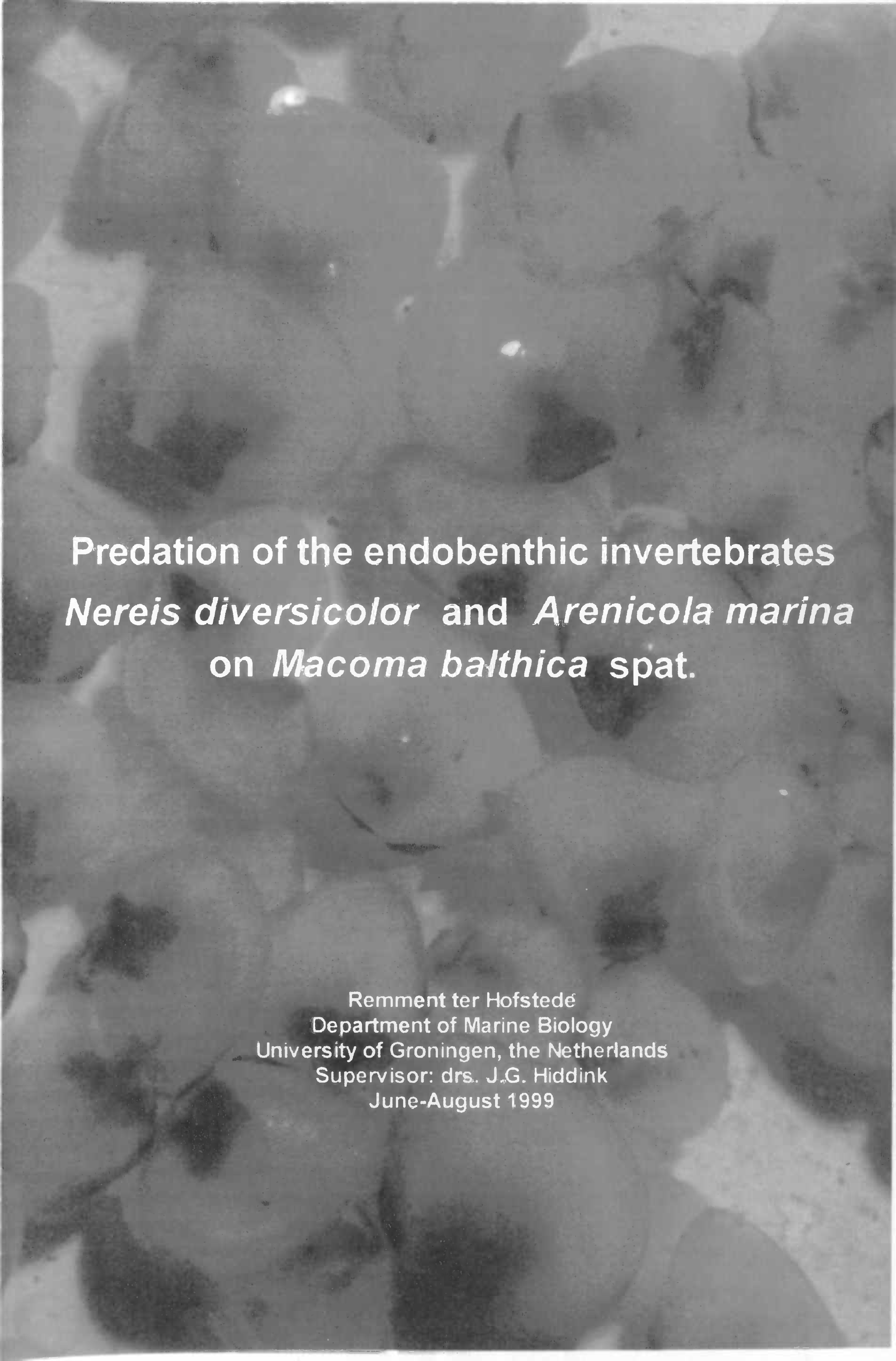

Predation of the endobenthic invertebrates *Nereis diversicolor* and *Arenicola marina* on *Macoma balthica* spat.

Remment ter Hofstede
Department of Marine Biology
University of Groningen, the Netherlands
Supervisor: drs. J.G. Hiddink
June-August 1999

Predation of the endobenthic invertebrates *Nereis diversicolor* (O.F.M.) and *Arenicola marina* (L.) on *Macoma balthica* (L.) spat.

Remment ter Hofstede
Department of Marine Biology
University of Groningen, the Netherlands
Supervisor: Drs. J.G. Hiddink
June-August 1999

Abstract

The tellinid bivalve *Macoma balthica* (L.) (Mollusca, Bivalvia) is a common and widespread macrobenthic species on the tidal flats of the Wadden Sea. In May, the barely settled juvenile *M. balthica* migrate to higher places on the tidal flats, so called nurseries. The juveniles leave these nurseries again when they grown up to 2-8 mm, at the onset of winter and return to the lower parts of the tidal flats where they stay the rest of their live. It is assumed that the choice of juvenile *M. balthica* to use nurseries is mainly determined by the difference in predation pressure at different tidal levels. Juveniles avoid the predation of aquatic epibenthos such as shrimp, crabs and flatfish on the lower tidal flats by migrating to nurseries. Adult *M. balthica* avoid the predation of birds by returning to the lower parts of the tidal flats.

This study showed that carnivorous endobenthic polychaetes *N. diversicolor* and *A. marina* also feed on *M. balthica* spat. Juvenile *M. balthica* has been recovered from both predators that were caught in the field after analyses of digestive tract contents. Also predation experiments in the laboratory have pointed out *N. diversicolor* and *A. marina* as endobenthic predators of juvenile *M. balthica.* A significant reduction of bivalves has been demonstrated in the predation experiments with both *N. diversicolor* and *A. marina* and also some of the consumed *M. balthica* have been retrieved in the predators after dissection.

Estimation of the mortality of *M. balthica* caused by these predators shows both *N. diversicolor* and *A. marina* to be of large impact on *M. balthica* population size.

Contents

Introduction

The tellinid bivalve *Macoma balthica* (L.) (Mollusca, Bivalvia) (see figure 1) is one of the most common and widespread macrobenthic species of the Wadden Sea, both on the tidal flats and in subtidal areas (Beukema, 1976; Dekker, 1989). *M. balthica* spends its adult and juvenile life on different parts of the tidal flats (Beukema, 1993). The adults live at intermediate tidal levels. Adult *M. balthica* spawns in spring. At the end of May, the pelagic eggs have grown into post-larvae with a size < 200 μm and initially settle on the low tidal flats (Armonies & Hellwig-Armonies, 1992; Günther, 1991). Subsequently, these juvenile bivalves (spat, 0-group specimens) re-suspend into the water column and migrate to higher places on the tidal flats, so called nurseries (Armonies & Hellwig-Armonies, 1992). The juveniles leave these nurseries again when they have reached a size up to 2-8 mm, at the onset of winter and return to the lower parts of the tidal flats (Günther, 1991; Beukema, 1993).
During both migration phases, the animals use a thin long mucus thread that increases the hydrodynamic drag, in order to facilitate the lift and transport through the water column by tidal currents (Armonies, 1994; Beukema & De Vlas, 1989).

The choice of nursery use by the 0-group specimens of *M. balthica* results from a trade-off between the advantages and the disadvantages. The migration itself to and out of nurseries through the water column is dangerous, because the animals are exposed to pelagic predators, and they may end up in unfavourable places: too high in the intertidal zone, or in deep channels. However, there has to be a reason for the juvenile *M. balthica* to migrate to and from the nurseries.
First of all, it is assumed that physical disturbance due to wave action and tidal currents is less in these nurseries (Beukema, 1993) and feeding conditions are better than at lower tidal levels (Armonies & Hellwig-Armonies, 1992).
Furthermore, the predation pressure at different tidal levels is considered a factor that determines the choice tot use nurseries. Nurseries are located high on the tidal flat in the intertidal zone and therefore are assumed to offer protection at low tide against predation by aquatic epibenthic organisms such as the shore crab (*Carcinus maenas*), the brown shrimp (*Crangon crangon*), and small fish like juveniles from goby (*Pomatoschistus sp.*), flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) (Beukema, 1992; Günther, 1990; Van der Veer, 1998). All these predator species inhabit the tidal flats of the Wadden Sea in large quantities and the diet of their juveniles is known to include *M. balthica* (Aarnio *et al.*, 1996; Jensen & Jensen; 1985; Pihl, 1985).
On the other hand, bivalves are much longer available to bird predation at these nurseries. Research on knots (*Calidris canutus*) (Piersma *et al.*, 1993) and oystercatchers (*Haematopus ostralegus*) (Hulscher, 1981), both abundant bird species on the tidal flats of the Wadden Sea, has shown that only adult *M. balthica* of size >10-mm is eaten by these predators. Therefore, the chance that the 0-group specimens are being eaten is rather small. This explains the difference in distribution on the tidal flats between juvenile and adult *M. balthica*. The juveniles avoid the predation of aquatic epibenthos by the use of nurseries. Adult *M. balthica* avoid the predation of birds by returning to the lower parts of the tidal flats.

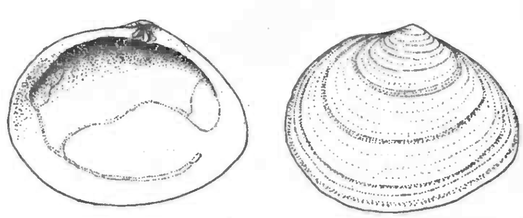

Figure 1: View on the inside (left) and outside (right)of the shell of M. balthica (from: Hayward & Ryland, 1995).

Predation by epibenthic organisms is a key factor in determining recruitment of *M. balthica* spat (Van der Veer *et al.*, 1998). In addition, carnivorous endobenthos on the tidal flats may also influence juvenile *M. balthica* recruitment. First, *M. balthica* spat has a relative small size, they belong to the meiofauna (200-1000 µm), and therefore are easy to swallow and consume by the endobenthic predators. Furthermore, these predators are distributed high on the tidal flats, so nurseries offer no protection for juvenile *M. balthica*. Even if the actual intake of juvenile *M. balthica* by endobenthic predators is low, the effect can be serious due to the high population density of these predators.

Reise (1979) found that in the Wadden Sea permanent meiofauna (Nematoda, Turbellaria, Ostracoda, Copepoda) is preyed heavily by some macrobenthic species, amongst which the endobenthic polychaete *Nereis diversicolor* (O.F.Müller) (see figure 2). This omnivorous ragworm is also known to prey on cockle spat (Reise, 1979) and pieces of mussel meat (Riisgård, 1991). Experiments by Lucas & Bertru (1997) show that *N. diversicolor* is able to degrade bacteria in its digestive tract, resulting in a high level of sediment intake, which may accidentally contain *M. balthica* spat. This ragworm can occur in very dense populations; densities more than 200 adults per m^2 have been observed high on the tidal flats in March (Dekker & De Bruin, 1998). This indicates that the predation pressure of *N. diversicolor* on *M. balthica* spat may certainly be of significance. A striking negative spatial correlation between a *N. diversicolor* population and meiofauna (Nematoda, Copepoda) has been described by Rees (1940).

Another polychaete that can influence the 0-group *M. balthica* densities, due to its numerous presence on the tidal flats, is the lugworm *Arenicola marina* (L.) (see figure 2). Population densities of more than 25 individuals per m^2 have been found throughout the year (Dekker & De Bruin, 1998). *A. marina* lives almost everywhere on the tidal flats of the Dutch Wadden Sea and accounts for about 20% of the benthic biomass (Beukema, 1976). They inhabit characteristically U-shaped burrows and have a substantial impact on the sediment by reworking it. Large volumes of sediment are ingested and notably bacteria and diatoms, but also meiofauna are digested. The undigested remains are subsequently deposited as faecal casts on the surface (Rijken, 1979; Retraubun *et al.*,1996).
Reise (1979) enclosed *A. marina*, resulting in a dramatic decrease of the meiofauna nematode abundance, relative to a control site where lugworms were excluded. This decrease was ascribed to physical factors and food limitations via habitat conditioning by these large sediment swallowers. Other field experiments involving manipulation of lugworm abundance by Flach (1992) showed that the presence of lugworms in densities of about 30 or more individuals per m^2 has a significantly negative effect on the abundance of *M. balthica* of spat size.

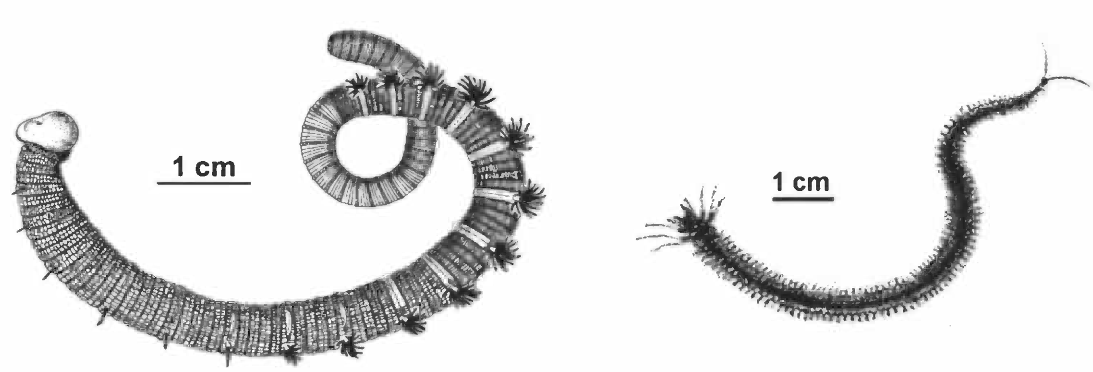

Figure 2: Habitus of A.marina (left) and N. diversicolor (right; from Barrett & Yonge, 1958).

These findings suggest that both *N. diversicolor* and *A. marina* may affect the *M. balthica* population through their influence on the 0-group bivalves. If *N. diversicolor* and *A. marina* actually appear to prey on the juveniles, influence of these predators on *M.balthica* spat is confirmed. When the predation appears to be of serious significance, the reason for migration by *M. balthica* spat to nurseries in order to avoid a high predation pressure will be invalidated.

The aim of this study is to show that both *N. diversicolor* and *A. marina* do affect the *M. balthica* population size through predation on the spat. This hypothesis will be tested by analysing the content of the digestive tracts of *N. diversicolor* and *A. marina* from the field, as well as by predation experiments in the laboratory. The significance of predation by *N. diversicolor* and *A. marina* on *M. balthica* spat will be estimated by determining the mortality of *M. balthica* caused by these two predators.

Materials and Methods

Study site

All animals were collected during low tide on the tidal flats in the eastern Dutch Wadden Sea near Noordpolderzijl (see figure 1). The invertebrate macro-fauna of the intertidal zone contained many of the elements of the classical *Macoma* community (Thorson, 1957). *Macoma balthica, Nereis diversicolor, Hydrobia ulvae, Retusa obtusa, Cerastoderma edule, Nephtys hombergii, Mya arenaria* and *Arenicola marina* were all present in the middle and upper shores.

Four sampling sites were located at different heights along a transect that was orientated perpendicular to the coastline (see figure 1).

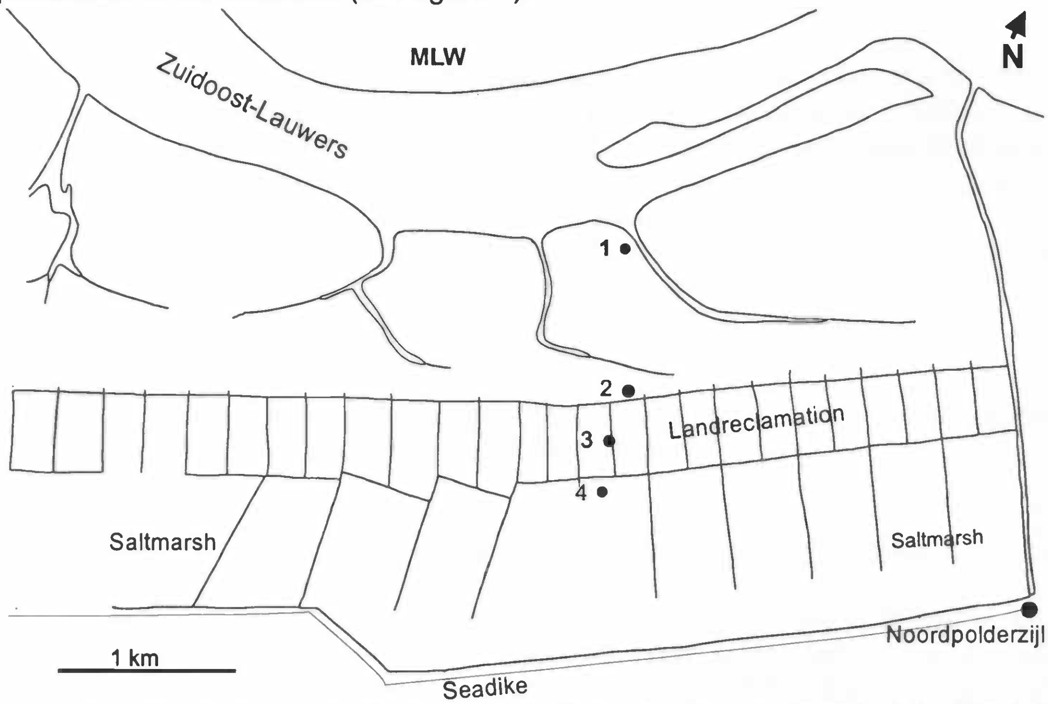

Figure 1: Overview of the study-site on the tidal flats near Noordpolderzijl of the Dutch Wadden Sea. The sampling sites are represented as numbered points (1-4). MLW = Mean Low Water level; N = North (after J.G. Hiddink, unpublished).

Population densities

Population densities of *M. balthica* spat, adult *N. diversicolor* and adult *A. marina* were determined along the transect at the four sampling sites. This was necessary to make an estimation about the predation impact of these polychaetes on the bivalve spat at different heights on the tidal flats. For each species a different sampling method was used. All measured densities were converted to a density of individuals m^{-2}.

Abundance of *M. balthica* spat was estimated by sampling sediment on the 10^{th} of June 1999, taking 5 replicate cores of 4.6-cm^2 cross-sectional area and 5 cm depth. In the laboratory the sediment was sieved over a 1000 µm, 500 µm and 300 µm mesh and the mean amount of individuals were calculated for each fraction (see appendix, table 1).

The density of *N. diversicolor* was established the 7^{th} of July 1999 at each sampling site, by taking 10 replicate cores (surface area 83 cm^2; 15 cm depth) and sieving the samples over a 1-mm mesh in the field. The collected specimens were counted and calculated per m^2 for each sampling site.

The quantity of *A. marina* present at each sample site was determined at the 19^{th} of August 1999 by counting the mounds of faecal casts on 4 replicate square areas of 0.25 m^2 (over 10 x 10 m). The assumption was made that one cast represents the presence of one *A. marina*. These amounts were summed for each sampling site.

Analysis of the digestive tract contents
To determine if predation by *N. diversicolor* and *A. marina* on *M. balthica* spat occurs in the field, the contents of the digestive tract of the potential predators were analysed.

Adult *N. diversicolor* was collected from sampling sites 2 and 4 on June the 9th, 15th and 23rd 1999, 10 individuals from each site. They were gathered by sieving mud samples over a 1-mm mesh in the field and subsequently killed in 4% formaline. After transport to the laboratory they were placed in 70% ethanol in order to preserve them.
Adults of *A. marina* were gathered on June the 9th 1999 (4 individuals) and July the 8th 1999 (10 individuals), respectively at sampling sites 1 and 3. Each individual was dug out of the sediment with a hayfork and like *N. diversicolor* killed in 4% formaline and preserved in 70% ethanol.

Prior to analysing the contents of the digestive tract of both species, the length of each individual was estimated to the nearest 0.1 cm. Next, the animal was completely cut open from rostral to caudal and the entire content of the digestive tract was spread out in a Petri-dish. All contents were carefully examined for recognisable shell fragments of *M. balthica* with the use of a 40x stereoscopic microscope.
Size of *M. balthica* in the digestive tract contents was estimated with the use of an eye piece micrometer.

Predation experiments
In order to gain evidence of predation by *N. diversicolor* and *A.marina* on *M. balthica* spat, predation experiments were conducted in the laboratory.

Collection of the animals
All animals used in these predation experiments, both prey and predators, were collected throughout the period June-August.
All juvenile *M. balthica* were gathered at sample site 3. They were obtained by collecting samples of the upper 5 cm sediment. After transport to the laboratory, the sediment including the *M. balthica* spat, was sieved over a 1000 µm, 500 µm and 300 µm mesh. By this method 3 fractions of juveniles were obtained: >1000 µm, >500 µm and >300 µm.
Adult *N. diversicolor* used in the predation experiments were sampled along the transect the same way the ones used for the analysis of the digestive tract contents. Live adults transported to the laboratory in plastic jars which were filled with seawater.
Adults of *A. marina* were individually dug out of the sediment with a fork. They were transported to the laboratory in a small bucket in their native sediment, after they had buried themselves.

Maintenance in the laboratory
Both *M. balthica* and *N. diversicolor* were maintained in the laboratory in a closed system aquarium, in separate compartments (15 x 10 x 13 cm). These compartments were filled with a small layer of sediment and covered with 1 mm mesh lids (after Marijnissen, 1998). The aerated aquarium was installed in a thermo-regulated room with day-night light regime (14-10), at 15.5 °C and filled with artificial seawater of 30‰.
M. balthica was fed every week with fresh algae (*Isochrysis galbana*). *N. diversicolor* was fed weekly with a mixture of dried algae (Tetramin). Before the predation experiment the selected individuals were starved for 2-12 days.
The predation experiment with *A. marina* was executed immediately after transport.

Experimental set-up

Before every predation experiment, a total of 100 juveniles of *M. balthica* were sorted out of different fractions: 30 individuals of >1000 µm, 50 of >500 µm and 20 of >300 µm.

Only live *M. balthica* were used which were recognised by activity of their siphon or protrusion of their foot.

- *N. diversicolor* as predator

For the predation experiments with *N. diversicolor* (see figure 3), the 100 bivalves were placed in an empty aerated experimental container (15 x 10 x 13 cm), which corresponds to a density of 0.67 specimen per cm^2. Subsequently, 5 randomly chosen individuals of *N. diversicolor* were added. All predators survived to the end of the experiment and were used only once. Each experiment lasted 24 hours and temperature, salinity and day-night light regime remained constant during the experiments.

After the experiment, the predators were removed, fixed in 4,5% formalin and preserved in 70% ethanol. In order to retrieve consumed *M. balthica*, the digestive tract content of each predator was examined, likewise the analysis of the digestive tract contents of the predators caught in the field.

The remaining *M. balthica* were counted and examined under a 40 x stereoscopic microscope. It was noted whether the retrieved bivalves were alive, cracked or empty (only the shell was present). In order to avoid harm to the retrieved *M. balthica* as much as possible, they were not sieved over different mesh size, so no subdivision in size-fraction could be made. The predation experiment with *N. diversicolor* was repeated 5 times.

Simultaneously to every predation experiment, an equal number of control experiments without predators was carried out, to determine any possible loss or mortality of *M. balthica* due to the treatment.

- *A. marina* as predator

For the experiment with *A. marina* (see figure 3), the 100 sorted *M. balthica* were added to the bucket with a layer of native sediment (10 cm) in which the 4 predators were already buried and transported. This way, after being caught the predators only had to bury into the sediment once, which is considered an intensive process. It was assumed that no *M. balthica* spat was present in the native sediment, considering the specific sample site (low on the tidal flat, no nursery) and late sample period (6^{th} of August, outgrown spat size).

The bucket had a surface area of 201 cm^2. The density of *M. balthica* was correspondingly 0.5 specimens per cm^2. The single experiment lasted 68 hours. After the experiment, the 4 specimens of *A. marina* were removed, fixed in 4,5% formalin and preserved in 70% ethanol. Like with *N. diversicolor*, all predators were subjected to an analysis of the digestive tract contents, in order to retrieve consumed *M. balthica*.

The sediment was sieved over a 1000 µm, 500 µm and 300 µm mesh and the number of surviving *M. balthica* of each fraction was recorded. The retrieved bivalves were subdivided into live, cracked and empty (only the shell was present).

Simultaneously to the predation experiment, a control experiment without predators was carried out.

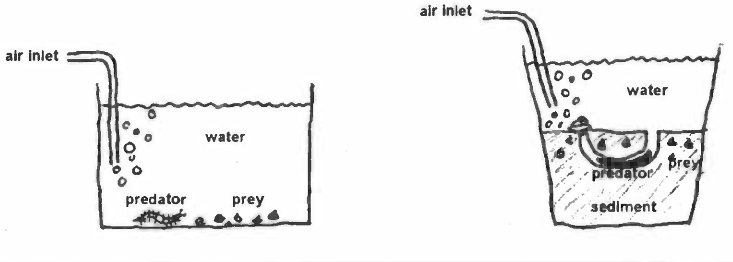

Figure 3: Schematic cross-section view of the container for predation experiment with N. diversicolor (left) and A. marina (right).

Statistical analysis

The retrieved *M. balthica* from the predation experiments were subdivided into the categories alive, cracked and empty (only the shell was present). Summing the number of retrieved *M. balthica* and comparing it with the number of added bivalves gives the number of lost *M. balthica*. It is assumed that the amount of consumed *M. balthica* is constituted by the total of lost and empty bivalves.

A Mann-Whitney W test was carried out to compare the medians (in stead of means) of the predation experiments with their controls.
If the P-value of the Mann-Whitney W test is less than 0.05, there is a statistically significant difference between the medians of the two samples at a 95.0% confidence level.

Results

Population densities

The population densities of *M. balthica*, *N. diversicolor* and *A. marina* were estimated at the four sampling sites and calculated to # per m^2. The heights of the sampling were measured with optical levelling instruments. The data are presented in table 1. Remarkable is that no A. marina was present at the highest sampling site (no. 4). Few *N. diversicolor* and *A. marina* have been found at respectively sampling site 1 and 2.

Table 1: Estimated densities of M. balthica, N. diversicolor and A. marina on the sampling sites (# per m^2). The height of the sampling sites (SS) is compared to NAP (Dutch Ordinance Level).

SS	height	# *M. balthica* per m^2	# *N. diversicolor* per m^2	# *A. marina* per m^2
	(cm)	(10/06/1999)	(07/07/1999)	(19/08/1999)
1	-65	870	1	16
2	-18	3013	34	1
3	35	4783	18	38
4	74	3478	28	0

Analysis of the digestive tract

The digestive tract contents of 20 *N. diversicolor* (6.3±1.6 cm) and 14 *A. marina* (5.9±2.3 cm) were examined for recognisable shell fragments of *M. balthica* (see table 2). The polychaetes were caught in the field at different sampling sites.

As shown in table 2, at both sampling sites, 10% of the dissected *N. diversicolor* contained *M. balthica*. The digestive tract of 10% of the dissected *A. marina* at sampling site 3 also contained bivalves. No *M. balthica* was found in the digestive tract of *A. marina* from sampling site 1.

Table 2: Analysis of the digestive tract contents of N. diversicolor and A. marina that were caught at different sampling sites (SS). Given are the mean size of the dissected predators, the percentage of predators that contained M. balthica, the number of consumed M. balthica, the mean size of the consumed M. balthica in mm and the mean number of M. balthica consumed by 1 predator. (N = Number of analysed individuals; - no data applicable).

SS	predator	N	Mean length of predator (cm)	% of predators that consumed *M. balthica*	# *M. balthica* consumed	Mean size of consumed *M. balthica* (mm)	mean # *M.balthica* consumed by 1 predator
1	*A. marina*	4	7.0±3.1	0	0	-	0.0
2	*N. diversicolor*	10	6.6±1.4	10	1	1.50	0.1
3	*A. marina*	10	5.5±2.0	10	2	0.85±0.12	0.2
4	*N. diversicolor*	10	5.9±1.7	10	2	0.17±0	0.2

Predation experiments

The graphs in figure 4 show the mean percentages of consumed *M. balthica* in the predation experiments with *N. diversicolor* and *A. marina*.

In the 5 experiments with *N. diversicolor* a total of 25 predators were used (5.5±1.0 cm) and they have consumed an average of 9.6±8.3% of the present *M. balthica*. During the control experiment 0.8±0.8% of *M. balthica* was consumed. According to the Mann-Whitney W test, there is a significant difference between the medians of the predation- and control experiments at a 95.0% confidence level ($p = 0.008$).

In the single predation experiment with 4 specimens of *A. marina*, 48% of the present *M. balthica* were consumed. In its control experiment, 23 % of the *M. balthica* appeared to be empty or lost (consumed).

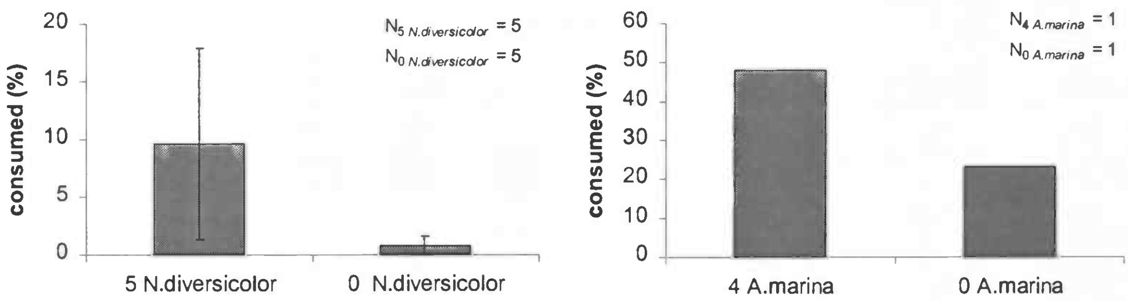

Figure 4: Mean percentages of consumed M. balthica in the predation experiments of N. diversicolor (left) and A. marina (right).

Table 3 gives the amount, size range and mean size of the consumed *M. balthica* that were retrieved from the predators after analysing the contents of their digestive tract. The table also shows the amount of predators in which *M. balthica* was found and the percentage of the predators from which the bivalves were retrieved.

Table 3: Size of M. balthica (mean ± standard deviation) in cm consumed by predators used in the predation experiments.

predator	# predators in which *M.balthica* was found	% predators in which *M.balthica* was found	# retrieved *M.balthica*	size range of retrieved *M.balthica* (mm)	mean size of consumed *M.balthica* (mm)
N. diversicolor	1	4	8	0.60 - 1.50	0.97 ± 0.35
A. marina	1	25	1	0.57	0.57

Because the *M. balthica* used in the predation experiment with *A. marina* was recovered from the sediment by sieving, a subdivision in size fraction could be made. By comparing the number of bivalves that was offered per size fraction with the number that was consumed, an estimation of size selection could be made.

The graph in figure 5 shows the percentages of consumed *M. balthica* of the fractions >1000 µm, >500 µm, >300 µm and its total amount for the predation experiment and the control experiment. Remarkable is that the largest fraction of *M. balthica* (>1000 µm) has hardly been consumed by *A. marina* (only 3%). Furthermore it is striking that from the smallest fraction (>300 µm) more *M. balthica* has been "consumed" (empty or lost) during the control experiment than in the actual predation experiment (respectively 65% and 50%).

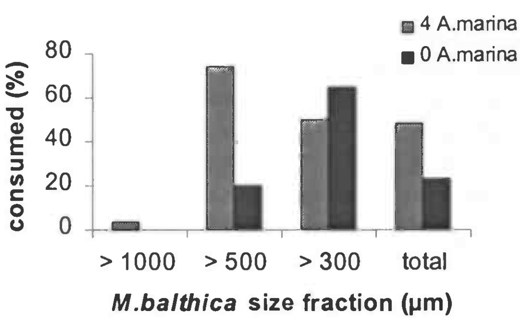

Figure 5: Percentages of consumed M. balthica of the fractions >1000 µm, >500 µm, >300 µm and its total amount for the predation experiment with A. marina and its control experiment.

The graph in figure 6 shows the percentage of *M. balthica* consumed by *N. diversicolor* against the starvation in days of the predators. The trendline gives a significant regression (R^2 = 0.77; $R^2_{0.05}$ = 0.77; n = 5), which means that the predator *N. diversicolor* consumed more *M. balthica* when it was starved for a longer period.

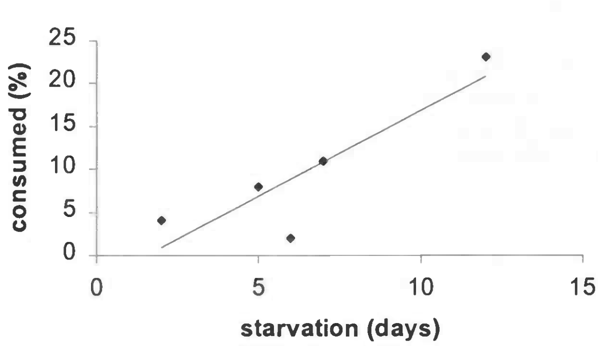

Figure 6: Percentage of consumed M. balthica by N. diversicolor against the starvation of the predators in days (R^2 = 0.77; n = 5).

Mortality

To make a prediction of the mortality of *M. balthica* spat in the field that is caused by predation *by N. diversicolor* and *A. marina,* one must know the predation pressure per unit of time, for example per day. Therefore the mean number of *M. balthica* that is recovered from the digestive tract of one predator in the field (table 3) had to be converted in values per day (table 4). This was managed by dividing the mean number of *M. balthica* that was retrieved from the digestive tract of one predator by the estimated digestion time of the predator.

The digestion time was estimated by dividing the mean number of retrieved *M. balthica* in the predation experiment by the mean percentage of consumed bivalves in the predation experiment, converted to days. It was assumed that the predators had a constant feeding rate during the predation experiment.

Table 4: The average number of M.balthica consumed by 1 predator in the field, the feeding rate of N. diversicolor and A. marina in the predation experiment per day and the average number of M. balthica consumed by 1 predator in the field per day.

SS	height	mean # *M. balthica* retrieved in digestive tract of 1 predator in field		digestion time of *M. balthica* in predator per day		calculated # *M. balthica* consumed by 1 predator per day	
	(cm)	*N.diversicolor*	*A.marina*	*N.diversicolor*	*A.marina*	*N.diversicolor*	*A.marina*
1	-65		0	0.17	0.059		0.00
2	-18	0.1		0.17	0.059	0.59	
3	35		0.2	0.17	0.059		3.39
4	74	0.2		0.17	0.059	1.18	

Combining the population densities (table 1) with the percentage of *N. diversicolor* and *A. marina* in the field that contained *M. balthica* (table 2) and the mean number of *M. balthica* consumed by 1 predator per day (table 4), results in a prediction of the percentage *of M. balthica* that is consumed per m^2 per day by these predators in the field (see table 5).

Table 5: Prediction of the percentage of M. balthica that is consumed per m^2 per day by N. diversicolor and A. marina at different heights on the tidal flat in the Dutch Wadden Sea near Noordpolderzijl (SS = Sampling Site).

SS	height	# predators per m^2		mean # *M. balthica* consumed by 1 predator per day		% of *M.balthica* consumed by predator m^{-2} per day	
	(cm)	*N. diversicolor*	*A.marina*	*N.diversicolor*	*A.marina*	*N.diversicolor*	*A.marina*
1	-65	1	16		0		0.00
2	-18	34	1	0.59		0.51	
3	35	18	38		3.39		2.69
4	74	28	0	1.18		0.95	

It is assumed that an exponential population decrease can be explained by the mortality. The mortality (Z) was calculated by:

$$Z = - \frac{\ln (N_t/N_0)}{t}$$

in which N_t = number of *M. balthica* that is alive at moment t, N_0 = mean number of *M. balthica* that is alive at moment the start of the time period and t = time in days. In this case, the mortality is measured per day (t=1). The mortality was calculated from combining the data from the predation experiments, population densities and data gained in analysing the digestive tract contents of predators caught in the field, is given in table 6 for different heights on the tidal flats.

Table 6: Estimated mortality of M. balthica caused by predation of N. diversicolor and A. marina on different heights on the tidal flats in the Wadden Sea near Noordpolderzijl.

Sampling Site	height	Mortality per day	
	(cm)	*N. diversicolor*	*A. marina*
1	-65		0.00000
2	-18	0.00511	
3	35		0.02737
4	74	0.00955	

Discussion

The analysis of the contents of the digestive tracts of *N. diversicolor* and *A. marina* that were caught in the field shown that these predators feed on *M. balthica* spat under natural conditions.
The predation experiments in this study confirm that both *N. diversicolor* and *A. marina* feed on *M. balthica* spat, because the bivalves have been retrieved from the digestive tract of some used predators. The predation on juvenile *M. balthica* by *N. diversicolor* has shown to be deliberate. This is sustained, because the *M. balthica* can not have accidentally be swallowed with for example sediment, because the experiment was conducted in a clean container, only filled with seawater.

Predation on *M. balthica* spat by *N. diversicolor* has previously been demonstrated by Ratcliffe *et al.* (1981), although they didn't analyse the contents of the digestive tracts of the predators. Their conclusions are solely based on observed reduction in spat numbers of 5 experiments where 20 individuals of *M. balthica* were offered to 2 *N. diversicolor*. The reduction of spat was 12% in 96 hours.
These values are comparable to the reduction of *M. balthica* in the predation experiments in this study (9.6%±8.3). It must be mentioned though, that the experiments in this study lasted only 24 hours and the number of offered *M. balthica* per predator was higher, 20 individuals compared to 10.

In an experiment by Reise (1979) *A. marina* was excluded from a 2-m^2 plot. In this plot the meiofauna (nematode) abundance decreased by 40 % within 20 days, relative to a control site with 90 lugworms. This decrease was ascribed to physical factors and food limitations via habitat conditioning by these large sediment swallowers. Field experiments involving manipulation of lugworm abundance by Flach (1992) showed that the presence of lugworms in densities of about 30 or more individuals per m^2 has a significantly negative effect on the abundance of *M. balthica* of spat size.

The predation experiment with *A. marina* was performed in sediment, and it must be taking into account that *M. balthica* has been swallowed by accidentally during burying behaviour of the predators. However, the fact that *M. balthica* was found in the digestive tract of the predator shows that *A. marina* does eat the *M. balthica* spat, intentionally or not.
The direct effects of the factor bioturbation in experiments with endobenthic predators like *A. marina* are questionable, because postlarvae of *M. balthica* needed to be buried up to 8-10 days before increased mortality became evident (Elmgren *et al.*, 1986). This suggests that effects of bioturbation on the *M. balthica* mortality in our experiment with *A. marina* were minor compared to those of direct predation.

Birds and aquatic epibenthos can only prey on *M. balthica* in the intertidal zone at respectively low and high tide. However, predation by endobenthic predators can occur at both high and low tide throughout the year, so the danger for *M. balthica* is continuously present. Once *M. balthica* spat have entered their first full summer's growth however, they will escape predation from these invertebrate predators because they will be too large.
In this predation experiments, the mean size of the retrieved *M. balthica* in *N. diversicolor* was 0.97±0.35 mm. Unfortunately, no subdivision in size-fraction had been made, so no preference for size could be determined.
The graph in figure 5 gives the consumption by *A. marina* of different sized *M. balthica*. From this graph it can be concluded that *A. marina* had a clear preference for *M. balthica* of the fraction >500 µm.

A rough estimate of the effects of predation on the *M. balthica* spat is yet available. It turns out that in the period June-August 1999, the estimated mortality factor per day of *M. balthica* caused by predation by *N. diversicolor* was 0.0051 on sampling site 2 (-18 cm) and 0.0096 on sampling site 4 (+74 cm). The mortality per day caused by *A. marina* on sampling site 3 (+35 cm) is estimated at 0.027.
A weak point in determining the mortality caused by these polychaetes is the small number of experiments that have been executed. The densities have been determined at only one day for each species and the digestion rate of *A. marina* is based in one single experiment. Still, The mortality values are in the same order magnitude as the mortality rates of *M. balthica* found in previous research, which vary from a mortality of 0.017 to 0.154 per day (Van der Veer ***et al.***, 1998).
Hiddink (in prep.) determined the mortality of *M. balthica* on the entire tidal flat during the period June-August 1998 at 0.014 per day. When assuming that the mortality during the period June-August 1999 was equal: this means that 36% of the total mortality of *M. balthica* in this period is caused by predation by *N. diversicolor* at height -18 cm. Higher on the tidal flats (at +74 cm), this influence even amounts to 69% of the mortality of *M. balthica*. The mortality of *M. balthica* caused by predation by *A. marina* at sampling site 3 (+35 cm) is very high, almost twice as much (193%) as Hiddink found for the entire transect. Partly this may have been caused by the very high density of *A. marina* at this height (38 individuals per m^2, see table 1). More likely is the fact that the estimated digestion time is too fast (see table 4).
Still, it can be concluded that the predation by the polychaetes *N. diversicolor* and *A. marina* is possibly of major importance to the *M. balthica* population

Experimental results of Desroy *et al.* (1998) support the hypothesis that infaunal predation is a structuring force in soft-bottom communities and can regulate populations of recruits.
In addition to direct predation, the activities of one group of benthic organisms, like bioturbation by *A. marina*, may indirectly influence the survival of another group. Such competition in the same niche may result in a relationship in which the feeding behaviour of one group adversely affects the survival of another. In the predation experiments in this study, only predators of one species at a time were used. Therefore, no interaction of different groups could occur, which might have had an abnormal effect on the predation compared with the field situation.

However, observing the estimated mortality of *M. balthica*, predation by *N. diversicolor* and *A.marina* on *M. balthica* spat can and should be considered as highly important factors that affect the *M. balthica* population size. For certain, the endobenthic predators should be included when performing research on use of nurseries by juvenile *M. balthica*. The presence of large amounts of endobenthic predators high on the tidal flats should be considered as a disadvantage in the trade-off of *M. balthica* spat whether to migrate to nurseries or not.

Further recommendations

First of all, research on the 0-group *M. balthica* should in the future be carried out in the period May-July, otherwise the juveniles will outgrow the experiments (especially fraction >300 to >500 µm).

Research on predation of *M. balthica* by *N. diversicolor* and *A. marina* like performed in this study should be continued in order to enlarge the n of the experiments. This way, more valuable predictions of mortality of *M. balthica* caused by these polychaete predators can be made.
More research should be done on determining preference of endobenthic predators for specific size of *M. balthica* spat. This will establish the period of their life in which *M. balthica* is at risk for predation by endobenthic predators. The duration of this period might be a key element in deciding whether to migrate to nurseries or not.

The predation experiments in this study with *N. diversicolor* restrict the dietary choices of the implanted predators depriving them of the normal range of prey items as well as any effect of changes to the organic carbon content of the sediments. Therefore, it is highly recommended to perform predation experiments under more natural conditions, in sediment and preferably in the field.

Field experiments can be executed by using exclosures and enclosures. The exclosures should be excluded from all predators. The enclosures should contain only one type of endobenthic predator, but also all endobenthic predators in order to observe competition between the endobenthic predators. Naturally, control experiments should be performed. When executing the experiments during the spring and summer at different tidal levels, one probably will find differences in population size of *M. balthica* due to difference in predation pressure by endobenthic organisms.
Considering the fact that endobenthic predators might influence the population size of *M. balthica*, one should monitor the spatial distribution of endobenthic predators throughout the season when juvenile *M. balthica* inhabit the nurseries, and relate it to spat abundance and spat size.

To close, the same experiments as with *N. diversicolor* and *A. marina* should be performed with the endobenthic snail *Retusa obtusa*. This snail is also highly abundant on the intertidal flats in the Dutch Wadden Sea and predation on *M. balthica* spat by *R. obtusa* has already been demonstrated by Ratcliffe *et al.* (1981). *R. obtusa* is also known to feed on whole foraminiferans and the youngest *Hydrobia ulvae* of 300 µm can be swallowed entirely by *R. obtusa* of 0.9 mm shell length (Berry & Thomson, 1990; Berry *et al.*, 1992). All this makes it a potential predator that may be an important factor that affects the *M. balthica* population size.

References

* Aarnio, K. & E. Bonsdorf & N. Rosenback (1996). Food and feeding of juvenile flunder *Platichthys flesus* (L.) and turbot *Scophthalmus maximus* (L.) in the Åland archipelago, northern Baltic Sea. *Journal of Sea Research*. Vol. 36: p. 311-320.

* Armonies, W. (1994). Turnover of postlarval bivalves in sediments of tidal flats in Königshafen (German Wadden Sea). *Helgoländer Meeresuntersuchungen*. Vol. 48: p. 291-297.

* Armonies, W. & M. Hellwig Armonies (1992). Passive settlement of *Macoma balthica* spat on tidal flats of the Wadden Sea and subsequent migration of juveniles. *Netherlands Journal of Sea Research*. Vol. 29: p. 371-378.

* Berry, A.J. & K.V. Radhakrishnan & K. Coward (1992). Is seasonal breeding in *Retusa obtusa* (Montagu) (Gastropoda: Opisthobranchia) merely the consequence of seasonal breeding in its prey, the mudsnail *Hydrobia ulvae* (Pennant)? *Journal of Experimental Marine Biology and Ecology*. Vol. 159: p. 179-189.

* Berry, A.J. & D.R. Thomson (1990). Changing prey size preferences in the annual cycle of *Retusa obtusa* (Montagu) (Opisthobranchia) feeding on *Hydrobia ulvae* (Pennant) (Prosobranchia). *Journal of Experimental Marine Biology and Ecology*. Vol. 141: p. 145-158.

* Beukema, J.J. (1976). Biomass and species richness of the macro-benthic animals living on the tidal flats of the Dutch Wadden Sea. *Netherlands Journal of Sea Research*. Vol. 10: p. 236-261.

* Beukema, J.J. (1987). Influence of the predatory polychaete *Nephtys hombergii* on the abundance of other polychaetes. *Marine Ecology Progress Series*. Vol. 40: p. 95-101.

* Beukema, J.J. (1992). Dynamics of juvenile shrimp *Crangon crangon* in a tidal-flat nursery of the Wadden Sea after mild and cold winters. *Marine Ecology Progress Series*. Vol. 83: p. 157-165.

* Beukema, J.J. (1993). Successive changes in distribution patterns as an adaptive strategy in the bivalve *Macoma balthica* (L.) in the Wadden Sea. Helgoländer Meeresuntersuchungen. Vol. 47: p. 287-304.

* Beukema J.J. & J. De Vlas (1989). Tidal-current transport of thread-drifting postlarval juveniles of the bivalve *Macoma balthica* from the Wadden Sea (Netherlands) to the North Sea. *Marine Ecology Progress Series*. Vol. 52: p. 193-200.

* Barrett, J. & C.M. Yonge (1958). Collins Pocket guide to the Sea Shore. *Collins Clear-Type Press, London*. 272 pp.

* Dekker, R. (1989). The macrozoobenthos of the subtidal western Dutch Wadden Sea. I. Biomass and species richness. *Netherlands Journal of Sea Research*. Vol. 23: p. 57-68.

* Dekker, R. & W. De Bruin (1998). Het macrozoobenthos op twaalf raaien in de Waddenzee en de Eems-Dollard in 1997. *NIOZ-rapport 1998-3*. 53 pp.

* Desroy, N. & C. Retiere & E. Thiebaut (1998). Infaunal predation regulates benthic recruitment: an experimental study of the influence of the predator *Nephtys hombergii*

(Savigny) on recruits of *Nereis diversicolor* (O.F. Muller). *Journal of Experimental Marine Biology and Ecology*. Vol. 228: p. 257-272.

* De Wilde, P.A.W.J. & E.M. Berghuis (1979). Laboratory experiments on growth of juvenile lugworms, *Arenicola marina*. *Netherlands Journal of Sea Research*. Vol. 13: p. 487-502.

* Elmgren, R. & S. Ankar & B. Marteleur & G. Edjung (1986). Adult interference with post-larvae in soft-bottom sediments: the *Pontoporeia-Macoma* example. *Ecology*. Vol. 67: p. 827-836.

* Flach, E.C. (1992). Disturbance of benthic infauna by sediment-reworking activities of the lugworm *Arenicola marina*. *Netherlands Journal of Sea Research*. Vol. 30: p. 81-89.

* Günther, C.P. (1990). Distribution patterns of juvenile macrofauna on an intertidal sandflat: an approach to the variability of predator/prey interactions. *In: Barnes, M. & Gibson, R.N. (eds.) Trophic relationships in the marine environment. Proceedings of the. 24th European Marine Biology Symposium; Aberdeen University Press*. p. 77-88.

* Günther, C.P. (1991). Settlement of *Macoma balthica* on an intertidal sandflat in the Wadden Sea. *Marine Ecology Progress Series*. Vol. 76: p. 73-79.

* Hayward, P.J. & J.S. Ryland (eds.) (1995). Handbook of the Marine Fauna of North-West Europe. *Oxford University Press Inc., New York*. 800 pp.

* Hiddink, J.G. (in prep.) Risks of nursery – related migrations for *Macoma balthica* (L.).

* Hulscher, J.B. (1981). The Oystercatcher *Haematopus ostralegus* as a predator of the bivalve *Macoma balthica* in the Dutch Wadden Sea. *Ardea*. Vol. 70: p. 89-152.

* Jensen, K.T. & J.N. Jensen (1985). The importance of some epibenthic predators on the density of juvenile benthic macrofauna in the Danish Wadden Sea. *Journal of Experimental Marine Biology and Ecology*. Vol. 89: p. 157-174.

* Lucas, F. & G. Bertru (1997). Bacteriolysis in the gut of *Nereis diversicolor* (O.F. Muller) and effect of the diet. *Journal of Experimental Marine Biology and Ecology*. Vol. 215: p. 235-245.

* Marijnissen, S.A.E. (1998). Size selective predation on the bivalve *Macoma balthica* by juvenile epibenthos. *MSc-report, unpublished*. 28 pp.

* Piersma, T. & R. Hoekstra & A. Dekinga & A. Koolhaas & P. Wolf & P. Battley & P. Wiersma (1993). Scale and intensity of intertidal habitat use by knots, *Calidris canutus*, in the Wadden Sea. *Netherlands Journal of Sea Research*. Vol. 31: p. 331-357.

* Pihl, L. (1985). Food selection and consumption of mobile epibenthic fauna in shallow marine areas. *Marine Ecology Progress Series*. Vol. 22: p. 169-179.

* Ratcliffe, P.J. & N.V. Jones & N.J. Walters (1981). The survival of *Macoma balthica* (L.) in mobile sediments. *In: Jones, N.V. & W.J. Wolff (eds.); Feeding and survival strategies of estuarine organisms; Plenum Press, New York.*

* Rees, C.B. (1940). A preliminary study of the ecology of a mudflat. *Journal of the Marine Biology Association U.K.* Vol. 24: p. 185-199.

* Reise, K. (1979). Moderate predation on meiofauna by the macrobentos of the Wadden Sea. *Helgoländer wissenschaftliche Meeresuntersuchungen*. Vol. 32: p. 453-465.

* Retraubun, A.S.W. & M. Dawson & S.M. Evans (1996). The role of the burrow funnel in feeding processes in the lugworm *Arenicola marina* (L.). *Journal of Experimental Marine Biology and Ecology*. Vol. 202: p. 107-118.

* Riisgard, H.U. (1991). Suspension feeding in the polychaete *Nereis diversicolor*. *Marine Ecology Progress Series*. Vol. 70: p. 29-37.

* Riisgard, H.U. (1994). Filter-feeding in the polychaete *Nereis diversicolor*: a review. *Netherlands Journal of Aquatic Ecology*. Vol. 28 (3-4): p. 453-458.

* Rijken, M. (1979). Food and food uptake in *Arenicola marina*. *Netherlands Journal of Sea Research*. Vol. 13 (3/4): p. 406-421.

* Ruppert, E.E. & Barnes, R.D. (1994). Invertebrate Zoology. *Saunders College Publishing*. Vol. 6: p. 950-966.

* Schubert, A. & K. Reise (1986). Predatory effects of *Nephtys hombergii* on other polychaetes in tidal flat sediments. *Marine Ecology Progress Series*. Vol. 34: p. 117-124.

* Thorson, G. (1957). Bottom communities (sub-littoral or shallow shelf). *In: Hedgepeth, J.D. (ed.); Treatise on marine ecology and paleoecology. Geological Society of the American Mem.* Vol: 67: p. 461-534. **non vidi**

* Van der Veer, H.W. & R.J. Feller & A. Weber & J.IJ. Witte (1998). Importance of predation by crustaceans upon bivalve spat in the Dutch Wadden Sea as revealed by immunological assays of gut contents. *Journal of Experimental Marine Biology and Ecology*. Vol. 231: p. 139-157.

* Witte, F. & P.A.W.J. de Wilde (1979). On the ecological relation *between Nereis diversicolor* and juvenile *Arenicola marina*. *Netherlands Journal of Sea Research*. Vol. 13 (3/4): p. 394-405.

Acknowledgements

With great gratitude I would like to thank Jan Geert Hiddink. As a result of his enthusiasm and knowledge about the mud flats I am now also infected with the wad-virus. Furthermore, he made me experienced with the practical, the theoretical and also the statistical side of research.

I wish to thank Wim Wolff and Ansje Lohr for the effort they put in collecting *A. marina* for me. Jan Geert Hiddink is acknowledged for providing me with the data of the densities of *N. diversicolor* and *A. marina* along the transect at the study site.

I would also like to thank Saskia Marijnissen for checking this essay for incorrect English spelling and grammar.

Appendix

Table 1: Estimated densities of M. balthica for the total spat and fractions >1000µm, >500 µm and >300 µm on the sampling sites (# per m²) at the 6th of June 1999. The height of the sampling sites (SS) is compared to NAP (Dutch Ordinance Level).

SS	height (cm)	fraction >1000µm	fraction > 500µm	fraction > 300µm)	total
1	-65	0	435	435	870
2	-18	879	1739	1304	3913
3	35	870	2609	1304	4783
4	74	1739	870	870	3478

Table 2: Recognised food items in the content of the digestive tract from each dissected predator that was caught in the field.

SS	species	length (cm)	contents
1	*A. marina*	6	Foraminifera
1	*A. marina*	11.5	Foraminifera, Ostracoda, faeces of *Hydrobia ulvae*
1	*A. marina*	6.2	Foraminifera, faeces of *Hydrobia ulvae*, Diatoms
1	*A. marina*	4.2	Foraminifera
2	*N. diversicolor*	8.5	Foraminifera
2	*N. diversicolor*	6.6	-
2	*N. diversicolor*	5.8	-
2	*N. diversicolor*	5.1	-
2	*N. diversicolor*	7.8	faeces of *Hydrobia ulvae*
2	*N. diversicolor*	8.3	Foraminifera
2	*N. diversicolor*	6.4	Foraminifera, faeces of *Hydrobia ulvae*, Diatoms
2	*N. diversicolor*	4.0	-
2	*N. diversicolor*	6.6	Foraminifera, faeces of *Hydrobia ulvae*, Diatoms
2	*N. diversicolor*	7.0	Foraminifera, faeces of *Hydrobia ulvae*, *Littorina littorea* (3.0 mm), *M. balthica* (1.5 mm)
3	*A. marina*	5.0	Foraminifera, Ostracoda, *M. balthica* (0.929 mm)
3	*A. marina*	3.5	Foraminifera, Ostracoda
3	*A. marina*	2.3	Foraminifera
3	*A. marina*	6.6	Foraminifera, Ostracoda
3	*A. marina*	7.8	Foraminifera, Other Bivalvia (2x)
3	*A. marina*	4.9	Foraminifera, Ostracoda
3	*A. marina*	7.0	Foraminifera
3	*A. marina*	7.2	Foraminifera
3	*A. marina*	7.0	Foraminifera, *M. balthica* (0.762 mm)
3	*A. marina*	3.1	Foraminifera
4	*N. diversicolor*	7.8	Foraminifera
4	*N. diversicolor*	6.1	Foraminifera
4	*N. diversicolor*	3.1	Foraminifera
4	*N. diversicolor*	8.4	Foraminifera, Ostracoda, faeces of *Hydrobia ulvae*
4	*N. diversicolor*	5.1	Foraminifera, Ostracoda, faeces of *Hydrobia ulvae*
4	*N. diversicolor*	3.5	Foraminifera, Ostracoda, faeces of *Hydrobia ulvae*
4	*N. diversicolor*	6.6	Foraminifera, faeces of *Hydrobia ulvae*
4	*N. diversicolor*	6.5	Foraminifera
4	*N. diversicolor*	4.8	Foraminifera
4	*N. diversicolor*	7.0	Foraminifera, Ostracoda, faeces of *Hydrobia ulvae*, *M. balthica* (2 x 0.167mm)