-20114

Eckert GL (2003) Effects of the planktonic period on marine population fluctuations. Ecology 84: 372-383

Eckman JE (1983) Hydrodynamic processes affecting benthic recruitment. Limnology and Oceanography 28: 241-257

Eckman JE, Nowell ARM, Jumars PA (1981) Sediment destabilization by animal tubes. Journal of Marine Research 39: 361–374

Eertman RMH, Kornman BA, Stikvoort E, Verbeek H (2002) Restoration of the Sieperda tidal marsh in the Scheldt estuary, the Netherlands. Restoration Ecology 10: 438-449

Elliott M, Burdon D, Hemingway KL, Apitz SE (2007) Estuarine, coastal and marine ecosystem restoration: Confusing management and science - A revision of concepts. Estuarine Coastal and Shelf Science 74: 349-366

Elliott M, Cutts ND (2004) Marine habitats: loss and gain, mitigation and compensation. Marine pollution bulletin 49: 671-674

Engstrom SJ, Marinelli RL (2005) Recruitment responses of benthic infauna to manipulated sediment geochemical properties in natural flows. Journal of Marine Research 63: 407-436

Esser F, Winterberg M, Sebesvari Z, Harder T (2008) Effects of halogenated metabolites from infaunal polychaetes on larval settlement of the spionid polychaete Streblospio benedicti. Marine Ecology Progress Series 355: 161-172

Evans PR, Ward RM, Bone M, Leakey M (1998) Creation of temperate-climate intertidal mudflats: Factors affecting colonization and use by benthic invertebrates and their bird predators. Marine Pollution Bulletin 37: 535-545

Fauchauld K, Jumars PA (1979) The diet of worms: a study of polychaete feeding guilds. Oceanography and Marine Biology: an Annual Review 17: 193-284

Fernandes S, Meysman FJR, P Sobral (2006) The influence of Cu contamination on Nereis diversicolor bioturbation. Marine Chemistry 102: 148-158.

Flach EC (1992) Disturbance of Benthic Infauna by Sediment-Reworking Activities of the Lugworm Arenicola-Marina. Netherlands Journal of Sea Research 30: 81-89

Flach EC (1996) The influence of the cockle, Cerastoderma edule, on the macrozoobenthic community of tidal flats in the Wadden Sea. PSZN I: Marine Ecology 17: 87–98

Flach EC (2003) The separate and combined effects of epibenthic predation and presence of macro-infauna on the recruitment success of bivalves in shallow soft-bottom areas on the Swedish west coast. Journal of Sea Research 49: 59-67

Flemming BW (2000) A revised textural classification of gravel-free muddy sediments on the basis of ternary diagrams. Continental Shelf Research 20: 1125-1137

Flemming BW, Delafontaine MT (2000) Mass physical properties of muddy intertidal sediments: some applications, misapplications and non-applications. Continental Shelf Research 20: 1179–1197

Forster RM, Creach V, Sabbe K, Vyverman W, Stal LJ (2006) Biodiversity-ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. Marine Ecology Progress Series 311: 191-201

Fortuin AW, Meijboom A, de Wolf L (1989) Het gebruik van de stormvloedkering bij de afbouwwerkzaamheden in de Oosterschelde: effecten van een aantal sluitingsscenario' s op bodemdieren. Rapporten en verslagen 1989 – 2, Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke, 79 pp.

Franco A, Torricelli P, Elliott M (2007) Preface: Biodiversity and ecosystem functioning in coastal and transitional waters. Edited by Anita Franco, Michael Elliott and Patrizia Torricelli. Estuarine Coastal and Shelf Science 75: 1-3

Fraschetti S, Giangrande A, Terlizzi A, Boero F (2002) Pre- and post-settlement events in benthic community dynamics. Oceanologica Acta 25: 285-295

Friedrichs M, Graf G, Springer B (2000) Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar Ecol Prog Ser 192: 219–228

Gallagher ED, Jumars PA, Trueblood DD (1983) Facilitation of soft-bottom benthic succession by tube builders. Ecology 64: 1200–1216

Gamito S (2006) Benthic ecology of semi-natural coastal lagoons, in the Ria Formosa (Southern Portugal), exposed to different water renewal regimes. Hydrobiologia 555: 75-87

Gatusso JP, Frankignoulle M, Wollast R (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. Annual review of ecology and systematics 29: 405-434

Gayanilo FC, Sparee P, Pauli D (1996) The FAO-ICLARM Stock Assessment Tools (FiSAT) Users' guide. FAO Computerized Information Series (Fisheries) no. 8. Rome, FAO 126 pp.

Gee JM, Warwick RM (1985) Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. Journal of Experimental Marine Biology and Ecology 91: 247-262

Gerino M (1990) The Effects of Bioturbation on Particle Redistribution in Mediterranean Coastal Sediment - Preliminary-Results. Hydrobiologia 207: 251-258

Gerino M, Aller RC, Lee C, Cochran JK, Aller JY, Green MA, Hirschberg D (1998) Comparison of different tracers and methods used to quantify bioturbation during a spring bloom: 234-thorium, luminophores and chlorophyll a. Estuarine Coastal and Shelf Science 46: 531-547

Gerino M, Stora G (1991) Invitro quantitative-analysis of the bioturbation induced by the polychaete Nereis diversicolor. Copmtes Rendus de l' Académie des Sciences Series III Life Sciences 313: 489–494

Gerino M, Stora G, Francois-Carcaillet F, Gilbert F, Poggiale JC, Mermillod-Blondin F, Desrosiers G, Vervier P (2003) Macro-invertebrate funcitonal groups in freshwater and marine sediments: a common mechanistic classification. Vie et milieu-life and environment 53: 221-231

Gilbert AJ, Janssen R (1998) Use of environmental functions to communicate the values of a mangrove ecosystem under different management regimes. Ecological Economics 25: 323-346

Gilbert F, Hulth S, Grossi V, Poggiale JC, Desrosiers G, Rosenberg R, Gerino M, Francois-Carcaillet F, Michaud E, Stora G (2007) Sediment reworking by marine benthic species from the Gullmar Fjord (Western Sweden): Importance of faunal biovolume. Journal of Experimental Marine Biology and Ecology 348: 133-144

Gimenez L, Borthagaray AI, Rodriguez M, Brazeiro A, Dimitriadis C (2005) Scale-dependent patterns of macrofaunal distribution in soft-sediment intertidal habitats along a large-scale estuarine gradient. Helgoland Marine Research 59: 224-236

Gimenez L, Dimitriadis C, Carranza A, Borthagaray AI, Rodriguez M (2006) Unravelling the complex structure of a benthic community: A multiscale-multianalytical approach to an estuarine sandflat. Estuarine Coastal and Shelf Science 68: 462-472

Gingras MK, Pemberton SG, Dashtgard S, Dafoe L (2008) How fast do marine invertebrates burrow? Palaeogeography, Palaeoclimatology, Palaeoecology 270: 280-286

Goodall DW (1954) Objective methods for the classification of vegetation – An essay in the use of factor analysis. Australian Journal of Botany 2: 304-324

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Marine Ecology Progress Series 146: 265-282

Grant J, Daborn G (1994) The effects of bioturbation on sediment transport on an intertidal mudflat. Netherlands Journal of Sea Research 32: 63–72

Grassle JP, Butman CA, Mills SW (1992) Active Habitat Selection by Capitella Sp-I Larvae .2. Multiple-Choice Experiments in Still Water and Flume Flows. Journal of Marine Research 50: 717-743

Graveland J (2005) Fysische en ecologische kennis en modellen voor de Westerschelde (Physical and ecological knowledge and models for the Westerschelde). Report RIKZ/2005.018. Rijkswaterstaat, Middelburg.

Green RH (1993) Application of repeated measures designs in environmental impact and monitoring studies. Australian Journal of Ecology 18: 81-98

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111: 1169-1194

Guerreiro J (1998) Growth and production of the bivalve Scrobicularia plana in two southern European estuaries. Vie Et Milieu-Life and Environment 48:121-131

Guillard RL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith LW, Chandley MH (Eds), Culture of Marine Invertebrate Animals. Plenum Press, New York

Günther C-P (1992) Dispersal of intertidal invertebrates: a strategy to react to disturbances of different scales? Netherlands Journal of Sea Research 30: 45–56

Hagerthey SE, Defew EC, Paterson DM (2002) Influence of Corophium volutator and Hydrobia ulvae on intertidal benthic diatom assemblages under different nutrient and temperature regimes. Marine Ecology Progress Series 245: 47-59

Halpern BS, Selkoe KA, Micheli F, Kappel CV (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conservation Biology 21: 1301-1315

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319: 948-952

Hampel H, Cattrijsse A, Elliott M (2005) Feeding habits of young predatory fishes in marsh creeks situated along the salinity gradient of the Schelde estuary, Belgium and the Netherlands. Helgoland Marine Research 59: 151-162

Hampel H, Cattrijsse A, Mees J (2004) Changes in marsh nekton communities along the salinity gradient of the Schelde river, Belgium and The Netherlands. Hydrobiologia 515: 137-146

Hampel H, Cattrijsse A, Vincx M (2003) Habitat value of a developing estuarine brackish marsh for fish and macrocrustaceans. Ices Journal of Marine Science 60: 278-289

Harder T, Lam C, Qian PY (2002) Induction of larval settlement in the polychaete Hydroides elegans by marine biofilms: an investigation of monospecific diatom films as settlement cues. Marine Ecology Progress Series 229: 105-112

Harriague AC, Albertelli G (2007) Environmental factors controlling macrofaunal assemblages on six microtidal beaches of the Ligurian Sea (NW Mediterranean). Estuarine Coastal and Shelf Science 73: 8-16

Haubois AG, Guarini JM, Richard P, Blanchard GF, Sauriau PG (2002) Spatio-temporal differentiation in the population structure of Hydrobia ulvae on an intertidal mudflat (Marennes-Oleron Bay, France). Journal of the marine biological association of the United Kingdom 82: 605-614

Haven DS, Morales-Alamo R (1966) Aspects of Biodeposition by Oysters and Other Invertebrate Filter Feeders. Limnology and Oceanography 11: 487-198

Hedges JI, Keil RG (1995) Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine Chemistry 49: 81-115

Hentschel BT (1998) Intraspecific variations in δ13C indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology 79: 1357–1370

Herman PMJ, Middelburg JJ, Heip CHR (2001) Benthic community structure and sediment processes on an intertidal flat: results from the ECOFLAT project. Continental Shelf Research 21: 2055–2071

Herman PMJ, Middelburg JJ, van de Koppel J, Heip CHR (1999) Ecology of estuarine macrobenthos. Advances in Ecological Research 29: 195-240

Herman PMJ, Middelburg JJ, Widdows J, Lucas CH, Heip CHR (2000) Stable isotopes' as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. Marine Ecology Progress Series 204: 79-92

Hester MW, Mendelssohn IA (1999) Long-term recovery of a Louisiana brackish marsh plant community from oil-spill impact: vegetation response and mitigating effects of marsh surface elevation. Marine Environmental Research 49: 233 – 254

Hiddink JG (2003) Modelling the adaptive value of intertidal migration and nursery use in the bivalve Macoma balthica. Marine Ecology Progress Series 252: 173-185

Hiddink JG, Jennings S, Kaiser MJ (2006) Indicators of the ecological impact of bottom-trawl disturbance on seabed communities. Ecosystems 9: 1190-1199

Hiddink JG, ter Hofstede R, Wolff WJ (2002) Predation of intertidal infauna on juveniles of the bivalve Macoma balthica. Journal of Sea Research 47: 141-159

Hiddink JG, Wolff WJ (2002) Changes in distribution and decrease in numbers during migration of the bivalve Macoma balthica. Marine Ecology Progress Series 233: 117-130

Hillebrand H, Duerselen C-D, Kirschtel D, Pollinger U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424

Hoagland KD, Rosowski JR, Gretz MR, Roemer SC (1993) Diatom extracellular polymeric substances: function, finestructure, chemistry, and physiology. Journal of Phycology 29: 537–566

Hobbs RJ, Nortan DA (1996) Toward a conceptual framework for restoration ecology. Restoration Ecology 4: 93-110

Hoffmann M, Adam S, Baert L, Bonte D, Chavatte N, Claus R, De Belder W, De Fré B, Degraer S, De Groote D, Dekoninck W, Desender K, Devos K, Engledow H, Grootaert P, Hardies N, Leliaert F, Maelfait JP, Monbaliu J, Pollet M, Provoost S, Stichelmans E, Toorman E, Van Nieuwenhuyse H, Vercruysse E, Vincx M, Wittoeck J (2005) Integrated monitoring of nature restoration along ecotones, the example of the Yser Estuary. In: Herrier, J.-L. et al. (Ed.) (2005). Proceedings 'Dunes and Estuaries 2005': International Conference on nature restoration practices in European coastal habitats, Koksijde, Belgium 19-23 September 2005. VLIZ Special Publication 19, pp 191-208

Holland AF, Zingmark RG, Dean JM (1974) Quantitative evidence concerning the stabilization of sediments by marine benthic diatoms. Marine Biology 27: 191–196

Honkoop PJC, Luttikhuizen PC, Piersma T (1999) Experimentally extending the spawning season of a marine bivalve using temperature change and fluoxetine as synergistic triggers. Marine Ecology Progress Series 180: 297-300

Honkoop PJC, Pearson GB, Lavaleye MSS, Piersma T (2006) Spatial variation of the intertidal sediments and macrozoo-benthic assemblages along Eighty-mile Beach, North-western Australia. Journal of Sea Research 55: 278-291

Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Shmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75: 3-35

Hulscher JB (1982) The Oystercatcher Haematopus-Ostralegus as a Predator of the Bivalve Macoma-Balthica in the Dutch Wadden Sea. Ardea 70: 89-152

Hummel H, Bogaards RH (1989) Changes in the reproductive cycle of the cockle Cerastoderma edule after disturbance by means of tidal manipulation. In: Ryland, J.S. & Tyler, P.A. (eds), Reproduction, genetics and distribution of marine organisms. Olsen & Olsen, Fredensborg, pp. 133 - 136.

Hummel H, Brummelhuis EBM, de Wolf L (1986) Effects on the benthic fauna of embanking an intertidal flat area (the Markiezaat, Eastern Scheldt estuary, the Netherlands). Netherlands Journal of Sea Research 20: 397 - 406

Hummel H, Fortuin AW, Bogaards RH, Meijboom A, de Wolf L (1994) The effects of prolonged emersion and submersion by tidal manipulation on marine macrobenthos. Hydrobiologia 282/283: 219 – 234

Hummel H, Fortuin AW, de Wolf L, Meijboom A (1988) Mortality of intertidal benthic animals after a period of prolonged emersion. Journal of Experimental Marine Biology and Ecology 121: 247-254

Hurlbert SH (1984) Pseudoreplication and design of ecological field experiments. Ecological monographs 45 (2): 187-211

Hyland J, Balthis L, Karakassis I, Magni P, Petrov A, Shine J, Vestergaard O, Warwick R (2005) Organic carbon content of sediments as an indicator of stress in the marine benthos. Marine Ecology Progress Series 295: 91-103

Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. Science 317: 58-62

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629-638

Jeffrey SW, Mantoura RFC, Wright SW (1997) Phytoplankton pigments in oceanography. United Nations International, Paris

Jenkins SR, Norton TA, Hawkins SJ (1999) Settlement and post-settlement interactions between Semibalanus balanoides (L.) (Crustacea : Cirripedia) and three species of fucoid canopy algae. Jounral of Experimental Marine Biology and Ecology 236: 49-67

Johnson JB, Omland KS (2004) Model selection in ecology and evolution. Trends in Ecology and Evolution 19: 101-108

Jones CG, Lawton JH, Shachac M (1997) Positve and negative effects of organisms as physical ecosystem engineers. Ecology 78: 1946-1957

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69: 373–386

Jones MB, Wigham GD (1993) Reproductive-biology of Orchestia-gammarellus (Crustacea, Amphipoda) living in a sewage-treatment works. Journal of the Marine Biological Association of the United Kingdom 73: 405 – 416

Jones PD (2006) Water quality and fisheries in the Mersey estuary, England: A historical perspective. Marine Pollution Bulletin 53: 144-154

Jonsson PR, Andre C, Lindegarth M (1991) Swimming Behavior of Marine Bivalve Larvae in a Flume Boundary-Layer Flow - Evidence for near-Bottom Confinement. Marine Ecology Progress Series 79: 67-76

Jouuchi T, Satuito CG, Kitamura H (2007) Sugar compound products of the periphytic diatom Navicula ramosissima induce larval settlement in the barnacle, Amphibalanus amphitrite. Marine Biology 152: 1065-1076

Jumars PA, Nowell ARM (1984a) Effects of benthos on sediment transport: difficulties with functional grouping. Continental Shelf Research 3: 115–130

Jumars PA, Nowell ARM (1984b) Fluid and sediment dynamic effects on marine benthic community structure. American Zoologist 24: 45–55

Kaiser MJ, Clarke KR, Hinz H, Austen MCV, Somerfield PJ, Karakassis I (2006) Global analysis of response and recovery of benthic biota to fishing. Marine Ecology Progress Series 311: 1-14

Kamermans P, van der Veer HW, Karczmarski L, Doeglas GW (1992) Competition in deposit-feeding and suspensionfeeding bivalves: experiments in controlled outdoor environments. Journal of Experimental Marine Biology and Ecology 162: 113–135

Kawamura T, Takami H (1995) Analysis of Feeding and Growth-Rate of Newly Metamorphosed Abalone Haliotis-Discus-Hannai Fed on 4 Species of Benthic Diatom. Fisheries Science 61: 357-358

Keough MJ, Raimondi PT (1995) Responses of Settling Invertebrate Larvae to Bioorganic Films - Effects of Different Types of Films. Journal of Experimental Marine Biology and Ecology 185: 235-253

Kesteloo JJ, van Stralen MR, Frey F, Jol J, Goudswaard PC (2008) Het kokkelbestand in de Nederlandse kustwateren in 2007. Imares rapport C071/07

Kesteloo JJ, van Stralen MR, Steenbergen JS (2007) Het kokkelbestand in de Nederlandse kustwateren in 2006. Imares rapport C054/06

Kimbro DL, Grosholz ED (2006) Disturbance influences oyster community richness and evenness, but not diversity. Ecology 87: 2378-2388

Kromkamp JC, Forster RM (2003) Developments in microphytobenthos primary productivity studies. In: Kromkamp JC, de Brouwer JFC, Blanchard GF, Forster RM, Creach V (eds) Functioning of microphtyobenthos in estuaries. Royal Netherlands Academy of Arts & Sciences (KNAW), Amsterdam, pp 9–30

Krumbein WE, Paterson DM, Stal LJ (1994) Biostabilization of sediments. Bibliotheks- und Informationssystem der Carl von Ossietzky Universitaet Oldenburg, Oldenburg

Lake PS, Bond N, Reich P (2007) Linking ecological theory with stream restoration. Freshwater Biology 52: 597-615

Lam C, Harder T, Qian PY (2005) Induction of larval settlement in the polychaete Hydroides elegans by extracellular polymers of benthic diatoms. Marine Ecology Progress Series 286: 145-154

Lam D, Harder T, Qian PY (2003) Induction of larval settlement in the polychaete Hydroides elegans by surface-associated settlement cues of marine benthic diatoms. Marine Ecology Progress Series 263: 83-92

Lant CL, Ruhl JB, Kraft SE (2008) The Tragedy of Ecosystem Services. Bioscience 58: 969-974

Larson F, Sundback K (2008) Role of microphytobenthos in recovery of functions in a shallow-water system after hypoxic events. Marine Ecology Progress Series 357: 1-16

Larson F, Sundbäck K (2008) Role of microphytobenthos in recovery of functions in a shallow-water sediment system after hypoxic events. Marine Ecology Progress Series 357: 1–16

Le Hir P, Monbet Y, Orvain F (2007) Sediment erodability in sediment transport modelling: Can we account for biota effects? Continental Shelf Research 27: 116–1142

Lenihan HS, Peterson CH, Kim SL, Conclan KE, Fairey R, McDonald C, Grabowski JH, Oliver JS (2003) Variation in marine benthic community composition allows discrimination of multiple stressors. Marine Ecology Progress Series 261: 63-73

Levin LA, Talley D, Thayer G (1996) Succession of macrobenthos in a created salt marsh. Marine Ecology Progress Series 141: 67-82

Lindeboom H (2002) The coastal zone: an ecosystem under pressure. In: Field JG, Hampel G, Summerhayes C (eds) Oceans 2020: Science, Trends and the Challenge of Sustainability. Island Press, Washington, pp 49-84

Lindegarth M, Jonsson PR (1991) Fluorescent microparticles: a new way of visualizing sedimentation and larval settlement. Limnology and Oceanography 36: 1471-1476

Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431: 1092-1095

Lohrer AM, Thrush SF, Lundquist CJ, Vopel K, Hewitt JE, Nicholls PE (2006) Deposition of terrigenous sediment on subtidal marine macirobenthos: response of two contrasting community types. Marine Ecology Progress Series 307: 115-125

Luckenbach MW (1986) Sediment stability around animal tubes: the roles of hydrodynamic processes and biotic activity. Limnology and Oceanography 31: 779-789

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: The Ecological Role of the "Secret Garden" of Unvegetated, Shallow-Water Marine Habitats. I. Distribution, Abundance and Primary Production. Estuaries 19: 186-201

Mackey RL, Currie DJ (2001) The diversity-disturbance relationship: Is it generally strong and peaked? Ecology 82: 3479-3492

Madgwick FJ, Jones TA (2002) Europe. In: Perrow MR, Davy AJ (eds) Handbook of Ecological Restoration. Restoration in Practice. Cambridge Univeristy Press, Cambridge: 22-56

Magni P, Micheletti S, Casu D, Floris A, Giordani G, Petrov AN, De Falco G, Castelli A (2005) Relationships between chemical characteristics of sediments and macrofaunal communities in the Cabras lagoon (Western Mediterranean, Italy). Hydrobiologia 550: 105-119

Mahaut ML, Graf G (1987) A Luminophore Tracer Technique for Bioturbation Studies. Oceanologica Acta 10: 323-328

Maire O, Duchêne JC, Grémare A, Malyuga VS, Meysman FJR (2007) A comparison of sediment reworking rates by the surface deposit-feeding bivalve Abra ovata during summertime and wintertime, with a comparison between two models of sediment reworking. Journal of Experimental Marine Biology and Ecology 343: 21-36

Maire O, Lecroart P, Meysman F, Rosenberg R, Duchene JC, Gremare A (2008) Quantification of sediment reworking rates in bioturbation research: a review. Aquatic Biology 2: 219-238

Mander L, Cutts ND, Allen J, Mazik K (2007) Assessing the development of newly created habitat for wintering estuarine birds. Estuarine Coastal and Shelf Science 75: 163-174

Marinelli RL, Williams TJ (2003) Evidence for density-dependent effects of infauna on sediment biogeochemistry and benthic-pelagic coupling in nearshore systems. Estuarine Coastal and Shelf Science 57: 179-192

Marinelli RL, Woodin SA (2002) Experimental evidence for linkages between infaunal recruitment, disturbance, and sediment surface chemistry. Limnology and Oceanography 47: 221-229

Marinelli RL, Woodin SA (2004) Disturbance and recruitment: a test of solute and substrate specificity using Mercenaria mercenaria and Capitella sp.1. Marine Ecology Progress Series 269: 209-221

Maris T, Cox T, Temmerman S, De Vleeschauwer P, Van Damme S, De Mulder T, Van den Bergh E, Meire P (2007) Tuning the tide: creating ecological conditions for tidal marsh development in a flood control area. Hydrobiologia 588: 31-43

Martini IP, Morrison RIG (1987) Regional Distribution of Macoma-Balthica and Hydrobia-Minuta on the Sub-Arctic Coasts of Hudson-Bay and James Bay, Ontario, Canada. Estuarine Coastal and Shelf Science 24: 47-68

Mazik K, Smith JE, Leighton A, Elliott M (2007) Physical and biological development of a newly breached managed realignment site, Humber estuary, UK. Marine Pollution Bulletin 55: 564-578

McDonald SJ, Wade TL, Brooks JM, McDonald TJ (1991) Assessing the exposure of fish to a petroleum spill in Galveston Bay, Texas. In: L.C. Worbel, C.A. Brebbia (eds.) Water Pollution, Modeling, Measuring, and Prediction, Computational

Mechanics Publication, Southampton and Elsevier Applied Science, London, pp. 707-718

McLusky DS, Elliott M (2004) The Estuarine Ecosystem – ecology, threats and management, 3th ed. Oxford University Press Inc., New York.

Meehan BW (1985) Genetic Comparison of Macoma-Balthica (Bivalvia, Telinidae) from the Eastern and Western North-Atlantic Ocean. Marine Ecology Progress Series 22: 69-76

Meire P, Van den Bergh E, Ysebaert T, Nijssen D (2002) Nature development along the river Scheidt: Combining ecosystem functions in the Kruibeke-Bazel-Rupelmonde polder. Pasture Landscapes and Nature Conservation: 173-185

Menge BA, Branch GM (2001) Rocky intertidal communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer, Sunderland, p 221-251.

Menge BA, Sutherland JP (1987) Community Regulation - Variation in Disturbance, Competition, and Predation in Relation to Environmental-Stress and Recruitment. American Naturalist 130: 730-757

Meysman FJR, Galaktionov ES, Gribsholt B, Middelburg JJ (2006) Bioirrigation in permeable sediments: Advective pore-water transport induced by burrow ventilation. Limnology and Oceanography 51: 142-156

Meysman FJR, Malyuga VS, Boudreau BP, Middelburg JJ (2008a) A generalized stochastic approach to particle dispersal in soils and sediments. Geochimica Et Cosmochimica Acta 72: 3460-3478

Meysman FJR, Malyuga VS, Boudreau BP, Middelburg JJ (2008b) Quantifying particle dispersal in aquatic sediments at short time scales: model selection. Aquatic Biology 2: 239-254

Meysman JFR, Middelburg JJ, Heip CHR (2006) Bioturbation: a fresh look at Darwin's last idea. Trends in Ecology and Evolution 21: 688-695

Michaud E, Desrosiers G, Mermillod-Blondin F, Sundby B, Stora G (2006) The functional group approach to bioturbation: II. The effects of the Macoma balthica community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. Journal of Experimental Marine Biology and Ecology 337: 178-189.

Micheli F, Halpern BS (2005) Low functional redundancy in coastal marine assemblages. Ecology Letters 8: 391-400

Mitchener H, Torfs H (1996) Erosion of sand/mud mixtures. Coastal Engineering 29: 1–25

Moens T, Verbeeck L, de Maeyer A, Swings J, Vincx M (1999) Selective attraction of marine bacterivorous nematodes to their bacterial food. Marine Ecology Progress Series 176: 165-178

Moens T, Vincx M (1997) Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 77: 211-227

Moles A, Rice S, Norcross BL (1994) Non-avoidance of hydrocarbon laden sediments by juvenile flatfishes. Netherlands Journal of Sea Research 32: 361-367

Montserrat F, Van Colen C, Degraer S, Ysebaert T, Herman PMJ (2008) Benthic community-mediated sediment dynamics. Marine Ecology Progress Series 372: 43-59

Mulsow S, Landrum PF, Robbins JA (2002) Biological mixing responses to sublethal concentrations of DDT in sediments by Heteromastus filiformis using a Cs-137 marker layer technique. Marine Ecology Progress Series 239: 181-191

Murray JMH, Meadows A, Meadows PS (2002) Biogeomorphological implications of microscale interactions between sediment geotechnics and marine benthos: a review. Geomorphology 47: 15–30

Naeem S (2002) Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. Ecology 83: 2925-2935

Naeem S, Thompson LJ, Lawler SP, Lawton JH, Woodfin RM (1994) Biodiversity in Model-Ecosystems - Reply. Nature 371: 565-565

National Research Council (1992) Restoration of aquatic ecosystems: science, technology, and the public. National Academy Press, Washington DC

Newell RC, Seiderer LJ, DR Hitchcock (1998) The impact of dredging works in coastal waters: a review of the sensitivity to disturbance and subsequent recovery of biological resources on the seabed. Oceanography and Marine Biology: an Annual Review 36: 127-178

Nilsson HC, Rosenberg R (1997) Benthic habitat quality assessment of an oxygen stressed fjord by surface and sediment profile images. Journal of Marine Systems 11: 249-264

Norkko A, Bonsdorff E (1996) Population responses of coastal zoobenthos to stress induced by drifting algal mats. Marine Ecology Progress Series 140: 141-151

Norkko A, Rosenberg R, Thrush SF, Whitlatch RB (2006) Scale- and intensity-dependent disturbance determines the magnitude of opportunistic response. Journal of Experimental Marine Biology and Ecology 330: 195–207

Nowell ARM, Jumars PA, Eckman JE (1981) Effects of Biological-Activity on the Entrainment of Marine-Sediments. Marine Geology 42: 133-153

Ólafsson EB (1989) Contrasting influences of suspension-feeding and deposit-feeding populations of Macoma balthica on infaunal recruitment. Marine Ecology Progress Series 55: 171-179

Ólafsson EB, Peterson CH, Ambrose WG (1994) Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments: the relative significance of pre- and post-settlement processes. Oceanography and Marine Biology: an Annual Review 32: 65-109

Olivier F, Tremblay R, Bourget E, Rittschof D (2000) Barnacle settlement: field experiments on the influence of larval supply, tidal level, biofilm quality and age on Balanus amphitrite cyprids. Marine Ecology Progress Series 199: 185-204

Oost AP (1995) Dynamics and sedimentary development of the Dutch Wadden Sea with emphasis on the Frisian Inlet. Universiteit Utrecht.

Orvain F, Sauriau PG, Bacher U, Prineau M (2006) The influence of sediment cohesiveness on bioturbation effects due to Hydrobia ulvae on the initial erosion of intertidal sediments: a study combining flume and model approaches. Journal of Sea Research 55: 54–73

Orvain F, Sauriau PG, Sygut A, Joassard L, Le Hir P (2004) Interacting effects of Hydrobia ulvae bioturbation and microphytobenthos on the erodibility of mudflat sediments. Marine Ecology Progress Series 278: 205–223

Osakabe M, Hongo K, Funayama K, Osumi S (2006) Amensalism via webs causes unidirectional shifts of dominance in spider mite communities. Oecologia 150: 496–505

Paarlberg AJ, Knaapen MAF, De Vries MB, Hulscher SJMH, Wang ZB (2005) Biological influences on morphology and bed composition of a intertidal flat. Estuarine Coastal and Shelf Science 64: 577-590

Paine RT (1966) Food web complexity and species diversity. American Naturalist 100: 65-75

Palomo G, Iribarne O (2000) Sediment bioturbation by polychaete feeding may promote sediment stability. Bulletin of Marine Sciences 67: 249–257

Paterson DM (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behavior of epipelic diatoms. Limnology and Oceanography 34: 223–234

Paterson DM, Black KS (1999) Water Flow, Sediment Dynamics and Benthic Biology. Advances in Ecological Research 29: 155-193

Patil JS, Anil AC (2005) Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle Balanus amphitrite. Marine Ecology Progress Series 301: 231-245

Pawlik JF (1992) Chemical ecology of the settlement of benthic marine invertebrates. Oceanography and Marine Ecology 30: 274-278

Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: an Annual Review 16: 229-311

Pearson TH, Rosenberg R (1987) Feast and famine: structuring factors in marine benthic communities. In: Gee JHR, Giller PS (eds) The 27th symposium of the Britisch Ecological Society, Aberystwyth 1986, Blackwell Scientific Publications, Oxford, pp 373-395

Peterson CH (1991) Intertidal zonation of marine invertebrates in sand and mud. American Scientist 79: 236-249

Pinedo S, Sarda R, Rey C, Bhaud M (2000) Effect of sediment particle size on recruitment of Owenia fusiformis in the Bay of Blanes (NW Mediterranean Sea): an experimental approach to explain field distribution. Marine Ecology Progress Series 203: 205-213

Pinto R, Pinto J, Patrício A, Baeta BDF, Neto JM, Marques JC (2009) Review of estuarine biotic indices to assess benthic condition. Ecological Indicators 9: 1–25

Pomfret JR, Elliott M, Oreilly MG, Phillips S (1991) Spatial and Temporal Patterns in the Fish Communities in 2 UK North-Sea Estuaries. Estuaries and Coasts: Spatial and Temporal Intercomparisons: 277-284

Qian PY, Lau SCK, Dahms HU, Dobretsov S, Harder T (2007) Marine biofilms as mediators of colonization by marine macroorganisms: Implications for antifouling and aquaculture. Marine Biotechnology 9: 399-410

Quinn G, Keough M (2002) Experimental design and Data analysis for Biologists. Cambridge University press, Cambridge, United Kingdom

Rabaut M, Guilini K, Van Hoey G, Magda V, Degraer S (2007) A bio-engineered soft-bottom environment: The impact of Lanice conchilega on the benthic species-specific densities and community structure. Estuarine Coastal and Shelf Science 75: 525-536

Raffaelli D, Hawkins S (1996) Intertidal ecology. Chapman & Hall, London

Rakocinski CF, Brown SS, Gaston GR, Heard RW, Walker WW, Summers JK (2000) Species-abundance-biomass responses by estuarine macrobenthos to sediment chemical contamination. Journal of Aquatic Ecosystem Stress and Recovery 7: 201-214

Ramsay K, Kaiser MJ, Hughes RN (1998) Responses of benthic scavengers to fishing disturbance by towed gears in different habitats. Journal of Experimental Marine Biology and Ecology 224: 73-89

Reading CJ (1979) Changes in the Downshore Distribution of Macoma-Balthica (L) in Relation to Shell Length. Estuarine and Coastal Marine Science 8: 1-13

Reise K (1985) Tidal flat ecology – an experimental approach to species interactions. Springer-Verlag, Berlin

Rhoads DC (1967) Biogenic reworking of intertidal and subtidal sediments in Barnstable Harbor and Buzzard Bay, Massachusetts. Journal of Geology 75: 461–476

Rhoads DC (1974) Organism-sediment relations on the muddy sea floor. Oceanography and Marine Biology: an Annual Review 12: 263-300

Rhoads DC, German JD (1986) Interpreting long-term changes in benthic community structure – a new protocol. Hydrobiologia 142: 291-308

Rhoads DC, Young DK (1970) The influence of depositfeeding organisms on sediment stability and community trophic structure. Journal of Marine Research 28: 150–178

Riisgård, H. U., and G. T. Banta. 1998. Irrigation and deposit feeding by the lugworm Arenicola marina, characteristics and secondary effects on the environment. A review of current knowledge. Vie et Milieu-Life and Environment 48: 243-257

Rodriquez, S.R., F.P. Ojeda and N.C. Inestrosa. 1993. Settlement of benthic marine invertebrates. Marine Ecology Progress Series 97: 193-207

Rosenberg R, Agrenius S, Hellman B, Nilsson HC, Norling K (2002) Recovery of marine benthic habitats and fauna in a Swedish fjord following improved oxygen conditions. Marine Ecology Progress Series 234: 43-53

Rossi F, Gribsholt B, Middelburg JJ, Heip C (2008) Context-dependent effects of suspension feeding on intertidal ecosystem functioning. Marine Ecology Progress Series 354: 47-57

Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation of delta C-13 and delta N-15 in deposit- and suspension-feeding bivalves (Macoma balthica and Cerastoderma edule): Evidence of ontogenetic changes in feeding mode of Macoma balthica. Limnology and Oceanography 49: 408-414

Rossi F, Middelburg JJ (in prep.) Use of microalgal carbon and diet shifts in early macrofauna consumers during community reassembly.

Rossi F, Vos M, Middelburg JJ (in press). Species idendity, diversity and microbial carbon flow in reassembling macrobenthic communities. Oikos

Rueda JL, Smaal AC (2002) Physiological response of Spisula subtruncata (da Costa, 1778) to different seston quantity and quality. Hydrobiologia 475: 505-511

Sabbe K, Vyverman W (1991) Distribution of benthic diatom assemblages in the Westerschelde (Zeeland, The Netherlands). Belgian journal of botany 124: 91-101

Sanders HL, Grassle JF, Hampson GR, Morse LS, Garner-Price S, Jones CC (1980) Anatomy of an oil spill, long term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. Journal of Marine Research 38: 265-380

Sandnes J, Forbes T, Hansen R, Sandnes B, Rygg B (2000) Bioturbation and irrigation in natural sediments, described by animal-community parameters. Marine Ecology Progress Series 197: 169-179

Schratzberger M, Warwick RM (1998) Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. Marine Ecology Progress Series 164: 83-94

Schratzberger M, Warwick RM (1999) Differential effects of various types of disturbances on the structure of nematode assemblages: an experimental approach. Marine Ecology Progress Series 181: 227-236

Schratzberger M, Whomersley P, Warr K, Bolam SG, Rees HL (2004) Colonisation of various types of sediment by estuarine nematodes via lateral infaunal migration: a laboratory study. Marine Biology 145: 69-78

Scoffin TP (1970) The trapping and binding of subtidal carbonate sediments by marine vegetation in Bimini Lagoon, Bahamas. Journal of Sedimentary Petrology 40: 249–273

Scrosati R, Heaven C (2007) Spatial trends in community richness, diversity, and evenness across rocky intertidal environmental stress gradients in eastern Canada. Marine Ecology Progress Series 342: 1-14

Sebesvari Z, Esser F, Harder T (2006) Sediment-associated cues for larval settlement of the infaunal spionid polychaetes Polydora cornuta and Streblospio benedicti. Journal of Experimental Marine Biology and Ecology 337: 109-120

Sistermans WCH, Escaravage V, Hummel H, Bergmeijer MA, Engelberts AGM, Dek L, van Hoessel OJA, .Markusse MM (2007) Het macrobenthos van de Westerschelde, de Oosterschelde, het Veerse Meer en het Grevelingenmeer in het voor- en najaar van 2006. KNAW Netherlands Institute of Ecology - Centre for Estuarine and Marine Ecology, Yerseke

Sistermans WCH, Hummel H, Engelberts A, Markusse MM (2005) Inventarisatie macrofauna Westerschelde 2004 - Rapportage in het kader van de evaluatie van de verdieping van de Westerschelde, NIOO-CEME (KNAW), Yerseke

Snelgrove P, Blackburn TH, Hutchings PA, Alongi DM, Grassle JF, Hummel H, King G, Koike I, Lambshead PJD, Ramsing NB, Solis-Weiss V (1997) The importance of marine sediment biodiversity in ecosystem precesses. Ambio 26: 578-583

Snelgrove PVR (1998) The biodiversity of macrofaunal organisms in marine sediments. Biodiversity and Conservation 7: 1123-1132

Snelgrove PVR (1999) Getting to the bottom of marine biodiversity: Sedimentary habitats - Ocean bottoms are the most widespread habitat on Earth and support high biodiversity and key ecosystem services. Bioscience 49: 129-138

Snelgrove PVR, Butman CA (1994) Animal sediment relationships revisited: cause versus effect. Oceanography and Marine Biology: an Annual Review 32: 111–177

Snelgrove PVR, Grassle JP, Butman CA (1998) Sediment choice by settling larvae of the bivalve, Spisula solidissima (Dillwyn), in flow and still water. Journal of Experimental Marine Biology and Ecology 231: 171-190

Snelgrove PVR, Grassle JP, Zimmer CA (2001) Adult macrofauna effects on Capitella sp I larval settlement: A laboratory flume study. Journal of Marine Research 59: 657-674

Soetaert K, Middelburg JJ, Heip C, Meire P, Van Damme S, Maris T (2006) Long-term change in dissolved inorganic nutrients in the heterotrophic Scheldt estuary (Belgium, The Netherlands). Limnology and Oceanography 51: 409-423

Sokal RR, Rohlf FJ (1995) Biometry. WH Freeman, New York

Solan M, Batty P, Bulling MT, Godbold JA (2008) How biodiversity affects ecosystem processes: implications for ecological revolutions and benthic ecosystem function. Aquatic Biology 2: 289-301

Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. Science 306: 1177-1180

Solan M, Wigham BD, Hudson IR, Kennedy R, Coulon CH, Norling K, Nilsson HC, Rosenberg R (2004) In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Marine Ecology Progress Series 271: 1-12.

Somerfield PJ, Clarke KR (1995) Taxonomic Levels, in Marine Community Studies, Revisited. Marine Ecology Progress Series 127: 113-119

Sousa ME (1979) Disturbance in marine intertidal boulder fields: the nonequlibrium maintenance of species diversity. Ecology 60: 1225-1239

Stachowicz JJ, Bruno JF, Duffe JE (2007) Understanding the effects of marine biodiversity on communities and ecosystems. Annual Review of Ecology and Systematics 38: 739-765

Steyaert M, Moodley L, Nadong T, Moens T, Soetaert K, Vincx M (2007) Responses of intertidal nematodes to short-term anoxic events. Journal of Experimental Marine Biology and Ecology 345: 175-184

Steyaert M, Vanaverbeke J, Vanreusel A, Barranguet C, Lucas C, Vincx M (2003) The importance of fine-scale, vertical profiles in characterizing nematode community structure. Estuarine Coastal and Shelf Science 58: 353-366

Strasser M, Reinwald T, Reise K (2001) Differential effects of the severe winter of 1995/96 on the intertidal bivalves Mytilus edulis, Cerastoderma edule and Mya arenaria in the Northern Wadden Sea. Helgoland Marine Research 55: 190-197

Struyf K, Degraer S. (2003) Quick Scan van de gevolgen van het afsluiten van Het Zwin op de ecologie – Herwerkte versie (oorspr. tekst: RIKZ/AB/2003/805x), Unpublished report.

Temmerman S, Bouma TJ, Van de Koppel J, Van der Wal DD, De Vries MB, Herman PMJ (2007) Vegetation causes channel erosion in a tidal landscape. Geology 35: 631–634

Tett P, Gowen R, Mills D, Fernandes T, Gilpin L, Huxham M, Kennington K, Read P, Service M, Wilkinson M, Malcolm S (2007) Defining and detecting undesirable disturbance in the context of marine eutrophication. Marine Pollution Bulletin 55: 282-297

Thistle D (1981) Natural Physical Disturbances and Communities of Marine Soft-Bottoms. Marine Ecology Progress Series 6: 223-228

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews 25: 1-45

Thrush SF, Coco G, Hewitt JE (2008) Complex positive connections between functional groups are revealed by neural network analysis of ecological time series. American Naturalist 171: 669-677

Thrush SF, Dayton PK (2002) Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. Annual Review of Ecology and Systematics 33: 449-473

Thrush SF, Hewitt JE, Funnell GA, Cummings VJ, Ellis J, Schultz D, Talley D, Norkko A (2002) Fishing disturbance and marine biodiversity: role of habitat structure in simple soft-sediment systems. Marine Ecology Progress Series 240: 296-296

Thrush SF, Hewitt JE, Hickey CW, Kelly S (2008) Multiple stressor effects identified from species abundance distributions: Interactions between urban contaminants and species habitat relationships. Journal of Experimental Marine Biology and Ecology 366: 160-168

Thrush SF, Pridmore RD, Hewitt JE, Cummings VJ (1992) Adult infauna as facilitators of colonization on intertidal sandflats. Journal of Experimental Marine Biology and Ecology 159: 253-265

Thrush SF, Whitlatch RB (2001) Recovery dynamics in benthic communities: balancing detail with simplification. In: Reise K (ed.) Ecological studies. Springer-Verlag, Berlin, pp 297-316

Thrush SF, Whitlatch RB, PridmoreRD, Hewitt JE, Cummings VJ, WilkinsonMR (1996) Scale-dependent recolonization: the role of sediment stability in a dynamic sandflat habitat. Ecology 77: 2472–2487

Thrush, S. F., J. E. Hewitt, M. Gibbs, C. Lundquist, Norkko A (2006) Functional role of large organisms in intertidal communities: Community effects and ecosystem function. Ecosystems 9: 1029-1040

Tolhurst TJ, Black KS, Shayler SA, Mather S, Black I, Baker K, Paterson DM (1999) Measuring the in situ erosion shear stress of intertidal sediments with the cohesive strength meter. Estuarine Coastal and Shelf Science 49: 281–294

Troost K, Kamermans P, Wolff WJ (2008) Larviphagy in native bivalves and an introduced oyster. Journal of Sea Research 60: 157-163

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Underwood GJC, Paterson DM (1993) Seasonal-changes in diatom biomass, sediment stability and biogenic stabilization in the Severn Estuary. Journal of the Marine Biological Association of the United Kingdom 73: 871–887

Van Colen C, Montserrat F, Herman PMJ, Vincx M, Ysebaert T, Degraer S (2008) Macrobenthic recovery from hypoxia in an estuarine tidal mudflat. Marine Ecology Progress Series 372: 31–42

Van Colen C, Vincx M, Degraer S (2006) Does medium-term emersion cause a mass extinction of tidal flat macrobenthos? – The case of the Tricolor oil pollution prevention in the Zwin nature reserve. Estuarine Coastal and Shelf Science 68: 343-347

van de Koppel J, Herman PMJ, Thoolen P, Heip CHR (2001) Do alternate stable states occur in natural ecosystems? Evidence from a tidal flat. Ecology 82: 3449–3461

van de Koppel J, van der Wal D, Bakker JP, Herman PMJ (2005) Self-organisation and vegetation collapse in salt marsh ecosystems. American Naturalist 165: E1–E12

Van der Meer J, Beukema JJ, Dekker R (2003) Large spatial variability in lifetime egg production in an intertidal Baltic tellin (Macoma batlhica) population. Helgoland marine research 56: 274-278

van Duren LA, Herman PMJ, Sandee AJJ, Heip CHR (2006) Effects of mussel filtering activity on boundary layer structure. Journal of Sea Research 55: 3–14

Van Ledden M (2003) Sand-mud segregation in estuaries and tidal basins. Communications on Hydraulic and Geotechnical Engineering. Delft University of Technology.

Van Ledden, M., W. G. M. Van Kesteren, and J. C. Winterwerp. 2004. A conceptual framework for the erosion behaviour of sand-mud mixtures. Continental Shelf Research 24: 1-11

van Oevelen D, Soetaert K, Middelburg JJ, Herman PMJ, Moodley L, Hamels I, Moens T, Heip CHR (2006) Carbon flows through a benthic food web: Integrating biomass, isotope and tracer data. Journal of Marine Research 64: 453-482

van Rijn LC (1993) Principles of sediment transport in rivers, estuaries and coastal seas. Aqua Publications, Amsterdam

van Wesenbeeck BK, van de Koppel J, Herman PMJ, Bakker JP, Bouma TJ (2007) Biomechanical warfare in ecology; negative interactions between species by habitat modification. Oikos 116: 742–750

Volkenborn N, Hedtkamp SIC, Van Beusekom JEE, Reise K (2007a) Effects of bioturbation and bioirrigation by lugworms (Arenicola marina) on physical and chemical sediment properties and implications for intertidal habitat succession. Estuarine Coastal and Shelf Science 74: 331-343

Volkenborn N, Polerecky L, Hedtkamp SIC, Van Beusekom JEE, De Beer D (2007b) Bioturbation and bioirrigation extend the open exchange regions in permeable sediments. Limnology and Oceanography 52: 1898-1909

Volkenborn N, Reise K (2007) Effects of Arenicola marina on polychaete functional diversity revealed by large-scale experimental lugworm exclusion. Journal of Sea Research 57: 78-88

Waldbusser GG, Marinelli RL (2006) Macrofaunal modification of porewater advection: role of species function, species interaction, and kinetics. Marine Ecology Progress Series 311: 217-231

Warwick RM, Clarke KR (1993) Comparing the Severity of Disturbance - a Metaanalysis of Marine Macrobenthic Community Data. Marine Ecology Progress Series 92: 221-231

Warwick RM, Goss-Custard JD, Kirby R, George CL, Pope ND, Rowden AA (1991) Static and dynamic environmental factors determining the community structure of estuarine macrobenthos in SW Britain: why is the Seven estuary different? Journal of Applied Ecology 28: 329–345

Wheacroft RA, Olmez I, Pink FX (1994) Particle Bioturbation in Massachusetts Bay - Preliminary-Results Using A New Deliberate Tracer Technique. Journal of Marine Research 52: 1129-1150

Wheatcroft RA, Martin WR (1996) Spatial variation in short-term (Th-234) sediment bioturbation intensity along an organic-carbon gradient. Journal of Marine Research 54: 763-792

Whitlatch RB, Lohrer AM, Thrush SF, Pridmore RD, Hewitt JE, Cummings VJ, Zajac RN (1998) Scale-dependent benthic recolonization dynamics: life stage-based dispersal and demographic consequences. Hydrobiologia 376: 217-226

Whitlatch RB, Zajac RN (1985) Biotic interactions among estuarine infaunal opportunistic species. Marine Ecology Progress Series 21: 299–311

Widbom B, Oviatt CA (1994) The 'World Prodigy oil spill in Narragansett Bay, Rhode Island, acute effects on macrobenthic crustacean populations. Hydrobiologia 291: 115-124

Widdicombe S, Austen MC (1999) Mesocosm investigation into the effects of bioturbation on the diversity and structure of a subtidal macrobenthic community. Marine Ecology Progress Series 189:181-193

Widdicombe S, Austen MC (2001) The interaction between physical disturbance and organic enrichment: An important element in structuring benthic communities. Limnology and Oceanography 46: 1720-1733

Widdows J, Blauw A, Heip CHR, Herman PMJ, Lucas CH, Middelburg JJ, Schmidt S, Brinsley MD, Twisk F, Verbeek H (2004) Role of physical and biological processes in sediment dynamics of a tidal flat in Westerschelde Estuary, SW Netherlands. Marine Ecology Progress Series 274: 41–56

Widdows J, Brinsley MD, Bowley N, Barrett C (1998) A benthic annular flume for in situ measurement of suspension feeding/biodeposition rates and erosion potential of intertidal cohesive sediments. Estuarine Coastal and Shelf Science 46: 27-38

Widdows J, Brinsley MD, Salkeld PN, Lucas CH (2000) Influence of biota on spatial and temporal variation in sediment erodability and material flux on a tidal flat (Westerschelde, The Netherlands). Marine Ecology Progress Series 194: 23–37

Widdows J, Friend PL, Bale AJ, Brinsley M, Pope ND, Thompson CEL (2007) Inter-comparison between five devices for determining erodability of intertidal sediments. Continental Shelf Research 27: 1174–1189

Widdows J, Lucas JS, Brinsley MD, Salkeld PN, Staff FJ (2002) Investigation of the effects of current velocity on mussel feeding and mussel bed stability using an annular flume. Helgoland Marine Research 56: 3-12

Wieczorek SK, Clare AS, Todd CD (1995) Inhibitory and Facilitatory Effects of Microbial Films on Settlement of Balanus Amphitrite Amphitrite Larvae. Marine Ecology Progress Series 119: 221-228

Williams ID, van der Meer J, Dekker R, Beukema JJ, Holmes SP (2004) Exploring interactions among intertidal macrozoobenthos of the Dutch Wadden Sea using population growth models. Journal of Sea Research 52: 307-319

Willows RI., Widdows J, Wood RG (1998) Influence of an infaunal bivalve on the erosion of an intertidal cohesive sediment: A flume and modeling study. Limnology and Oceanography 43: 1332-1343

Winterwerp JC, van Kesteren WGM (2004) Introduction to the physics of cohesive sediment in the marine environment, Vol 56. Elsevier, Amsterdam

Wolanski E. (2007) Estuarine ecohydrology, 1st ed. Elsevier, Amsterdam.

Wolfstein K, de Brouwer JFC, Stal LJ (2002) Biochemical partitioning of photosynthetically fixed carbon by benthic diatoms during short-term incubations at different irradiances. Marine Ecology Progress Series 245: 21–31

Wood R, Widdows J (2002) A model of sediment transport over an intertidal transect, comparing the influences of biological and physical factors. Limnology and Oceanography 47: 848–855

Woodin SA (1978) Refuges, disturbance, and community structure—marine soft-bottom example. Ecology 59: 274–284

Woodin SA, Jackson JBC (1979) Interphyletic competition among marine benthos. American Zoologist 19: 1029–1043

Woodin SA, Lindsay SM, Wethey DS (1995) Process-Specific Recruitment Cues in Marine Sedimentary Systems. Biological Bulletin 189: 49-58

Woodin SA, Marinelli RL, Lincoln DE (1993) Allelochemical Inhibition of Recruitment in a Sedimentary Assemblage. Journal of Chemical Ecology 19: 517-530

Woodin SA, Marinelli RL, Lindsay SM (1998) Process-specific cues for recruitment in sedimentary environments: Geochemical signals? Journal of Marine Research 56: 535-558

Wootton JT (2002) Mecanisms of successional dynamics: consumers and the rise and fall of species dominance. Ecological Research 17: 249-260

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314: 787-790

Ysebaert T (2000) Macrozoobenthos and waterbirds in the estuarine environment: spatio-temporal patterns at different scales. PhD Thesis Antwerp university - Communications of the Institute of Nature Conservation

Ysebaert T, Herman PMJ (2002) Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. Marine Ecology Progress Series 244: 105–124

Ysebaert T, Herman PMJ, Meire P, Craeymeersch J, Verbeek H, Heip CHR (2003) Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary, NW Europe. Estuarine Coastal and Shelf Science 57: 335-355

Ysebaert T, Meire P, Herman PMJ, Verbeek H (2002) Macrobenthic species response surfaces along estuarine gradients: prediction by logistic regression. Marine Ecology Progress Series 225: 79-95

Zajac RN (1991) Population ecology of Polydora ligni (Polychaeta: Spionidae). I. Seasonal variation in population characteristics and reproductive activity. Marine Ecology Progress Series 77: 197-206

Zajac RN, Whitlatch RB, Thrush SF (1998) Recolonization and succession in soft-sediment infaunal communities: the scale of controlling factors. Hydrobiologia 375/376: 227-240

Zardus JD, Nedved BT, Huang Y, Tran C, Hadfield MG (2008) Microbial biofilms facilitate adhesion in biofouling invertebrates. Biological Bulletin 214: 91-98

Zedler JB (2001) Handbook for Restoring Tidal Wetlands. CRC Press LLC, Florida

Zwarts L, Blomert AM (1992) Why knot Calidris Canutus take medium-sized Macoma Balthica when 6 prey species are available. Marine Ecology Progress Series 83: 113-128

SUMMARY – SAMENVATTING

Summary

Estuaries and the adjacent intertidal habitats fulfil several important ecosystem functions (e.g. high productivity, nursery and feeding habitats for epibenthic fishes, crustaceans and birds) and services (e.g. pollution filter, counteracting coastline erosion) (McLusky & Elliott 2004). However, along with the expanding human population, the diversity and intensity of anthropogenic stressors (e.g. dredging, eutrophication, fishing) has increasingly diminished the quality of these ecosystems (Worm *et al.* 2006, Wolanski 2007). Consequently, there is an increasing need for restoration of these habitats in order to regain the essential ecosystem function and service losses that we take for granted. It is now in many cases legally demanded that anthropogenic activities that damage ecosystems require mitigation measures (e.g. the Water Framework Directive, Anon. 2000). As an integral part of management practice, monitoring should occur in order to detect change and to determine whether management is having the desired effect (McLusky & Elliott 2004). The macrobenthos (i.e. bottom fauna commonly defined as organisms retained on a 1 (0.5) mm mesh sized sieve) of estuarine tidal flats recycles nutrients (Lohrer *et al.* 2004), alters sediment transport processes considerably (Solan *et al.* 2008) and represents an important trophic linkage, being a food source for epibenthic crustaceans, fish and birds (Hampel *et al.* 2004) and feeding on benthic algae and bacteria (Herman *et al.* 2000, Van Oevelen *et al.* 2006). Consequently, given its central role in the functioning of the tidal flat ecosystem, monitoring of the macrobenthos should take part in an integrated evaluation of the progress of marine and estuarine restoration projects. In order to enhance our understanding of restoration of tidal flat sediments, this thesis aimed to investigate the role of macrobenthos-environmental interactions in determining benthic settlement, community recovery dynamics (i.e. succession) and benthic community structure, in general.

The macrobenthos community structure along the cross-shore gradient of the Paulinapolder tidal flat was investigated in **Chapter 2** of this Ph.D. manuscript. This

tidal flat, located in the polyhaline part of the Westerschelde estuary (SW the Netherlands), has a semi-diurnal tidal regime with a mean tidal range of 3.9 and the bed material generally consists of mud (average median particle size = 65 μm, average mud content = 51 %). The established macrofauna community is rich, consisting of, on average, 20000 individuals of 10 different species which are characterized by a wide variety of biological traits. Macrobenthos diversity was highest in the high intertidal zone and lowest in the highly hydrodynamically stressed low intertidal. Further, the relative biomass and abundance of suspension feeders peaked in the mid intertidal, whereas the relative biomass and abundance of surface deposit feeders and the relative abundance of species with a lecitotrophic larval development were lowest in this region. Surface deposit feeding species and species with a non-pelagic lecitotrophic development are considered most vulnerable to superficial sediment disturbance. Hence, our results suggest that disturbance of the superficial sediment by the suspension feeder *Cerastoderma edule* (cockle), affects the macrobenthos community structure and diversity considerably. Consequently, inhibitory benthos-physical interactions that affect ecosystem diversity should be incorporated into tidal flat biodiversity-stress models. The sediment disturbance of the cockle was assessed into detail in **Chapter 8**, using two size classes UV-fluorescent sediment fraction tracers (luminophores). Image analysis of the vertical distribution of the different luminophore size classes in the field and in an additional mesocosm experiment showed that cockles selectively remove fine material from the sediment, thereby rendering it less muddy.

Macrobenthos recolonisation patterns after complete mortality resulting from experimentally induced hypoxia were investigated in **Chapter 3, 4** and 7. Hypoxic conditions were created by covering replicated 16 m^2 sediment patches at the Paulinapolder tidal flat with a waterproof polyethylene sheet during a 40-day incubation period. Subsequently, from spring 2005 onwards, the macrobenthos recolonisation was assessed and compared with control sediments during 3 years, focussing on how the temporal scale of macrobenthic recovery interacted with the temporal scale of biotic (e.g. microphytobenthos, meiobenthos) and sedimentological developments (e.g. grain size, bed level, erosion threshold).

Macrobenthic recovery was predominantly determined by juvenile recruitment and the time scales of changes in microphytobenthos and abiotic characteristics of the sediment were largely set by the time scale of macrofauna recovery. During the first 2 years, a Pearson-Rosenberg type of community recovery took place along with the improving bottom water oxygen conditions. After 3 months, spionid polychaetes became highly abundant, followed rapidly by a steep decline. Subsequently, a steep increase in biomass, related to the growth of the long-lived species occurred. Transitions between different succession stages appeared to be related to recruitment of species and the congruous changes in sediment characteristics (oxygenation state of the sediment, bio(de)stabilization, food availability) inhibiting or facilitating early and late macrobenthic colonizers. Such biotic-environmental interactions may have far-reaching consequences for recovery of disturbed tidal flat sediments. For example, **Chapter 7** shows that recovering communities may diverge from control conditions at the long term whenever the impact of species which are affected by biotic-environmental interactions occurring at early recovery stages becomes (more) important. Moreover, benthic-mediated changes in benthic primary production and susceptibility to physical stress were found to significantly affect the recovery of nematode communities from hypoxia (**Chapter 6**) and the growth and production of benthic recruits (**Chapter 5**). Additionally, **Chapter 9** shows that benthic-mediated differences in benthic primary production may also directly affect macrobenthos recovery by affecting the settlement success of macrobenthos larvae.

In summary, this thesis highlights that the macrobenthos plays a critical role in the biotic and abiotic (recovery) dynamics of estuarine intertidal sediments and that macrobenthic recovery in a tidal mudflat habitat should be considered as a dynamic process, related to the natural temporal variation, the life history traits and the bio-engineering capacities of the colonizing species. Consequently, scientific information on these aspects, as discussed in **Chapter 10**, should be addressed to consider and to evaluate habitat degradation and restoration.

Samenvatting

Estuaria en meer bepaald hun aangrenzende slikken zijn zeer productieve habitats, die onder andere functioneren als pollutiefilter en kustlijn erosie verminderen. Bovendien vervullen deze ecosystemen belangrijke functies zoals het voorzien van broed -en voedingsgebied voor vogels, vis en schaaldieren (McLusky & Elliott 2004). Gedurende de laatste 40 jaar heeft de toenemende verscheidenheid en intensiteit aan antropogene verstoringen, zoals overbevissing, eutrofiëring en baggerwerken de kwaliteit van deze ecosystemen aanzienlijk doen afnemen (Worm *et al.* 2006, Wolanski 2007). Bijgevolg is er een toenemende nood aan maatregelen om de functies en diensten van slikken te herstellen. Het macrobenthos, mariene bodemdieren > 1 (0.5) mm, van slikken recycleert nutriënten (Lohrer *et al.* 2004), beïnvloedt het sediment transport aanzienlijk (Solan *et al.* 2008) en is een belangrijke trofische link omdat het zich voedt met benthische algen en bacteriën en tevens voedsel is voor vogels, vis en schaaldieren (Reise 1985). Het macrobenthos vervult dus een belangrijke rol in het functioneren van het slikke-ecosysteem en monitoring van het herstel van het macrobenthos is daarom noodzakelijk om te evalueren of de, vaak wettelijk verplichte, mitigerende maatregelen het gewenste effect veroorzaken (McLusky & Elliott 2004). Om beter te begrijpen hoe dergelijke herstelprocessen in slikken verlopen werd in dit doctoraatsproefschrift het specifieke belang van interacties tussen het macrobenthos en zijn omgeving tijdens het herstel van een slik onderzocht.

Hoofdstuk 2 behandelt de gemeenschapsstructuur van het macrobenthos langs de intergetijdengradient van een zout slik langs het Westerschelde estuarium (Paulinapolder, Nederland). De macrobenthos gemeenschap van het onderzochte slik bestaat gemiddeld uit 20000 individuen per vierkante meter die behoren tot 10 verschillende soorten. De meest diverse macrobenthos gemeenschap werd bereikt in de hoogste intergetijdenzone en de minst diverse in de laagste intergetijdenzone. Het macrobenthos dat zich voedt met micro-algen uit de waterkolom is het meest talrijk in de middelste getijdenzone terwijl soorten die het meest kwetsbaar zijn aan verstoring van de bovenste sedimentlaag, zoals oppervlakte depositie voeders of soorten met een lecitotrofe ontwikkeling, in deze zone net hun laagste relatieve abundantie bereikten. In het onderzochte slik is *Cerastoderma edule* (de kokkel) de belangrijkste soort die zich voedt met micro-algen uit de waterkolom. De verstoring van het sediment door deze soort werd in detail bestudeerd met behulp van fluorescerende sediment partikels in **Hoofdstuk** 8 en toonde aan dat de kokkel de bovenste lagen van het sediment aanzienlijk verstoort door selectief de kleinste sedimentpartikels te verwijderen. De bevindingen uit **Hoofstuk 2** en **8** tonen dus aan dat interacties tussen het macrobenthos en hun omgeving belangrijk zijn voor het bepalen van de structuur en diversiteit van hun gemeenschap.

Herkolonisatie van het macrobenthos na volledige mortaliteit ten gevolge van hypoxie (d.i. zuurstofconcentratie van het bodemwater < 2 mg l^{-1}) werd onderzocht in **Hoofdstuk 3, 4** en 7. Lage zuurstofconcentraties werden bekomen door grote stukken sediment (16 m^2) af te dekken met een waterdichte folie gedurende 40 dagen. Gedurende 3 jaar, startend vanaf de lente van 2005, werd het herstel van het macrobenthos en de invloed van de hertstellende macrobenthos gemeenschap op het sediment opgevolgd. Herstel van het macrobenthos gebeurde voornamelijk door de rekrutering van juveniele organismen. Het tijdsverloop van de veranderingen in benthische micro-algen en het sediment was sterk gerelateerd aan het macrobenthos herstel. Na 3 maanden van herstel domineerden kleine kokerbouwende borstelwormen de macrobenthische gemeenschap en na de ineenstorting van deze opportunistische populaties nam de totale macrobenthos biomassa sterk toe door de groei van langlevende soorten. Tijdens de herstelperiode bleken de veranderingen tussen de verschillende macrobenthosgemeenschappen sterk gerelateerd aan de rekrutering van verschillende macrobenthische soorten en de daarmee verbonden faciliterende of inhiberende invloed op de sedimentkarakteristieken, zoals het zuurstofgehalte, de stabiliteit en het voedselaanbod. Bovendien bleken dergelijke interacties tussen het macrobenthos en hun omgeving ook belangrijke gevolgen te kunnen hebben voor het herstel op lange termijn omdat de rekrutering van een soort bijvoorbeeld in dergelijke mate bevoordeeld kan worden waardoor deze soort de herstellende gemeenschap na verloop van tijd sterk gaat domineren (**Hoofdstuk** 7). Verder werd in de **Hoofdstukken**

5 en **6** aangetoond dat veranderingen in primaire productie en de stabiliteit van het sediment, veroorzaakt door de kolonisatie van het macrobenthos, de groei en productie van het juveniele macrobenthos en het herstel van meiobenthosgemeenschap (mariene bodemdieren < 1 mm en > 32 (38) µm) beïnvloedt. Ook werd, aan de hand van labo-experimenten, aangetoond dat veranderingen in primaire productie de settlement van macrobenthos larven kan beïnvloeden (**Hoofdstuk 9**).

Dit doctoraatsproefschrift toont dus aan dat het macrobenthos een cruciale rol speelt in het biotische en het abiotische herstel van slikken en dat herstel van het macrobenthos (bvb. na hypoxie) beschouwd moet worden als een dynamisch proces waarbij natuurlijke temporele variatie, soortskarakteristieken en de interacties van het macrobenthos met zijn omgeving een belangrijke rol spelen. Wetenschappelijke informatie over deze verschillende aspecten, zoals bediscussieerd in **Hoofdstuk 10**, is daarom cruciaal om de gevolgen van verstoringen en het herstel van slikken te interpreteren en te evalueren.

PUBLICATION LIST

Van Colen C., Vincx M., Degraer S. (2006) Does medium-term emersion cause a mass extinction of tidal flat macrobenthos? - The case of the *Tricolor* oil pollution prevention in the Zwin nature reserve (Belgium and The Netherlands). *Estuarine Coastal and Shelf Science* 68: 343-347

Van Colen C., Montserrat F., Vincx M., Herman P.M.J., Ysebaert T., Degraer S. (2008) Macrobenthic recovery from hypoxia in an estuarine tidal mudflat. *Marine ecology progress series* 372: 31-42

Montserrat F., Van Colen C., Degraer S., Ysebaert T., Herman P.M.J. (2008) Benthic community-mediated sediment dynamics. *Marine ecology progress series* 372: 43-59

Van Colen C., Snoeck F., Struyf K., Vincx M., Degraer S. (in press) Macrobenthic community structure and distribution in the Zwin tidal lagoon (Belgium and the Netherlands). *Journal of the Marine Biological Association of the UK*

Bert W., Manhout J., Van Colen C., Borgonie G., Decraemer W. (in press) Nematode assemblages in a nature reserve with historical pollution. *Belgian Journal of Zoology*

Van Colen C., Montserrat F., Verbist K., Vincx M., Steyaert M., Vanaverbeke J., Herman P.M.J , Degraer S, Ysebaert T. (in press) Tidal flat nematode response to hypoxia and subsequent macrofauna-mediated altered sediment properties. *Marine ecology progress series*

Van Colen C., Lenoir J., De Backer A, Vanelslander B., Vincx M., Degraer S.,Ysebaert T. (accepted with minor revision) Settlement of *Macoma balthica* larvae in response to benthic diatom films. *Marine Biology*

De Backer A., Van Coillie F., Provoost P., Van Colen C., Vincx M., Degraer S. (submitted) The bioturbation effects of *Corophium volutator*: importance of density and biological activity.

Montserrat F., Van Colen C., Provoost P., Ponti M., vanden Meersche K., Ysebaert T., Herman P.M.J. (submitted) Sediment segregation by biodiffusing bivalves.

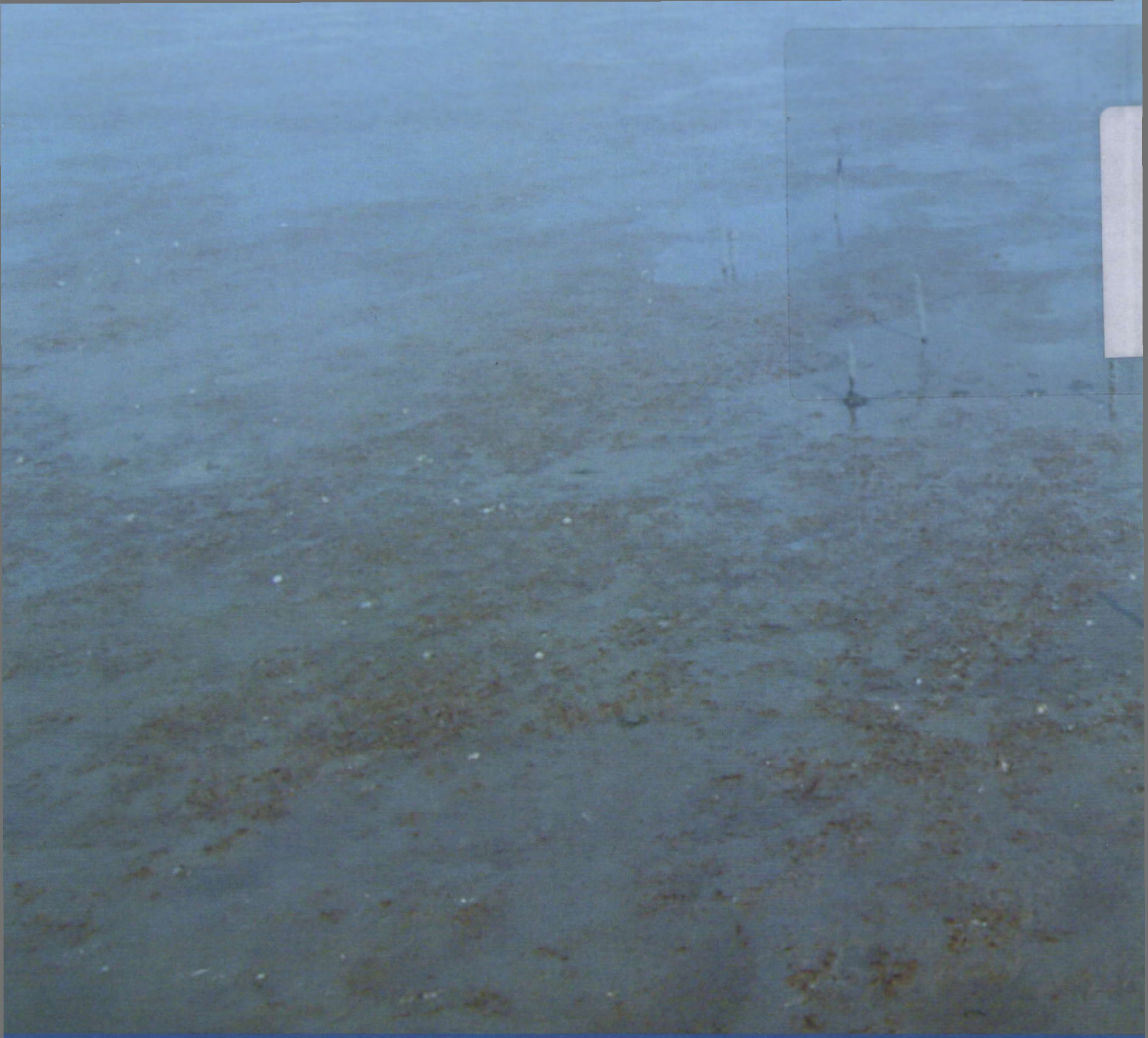
Van Colen C., Montserrat F., Vincx M., Herman P.M.J, Ysebaert T., Degraer S. (in prep.) A long-term study of the macrobenthos recovery following hypoxia-induced mass mortality.

Van Colen C., Meulepas G., De Backer A., Vanderwal D., Vincx M., Degraer S., Ysebaert T. (in prep.) Benthic community structure, diversity and trait displacement across an estuarine tidal flat hydrodynamic stress gradient.

Van Colen C., Montserrat F., Vincx M., Herman P.M.J, Ysebaert T., Degraer S. (in prep.) Benthos-mediated sediment dynamics: implications for growth and production of juvenile recruits.

Rabaut M., Van Colen C, Vincx M., Degraer S. (in prep.) Active selection for *Lanice conchilega* reefs.

De Backer A, Van Colen C., Vincx M., Degraer S. (in prep.) Seasonal sedimentation-accretion processes and the influence of biotic factors.



ISBN 978-90-902416-6-1

9 789090 241661 >