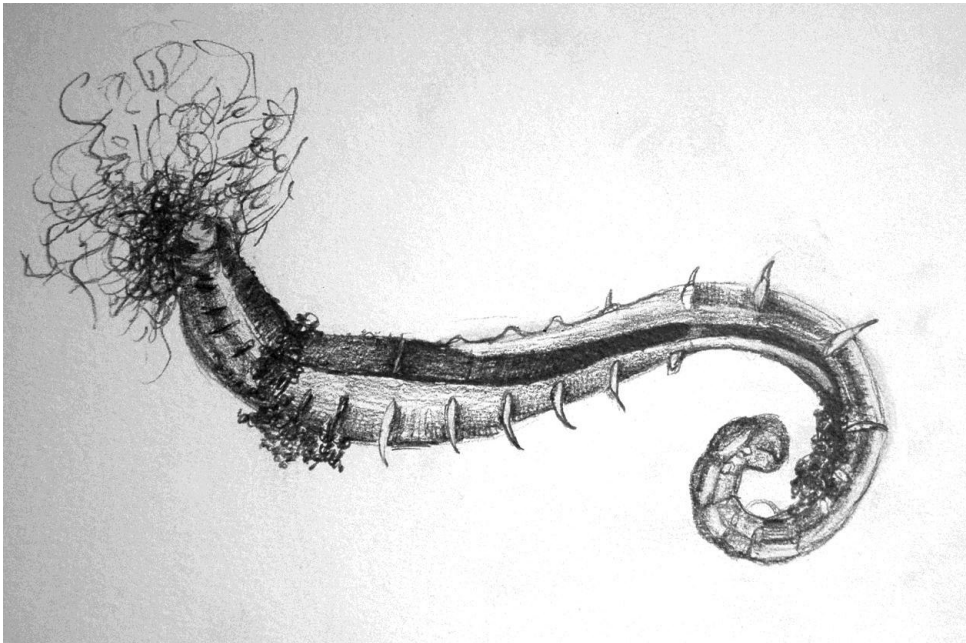


CHAPTER 7

Importance of eco-engineered inshore habitats for juvenile flatfish



Submitted as:

Rabaut, M., Van de Moortel, L., van Dalfsen, J., Vincx, M. and Degraer, S. In prep.
Importance of eco-engineered inshore habitats for juvenile flatfish.
Estuarine Coastal and Shelf Science

PART III – CHAPTER 7

Abstract

Within coastal nurseries, the distribution of juvenile flatfish may depend on small-scale habitat variability. The present study investigates the relation between the distribution of two juvenile flatfish species (*Pleuronectes platessa* and *Limanda limanda*) with two sessile tube dwelling polychaetes that create specific habitats at high densities. Their modulating effects make them classify as ecosystem engineers. *Lanice conchilega* and *Owenia fusiformis*, both frequently occurring in the coastal zones of the North Sea are the studied ecosystem engineers. These two benthic tube worm systems are investigated for their function as 'essential juvenile habitat' (EJH) in two geographical areas (the Belgian part of North Sea and the Dutch part of the Wadden Sea). General responses were identified by comparing relative differences between ecosystem engineered habitats and adjacent bare sand (*i.e.* non-ecosystem engineered) habitats. Results show that both flatfish species select for the ecosystem engineered habitat. This behaviour was further investigated using stomach content analyses. For *P. platessa* occurring in *L. conchilega* habitat, this selection was explained as feeding behaviour. For the habitats created by *O. fusiformis*, no such a relation was found. For *L. limanda* higher densities within the ecosystem engineered habitats cannot be explained by feeding advantage but by the use of this habitat as a shelter. Therefore, higher flatfish densities could be explained by an antipredation behaviour. *Lanice conchilega* aggregations may be more important as feeding area for juvenile flatfish species in comparison with *O. fusiformis* aggregations. The indirect impacts of bottom trawling on benthic tube worm aggregations by reducing the suitability of the areas for juvenile flatfishes are discussed. We conclude that the emergent structures in the flatfish nursery area play an important role in the ecology of the juvenile flatfishes as feeding ground and/or as refuge from predation. These small-scale aspects of nursery grounds can be considered as EJH and merit attention in habitat suitability models as well as in marine conservation.

Key words

Pleuronectes platessa, *Limanda limanda*, *Lanice conchilega*, *Owenia fusiformis*, ecosystem engineers, flatfish, essential juvenile habitat, nursery area, North Sea

Introduction

Larger scale distribution patterns of juvenile flatfish are explained by temperature, depth, salinity and sediment characteristics (Gibson and Robb, 2000, McConnaughey and Smith, 2000, Moles and Norcross, 1995, Rijnsdorp *et al.*, 1990, Riou *et al.*, 2001, Rogers, 1992). The early life stages of many marine fishes migrate from the spawning grounds to the nursery areas and finally to the adult feeding ground (Harden Jones, 1968). For some flatfish species such as *Pleuronectes platessa*, no relation between age class and sediment-defined habitat is found (Gibson and Robb, 2000). For many species, nursery areas are characterized by few predators and high structural complexity (Wennhage, 2002). Moreover, emergent structures in otherwise low-relief benthic habitats, may play an important role in the ecology and population dynamics of some juvenile flatfishes (Pappal, 2006) as structurally complex benthic habitats reduce predation risk (Auster *et al.*, 1997, Joseph *et al.*, 2006, Ryer *et al.*, 2004).

Marine biogenic structures that reach a few centimetres into the water column can have a profound effect on the structure and functioning of marine ecosystems. These systems are heavily used by a variety of taxa, including post-settlement juveniles of commercially important fish species (Watling and Norse, 1998). Furthermore, food availability can be an important factor explaining flatfish distribution in the nursery (Beyst *et al.*, 1999) and can even override abiotic habitat preferences (Phelan *et al.*, 2001). Flatfish nursery areas are generally characterized by higher densities and biomass of macrobenthic species in comparison with the adjacent non-nursery areas (Wouters and Cabral, 2009). It is, however, not always clear whether the preference for structured habitats is because of either the shelter or the feeding advantage, as was experimentally shown by Sogard (1992). Recently it has been recognized that in order to better understand patterns in habitat use of fish within nursery areas, a small-scale approach is desirable (Shucksmith *et al.*, 2006, Vinagre *et al.*, 2009).

The biogenic structures formed by sessile emergent tube dwelling polychaetes are of potential ecological importance because these act as refuge for juvenile fish (Auster *et al.*, 1997, Ryer *et al.*, 2004, Walters and Juanes, 1993). Moreover, these are associated with a diverse assemblage of fauna that provide important prey (Kaiser *et al.*, 1999a, Peattie and Hoare, 1981). The target biogenic structures of this study are those formed by *Lanice conchilega* and by *Owenia fusiformis*. These are sessile, tube dwelling polychaetes occurring at

high density aggregations and well known from the coastal zones of the North Sea (Van Hoey *et al.*, 2008). These considerably increase abundance and diversity of the benthic community through their ecosystem engineering impact.

Lanice conchilega (Polychaeta, Terebellidae) builds a tube to a maximum of 5 mm in diameter and a length up to 65 cm, composed of grains and other solid particles. The top end protrudes above the sediment by 1-4 cm and ends in a fringe of filaments of mucus-bound sand grains, which traps suspended particles. For the macrobenthic community, the habitat modifying capacity of *L. conchilega* has been suggested to lie in the creation and regulation of safe havens for species, in influencing the interactions between local species and in changing the physical environment (Rabaut *et al.*, 2007, Van Hoey *et al.*, 2008). Therefore, the species has been described as an important ecosystem engineer. Its effect on biodiversity has been described extensively (Callaway, 2006, Carey, 1987, Dittmann, 1999, Féral, 1989, Rabaut *et al.*, 2007, Van Hoey, 2006, Zühlke *et al.*, 1998). *Lanice conchilega* has the capacity to double the biodiversity in the richest soft-sedimented macrobenthic habitat of the Belgian part of the North Sea (BPNS) (*i.e.* the *Abra alba* – *Kurtiella bidentata* community *sensu* Van Hoey *et al.* (2004)). Furthermore, several studies describe in detail how *L. conchilega* affects the abiotic environment (Braeckman *et al.*, accepted, Forster and Graf, 1995). Recently, the species has been defined as a true reef builder (Rabaut *et al.*, 2009b).

The tube building polychaete *O. fusiformis* (Polychaeta, Oweniidae) occurs in the same macrobenthic community (*Abra alba* – *Kurtiella bidentata*) as *L. conchilega*. *Owenia fusiformis* is a thin cylindrical, segmented worm, up to 10 cm long, that lives in a tough though flexible tube buried in the sand (Pinedo *et al.*, 2000). The tube abruptly widens from the initial part towards the top end, increasing the external diameter from ca. 1 mm to 4 mm (Noffke *et al.*, 2009) and is composed of sand grains or shell fragments glued together in an overlapping, imbricate fashion. The tube is slightly longer than the worm and its top end may protrude to up to 2 cm from the surface. The species has an adult life span of three to four years (Menard *et al.*, 1989), as opposed to *L. conchilega* which has a lifespan of about one year in Belgian waters (Van Hoey, 2006). The species is widely distributed in coastal regions throughout North-Western Europe, the Mediterranean, the Indian Ocean and the Pacific and occurs in fine to coarse sediments, reaching only high densities in finer sediments (Pinedo *et al.*, 2000,

Somaschini, 1993). In a recent study *O. fusiformis* proves to be an ecosystem engineer that stabilizes sand bank systems (Rabaut *et al.*, in prep.). This has clear consequences for the biotic (benthic) community structure that profits from a stable small scale niche in an otherwise highly dynamic area.

Both ecosystem engineered habitats of *L. conchilega* and *O. fusiformis* have been extensively studied as for their macrobenthic species diversity and ecosystem functioning (Callaway, 2006, Carey, 1987, Dittmann, 1999, Féral, 1989, Rabaut *et al.*, in prep., Rabaut *et al.*, 2007, Somaschini, 1993, Van Hoey *et al.*, 2008, Zühlke *et al.*, 1998). It is, however, not clear whether these hotspots of biodiversity are important for juvenile flatfish dwelling in the nursery area. The areas of interest to this study are shallow coastal zones in the North Sea that serve as nursery grounds (Le Pape *et al.*, 2003a, Miller *et al.*, 1988, van der Veer *et al.*, 2001). Our study focuses on the juveniles of two flatfish species that occur in high densities (*Limanda limanda* and *P. platessa*). The general aim of this study is to investigate whether these juvenile flatfishes select for such small scale engineered habitats and whether this behaviour represents an antipredation and/or a feeding strategy. The two benthic tube worm engineered habitats are investigated in two different geographical areas. We hypothesize that general responses of these juvenile flatfish species to the presence of high densities of the two polychaetes can be identified when relative differences in abundance and stomach content between engineered habitats and adjacent non-engineered habitats are compared. The results are discussed in the framework of 'Effective Juvenile Habitats' (EJHs) (*sensu* Dahlgren *et al.* (2006)), together with the potential vulnerability towards mobile fishing gear.

Methodology

Sampling and sample treatment

One area of investigation was located in the Belgian part of the North Sea (BPNS) (51°21'N, 2°57'E), further referred to as Area 1 (Figure 1). A second area was located in the Dutch part of the Wadden Sea (DPWS) (54°00'N, 7°50'E), further referred to as Area 2 (Figure 1). In both areas two subzones were identified based on differences in density of the ecosystem engineer as quantified from Van Veen grab samples. Densities of the ecosystem engineer were averaged. Densities of > 150 ind m⁻² were considered to be high and qualify as an ecosystem

engineered habitat (Rabaut *et al.*, 2007, Rabaut *et al.*, 2009b), whereas averages < 50 ind m^{-2} were considered as non-engineered habitat. The densities of the ecosystem engineer in the different subzones (ecosystem engineered habitat versus non-ecosystem engineered habitat) were investigated for each area and differences between subzones were tested in generalized linear models with habitat as a fixed factor and the ecosystem engineer densities as the response variable. Both habitats in Area 1 differed significantly ($p < 0.0001$) with average *O. fusiformis* densities of 925 ± 135 SE ind m^{-2} within the ecosystem engineered area and 31 ± 21 SE ind m^{-2} outside. In Area 2, *L. conchilega* densities differed significantly ($p < 0.0001$) with average densities of 242 ± 90 SE ind m^{-2} and 12 ± 1 SE ind m^{-2} respectively. In Area 1, the dominant ecosystem engineer found was *O. fusiformis* (associated *L. conchilega* was on average only 16% of *O. fusiformis* densities) while in Area 2 it was *L. conchilega* (associated *O. fusiformis* was on average only 8% of *L. conchilega* densities). Both geographical areas were sampled for flatfish with a beam trawl, each time with sampling in both subzones (*i.e.* the ecosystem engineered subzone and the non-ecosystem engineered subzone; Figure 1). These subzones represent the fixed effect factor 'habitat'. Area 1 was sampled in October 2008 while Area 2 was sampled in June 2007. Sampling depths were respectively ca. 10 m and ca. 6 m. Flatfish were collected with a beam trawl. The beam trawl used in Area 1 had a width of 4 m and was deployed from the RV 'Zeeleeuw' while the trawl used in Area 2 was a 2 m beam trawl deployed from the RV 'WR76 Herman Simon'. Within each particular geographical area, the two habitats were sampled with exactly the same gear, to make flatfish density comparisons within the area possible (*cf.* sampling period and beam trawl used). Fish were identified, counted and measured on board. In the field, flatfish was anaesthetized in a benzocaine (ethyl amino-4-benzoate) solution to prevent regurgitation of the stomach contents and preserved in an 8% buffered formalin solution. Flatfishes were divided in two age classes: age class 0, containing individuals between 5 and 9 cm and age class 1 containing individuals between 9.1 and 13 cm (Amara *et al.*, 2001, Beyst *et al.*, 1999). For age class 0, no individuals smaller than 5 cm were subjected to analysis as these small individuals are mainly feed on meiobenthos, while larger individuals shift to macrobenthos (Aarnio *et al.*, 1996). In the laboratory, a minimum of ten individuals, per area and in each habitat, of *P. platessa* and *L. limanda* was selected for further stomach analyses (Rijnsdorp and Vingerhoed, 2001). All prey items in the stomachs were counted and identified to the lowest

possible taxonomic level (further referred to as species). The biomass (mg AFDW) of each prey item present in the stomach as well as the flatfish biomass was measured.

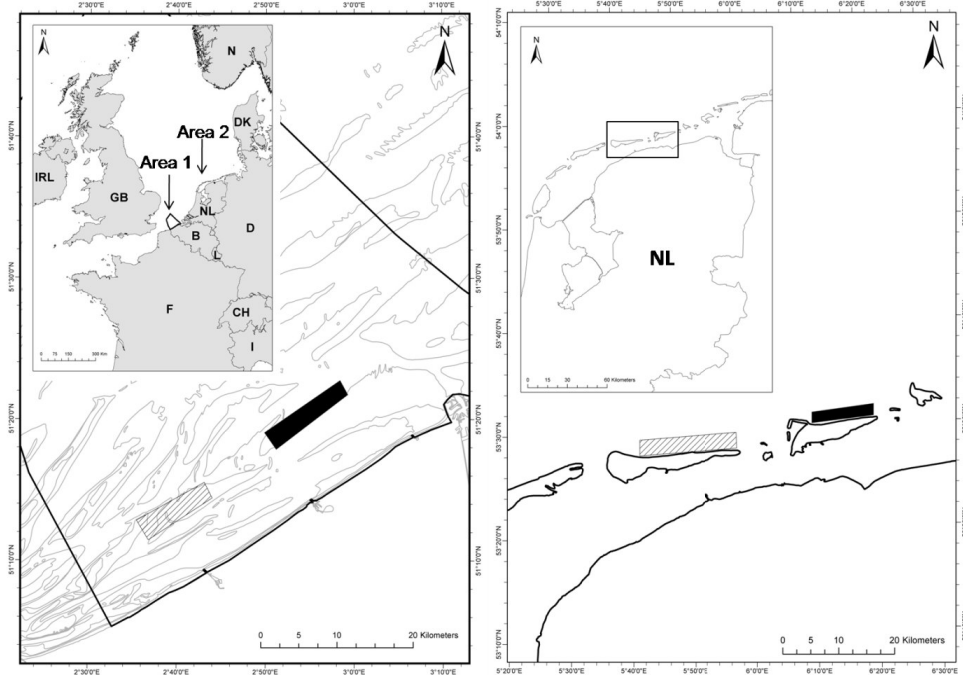


Figure 1. Study areas. Area 1 is located in the Belgian part of the North Sea, while Area 2 is located in the Dutch part of the Wadden Sea (in front of the islands Ameland and Schiermonnikoog). Non-ecosystem engineered subzones are shaded; black zones represent the ecosystem engineered subzones.

Data analysis

The relative importance of prey in the diet (*i.e.* stomach content) was expressed as percent of numerical abundance (N%), weight (G%), and fullness (FI%). N% and G% were calculated on non empty stomachs (Frid *et al.*, 1999), while FI% was calculated on all stomachs (including empty). Feeding activity was evaluated by the vacuity index (V%). These percentages were calculated for groups of samples as follows:

$$N\% = \frac{\text{Number of food items in a stomach}}{\text{Total number of food items in group}} \times 100$$

$$G\% = \frac{\text{Total biomass in a stomach}}{\text{Total biomass in group}} \times 100$$

$$FI\% = \frac{\text{AFDW of stomach content}}{\text{AFDW of fish}} \times 100$$

$$V\% = \frac{\text{Number of empty stomachs in group}}{\text{Total number of analyzed stomachs in group}} \times 100$$

To analyze more in detail prey selectivity, numerical, gravimetric percentages and the frequency of occurrence (FO%) were further calculated per species within each sampling group as:

$$N_i\% = \frac{\text{Number of prey type } i}{\text{Total number of prey items in the stomach}} \times 100$$

$$F_i\% = \frac{\text{AFDW of prey type } i}{\text{Total AFDW of ingested food}} \times 100$$

$$FO_i\% = \frac{\text{Number of stomachs containing prey item } i}{\text{All stomachs}} \times 100$$

Statistical analyses

Flatfish densities

Flatfish densities were compared between habitats within each geographical area, as different beam trawl types may have different catch efficiencies. All densities were recalculated to individuals per 100 m². Differences were calculated using a generalized linear model in which the fixed factors habitat, age and their interaction effect were related to the flatfish densities. As the response variables are count data, the residual error structure was assumed to follow a Poisson distribution with the variance multiplied by an overdispersion

parameter. Because the predictor and the mean response are not linearly related to each other, the relationship was specified by a log link function. The fixed effects structure was reduced in a backward stepwise manner.

Stomach analyses

As the response variables are expressed as relative percentages, the residual error structure was assumed to follow a Binomial distribution. Hence, a generalized linear model was used to evaluate the significance of the fixed effect habitat, age and their interaction effect. The relationship was specified by a logit link function. In a first overall analysis all (non empty) stomachs of Area 1 and Area 2 were analyzed together. To account for the statistical dependence of observations from the same Area, this factor was included as a random effect (using a mixed model). In a second analysis, differences between habitats were analysed for each area and age separately (generalized linear model with habitat as a fixed effect). This analysis could only be performed for groups where stomachs of both habitats were present (*i.e.* for Area 1: both age classes of *L. limanda* and age class 1 of *P. platessa*; for Area 2: both age classes of *P. platessa*). For these groups, a detailed analysis on species composition was performed. Differences in prey species composition were analysed using multivariate ANOSIM and SIMPER analysis (Clarke and Warwick, 2001). When two factors (habitat and age) were analysed together, two way crossed ANOSIM and SIMPER routines were run (Primer v6).

The most important prey species were found on the basis of the frequency of occurrence (FO%) and the relative contribution of individual prey species to the dissimilarity between groups (SIMPER analysis; two way crossed when two factors involved). These analyses were performed both on numerical and gravimetric indices. Differences in diversity of prey species were based on differences in Shannon Wiener index (Hampel *et al.*, 2005), which was tested with a general linear model if there was homogeneity of variances (Levene's test) and if the residual error structure followed a Normal distribution (Shapiro-Wilk's test). If assumptions were not met, non parametric tests on Shannon Wiener index were performed (Wilcoxon).

Results

Flatfish densities

Where both age classes are analysed together, the two flatfish species occur in significant higher densities in the ecosystem engineered subzones (Figure 2; Table 1). Analyzing per age class reveals that for *L. limanda* only age class 0 occurs in significantly higher densities in the *O. fusiformis* area, while for *P. platessa* each age class occurs in higher densities in the *L. conchilega* areas, but no difference was found for age class 1 in the *O. fusiformis* areas (Area 1). No age effects or interaction effects were found (Table 1).

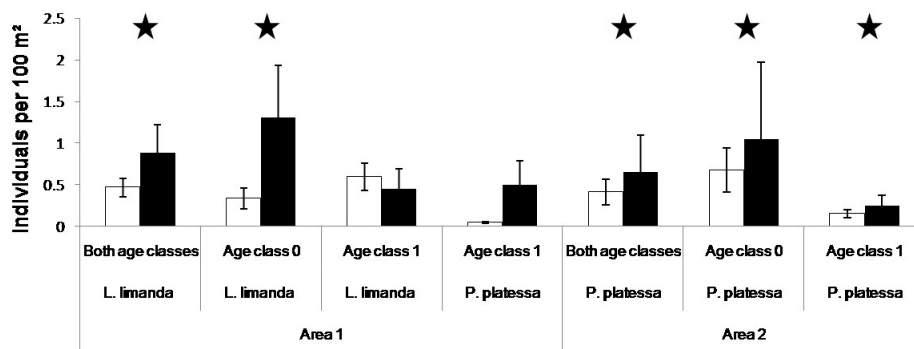


Figure 2. Flatfish densities. Both flatfish species are represented per geographical area and per age class. White bars represent flatfish densities in subzones without the ecosystem engineer; black bars represent flatfish densities in ecosystem engineered subzones. Significant differences between habitats are indicated with a star.

Table 1. Differences in flatfish densities between subzones, age and their interaction effect (p-values). Asterisks indicate significant differences. Differences between subzones represent differences between ecosystem engineered and non-ecosystem engineered habitats (referred to as the 'Habitat' effect).

		<i>Limanda limanda</i>			<i>Pleuronectes platessa</i>		
		Habitat	Habitat x Age	Age	Habitat	Habitat x Age	Age
Area 1	Both age classes	0.0001*	0.5760	0.8368	-	-	-
	Age class 0	0.0002*	-	-	-	-	-
	Age class 1	0.1248	-	-	0.8428	-	-
Area 2	Both age classes	-	-	-	0.0001*	0.9699	0.1949
	Age class 0	-	-	-	0.0024*	-	-
	Age class 1	-	-	-	0.0076*	-	-

Stomach analyses

In the overall analyses per flatfish species (*i.e.* both study areas together), the fullness index (FI%) is significantly higher within ecosystem engineered areas for *P. platessa* of age class 0. For *L. limanda*, significantly lower FI% was found for age class 0 individuals caught within the ecosystem engineered subzone (Figure 3). Numerically, all responses turn out to be significant, with relatively more prey in the stomachs of *L. limanda* caught in the ecosystem engineered subzone and the opposite for *P. platessa*. Gravimetrically, the response is different with significantly more prey biomass in flatfish caught in the non-ecosystem engineered subzone (*L. limanda* age class one and *P. platessa*) (Figure 3, Table 2). Analyzing the same parameters per area, in Area 1, *L. limanda* age class 0 has a significantly higher fullness index and a gravimetrically higher stomach content outside the ecosystem engineered subzone as well, while the age 1 group caught within the ecosystem engineered subzone has numerically a higher stomach content (Figure 3, Table 2). In Area 2, both age classes of *P. platessa* have a higher fullness index and age class 0 has a gravimetrically higher stomach content within the ecosystem engineered subzone. Numerically, age class 0 of this flatfish species has a higher stomach content in the non-ecosystem engineered area. Significance levels are summarized in Tables 2 and 3.

Table 2. Differences (expressed as p-values) in stomach contents between subzones, age and their interaction effect for numerical, gravimetric and fullness indices (independent of geographical area). Asterisks indicate significant differences. Differences between subzones represent differences between ecosystem engineered and non-ecosystem engineered habitats (referred to as the 'Habitat' effect). In the analyses with both age classes, the results on individual factors 'age' and 'habitat' cannot further be interpreted when their interaction is significant (no asterisks).

		<i>L. limanda</i>			<i>P. platessa</i>		
		Habitat	Habitat x Age	Age	Habitat	Habitat x Age	Age
N%	Both age classes	<0.0001	0.0007*	<0.0001	<0.0001	0.0069*	0.134
	Age class 0	<0.0001*	-	-	<0.0001*	-	-
	Age class 1	<0.0001*	-	-	0.0002*	-	-
G%	Both age classes	0.0005*	0.9132	<0.0001*	0.0232	<0.0001*	<0.0001
	Age class 0	0.9995	-	-	0.9991	-	-
	Age class 1	0.0431*	-	-	0.8927	-	-
FI%	Both age classes	0.0019*	0.0892	0.6221	0.0732	<0.0001*	0.4691
	Age class 0	0.0069*	-	-	<0.0001*	-	-
	Age class 1	0.0746	-	-	<0.0001*	-	-

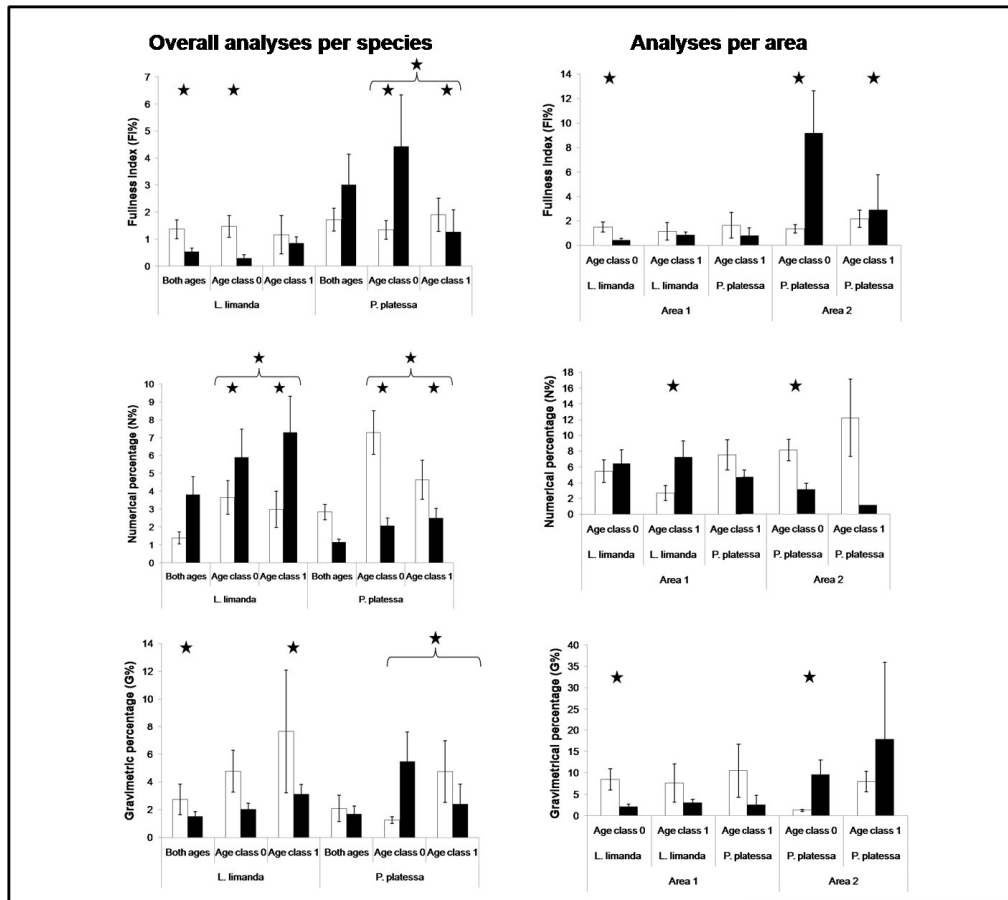


Figure 3. Overall stomach analysis per species (left) and per geographical area and species (right) of the fullness index (FI%), proportional numerical differences (N%) and proportional gravimetric differences (G%). Non-ecosystem engineered subzones are represented with white bars; ecosystem engineered subzones are visualized as black bars. Significant differences between subzones are indicated with a star. Interaction effects (habitat x age) are indicated with a brace.

Table 3. Differences (expressed as p-values) in stomach content per area and per age class. Differences for numerical, gravimetric and fullness indices are shown. Asterisks indicate significant differences.

			N%	G%	FI%
Area 1	<i>L. limanda</i>	Age class 0	0.6473	0.0030*	0.0018*
		Age class 1	0.0311*	0.1382	0.4483
	<i>P. platessa</i>	Age class 1	0.1317	0.0864	0.1723
Area 2	<i>P. platessa</i>	Age class 0	0.0030*	0.0005*	0.0002*
		Age class 1	0.1419	0.2806	0.0089*

The feeding activity was generally lower in ecosystem engineered habitats, as shown by the vacuity index (V%) (Table 4).

Table 4. Feeding activity (V%) within and outside ecosystem engineered subzones.

		Non-ecosystem engineered subzone	Ecosystem engineered subzone
<i>L. limanda</i>	Both age classes	10	29.16
	Age class 0	10	50
	Age class 1	10	0
<i>P. platessa</i>	Both age classes	16.67	24.32
	Age class 0	0	20
	Age class 1	25	29.41

In Area 1, two way crossed ANOSIM and SIMPER results for N% and G% show that there is a habitat effect when gravimetric percentages are used (table 5). Based on the frequency of occurrence (FO%) and on multivariate analyses on G% and N% data, the four most important prey species were selected: Caprellidea spec., *Microprotopus maculatus*, Hydrozoa and *L. conchilega* (Table 6).

Table 5. Prey species community composition. ANOSIM (R-values) and SIMPER dissimilarities between prey communities of flatfish caught inside ecosystem engineered subzones and those from outside and between age classes (two way crossed analyses when both ages are involved). ANOSIM R and correspondent p-values are given for numerical (N%) as well as for gravimetric (G%) percentages. SIMPER dissimilarities indicate differences in prey composition. Significant differences are indicated with an asterisk.

			N%			G%		
			R	p	Dissimilarity	R	p	Dissimilarity
Area 1	<i>L. limanda</i>	age	0.044	0.112	36.71	0.025	0.29	48.46
		habitat	0.009	0.375	36.55	0.362	0.001*	62.94
	<i>P. platessa</i>	habitat	0.15	0.072*	74.2	0.152	0.039*	82.65
Area 2	<i>P. platessa</i>	age	0.351	0.005*	71.46	0.311	0.001*	73.76
		habitat	0.581	0.001*	90.57	0.507	0.001*	94.12

Caprellidea spec. was gravimetrically more abundant as prey in *L. limanda* caught in areas with the ecosystem engineer, while *M. maculatus* was numerically more important as prey in areas without the ecosystem engineer. Hydrozoa seemed to be far more important as prey item in areas without ecosystem engineers. *Lanice conchilega* was found to be numerically more abundant as prey in *L. limanda* caught in the non-ecosystem engineered subzone (Figure 4). Differences linked to age class were found only for *M. maculatus* (%N, p = 0.0012)

and for Hydrozoa (%G, $p = 0.0417$) (Table 6). There were no significant differences in Shannon Wiener index because of habitat ($p = 0.6507$) or age ($p = 0.9627$).

For *P. platessa* in Area 1 (age class 1 only), SIMPER results for N% and G% show a large dissimilarity between prey item assemblage of *P. platessa* caught in areas with and without ecosystem engineer (Table 5). Moreover, ANOSIM results show that there is a significant habitat effect when gravimetric percentages are used (Table 5). The four most important prey species are Caprellidea spec., *Phyllodoce (Anaitides) mucosa*, *L. conchilega* and Anthozoa spec. Caprellidea spec. was numerically more abundant as prey in *P. platessa* caught in the ecosystem engineered subzone, while *L. conchilega* was numerically more important as prey in non-ecosystem engineered subzones. Gravimetrically, only Anthozoa spec. differed significantly between habitats (more Anthozoa in areas without ecosystem engineer) (Figure 4, Table 6). There were no significant differences in Shannon Wiener index because of habitat ($p = 0.1333$).

In Area 2, two way crossed SIMPER results show a large dissimilarity between prey item assemblage of *P. platessa* (both age classes) caught in areas with and without ecosystem engineer (Table 5). Moreover, two way crossed ANOSIM results show that there is a significant habitat and age effect, both numerically and gravimetrically (Table 6). Based on frequency of occurrence (FO%) and on multivariate analyses on G% and N% data, the four most important preys are *Spio* spec., *L. conchilega*, *Pontocrates altamarinus* and *Nephtys hombergii* (Table 6).

Spio spec. is an important prey for *P. platessa* in the non-ecosystem engineered subzone (both numerically and gravimetrically), while *L. conchilega* seems to be an important prey in the ecosystem engineered subzones. No significant effects were found for *P. altamarinus* and *N. hombergii* (Figure 4, Table 6). Differences because of age classes were found for *Spio spec.*, which occurred in significantly higher densities in age class 0 (N%, $p = 0.0266$; G%, $p = 0.002$) and for *L. conchilega*, which was more frequently eaten by *P. platessa* of age class 1 (only gravimetrically, $p = 0.0221$). In terms of diversity of species, no significant differences were found between habitats ($p = 0.2633$) or ages ($p = 0.5094$).

Table 6. Most dominant prey items per species per area. Importance of prey items is based on frequency of occurrence (FO%), species contribution to the dissimilarity of community composition between subzones (SIMPER; two way crossed SIMPER when both ages are involved). Numerical and gravimetric differences of prey species between subzones is indicated with significance level (Asterisks indicate significant differences)

	species	FO%		SIMPER		p level	
		Non-ecosystem engineered subzones	Ecosystem engineered subzones	% contribution (N%)	% contribution (G%)	N%	G%
Area 1	<i>L. limanda</i>	100	87.86	28.03	22.47	0.0834	<0.0001*
	<i>M. maculatus</i>	32.2	42.14	19.24	6.69	0.0215*	0.1246
	Hydrozoa	95	15	-	30.18	-	<0.0001*
	<i>L. conchilega</i>	10.55	0	3.16	4.55	0.037*	0.0993
	<i>P. platessa</i>	14.24	50	18.89	13.15	0.0161*	0.2978
Area 2	<i>L. conchilega</i>	42.85	20	10.17	11.79	0.0323*	0.3885
	Anthozoa sp.	14.28	0	8.77	7.53	0.1016	0.0173*
	<i>Spio</i> sp.	68.75	25	39.51	39.56	<0.0001*	<0.0001*
	<i>L. conchilega</i>	6.25	16.67	33.43	24.75	<0.0001*	<0.0001*
	<i>P. altamarinus</i>	10	11.11	11.21	0.05	0.069	0.9828
	<i>N. hombergii</i>	21.25	0	3.27	2.8	0.2926	0.0501

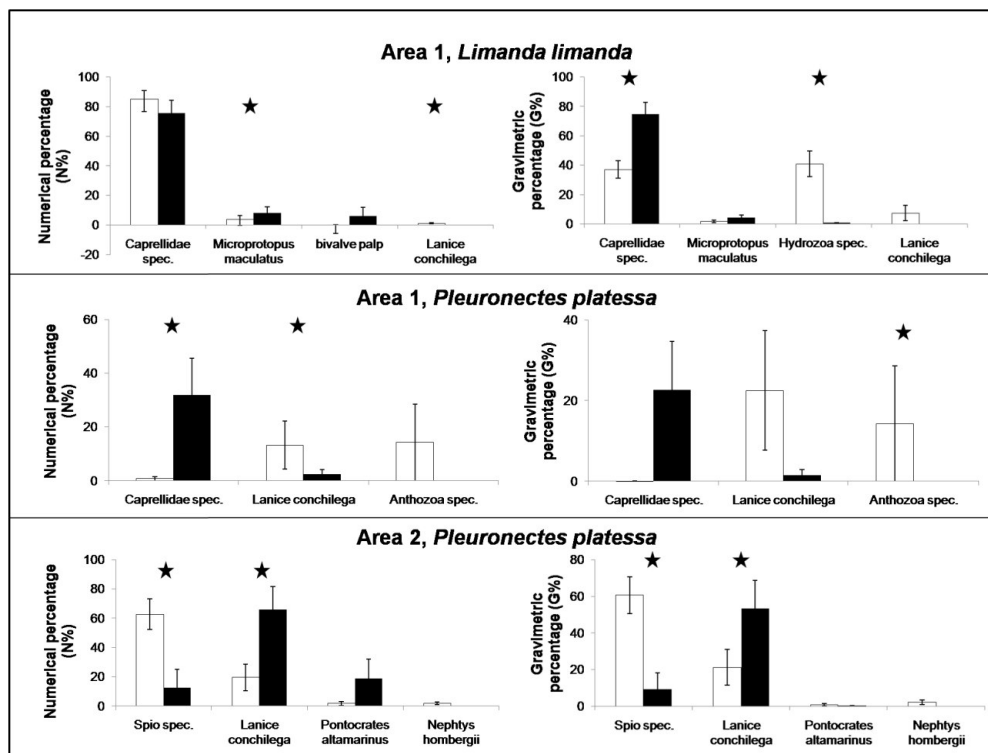


Figure 4. Prey species analyses per area and per flatfish species. Numerical (N%) and gravimetric (G%) differences for the most important prey species. Non-ecosystem engineered subzones are represented with white bars; ecosystem engineered subzones are visualized as black bars. Significant differences between habitats are indicated with a star.

Discussion

Flatfish densities and ecosystem engineered habitats

The ecosystem engineered habitats in this study attract juveniles of the flatfish species *L. limanda* and *P. platessa*. With *O. fusiformis* present as ecosystem engineer, *L. limanda* occurs at higher densities than outside the *O. fusiformis* area. The same pattern is found for *P. platessa* in *L. conchilega* habitat. Shucksmith *et al.* (2006) indicate that *P. platessa* densities correlate specifically with *L. conchilega* and *Chaetopterus* spec. Both *L. limanda* and *P. platessa* have been described before as being tightly associated with assemblages containing structuring epifauna such as hydroids, *Alcyonium digitum*, *A. diaphanum* and *Flustra foliacea* (Kaiser *et al.*, 1999b). However, no significantly higher densities of *P. platessa* age class 1 could be found in

O. fusiformis engineered areas. Flatfish species have a burial behavior to protect themselves against predation and therefore they generally avoid habitats that prevent burial (Phelan *et al.*, 2001), which suggest that the biogenic habitats investigated here provide enough refuge capacity. This is probably related to the sheltering function of the tubes itself. The generally higher densities of *P. platessa* and *L. limanda* suggest that the ecosystem engineered areas contribute with a greater proportion of individuals to the adult population than the mean level contributed by all habitats used by juveniles, regardless of area coverage. Therefore, these biogenic habitats may be considered as an 'Effective Juvenile Habitat (EJH)' (*sensu* Dahlgren *et al.* (2006)). However, the preference of juvenile flatfish for the biogenic habitats does not provide insight in the eventual survival of the juvenile flatfish species (*cf.* discussed below).

Pleuronectes platessa in and outside the biogenic habitat

Whether analysed together or by geographical area (*i.e.* per ecosystem engineer), *P. platessa* has a higher fullness index within the ecosystem engineered areas. This is also reflected in the gravimetric percentage for age class 0 individuals of Area 2. The numerical percentage, however, shows a totally opposite trend. The higher gravimetric response of *P. platessa* in Area 2 is totally attributed to the predation on the ecosystem engineer concerned, *L. conchilega*. *Lanice conchilega* has previously been described as the predominant prey species of age class 0 *P. platessa* (Amara *et al.*, 2001). The higher number of prey items in areas without the ecosystem engineer was fully attributed to *Spio spec.* in Area 2. It seems that in the absence of the ecosystem engineer *L. conchilega*, *P. platessa* turns to *Spio spec.* as main prey item, which is an important item both numerically and gravimetrically. *Spio spec.* are described as positively associated with *L. conchilega* (Rabaut *et al.*, 2007), but it appears that *P. platessa* feed less on it when *L. conchilega* is available as prey. Although *Pontocrates altamarinus* is known to be negatively associated with *L. conchilega* (Rabaut *et al.*, 2007), it occurs more frequently as prey in flatfish caught in *L. conchilega* aggregations. In another study, *P. platessa* was found to be less vulnerable to predation in areas with filamentous green macroalgae in comparison with bare sand (Wennhage, 2002), suggesting that the biogenic habitats may be used as a refuge as well.

The *L. conchilega* aggregations in this study provide an important food source and relate to flatfish density. This might have consequences for growth rate as growth rate of *P. platessa* age class 0 was found to be influenced by food abundance and quality (van der Veer and Witte, 1993). These authors suggested that the differences in growth rate do not necessarily reflect competition for food, but might reflect differences in food composition and availability (searching and handling time). This particular feeding behaviour plays an important part in determining the distribution of flatfishes, though it remains unknown on which basis flatfishes select and subsequently maintain their position in their juvenile habitat (Gibson, 1997).

For *P. platessa* age class 1 of Area 1, there were no overall significant differences found, though there is an observation towards higher prey consumption in areas without the ecosystem engineer, *O. fusiformis*. Numerically, *L. conchilega* seems to be an important item outside the *O. fusiformis* engineered habitat while Caprellidea spec. were both numerically and gravimetrically much more important within the ecosystem engineered subzones. Although *L. conchilega* has been described as highly associated with *O. fusiformis* (Rabaut *et al.*, in prep.), it was not found to be important as prey item. This might be explained by the tube characteristics of this species. Hence, it is possible that juvenile *P. platessa* for reasons of food availability do select for biogenic structures created by *L. conchilega*, but not by *O. fusiformis*. On the contrary, the importance of Caprellidea spec. as prey is directly related to the presence of the *O. fusiformis* engineered habitat. These amphipod species have been observed to perform a clinging behaviour in which they attach to small-scale structures with adapted grasping appendages (Aikins and Kikuchi, 2001, Guerra-Garcia *et al.*, 2002). Therefore, they are probably easy to catch in biogenically structured habitats where there is plenty of structures to attach to. The importance of Anthozoa spec. for *P. platessa* is not very clear as the differences found are attributed to a high abundance of Anthozoan individuals in just one *P. platessa* individual.

Limanda limanda in and outside the biogenic habitat

Juveniles of *L. limanda* have a lower fullness index of the stomach when caught inside the ecosystem engineered habitats and a higher relative prey biomass outside the ecosystem

engineered areas. The number of species in the stomach shows the inverse trend. Differences in food were mostly attributed Caprellidea spec. (higher prey biomass in ecosystem engineered subzone) and Hydrozoa spec. (higher prey biomass in non-ecosystem engineered subzone). *Microprotopus maculatus* is an important prey both inside and outside ecosystem engineered habitats, but was preyed on significantly more in the ecosystem engineered habitat. Amara *et al.* (2001) found polychaetes, hydrozoa and amphipods to be the most important prey items for *L. limanda*. They mention that in both *P. platessa* and *L. limanda* specimens, *L. conchilega* was consumed in high proportions.

Ecosystem engineer and juvenile flatfish

Overall, juveniles of both flatfish species select for ecosystem engineered habitats. This attraction can be partly explained by prey availability, which is certainly the case for *P. platessa* feeding in *L. conchilega* habitat. It has been suggested that prey availability might be the driving force for habitat selection of *P. platessa* within sandy habitats (Shucksmith *et al.*, 2006). The composition and quantity of juvenile *P. platessa* gut contents varies over a small spatial scale (meters) (De Raedemaeker *et al.*, pers. comm.), while food availability in the nursery areas was not found to be related to *P. platessa* densities (Pihl and Vanderveer, 1992). The intrinsic patchiness of *L. conchilega* reefs and the habitats formed by *O. fusiformis* (Rabaut *et al.*, accepted, Rabaut *et al.*, 2009b) is probably a key characteristic of these habitats as differences in nursery grounds could be a reflection of the spatial heterogeneity (Wouters and Cabral, 2009). For the habitats created by *O. fusiformis*, no such a relation could be found with *P. platessa*. The significantly higher densities of *L. limanda* in the *O. fusiformis* ecosystem engineered area were not explained by feeding advantage. The juveniles of this flatfish species might therefore enjoy other advantages in selecting the ecosystem engineered areas as preferred juvenile habitat. We hypothesize that juveniles of this flatfish species use the habitat as a shelter (refuge for predation) and prefer to feed outside these areas. Burying in sand provides only a partial refuge from predation (Ansell and Gibson, 1993) and the ecosystem engineering habitat may contribute to a reduced predation pressure. In this study, indications are found which suggest that *L. conchilega* aggregations are more important as feeding area for juvenile flatfish species than just providing shelter. However, as no feeding *L. limanda* juveniles have been caught in the *L. conchilega* habitat, it is not sure whether this

flatfish species would profit from *L. conchilega* aggregations as food source. Surprisingly, the feeding activity of both flatfish species seems lower within the ecosystem engineered habitat, an effect that is more pronounced for *L. limanda* than for *P. platessa* which suggests there are probably multiple functions of the biogenic habitats.

Pleuronectes platessa generally prefers to consume common species and *L. limanda* utilizes any food source available (Lockwood, 1984, Wyche and Shackley, 1986). Therefore, we hypothesize in this study that the selectivity of juvenile flatfish to feed within biogenically created habitats is a result of their generally opportunistic feeding behaviour (Beyst *et al.*, 1999, Dolbeth *et al.*, 2008, Holmes and Gibson, 1983). Increasing abundances of relatively easy-to-catch prey attract these opportunistic feeders.

The present study demonstrates that biotic factors such as biogenically created habitats play an important role in juvenile flatfish distribution, being either a food source or providing shelter. These responses to biogenically created habitats only seem to occur for juveniles as in a study of different adult flatfish species (including *P. platessa*), no clear association was found between flatfish abundance, structuring epifauna, and prey availability (Hinz *et al.*, 2006).

Vulnerability towards beam trawling

Pleuronectes platessa and *L. limanda* are both commercial flatfishes that are exploited with bottom-fishing gear. These fisheries do not only have a direct impact on the adult flatfish stocks, but have also indirect effects. Bottom trawling can pose a significant threat to the integrity of complex benthic habitats, particularly those formed by sessile emergent fauna (Collie *et al.*, 1997, Kaiser *et al.*, 2000b, Rabaut *et al.*, 2008, Thrush *et al.*, 1998, Watling and Norse, 1998). For *L. conchilega* reefs, it has been demonstrated that the system degrades after one disturbance through the impact on the closely associated species (Gamarra, 2008, Rabaut *et al.*, 2008), while the whole reef system will collapse under continued high frequency disturbances (Rabaut *et al.*, *subm.*). The finding that bottom trawling may have a positive effect through an increase of small, soft-bodied organisms (as food for juvenile *P. platessa*) (Hiddink *et al.*, 2008b), does not hold for the ecosystem engineered habitats of this study, as it is the small bodied associated fauna that is removed by trawlers (Rabaut *et al.*, 2008). Thus,

bottom trawling activities are able to reduce the suitability of the area as feeding and shelter zones to species of commercial importance (Sainsbury, 1987; Kaiser *et al.*, 1999).

The inclusion of the abundance of benthic fauna in habitat suitability models has been proven to be important in distribution predictions (Le Pape *et al.*, 2007, Nicolas *et al.*, 2007) and Ryer *et al.* (2004) suggest to incorporate considerations of emergent structures of low-relief benthic habitats that impart structural complexity into models for flatfish habitat-suitability. The important conservation stake of these biogenic systems has recently been advocated because of their particular functional value (Godet *et al.*, 2008), while it has been suggested that protection is possible under the EU Habitats Directive as habitat type ‘reef’ (Rabaut *et al.*, 2009b). Janssen *et al.* (2008) propose to include the surf zone in conservation management strategies, as these zones proved to be highly diverse, contains dense patches of *L. conchilega* and sustain juvenile flatfish populations. The availability of mostly less preferred habitat may decrease survival rates through increased losses to predation (Ryer *et al.*, 2004) or to suboptimal feeding. Therefore, a limitation in the extent of these emergent structures as nursery grounds could create a bottleneck in recruitment and determine the overall population size (Gibson, 1994) as mortality of late-larval and early-juvenile stages may affect year-class strength (Myers and Cadigan, 1993, van der Veer *et al.*, 1990). Competition for nursery grounds (or EJH) may therefore represent an important factor affecting the relative abundance of flatfish (Rijnsdorp *et al.*, 1990). The present study shows that small scale variability can be induced by biological factors and play indeed a significant role in the distribution of juveniles of *L. limanda* and *P. platessa*.

Conclusion

The ecosystem engineered habitats created by *O. fusiformis* and *L. conchilega* are found to be related to higher densities of juveniles of both the flatfish species *P. platessa* and *L. limanda*. These emergent structures in the flatfish nursery area are assumed to play an important role in the ecology of the juvenile flatfishes. The ecological role is explained by the provision of a food source and of shelter as refuge from predation. The ecosystem engineering shelter effect seems to be of more importance in comparison with the trophic interaction effect. The small-scale aspects of larger nursery grounds can be considered as EJH and merit attention in habitat suitability models as well as in marine conservation measures. The study confirms

the particular functional value of these systems. Bottom trawling has been described to affect benthic tube worm aggregations and therefore the indirect impacts on the commercial flatfish stock need further study.

Acknowledgements

We would like to thank the master and crew of both the RV 'Herman Simon' and the RV 'Zeeleeuw' for their patient and skilful help during sampling campaigns. We are very grateful for the assistance during AFDW measurements by D. Schram. We further thank L. Hiele for the valuable linguistic comments. This research was carried out within the Ghent University *BBSea* project (contract no. GOA 01G00705; <http://biology.ugent.be/bbsea>) and within the WESTBANKS project (understanding benthic, pelagic and air-borne ecosystem interactions in shallow coastal seas; <http://www.vliz.be/projects/westbanks>) which is supported by the Belgian Science Policy (BELSPO; contract no. SD/BN/01). The authors further acknowledge the support by the MarBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning' (<http://www.marbef.org>) which is funded by the Sustainable Development, Global Change and Ecosystems Programme of the European Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446). The first author acknowledges a fellowship from the Research Foundation – Flanders (*FWO-Vlaanderen*), Belgium.