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).

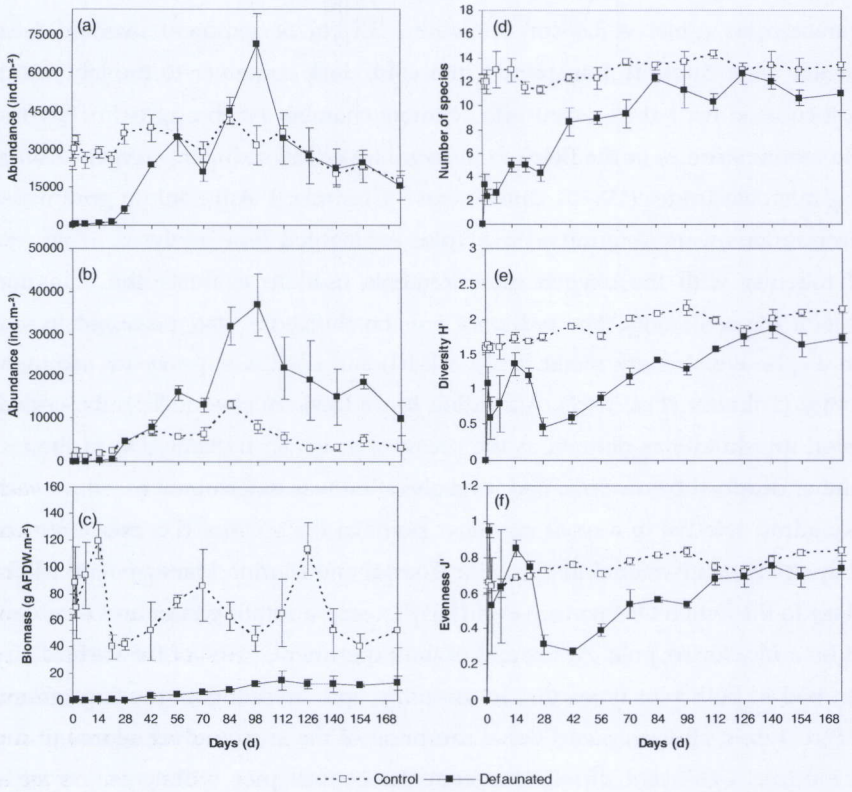

Fig. 1. Temporal variation of (a) total abundance, (b) total juvenile abundance, (c) total biomass, (d) number of species, (e) Shannon-Wiener diversity and (f) Pielou's evenness in defaunated and control plots. Values are means (n = 3) ± 1 SE.

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).

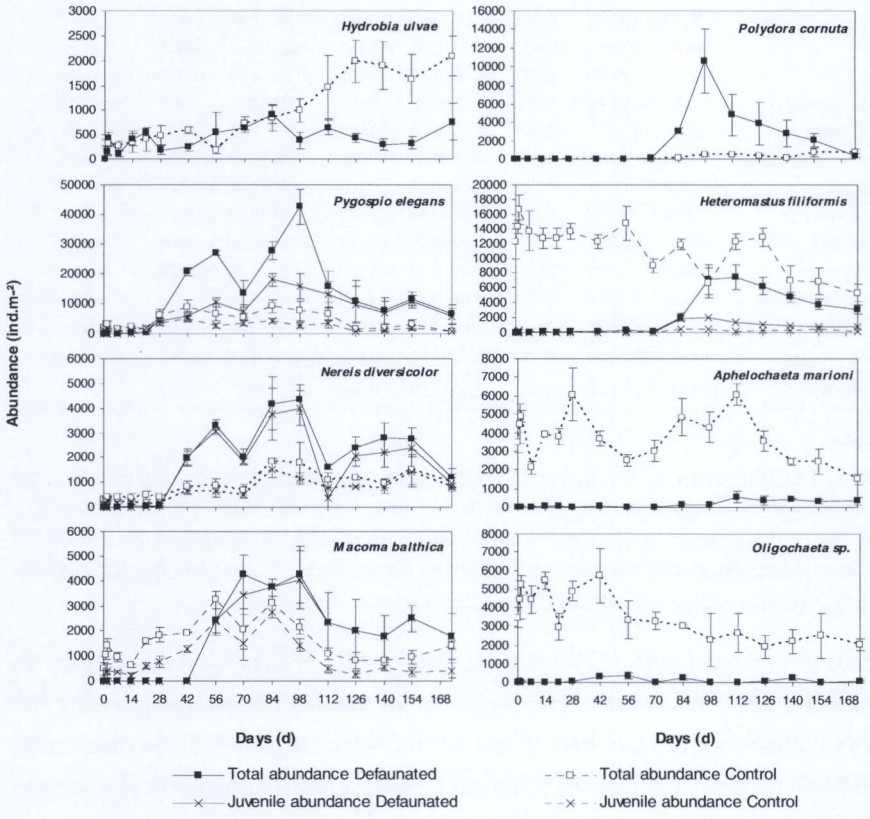

Fig. 2. Hydrobia ulvae, Pygospio elegans, Nereis diversicolor, Macoma balthica, Polydora cornuta, Heteromastus filiformis, Aphelochaeta marioni *and* Oligochaeta. *Temporal variation in mean abundance (n = 3) ± 1 SE in defaunated and control plots.*

	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; ρ = 0.651). Adding other variables resulted in a lower correlation (e.g. mud content, water content, MPB biomass and NH_4^+ concentration: ρ = 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).

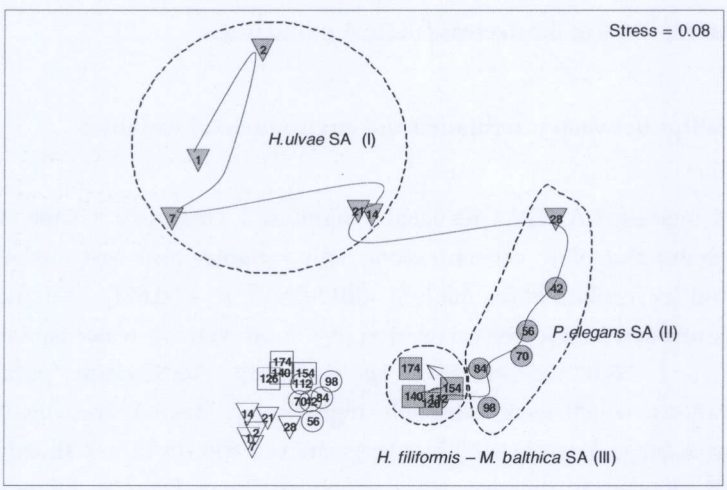

Fig. 3. *Multi-dimensional scaling plots of species assemblages (SA) in defaunated and control plots over time based on Bray-Curtis similarities of standardised square-root abundance species data. Shading indicates treatments (grey = defaunated; white = control) and symbols indicate seasonality (triangles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

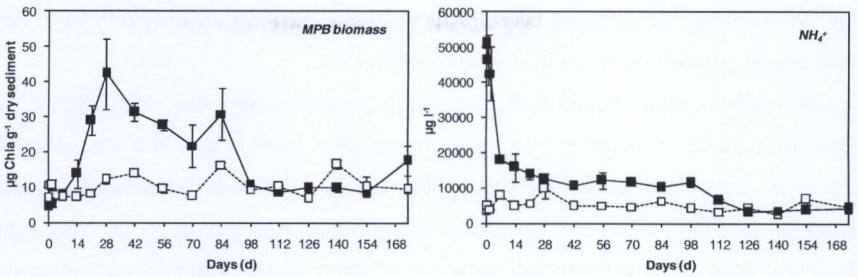

Fig. 4. Temporal variation in (a) microphytobenthos (MPB) biomass (µg chl a g^{-1} dry sediment) and (b) NH_4^+ pore water concentration. Means (n = 3) ± 1 SE in both defaunated and control plots.

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.

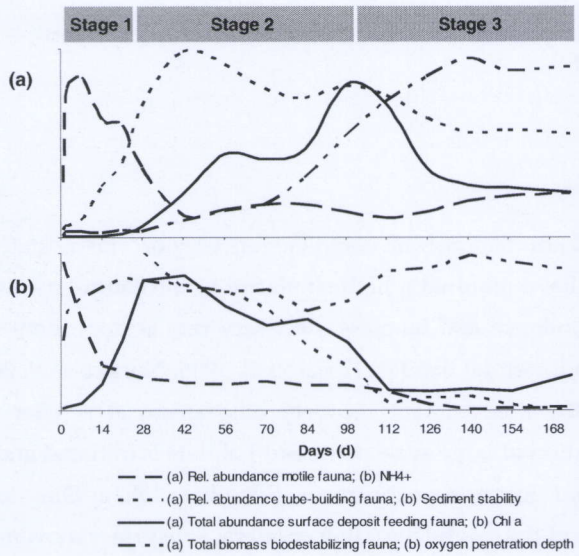

Fig. 5. *(a) Temporal variation of surface deposit feeding population abundance, biomass of biodestabilising populations, relative abundance of tube-building populations and motile infauna populations during succession. (b) Temporal variation of microphytobenthos biomass, sediment stability, oxygen penetration 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*).

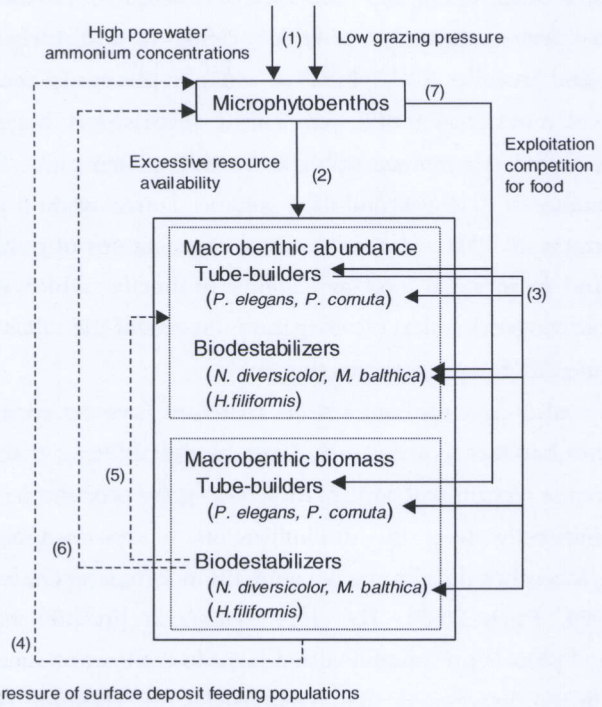

Fig. 6. *Schematic representation of the hypothesised facilitative (solid lines) and inhibitory (dashed lines) interactions during this succession study. Following defaunation resulting from hypoxia, the low grazing pressure of surface deposit feeding populations and the high pore water ammonium concentrations 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).