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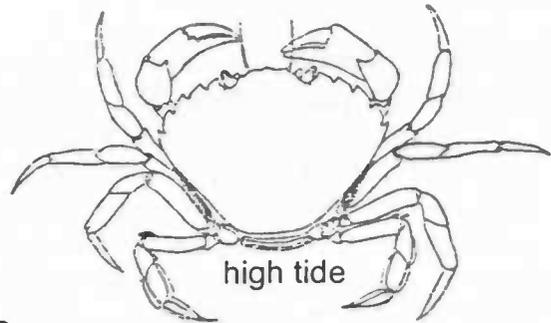
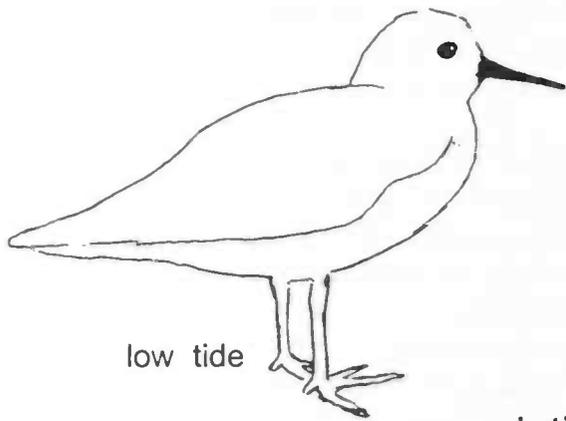
# Phenotypic plasticity of the burying depth of *Macoma balthica*

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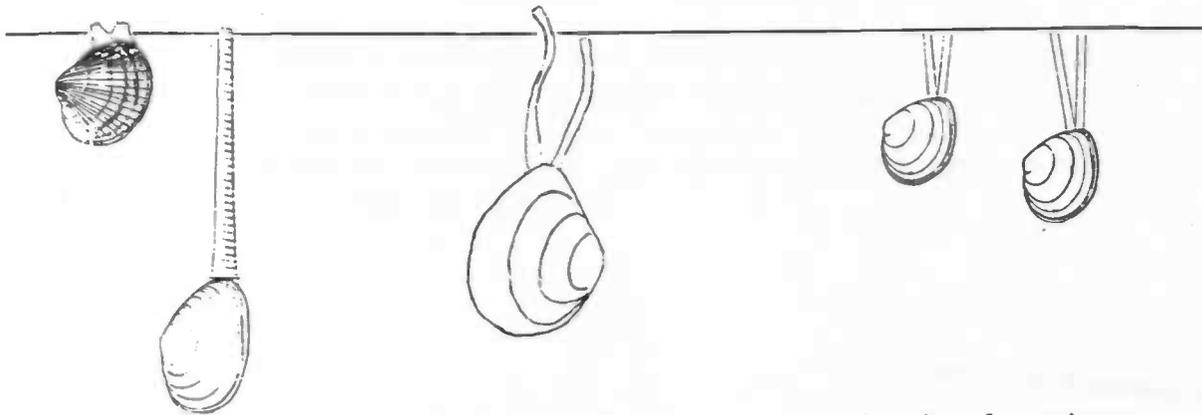
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NIOZ



predation



density of other clams

individual

density of specimen

## Summary

The bivalve *Macoma balthica* has to deal with a trade-off between predation risk and food availability in order to determine its burying depth. To know more about the phenotypic plasticity of the burying depth of *Macoma*, experiments are done in the field with regard to predator presence of the Shore crab *Carcinus maenas* and the shorebirds the Knot *Calidris canutus* and the Oystercatcher *Haematopus ostralegus*. By means of transplantation experiments it is determined whether the origin and the environment of the clam are important factors influencing the burying depth.

The origin of a clam has an effect; the clams originating from near the gully bury significantly deeper at the same location than the clams originating from high on the tidal flat. The environment of the clam seems to have no effect upon the burying depth of *Macoma*, only little variation in burying depth between the treatments occurred during the second experiment. This might be due to the fact that the clams were already buried deep; possibly safer from predation. To know the effect of the environment of the clam upon the burying depth, it is recommended to repeat this experiment. A crab predator seems to have an effect upon the burying depth of *Macoma*. In the presence of a predator the clams seem to bury deeper, but this effect is not significant. The same results come out of the experiment with the shorebirds, now only the effect of predator presence is tested. Another outcome of both experiments seem to be that the condition of a clam has a positive effect upon the burying depth of *Macoma*. With increasing condition the burying depth is increasing.

Another experiment is done to look at the effect of clam density upon the burying depth of *Macoma*. *Macoma* living in a low density bury significantly deeper than those living under medium- or high-density categories do.

Besides these experiments an observation on the burying depth of *Macoma* at different locations on the tidal flat during the season is done. The burying depth of *Macoma* is dependent on the time of the year, but also on the initial depth. *Macoma* that are buried deep do not react much on change in time, while the shallow living ones bury deeper. Thus differences in burying depth between *Macoma* of different locations change in time.

As predator presence seems to have an effect on the burying depth of *Macoma*, it is recommended to repeat this experiment and to test again the effect of 'environment' besides predator presence. The origin of a clam is of importance; it would be interesting to look whether the difference in burying depth can be inherited by their offspring.

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## Introduction

In nature the principle 'eaten or being eaten' is often in force. According to this principle many organisms are as well predator as prey. An organism has to deal with both the different interests; not one of them can be ignored. The predator-part of an organism tries to be as good as possible in capturing prey, while the prey-part tries to be as good as possible in avoiding predation. This conflict enacts not only within an organism, but also between two organisms related to each other as predator and prey. Both tries to do best and win the contest that has develop between them by doing so. In a stable environment the best solution for both organisms is to be completely adapted to their environment. However in a variable environment, where it is uncertain where your offspring will be raised, it is better to develop certain plasticity of a trait; the possibility to have flexibility in phenotype in reaction to environmental factors. For instance the snail *Littorina obtusata* has a thicker shell, when living in an area with a lot of crab predators, while those living without crab predator remain to have a thin shell (Trussell 1994). However phenotypic plasticity is costly (DeWitt *et al* 1998) e.g. the characteristic is not always irreversible; the snail *Littorina obtusata* can not get again a thinner shell when moving to an area without predation risk of crabs. Therefore an organism has to weigh the consequences of having plasticity.

During this project will be looked whether there is phenotypic plasticity of the burying depth of a prey; the bivalve *Macoma balthica*, in relation to its predators.

This bivalve, with a maximal shell length of 30 mm, lives burrowed in the sediment of the intertidal zone or the shallow subtidal zone (Steur *et al.* 1996). The clam has a wide arctic-boreal distribution, that might be a result of the few restrictions of environmental factors the clam has (like sediment composition, intertidal level or salinity), as seen in the Wadden Sea (Beukema 1981).

Burrowed in the sediment *Macoma* has contact with the above water column through its siphons; the siphons are at the posterior end of the clam, thus the clam is buried upside down in the sediment. One siphon is for the food supply, the inhalant, while the other gets rid of the waste products (the exhalant). The clam is a facultative suspension and deposit feeder (Brafield and Newell 1961; Olafsson 1986; Zwarts and Wanink 1989), foraging on planktonic and benthic microalgae. During suspension feeding the clam holds the inhalant siphon just outside the sediment surface and filters food from the above water column. When deposit feeding the inhalant siphon is stretched out over the sediment surface, sucking away detritus. By this way of foraging the clam has to expose its siphon more outside the sediment than by suspension feeding. Since siphon length determines the maximal burying depth (Reading and McGrorty 1978), deposit feeders have to live shallower in the sediment than suspension feeders. Although it is not clear yet how *Macoma* does switch between these two ways of foraging and which technique is more profitable; it is thought that deposit feeding yields more

(Zwarts 1986). Furthermore De Goeij and Luttikhuisen (1998) have proven that with increasing depth the growth rate and the condition of the clam is decreasing. Therefore one might expect that *Macoma* would live shallow in the sediment. However in the field situation, many *Macoma* do not live buried shallowly. Obviously there is another factor which interfere with the advantage of living shallow, that make the *Macoma* bury deeper than expected due to foraging method. This probably is predation risk (Zwarts and Wanink 1989).

By living in the tidal zone *Macoma* has to fear different kinds of predators during the tidal cycle. At low tide, when the mudflat falls dry, the main predators are two bivalve-eating shorebirds; the Red Knot *Calidris canutus* and the Oystercatcher *Haematopus ostralegus* (Zwarts and Blomert 1992). During high tide the predators are no longer birds, but only marine predators like shrimps, fishes and crabs (Steur *et al.* 1996).

All predators have the same kind of demands on their prey *Macoma*. The buried clam has to live within reach of the predator, will the predator be able to prey upon it. *Macoma*, which are buried too deep - out of reach - are therefore saving from predation. Not all *Macoma*, living in reach of their predator will be eaten. Some size classes, which theoretically can be eaten are ignored, as they are not lucrative to harvest, being either too small or too large (Zwarts *et al.* 1992). The predator has to weigh the yield of such a prey with regard to the time of handling the prey. Since all types of predators have their own possibilities and restrictions they mostly prey upon a certain size class or size range. This will lead to size-dependent predation.

Their bill length restricts avian predators, when they try to reach a clam living deeper than their bill is long. Knots feeding on *Macoma*, can, with their 4 cm long bill, only reach the clams living in the upper 2 till 3 cm (Zwarts *et al.* 1992). Since they swallow their prey as a whole to crush it in the stomach (Goss-Gustard *et al.* 1977), the maximum prey size of 16 millimetre (Zwarts *et al.* 1992) is determined by their bill opening.

Oystercatchers can, with their 70 to 80 millimetre long bill (Zwarts 1996), reach deeper living *Macoma* than Knots since their bill is longer. Although there is no restriction on shell size, Oystercatchers prefer certain sizes of clams to others since they are more lucrative to harvest.

During high tide and for subtidal living *Macoma* the clams live in risk of being eaten by only marine (epibenthic) predators like shrimps, fishes and shore crabs. Shrimps mainly prey upon brood of *Macoma* (Steur *et al.* 1996). Fishes, especially juvenile flatfishes can be seen as partial predators, because they only prey upon the inhalant siphon of *Macoma* called siphon nipping (De Vlas 1979). Although siphon nipping is not lethal, it can diminish the working of the siphon, causing a lower intake rate. Since the siphon needs to be regenerated, possibly at the expense of other energy demanding processes, it can cause reduction of the fitness of the clam (Kamermans and Huitema 1994). Beside this, the predation risk will be higher as the clam has to live shallower in the sediment with its shorter siphon (Blundon and Kennedy 1982; De Vlas 1979; Zwarts and Wanink 1989).

An important marine epibenthic predator is the Shore crab *Carcinus maenas*. It can detect its prey by the chemical receptors on the antennae and by

probing the walking legs, also with chemical receptors, into the sediment (Elner and Hughes 1978; Scherer and Reise 1981). Till what depth the Shore crabs dig for their prey is not described. However observations by Marijnissen (1998) during crab experiments have revealed that that the Shore crabs dig for their prey to a depth of four centimetres (estimated). When coming upon a *Macoma*, the crab can easily crush the small specimen with the chelae. The larger clams are forced open at the weakest spot with the aid of the thumb of the chelal, while holding the clam tight to the body with the other chelal (Scherer and Reise 1981).

When considering the risk of predation for *Macoma* it is clear that *Macoma* have to try to escape predation by moving to a safer place; burying deeper into the sediment (Virnstein 1977; Blundon and Kennedy 1982), out of the reach of its predators. However as a forager the clam has to live as shallow as possible to gain most energy supply at the lowest energy costs (De Goei and Luttikhuisen 1998; Zwarts 1986). *Macoma* has to deal with these clashing interests. However not only predation and the costs of living determine the burying depth, also other factors are of importance. The depth of the burrow of *Macoma* is dependent on the time of year (Reading and McGroarty 1978; Zwarts and Wanink 1989). In winter they live in a deep burrow; quite inactive in severe winters and more active during mild winters. In the short period of growth between March and July they bury shallow in the sediment. At the end of the summer they burrow deep again.

As individuals of a population are not identical, the burying depth is also dependent on individual characteristics as the shell length and the age of a clam and the presence of parasites inside the clam. With increasing shell length, and age, the maximum burying depth increases, giving a S-shaped curve between increasing shell length and burying depth (Zwarts and Wanink 1993). However at a certain size of *Macoma* the burying depth will decrease again (field observation P. Edelaar and M. de Heij). The trematode parasite *Parvatrema affinis* uses *Macoma* as an intermediate host. Since shorebirds are the end host, the parasite make the clam to live shallower or even live on top of the sediment, to be certain the clam will be prey upon; thus to complete its life cycle (Swennen and Ching 1974).

On population level an increase of density of either other *Macoma* or other clam species like the cockle *Cerastoderma edule* and the Soft-shell clam *Mya arenaria* will lead to an increasing competition for food and finally to an decrease in burying depth (Lin and Hines 1994). But the presence of many other individuals might have a positive effect as well, since the chance per individual of getting caught by a predator is smaller with a higher density than with a lower density. This might have its influence on the trade-off resulting in a shallower buried clam. However this advantage will be annulled when predators only forage on high-density patches.

Thus it is clear that many factors can have their influence upon the burying depth of *Macoma*. And since there are size dependent processes - like predation - the solution of the trade-offs can be different per size class. To understand why a certain *Macoma* is at the depth in the field it is important to unravel these factors and to quantify the influence of all these factors.

Thus the main aim of this project is to reveal the importance of phenotypic plasticity of the burying depth of the bivalve *Macoma balthica* in reaction to its predators.

I try to achieve this goal by solving the smaller questions:

- does the presence of a predator as the common shore crab *Carcinus maenas* have an effect upon the burying depth of *Macoma balthica*,
- does the origin of a clam (the location on the tidal flat) have an effect upon the reaction (in burying depth) of the clam to predator presence of a crab,
- does the presence of avian predators like the Red Knot *Calidris canutus* and the Oystercatcher *Haematopus ostralegus* have an effect upon the burying depth of *Macoma balthica*,
- does the density of other clams have an effect upon burying depth of a *Macoma*,
- does the variation in burying depth between different locations on the tidal flat change in time?

Part 1: The effect of an epibenthic predator on the burying depth of *Macoma*

In the field *Macoma* populations living near the gully are buried deeper in the sediment than those living high on the tidal flat (Hulscher 1973). One of many reasons for this might be that low on the tidal flat and in the gully the crabs are more abundant than high on the tidal flat (Klein Breteler 1976). As the crab abundance is higher, it is expected that the predation risk of the predator on *Macoma* is highest for the *Macoma* living near the gully. Lab experiments have shown that *Macoma* reacts on the presence of crab by burying deeper in the sediment. Thus the difference in burying depth might be explained by the difference in predator presence of the crabs.

The lab experiments will be repeated in the field to look at the importance of the crab presence on the burying depth in nature. Thus during this experiment it is tested in the field whether *Macoma balthica* varies its burying depth in the presence of the Shore crab *Carcinus maenas*. If this is true, do all *Macoma* on the tidal flat react the same? Therefore it is also examined, whether *Macoma* of different origin (locations on the tidal flat) react (in burying depth) differently to crab presence.

## Method

### General experimental set-up

Does the presence of the predator the Shore crab *Carcinus maenas* have an effect upon the burying depth of *Macoma balthica*?

To examine this an experiment was set-up in which two treatments with difference in predator presence was created to compare the burying depth at the end of the experiment. Some *Macoma* were kept in a cage in the presence of one adult crab (crab enclosure), whereas others were kept in a cage without a crab predator (crab enclosure). The design of the both cages was the same. To have a stronger statistical test each treatment had five replicates and within each treatment 20 experimental *Macoma* were placed.

Does the origin of a clam (the location on the tidal flat) have an effect upon the reaction (in burying depth) of the clam to predator presence of a crab?

To examine this, the experiment was done twice at two locations on the tidal flat at the same time; one near the gully (further referred to as 'low') and the other high on the tidal flat ('high')(see Appendix II). Experimental *Macoma* of each location were transplanted to both the other location as the location of their origin, to examine the differences in burying depth at the end of the experiment. If differences in burying depth of the clams between the two locations would occur, the explanation should be found in environmental factors, other than predation. If at each of the two locations the burying depth of *Macoma* of the two origins would differ, the explanation should be found inside the *Macoma*; "genetically". The *Macoma* would be in the same environment, but reacted differently on the same environmental factors or did not react at all.

The two experiments were carried out in the same cage, to reduce the number of cages per location. Thus 5 \* 2 treatments instead of 5 \* 4 treatments were used per location with 20 experimental *Macoma* per cage; 10 \* 2 origins (see table 1-1 and figure 1-1).

Table 1-1: Summary of the experimental design

location	crab presence	# replicate cages	origin of clams	# clams per origin per cage
high	+	5	high	10
			low	10
	-	5	high	10
			low	10
low	+	5	high	10
			low	10
	-	5	high	10
			low	10

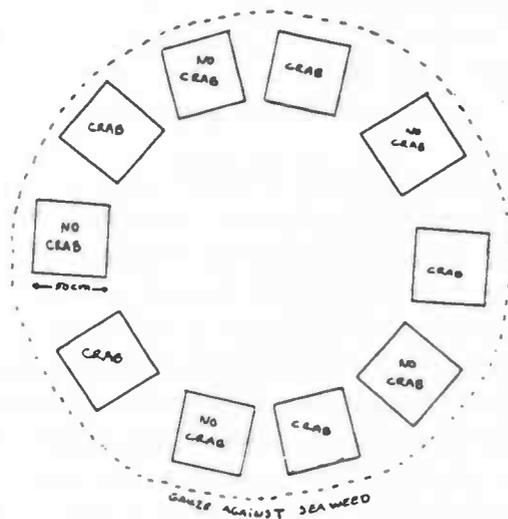


Figure 1-I: Design of the experiment at one location

With a pilot study information was gathered on the depth distribution of the *Macoma* population at both locations (for method see appendix I), to check whether there was variation in burying depth between the two locations (figure 1-II). Also a pilot study was done on the presence of the trematode parasite *Parvatrema affinis* in the *Macoma* of the two selected locations (see table 1-II), seeing that this parasite influence the burying behaviour of *Macoma* by making them bury shallower in the sediment (Swennen and Ching, 1974). It would therefore have given an unwanted artefact to the experiment. Since this parasite was not abundant at the two locations, both selected locations were kept as part of the experiment.

Table 1-II: measure of parasites at the two locations

location on the tidal flat	number of clams checked	number of clams with parasites	number of clams without parasites
high	75	4.0 %	96.0 %
low	41	2.4 %	97.6 %

The abiotic factors as well as the differences in population characteristics at both locations were investigated. The results are summarised in "Description of the two locations and the two *Macoma* populations".

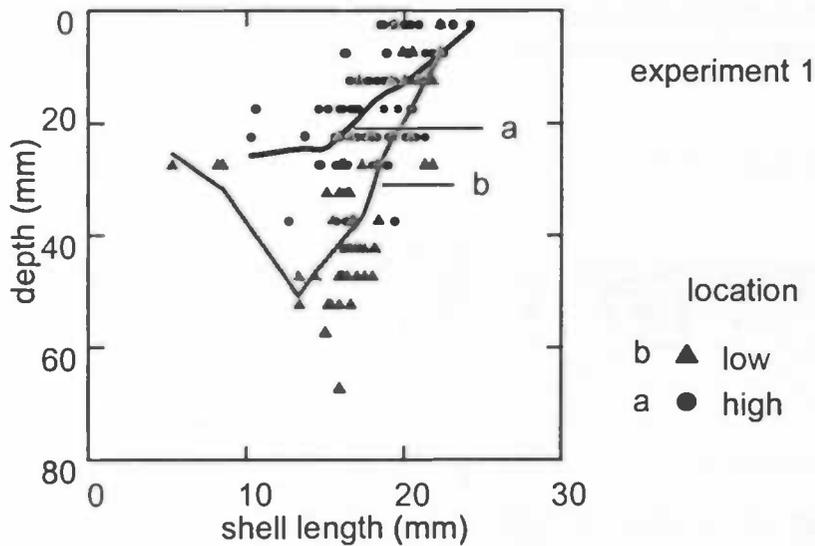


Figure 1-II: The depth distribution of the *Macoma* population at the two experimental locations taken a few days before the first experiment. Note the large difference in burying depth for the *Macoma* from different locations with shell length between 13 and 18 millimetre.

At the start of the experiment 10 cages were placed at both experimental locations (see appendix II). The cages were 50 by 50 by 25 centimetre, made of small gauze (mesh width = 1.2 cm) with a lid from coarser gauze (mesh width = 2.5 cm) (see figure 1-III).

The cages were anchored in the corners with wooden tent pegs to keep them at their place. Tent pegs were used instead of wooden pickets to prevent erosions around the corners. The cages were numbered and placed in a circle surrounded by a circle of big gauze (mesh width at least 5 cm) to prevent them from coverage with sea weed *Ulva spec.* The experimental *Macoma* were placed into the sediment of the cage after three days; this to let the sediment recover from the experimental set-up. However the sediment in and around the cages was still exposed to erosion and sedimentation; at low more than at high.

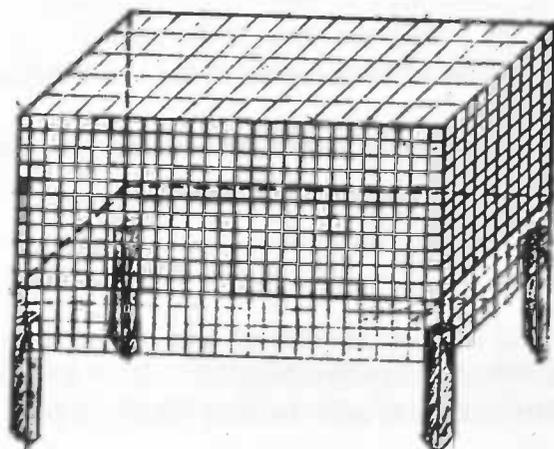


Figure 1-III: Design of the cages.

In the days before the experiment started the experiment clams within the size-range of 15 till 19 millimetre were collected at each experimental location; they were collected with aid of a sampling tube and a sieve. The shell size was measured to the nearest millimetre and the clams were subdivided in size classes (for example; size class 15 = 14.5 mm up to and including 15.4 mm). The size range of shell length between 15 and 19 millimetre was used, as the difference in burying depth at the two locations was found to be largest for these sizes (see figure I-II). The clams of different size classes were evenly divided over the 20 cages, so that each cage contained 10 clams of each location.

Until the clams were used in the experiment they were maintained in a tray with a little seawater. The day before the clams were inserted into the sediment of the cages, they were labelled by the method Zwarts (1986) used; one end of a nylon thread of approximately 10 centimetre was attached to the right shell valve with a piece of tape and super glue gel. The thread was attached in such a way that it was perpendicular to the flat side of the posterior end of the shell (figure 1-IV). Particularly the right valve of the shell was taken, since *Macoma* hang slightly over to the left when buried into the sediment (field observations Pim Edelaar). At the other end of the thread a plastic label with a unique number was attached to be able to distinguish individuals.



In each cage 20 labelled *Macoma* were inserted into the sediment till the posterior side was just below the sediment surface; 10 of the clams coming from 'low' and the other ten from 'high'. The clams were inserted in four rows of five clams, divided among them with equal distance. The clams of different origin were alternated inserted. This was done to make sure that the clams of the two origins were spread equally over the surface of the cage and also to be able to find the labels back at the end of the experiment.

After a day one crab was put into 5 of the 10 cages per site; thus these cages (even numbered) became the crab enclosures. All crabs were of equal size (between 50 and 65 millimetre) to keep the variation in treatment as low as possible. Since male crab mainly prey upon bivalves, while female crab rather eat softer prey types as annelids (Scherer and Reise 1981), male crabs were used in the experiment (Blundon and Kennedy 1982; Elner and Hughes 1978; Scherer and Reise 1981). In the period prior to this day the crabs were collected and kept in a bucket. They were fed with crushed *Macoma* to get them used to this way of foraging.

Each day of the experiment the cages were made free of seaweed and the crabs were fed with 8 crushed *Macoma*; in the hope they would not prey upon the labelled *Macoma*.

At the end of the experiment the labelled *Macoma* were carefully pulled out of the sediment by taking the thread just at the sediment surface. The burying

depth was measured with a ruler to the nearest millimetre; from the flat side of the posterior end of the shell till the place where the thread was hold (figure 1-V).

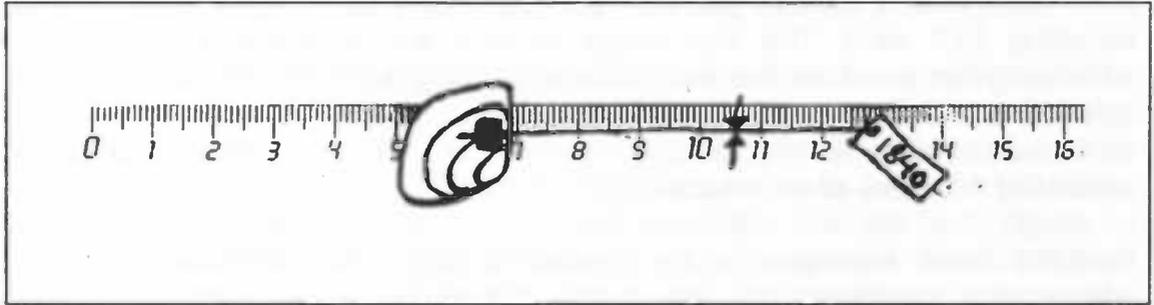


Figure 1-V: With a ruler the length (= burying depth) of the thread was measured from the clam till the point where the thread came out of the sediment.

### Experiment 1

The first experiment was started on Sunday the 16<sup>th</sup> of August 1998 by placing the cages. The clams were collected on the fourth and second day previous to the begin of the experiment. One day after the start of the experiment the crabs were put into the crab enclosures. The crabs were collected on the tidal flat in the period prior to this day and kept in a bucket. During the experiment the crabs were fed every day. Only on one day the crabs of the experiment at 'low' were not fed, since the water level was to high to be able to reach the experiment.

During the experiment the big gauze circle that was rushed upon tore 6 cages at 'low' (4 enclosures and 2 exclosures) loose. Since too few cages were left, this part of the experiment was not taken into account with the analysis. After eight days the burying depth of the clams of the first few cages at both locations was measured. Due to a thick silt layer that covered the sediment, the rest of the burying depth of the clams was measured two days later instead of the day after. Since not all labels of the experimental clams were visible, it was tried to find them by taking away a layer of sediment. Since the actual burying depth could not be measured, it was corrected for with the thickness of the removed sediment layer.

The shell length and age of the labelled clams were determined and the clams were screened on parasites.

### Experiment 2

The experiment was done a second time, started on the 5<sup>th</sup> of September. The experimental set-up was the same; only the way to carry it out had changed a little.

The cages at 'high' were already at their place and were not replaced. Only to make a hold to the sedimentation in the middle of the circle the sediment level

was repaired. However during the experiment the sediment was still exposed to erosion and sedimentation. The cages at 'low' were placed further away from the gully to make the place more controllable during periods of high water levels (see Appendix II), although also this time it was impossible to feed the crab every day during the experiment due to the water level.

After a few days to let the sediment recover from the experimental set-up, the experimental clams were inserted in the sediment. Twenty percent of the 164 clams in the first experiment were found to be dead; both natural and predation causes. It was thought that the mortality of the clams might be due to the long handling time of 4 days, before they were used in the experiment. To reduce the mortality, the clams were collected and labelled this time at the same day and inserted in the sediment the day after; keeping them overnight in the same way as before. Also to reduce the mortality of the clams during the experiment the clams were left to bury themselves deeper into the sediment for two days instead of one, before the crabs were placed into the cages. In the week before the crabs were collected in the gully with aid of a drag net and kept in a bucket.

During the experiment another measurement of the depth distribution of the total *Macoma* population was taken (figure 1-VI). The depth distribution of both *Macoma* populations at the two locations was almost the same.

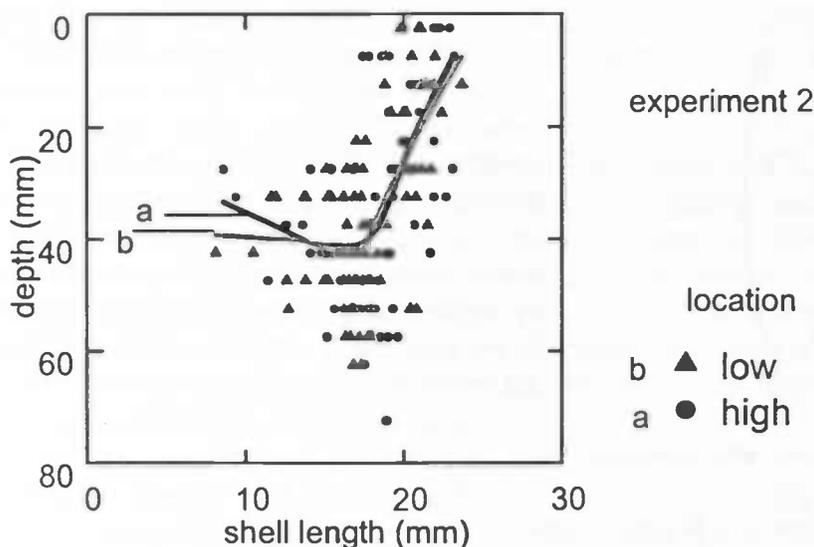


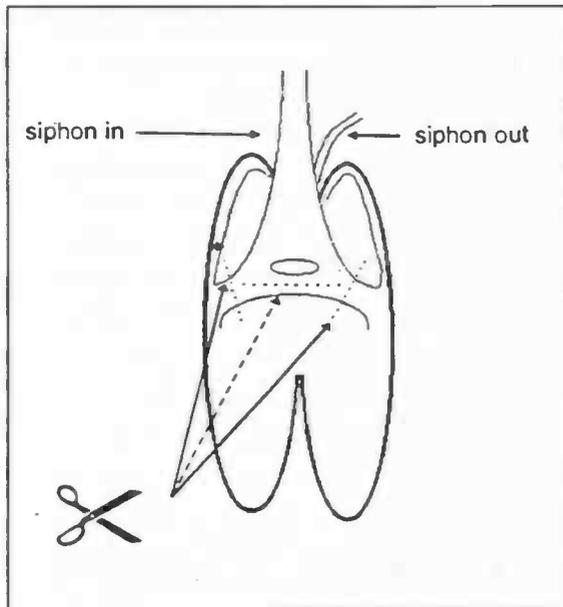
Figure 1-VI: The depth distribution of the *Macoma* population at the two experimental locations taken during the second experiment. Note that the differences that occur during the first sampling of the depth distribution have disappeared.

Unfortunately a storm on the ninth day of the experiment swept away the big gauze circle and one enclosure at 'high'. The experiment at 'high' was brought to an end after eleven days by taking out the labelled *Macoma* and measuring their depth. Since the sediment inside the cages of 'low' was heavily exposed to sedimentation due to the storm, the experiment went on for another eight days to let the sediment

recover from the disturbance; the big gauze circle was removed in the hope the sediment would recover sooner.

### Lab

All labelled clams of the second experiment were stored in a refrigerator to take them to the lab to measure the shell length to the nearest 0.01 millimetre -from the posterior end to the anterior end, to determine the age and to screen them on parasites. The flesh was removed from the shell, after the inhalant siphon was cut off to be weighted separately from the body (figure 1-VII). Siphon weight was measured instead of siphon length; the material of the siphon is rather elastic, which makes the length difficult to measure (Reading and McGrorty 1978).



The flesh of the body and the siphon were dried at 60 degrees Celsius for minimal three days before the dry weight, including the crucible, was determined. The samples were burned in a furnace at 550 degree Celsius for 5 hours to determine the ash weight, inclusive crucible. The ash-free dry weight (AFDW) was computed by subtracting the ash weight from the dry weight. For the total body dry weight, the dry- and ash weight was determined by subtracting the weight of the crucible. From the shells only the dry weight was determined.

Figure 1-VII: The inside of a *Macoma*, after opening of the shell valves. The place of incision to separate the siphon of the body is showed.

### Analysis

Before testing the data, the data from the lab and the field were merged and a few parameters were calculated. The condition, Body Mass Index (BMI) was calculated as the AFDW of the body (in mg) divided by the shell length (in cm) to the third power. The ratio ash weight - AFDW of the body was calculated as a measure for the foraging method -suspension- or deposit feeding. More

ash in the body weight means that the *Macoma* got more sediment particles inside; thus that the clam was a deposit feeder (Lin and Hines 1994).

Clams with parasites and with missing values in one of the variables were left out of the analysis. In experiment 2 only one clam was left in enclosure 1, also this case is not taken into account.

For both experiments it was tested whether depth was normally distributed and whether the division of *Macoma* over the experimental set-up was balanced by testing whether the clams of all cages had the same characteristics. This was to exclude the fact that other variables than predation presence, for instance siphon length (second experiment), determined the burying depth between the two treatments. The depth distribution was normal and also the clams were identical with respect to the shell length, the age, the condition (BMI), the AFDW of the siphon, the shell weight and the ratio ash weight- AFDW of the body of the clam.

In the crab enclosures it could occur that the crab had eaten *Macoma*. Starting from the conservative principle that the predator will prey upon the shallowest living *Macoma*, the same amount of clams of the crab enclosures with the shallowest burying depth were excluded from the statistical test to make up for all cracked clams. This was done for both experiments.

For the first experiment it was tested if the variables crab, origin and the interaction between the two were influenced the burying depth. It was tested with the mean burying depth of the 10 *Macoma* of the same origin per cage. Not the individual burying depth of the 10 *Macoma* per cage was used, since clams in the same cage were statistically dependent of each other.

In the second experiment other variables than origin and crab presence were taken into account, like the location where the clams were buried and the individual characteristics as shell length, age, condition (BMI), AFDW of the siphon and shell weight of a clam. The ratio ash weight- AFDW of the body was not taken in the analysis, but tested apart without the variable 'burying depth', since it could be that the burying depth of *Macoma* and the foraging method are correlated; when a clam buries deeper to avoid predation it can also decide to switch its way of foraging.

The siphon weight had no relation with shell length, contradictory to the findings of Zwarts and Wanink (1989). Therefore the data of siphon weight were not corrected.

All tests were done with the statistical program SYSTAT 7.0.1 (Wilkinson 1997).

## Results

## Experiment 1

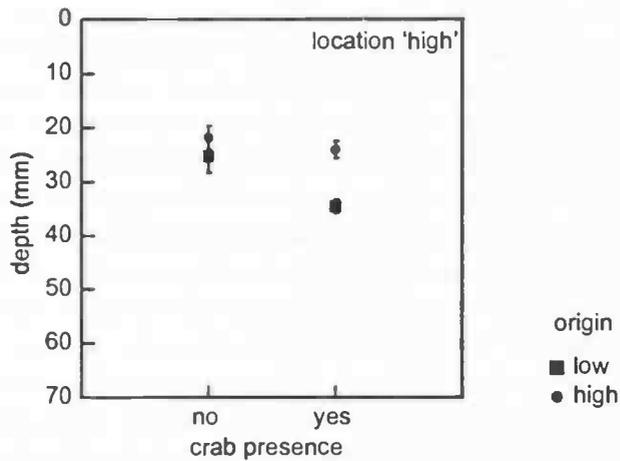


Figure 1-VIII: The difference in burying depth of *Macoma* of two different origin in and without the presence of a crab as result of the first experiment at location 'high'.

The clams in crab enclosures seem to bury deeper than those in the crab enclosures (figure 1-VIII); although this effect is not significant (table 1-III). Clams originated from 'low' are buried significant deeper than clams originating from 'high'. Although the interaction between crab presence and origin is not significant, it seems that clams originating from 'low' (near the gully) react sharper to the presence of a crab than clams from 'high'.

Table 1- III: Statistical output of the GLM test

N=20 R <sup>2</sup> = 0.424	Sum of Squares	Degrees of freedom	P-value
Regression	468.473	3	0.028
-origin	243.544	1	0.025
-crab	164.001	1	0.060
-interaction crab* origin	60.920	1	0.234
Error	636.437	16	-

Experiment 2

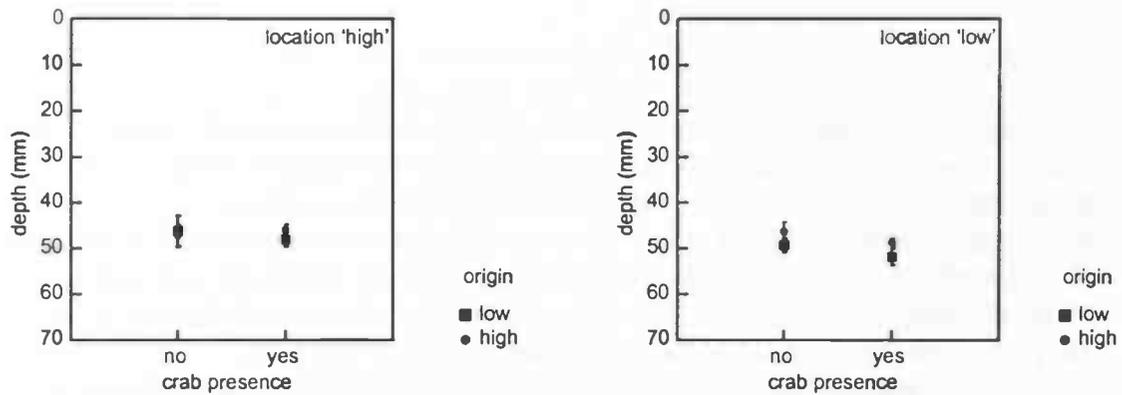


Figure 1-IX: The difference in burying depth of *Macoma* of two different origins in and without the presence of a crab, the second experiment at location 'high' and 'low'.

Figure 1-IX and table 1-IV shows that no variable can explain the variation in burying depth of the experimental clams. Only the origin of the clam seems to influence the burying depth; although not significant. The clams originating from low seem to bury deeper than those originating from high on the tidal flat do.

The other variables shown in table 1-IV have no effect upon the burying depth of *Macoma*.

Table 1-IV : Statistical output of the GLM test.

N=36 R <sup>2</sup> = 0.160	Sum of Squares	Degrees of freedom	P-value
Regression	150.960	9	0.823
-origin	40.736	1	0.134
-condition (BMI)	33.235	1	0.254
-shell weight	15.112	1	0.484
-crab	30.682	1	0.522
-location	13.393	1	0.568
-age	9.482	1	0.645
-AFDW of siphon	5.292	1	0.736
-shell length	5.010	1	0.765
-interaction crab* origin	0.062	1	0.786
Error	1092.636	27	-

## Discussion

### Experiment 1

Although not significant, crab presence seems to have an influence upon the burying depth of *Macoma*. In the presence of a crab *Macoma* bury deeper than when a crab is absent. They seem to know the danger of the presence of a crab and they seem to choose for a safer place; deeper in the sediment. To prove the effect of crab presence on the burying depth the experiment has to be repeated. Whether the density of predators has an influence upon the burying depth would be an interesting study for the future.

During the experiment the *Macoma* was fed with crushed *Macoma* in the hope they would not prey upon the labelled *Macoma*. Since the way on which *Macoma* observe the presence of danger is not known yet, it can not be excluded that the clams react on the presence of the crushed specimen, as Rawlings (1994) found for the gastropod *Nucella emarginata* and not on the crab presence. Therefore it must be concluded by this way of experimental set-up that *Macoma* seems to react to the combination of the presence of a crab and crushed *Macoma*. How *Macoma* notice the presence of the predator will be an interesting feature to look at.

The origin of a *Macoma* has proven to have an effect upon the burying depth. In the same environment the clams originated from the location near the gully ('low') bury deeper in the sediment than those clams originated from the location high on the tidal flat ('high'). Moreover it seems that the origin of a clam determine the strength of the reaction on the presence of the crab; although this interaction is not significant. The *Macoma* originating from the location near the gully seems to react stronger on the crab than the *Macoma* originating from the location high on the tidal flat. Can it be that the clams coming from the location near the gully with high crab abundance (Klein Breteler 1976) know the danger of predation better than the *Macoma* coming from a location where crabs are scarce, thus that the 'history' (the location where *Macoma* grows up) of a clam is important. Or is it that the differences in crab abundance at the two locations provide different selection pressures by which at the location near the gully only the deepest *Macoma* is selected. Whether the difference in burying depth by origin is caused by the 'history' of a clam or by the genetics of its parents is an interesting problem that need to be investigated. Since only data is available of the experiment at 'high', nothing can be concluded about the effect of environmental factors on the burying depth of *Macoma*. Thus from this experiment can be concluded that the origin of a clam is one factor that has an influence upon the burying depth, whether location and crab are of importance must still be examined by repeating the same experiment.

## Experiment 2

No variables can explain the small variation in burying depth that occurred during the second experiment. Only the origin of a clam seem to have an effect on the burying depth, corresponding with the results of the first experiment clams originating from the location near the gully seem to bury deeper in the sediment than those high on the tidal flat. A possible explanation for the small variation in burying depth in the different treatments of the experiment can be that the experiment is done too late in the season. The variation between the locations, which occurs during the spring and early summer, might disappear during winter; as the second depth sampling already indicated. Comparing the mean burying depth of the *Macoma* of the first experiment with those of the second experiment at only location 'high' (see figure 1-X), it is obvious that the clams - independent of origin- are buried deeper the second time; the clams of experiment 1 have a mean burying depth of 26.4 millimetre, while the clams of the second experiment at the same location have a mean burying depth of 46.9 millimetre. This is almost more than two centimetre.

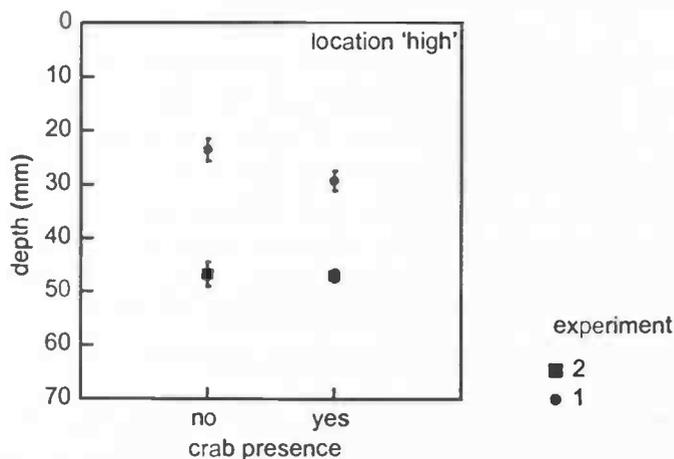


Figure 1-X: The difference in mean burying depth of *Macoma* in and without the presence of a crab; comparing the result of the first and second experiment at the location 'high'.

If predation risk indeed decreases with burying depth as Blundon and Kennedy (1982), Zwarts and Wanink (1989) have investigated, the *Macoma* in the second experiment are already safer from predation than those in the first experiment. Therefore it is possible that the clams in the second experiment do not react much on the presence of a crab. It will be interesting to do this experiment again at different periods of the year to investigate whether the reaction of clams on the presence of a crab is dependent on the time of year.

Till what depth the Shore crab digs for its prey, thus at what depth clams are save from predation by a crab is not known yet. It would still be interesting to quantify the predation risk in relation with burying depth. This can be done by observing what *Macoma* at what depth were eaten by the crab, when

inserting marked *Macoma* at a certain depth, not capable to bury differently than they are inserted.

Another outcome of experiment 2 (figure 1-XI and table 1-V) seems to be that the condition of a *Macoma* is dependent of both the locations on the tidal flat as the origin of the *Macoma*. *Macoma* of both origins seem to do better at the location near the gully than at the location high on the tidal flat. When the *Macoma* originating from the location near the gully are transplanted to the location high on the tidal flat, they seem to do worse; their condition declines. While *Macoma* originating from 'high' seems to do better when transplanted to the location near the gully; their condition increases possibly due to growth. A better food supply or food quality at this location might be a reason, therefore it would be interesting to qualify and quantify the food supply at both locations taking the time of foraging into account.

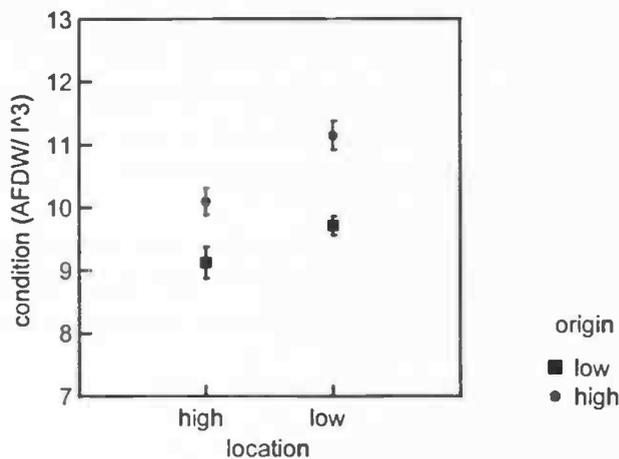


Figure 1-XI: Difference in condition of the clams at the two locations per origin.

Also the origin of the clam seems to be of importance. The clams originating from the location high on the tidal flat seem to have a better condition at both locations than those originating from the location near the gully. Could it be that the clams differ in foraging technique? Since deposit feeding seems to be the most profitable foraging technique comparing to suspension feeding (Zwarts 1986, De Goeij and Luttkhuizen 1998) this might be a possible explanation for the results found.

Table 1-V: Statistical output of the GLM test

N=36 R <sup>2</sup> = 0.798	Sum of squares	Coefficient	Degrees of freedom	P-value
Regression	36.472	-	5	0.000
-origin (high)	-	0.795	1	0.000
-location (high)	-	-0.274	1	0.008
-age	-	-2.490	1	0.000
-weight of shell	-	9.939	1	0.007
-ratio	-	-7.464	1	0.000
Error	9.222	-	30	-

Therefore the way of foraging of the experimental clams is investigated by looking at the ratio ash weight/ AFDW of the body. More ash in the body weight means that the *Macoma* got more sediment particles inside; thus that the clam is a deposit feeder (Lin and Hines 1994). The way of foraging (ratio) and the condition of a clam appear to be highly correlated.

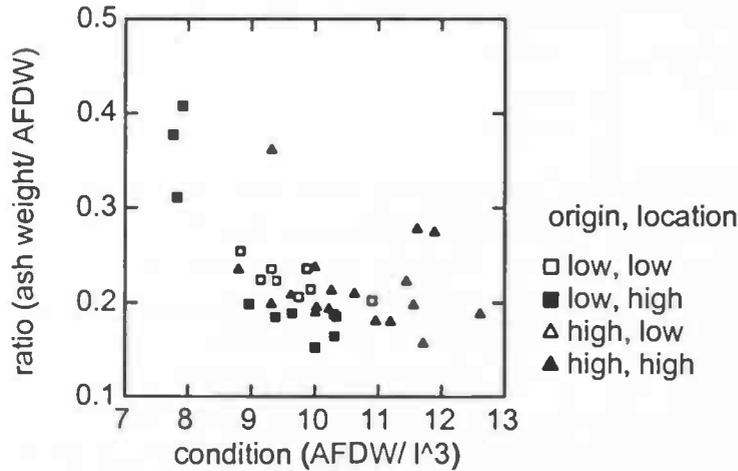


Figure 1-XII: The ratio ash/ AFDW of a clam is depending on its condition.

The clams originating from near the gully have a higher ratio than (more ash) the clams high on the tidal flat (see figure 1-XII and table 1-VI). However these clams are also in a worse condition. As the condition of a clam is correlated with ratio ash weight/ AFDW, there have to be corrected for condition. Comparing the clams of both origin with the same condition, the result seems to be that the clams originated from the location high on the tidal flat have a higher ash/AFDW ratio; thus are deposit feeders.

Table 1-VI: Statistical output of the GLM test

N=36 R <sup>2</sup> = 0.434	Sum of squares	Coefficient	Degrees of freedom	P-value
Regression	0.052	-	3	0.000
-origin (high)	-	0.022	1	0.030
-age	-	-0.104	1	0.012
-condition (BMI)	-	-0.043	1	0.000
Error	0.068	-	32	-

Zwarts and Wanink (1991) had the idea that *Macoma* in good condition can afford to bury deep in the sediment. The outcome of this experiment (figure 1-XIII) seems to correspond with this idea. *Macoma* in bad condition are buried shallower than those *Macoma* in better condition as. The effect of condition on the burying depth of *Macoma* seems to be important when the clam is in a bad condition; it levels off at a condition of about 10 AFDW / I<sup>3</sup>.

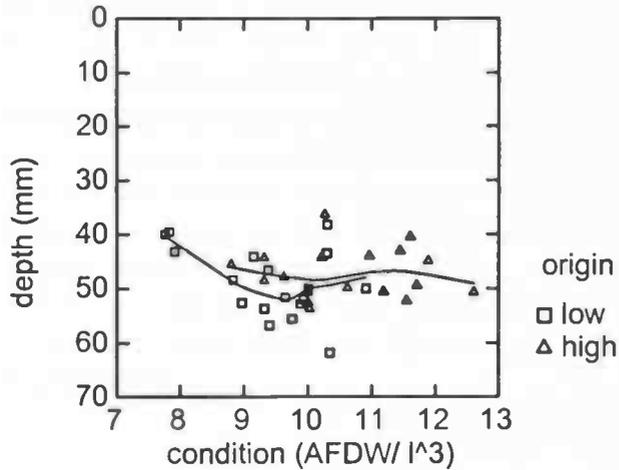


Figure 1-XIII: With increasing condition of *Macoma* the depth is increasing. At a certain condition the graph levels off.

Siphon length did not correlate with shell length although Zwarts and Wanink (1989) found it. This can be the result of both the small length ranges as the levelling off of this part of the graph.

During the first experiment the mortality of the clams was about 20 %. During the second experiment this decreases to 10 %. The improved treatment could be the reason, however there was no real proof for that.

## Description of the two locations and the two *Macoma* populations

The abiotic factors at both locations and the characteristics of both populations were investigated. This was to qualify the differences of the environment where the *Macoma* were living in and to see if the populations of the two locations were significantly different in their structure.

### Immersion time

The immersion time was determined, to investigate what the differences in feeding time of both populations at the two locations. The immersion time was important for the amount of food that can be obtained during the period of tide. Honkoop and Beukema (1997) have experimentally proven that with increasing immersion time the body mass is increasing. By noting the time when the locations were exposed or flooded again, the mean height of that location with regard to NAP could be calculated from data of the water level in Harlingen register by measuring-instruments of Rijkswaterstaat. Globally the mean period of exposure time was determined (by subtracting this number from 24 hours the mean immersion time was calculated for a normal day).

### Results

The location near the gully was located less high and will therefore be exposed for a shorter period of time. The location high on the tidal flat was exposed for a longer period (table 1-VII).

Table 1-VII: Mean exposure time per location

location	mean exposure time (hours)
high	5
low	1

With low water level due to spring tide and the weather, the immersion time will be shorter for both locations. By high water level the immersion time will increase. It could even occur that the location near the gully would not be exposed at all. Moreover during very bad weather it could occur that the whole tidal flat would not (or just for a very short period of time) be exposed. From the data of Rijkswaterstaat it was calculated that the location 'low' did not fall dry twice as much as the location high on the tidal flat.

### Discussion

The clams living near the gully have a longer time to forage than the clams living high on the tidal flat, since they have a longer immersion time. How long a *Macoma* spent its time on foraging and therefore will benefit from this longer forage time, still has to be investigated.

## Sediment

Quantitatively the differences between the both sediment structures were not determined. But globally can be said that the sediment near the gully has a lower silt/sand ratio (i.e. contains less silt and more sand) than the sediment high on the tidal flat. It is thought that *Macoma* in sandy environments mainly suspension feed as the sand is deprived of organic material, while *Macoma* deposit feed at a location with a high silt/sand ratio (Steur *et al.* 1996). Therefore it is thought that *Macoma* at the location near the gully will mainly suspension feed and the *Macoma* at the location high on the tidal flat will deposit feed.

## Population structure

### Method

At each location sediment cores were taken with aid of a tube and sieved. All clams were collected for measurement. The shell length was measured to the nearest millimetre and the age was determined.

### Analysis

In the analysis the Von Bertalanffy growth curve (SYSTAT, Wilkinson 1997) was used to calculate the parameters  $k$  (growth rate) and  $L_{\max}$  (maximum length of population) and their confidence interval, using the data of the length and age of the clams per location. The survival of a year class to the next year ( $l_x$ ) was calculated by dividing the number of a year class ( $n_x$ ) by the number of a year class younger ( $n_{x-1}$ ). The chance on survival to a certain age ( $a$ ) is achieved by the product of all  $l_x$  (with  $x$  smaller than  $a$ ). As no one year old *Macoma* for both populations and no two year old *Macoma* of the population near the gully were found, the change of survival is calculated starting from year class three.

Results

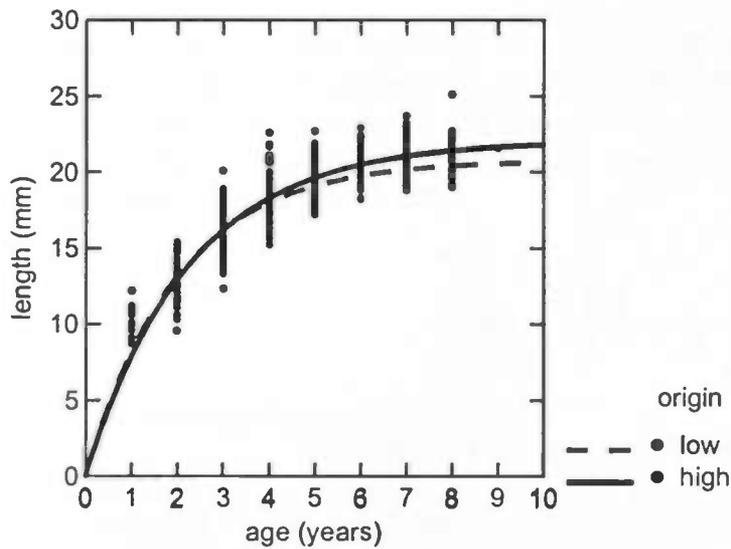


Figure 1-XIV: The growth curve of the *Macoma* population at both locations.

Table 1-VIII: Output of the Von Bertalanffy test

Origin	Parameters	< 95% >	
high	$l_{max}$	22.063	21.713 22.413
	k	0.440	0.420 0.459
	n	423	
low	$l_{max}$	20.786	20.343 21.230
	k	0.499	0.468 0.530
	n	295	

The growth rate and the maximum length of the populations were significantly different from each other, as can be seen in figure 1-XIV and table 1-VIII. The population high on the mudflat has a lower growth rate, but a higher maximum length than the *Macoma* population near the gully.

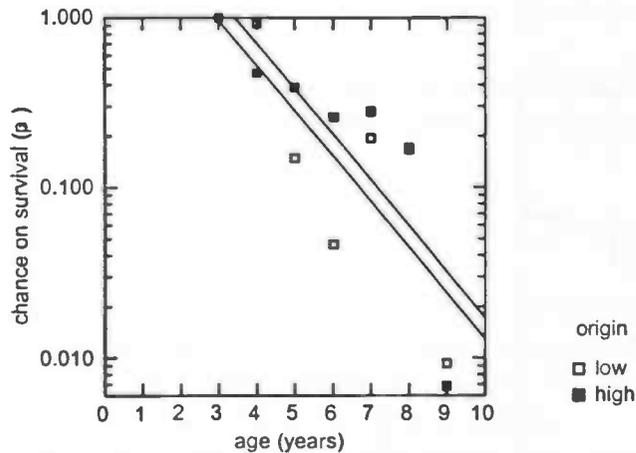


Figure 1-XV: Relative number of clams on log-scale per year.

The survival of a clam is neither dependent on the origin of the clam, nor on the interaction between the age and the origin of a clam, but it is dependent on the age of a clam; with increasing age the change of survival is decreasing (see both figure 1-XV and table 1-VIX).

Table 1-VIX: Statistical output of the GLM-test.

N=14 R <sup>2</sup> = 0.67	Sum of Squares	Degrees of freedom	P-value
Regression	21.606	3	0.008
-age	21.298	1	0.001
-origin	0.037	1	0.853
-interaction age* origin	0.000	1	0.987
Error	10.215	10	-

## Discussion

The *Macoma* population near the gully grows faster than the population high on the mudflat, but remain smaller. It might be for two reasons that they grow faster. The location is more profitable, which makes high growth rates possible or this is the life- history of this population due to predation. However there are no results from this research project that support one of these solutions.

The survival of a clam is neither dependent on the origin of the clam, nor on the interaction between the age and the origin of a clam. Thus there seem to be no difference between the chance on survival between the two locations. However it should be noted that the survival curve is only calculated out of data from one-year sampling. It would be better to calculate this from sampling data of more years, since the chance of survival can be variable.

The absent of the one and two year old *Macoma* can be due to 100 percent mortality, which is not likely or due to emigration from these small *Macoma* to another place.

## Density

From the same samples used for the calculation of the growth rate (see 'Population structure' in 'Description of the two locations and the two *Macoma* populations' (25)) the density of the clams at the locations was calculated by dividing the number of clams collected by the surface of the sampled area; surface one core ( $\pi r^2$ ) ( $r=7.5$  cm).

## Results

Table 1-VXI: Density of clams per location.

origin	number of clams	number of cores (surface = $176.7 \text{ cm}^2$ )	number of clams/ $\text{m}^2$
high	423	60	398.98
low	295	90	185.50

A high density is found at the location high on the tidal flat. This density is more than twice as high as the density from 'low' (table 1-VX).

## Discussion

The *Macoma* high on the tidal flat are living in a much higher density than the clams near the gully; even more than twice as high. Probably there is an advantaged of living there that so many *Macoma* do. Can it be that the trade-off between predation risk and food availability is favourable at this location?

## Distribution of the Shore crab on the tidal flat

Adult crabs were mainly living in the gully and only migrate higher on the tidal flat to forage (Klein Breteler 1976). Local crab densities were measured to confirm this statement.

### Method

Fishing for crab occurred over three parallel traces from the gully to high on the tidal flat (figure 1-XVI). In spite of the fact that the time of fishing was near high tide, the highest point of the tidal flat could not be reached, because of the low water level due to the spring tide.

At each sampling point a 100 metre long trace was taken with a width of the net of two metre; the sampling area was 200 square metre. At every sampling point the position (measured with the GPS) and the water level beneath the boat was written down to be able to estimate the depth of that point. Only crabs larger than 2 centimetre were collected, since smaller crabs will not prey upon large *Macoma*; they prefer *Macoma* of about 5.5 millimetre (Marijnissen 1998). The crabs were measured with a calliper to the nearest millimetre and sexed.

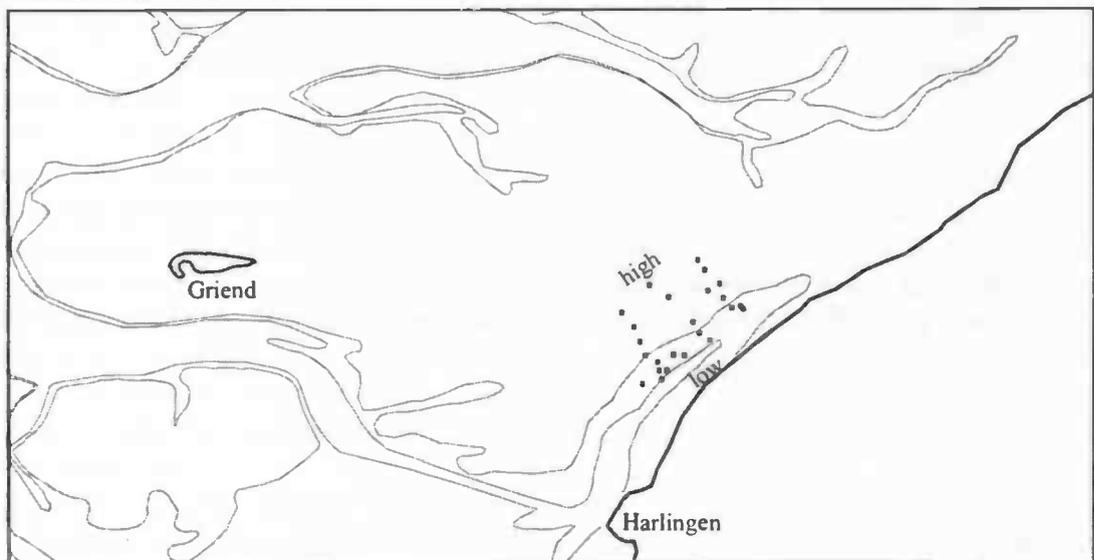


Figure 1-XVI: Location of the sampling points.

### Analysis

Although the depth beneath the boat was measured the exact depth could not be calculated, because no data was available about the exact time of sampling. Therefore the depth was estimated with aid of the contour lines in a map of the western Wadden Sea (Rijkswaterstaat). The density of crabs was not normally distributed, therefore the density was transformed with a square root transformation:

$$\text{Transformed depth } (X') = \sqrt{X} + \sqrt{X + 1}$$

With regression (SYSTAT, Wilkinson 1997) the relation between the transformed crab density and the estimated depth was determined.

Results

As figure 1-XVII shows, a positive relation exist between the density of crabs and the estimated depth (see also table 1-V). With increasing depth the number of crabs per 200 square metre is also increasing.

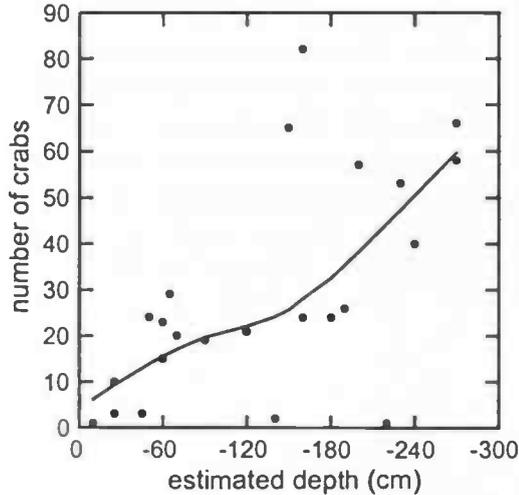


Figure 1-XVII: The number of crabs per 200 square metre. With increasing depth the number of crabs was increasing.

Table 1-XI: Statistical output of the regression test.

Regression:  $Y = -0.016962 * \text{estimated depth (X)} + 2.605324$

$$\text{crab density} = \sqrt{y} + \sqrt{y + 1}$$

	Sum of Squares	Degrees of Freedom	P-value
N = 23 R <sup>2</sup> = 0.344			
Regression	43.739	1	0.003
Error	83.511	21	-

Discussion

The density of crabs is positive related to the estimated depth. With increasing depth the density of crabs is increasing. Thus the predation risk shall be highest for the *Macoma* living near the gully, where a lot of crabs can be found. This is according to the results of Klein Breteler (1976), the assumption made in the experiment with crab presence is therefore correct. Even if the number of crabs would be equally divided over the tidal flat during high tide, the predation pressure would be highest near the gully, since the crabs can forage there longer due to the tidal movements (i.e. longer immersion time).

Part 2: The effect of avian predators on the burying depth of *Macoma*

## Method

To see whether *Macoma* reacts on the presence of shorebirds by burying deeper into the sediment, five bird exclosures were built at a place on the tidal flat where many shorebirds were observed the days before the experiment (see appendix II). The bird exclosures were made by placing four wooden pickets in a square (surface of about one square metre each) with a rope around them at the height of a bird. The five exclosures were placed near each other with a distance of circa twenty-five metres in between (figure 2-1).

On the location of the exclosures *Macoma* were collected. Specimens with shell length from 17.5 till 20.5 millimetres were selected and labelled (in the way described in 'The effect of an epibenthic predator on the burying depth of *Macoma* (part 1)'). The day after collecting them, they were inserted into the sediment, ten clams inside and ten clams outside the exclosure; about five metres away from the exclosures. The clams were inserted with their anterior end just below the sediment surface. During the experiment the reliability of the exclosures was checked by doing observations of birds' presence and of their foot prints inside the exclosures; no bird or indications of the presence of a bird was observed.

After ten days the clams were taken out and the depth was measured in the same way as in the crab predation experiment. Unfortunately not all *Macoma* were found back. This might be due to predation, but also due to sedimentation of the labels. During the first days of the experiment the number of labels visible per position was noted. The numbers of labels found each day did vary strongly.

## Lab

All labelled clams were stored in a refrigerator and taken to the lab to measure the shell length, the shell dry weight, the ash weight and the AFDW of the body and the AFDW of the siphon. Also the age of the clams was determined and they were screened on parasites.

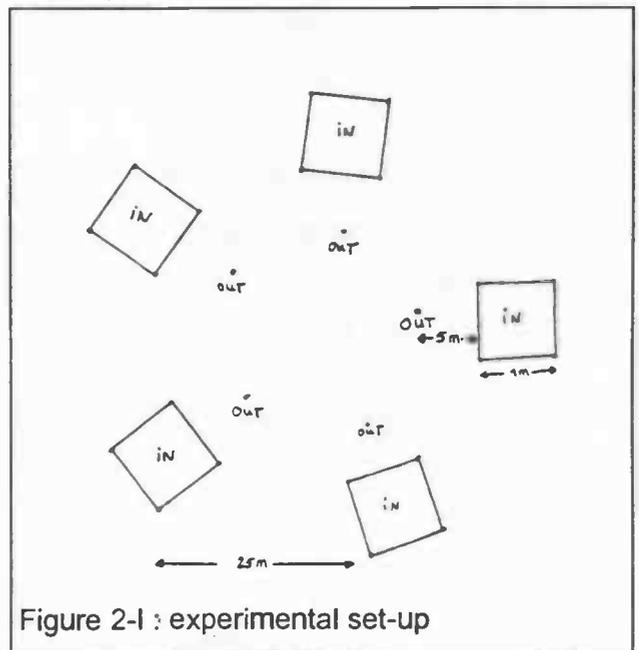


Figure 2-1 : experimental set-up

## Analysis

The data from the lab were merged with the data from the depth measurements in the field and a few parameters were calculated, like condition and ratio (in the same way as described in part 1). In this experiment depth was not normally distributed and corrected for with a square root transformation (see part 1: Distribution of the Shore crab on the tidal flat). Since the individuals inside and outside an enclosure are dependent, the mean depth per position was used in the test of variance (each enclosure two possibilities; in and out). Too few cases were left to test all variables within one model. Therefore all variables were tested separately to examine their influence on burying depth. Only the variables of influence (position (in/out), length, age, shell weight, AFDW of the siphon and condition (BMI)) were tested together against burying depth in the General Linear Model (SYSTAT 7.0.1, Wilkinson 1997).

## Results

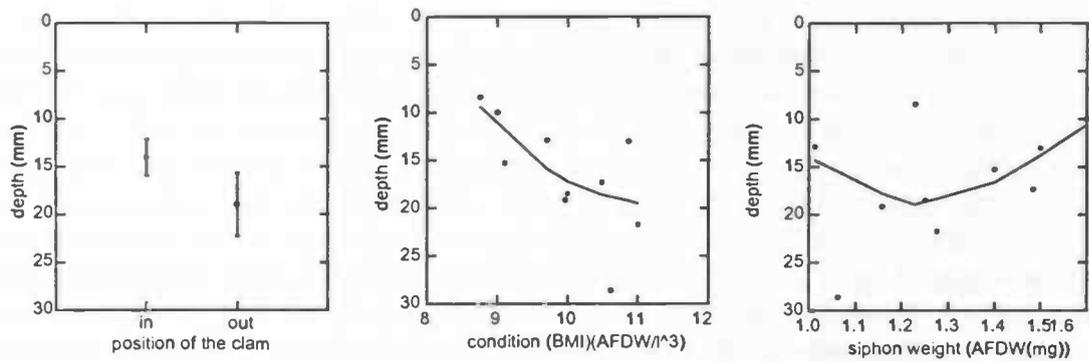


Figure 2-II: Depth against position of the clam (a), depth against condition (BMI) of the clam (b) and depth against siphon weight of the clam (c).

The position of the clam (figure 2-IIa), the condition (BMI) of the *Macoma* (figure 2-IIb) and the AFDW of the siphon (figure 2-IIc) turn out to have an influence upon burying depth, although the condition of a clam is the only variable with a significant effect (see table 2-1); with increasing condition the burying depth increases. Although the effect of position (in or out an enclosure) is not significant, the position of *Macoma* seems to have an effect upon burying depth; outside the enclosure the *Macoma* seems to bury deeper into the sediment than inside the enclosure. Against the expectation the siphon weight of a clam seems to have a negative relation with burying depth.

Table 2-1: Statistical output of the GLM test

	Sum of Squares	Degrees of Freedom	P-value
N = 10 R <sup>2</sup> = 0.822			
Regression	14.829	3	0.012
-position	3.120	1	0.052
-AFDW of siphon	2.802	1	0.062
-condition (BMI)	8.205	1	0.008
Error	3.219	6	-

## Discussion

There seem to be an effect of the position, the condition (BMI) and the AFDW of the siphon of the clam on burying depth of *Macoma*; although the condition of a clam is the only variable with a significant effect. The clams that are positioned outside the exclosures, where avian predation could occur, tend to bury deeper in the sediment than the *Macoma* inside the enclosure. Thus the clams seem to react on the presence of avian predators by burying deeper into the sediment. Since not all clams are found back, outside less than inside the enclosure, it could be that birds have eaten away the shallowest living *Macoma* which means that the result of the experiment is not reliable. However the clams outside the enclosures tend to be larger than the clams inside. With the assumption that the clams of different length classes were equally divided in- and outside the enclosures, the birds have to have eaten away the smallest *Macoma*. Since the smallest clams live deeper in the sediment it could be concluded that, when no depletion have taken place the effect of predation by avian predators could have been even stronger than found with these data. Perhaps the depletion is not due to predation, but due to disappearance of the labels, because the clams are buried too deep. Therefore they could not be found. It is recommended that the experiment is done another time with another length range (not including the largest *Macoma* as they bury shallower) and to do the experiment again with more individuals per enclosure or/ and with more enclosures.

The condition of a clam has a positive relation with the burying depth. *Macoma* in good condition are buried deeper in the sediment than those in bad condition. De Goeij and Luttikhuisen (1998) have proven that with increasing depth the growth rate and the condition of the clam is decreasing. Thus the environmental conditions do not seem to be optimal there. Obviously the clams in good condition that are living deep can afford to live deep, as Zwartz and Wanink (1991) already thought. It could be that they have more to lose and therefore do not choose to take the risk of being eaten, but choose to live on lower intake rate.

Also a relation between the burying depth and the siphon weight of a clam occurs. Looking at individuals the relation between siphon weight and burying depth is positive. This is also expected; with increasing depth the siphon length has to increase and with it the siphon weight. However working with the mean data per enclosure there seems to be a negative effect. This might be due to the small data set, by which means that can be concluded that the experiment have to be repeated with more individuals or due to the fact that siphon weight might be related with shell length; then there need to be corrected for.

Part 3: The effect of clam density upon the burying depth of *Macoma*

The effect of population density on burying depth is examined by measuring the burying depth from *Macoma* living under different densities.

Method

Eleven plots of 0.25 square metre (50 by 50 cm) were used. The plots (see appendix II for the location), together with a border of approximately 10 centimetre around them, were made free of all bivalves and valves of dead individuals larger than 4 millimetre, by sieving the sediment over a sieve with a mesh width of (at the most) 4 millimetre (see figure 3-1). The living *Macoma* found in the plots, were collected and their shell length was measured.

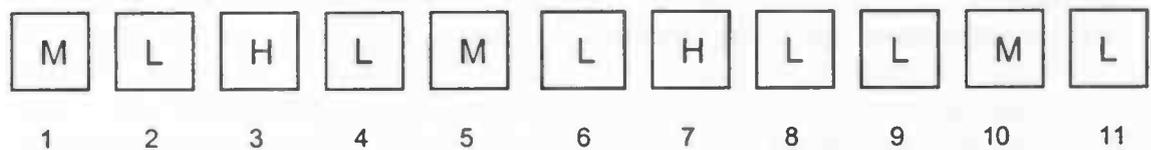


Figure 3-1: Distribution of the density categories over the eleven plots

Length classes were made (example size class 17;  $16.5 \leq 17 < 17.5$ ) and 5 of these length classes within the size range from 15 till 21 millimetre were used (15, 16, 17, 18 and 21). The length classes 19 and 20 were not used, because of the lack of enough individuals. In length classes 15 and 21 a few individuals of respectively length classes 14 and 22 were included, to have enough individuals. The number of individuals were equally distributed over the several length classes.

The tide after collecting them, the clams were inserted into the sediment in three different density levels; six plots with 15 clams (low density), three plots with 60 clams (medium density) and two plots with 240 clams (high density). This represents densities of respectively 60, 240 and 960 *Macoma* per square metre. The different densities were randomly divided over the 11 plots. The *Macoma* of the plots with low and medium density were inserted randomly into the sediment with their anterior end just below the sediment surface, while *Macoma* of the largest density were spread over the plot. For the plots with low density, only 3 size classes were used (15, 18 and 21). In this way 5 individuals per length class were used instead of 3 individuals per length class. This higher amount of replica per size class made the test statistically stronger.

After 9 days a begin was made of measuring the burying depth of the *Macoma* in each plot (see for method appendix I). It took three days to measure them all. The depth of a *Macoma* was measured to the nearest millimetre from the shell top to the sediment surface. The *Macoma* are collected in depth categories of 0.5 centimetre. On both sides of the clam the depth in millimetre was written down. Not all clams were found back.

The clams were stored in the refrigerator and taken to the laboratory. There the shell length and the age of the clam was determined. If the inscription on the shell valves could be read, this accurate depth in millimetre was used. Otherwise the depth category in 0.5 centimetre was used. This occurred just one time.

### Analysis

Although the final density was not identical to the initial density, the density categories could still be categorized as low, medium and high (table 3-1). Per density category and per length class the mean depth was calculated. The depth distribution of the clams was normal, therefore a transformation was unnecessary. With a General Linear Model (SYSTAT 7.0.1, Wilkinson 1997) it was tested whether depth was depended on density category and on length class. The interaction could not be tested, because of a lack of degrees of freedom due to the number of data.

Table 3-1: Initial and final density of the different plots divided over the three density categories

density category 'low'		density category 'medium'		density category 'high'	
initial density	final density	initial density	final density	initial density	final density
15	11	60	27	240	99
"	12	"	30	"	125
"	12	"	37		
"	13				
"	16				
	20				

## Results

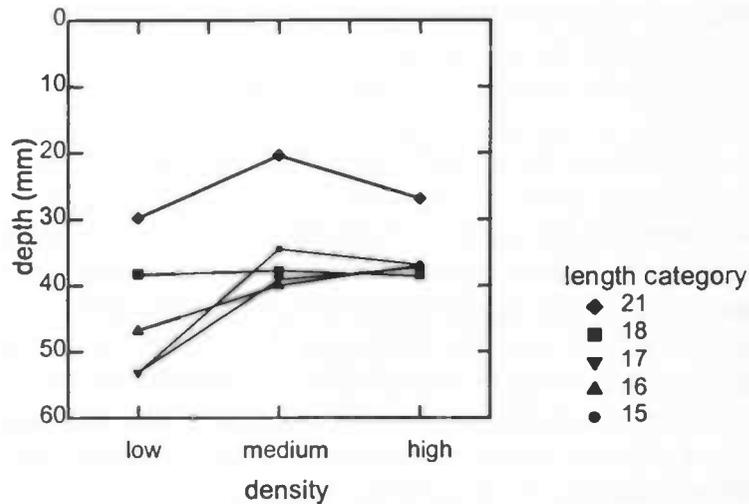


Figure 3-II: The burying depth of *Macoma* per length class and density category.

Both table 3-II and figure 3-II show that both density category and length class turn out to have an influence upon burying depth. There is no linear relation between burying depth and density. With low density the clams are buried deeper in the sediment than with the density levels medium and high. Difference in burying depth between medium and high density do not seem that strong. Length class has an influence upon burying depth as well; the largest *Macoma* (length class = 21 mm) are buried shallower than the other length classes. The three smallest length classes (15, 16 and 17) look rather alike and are buried deepest, while length class of clams of 18 millimetre are in between.

Table 3-II: Statistical output of the GLM test

	Sum of Squares	Degrees of Freedom	P-value
N = 15 R <sup>2</sup> = 0.852			
Regression	901.772	6	0.006
-density category	297.692	2	0.014
-length category	604.081	4	0.008
Error	157.180	8	-

## Discussion

Density turns out to have an influence upon burying depth. At low density the clams live deeper in the sediment, than at density category medium and high. If this is due to food competition (Lin and Hines 1994) or due to the decrease of predation risk per capita cannot be concluded from this experiment. By looking at the ratio ash weight - AFDW of the clam it might be possible to split up the food component from the predation component: a deposit feeding *Macoma* is expected to have a higher percentage of ash weight in regard with a suspension feeding *Macoma* due to the intake of more sediment particles (Lin and Hines 1994). However in this experiment the ratio (ash weight - AFDW of the clam) is not measured, therefore nothing can be said about the food component. This should be examined in further experiments. Even as the suggestion that the change in predation risk per capita can lead to shallower living clams. Knowing that birds forage on patches with high density of clams (Charnov 1976), the range of density using in the experiment have to be dependent on this density.

Length classes have an influence upon burying depth. The smallest three length classes are buried deepest in the sediment. With length classes 18 and 21 the burying depth is decreasing. As seen in the results of earlier field observation (Pim Edelaar) and other experiments during this project the burying depth of *Macoma* decreases from a certain shell length. This is approximately the length of 18 millimetre. The reason for this is a interesting feature for further research.

Although the interaction is not tested it seems that length categories react differently on different density levels. All *Macoma* react strongly, except the clams with a shell length of 18 millimetre; they do not react at all. It will be interesting to look at the effect of density on the burying depth of *Macoma* in more detail (looking at the condition (BMI) of the clam and its foraging behavior (ratio ash weight/ AFDW), using smaller length classes, to be able to understand more of the results of this experiment.

Part 4: Depth distribution of *Macoma* during the season for four different locations on the tidal flat

It is known that the burying depth of *Macoma* changes during season (Reading and McGrorty 1987). In winter the clams live deep. In spring the clams go up in the sediment and bury deep again at the end of the summer (about September/ October). The depth distribution of a *Macoma* population at a location on the tidal flat will be different between two sampling periods during the season. But do the clams of different locations on the tidal flat differ in their reaction to the season? And is the burying depth at the end of the season independently of the depth during the summer?

#### Method

Four sample areas were selected for their location (high, medium or low) on the tidal flat (see appendix II). During the field period (end of July till the end of September) samples were taken two times with an interval of about a month. First sampling took place on the 22 of August and the second on the 24 of September. Per sampling the depth distribution (as described in appendix I) of about 80 *Macoma* of the population was measured. About the same number of burying depth of clams were measured each time to make the variation less, which makes the statistical test stronger. The shell length of the collected clams was measured and the age of them was determined.

#### Analysis

With a paired t-test it was tested whether the depth distribution of the second measurement changed in respect to the first measurement. With a GLM - model (SYSTAT 7.0.1, Wilkinson 1997) it was tested whether the depth changes between the final and initial depth could be explained by length class, the initial depth (depth measured the first time) and their interaction. Length classes were used to see whether clams of different shell length react otherwise on change in time; four length classes were used 15 ( $\leq 15$ ), 16.25 ( $15 < x \leq 17.5$ ), 18.75 ( $17.5 < x \leq 20$ ), 20 ( $x > 20$ ).

Results

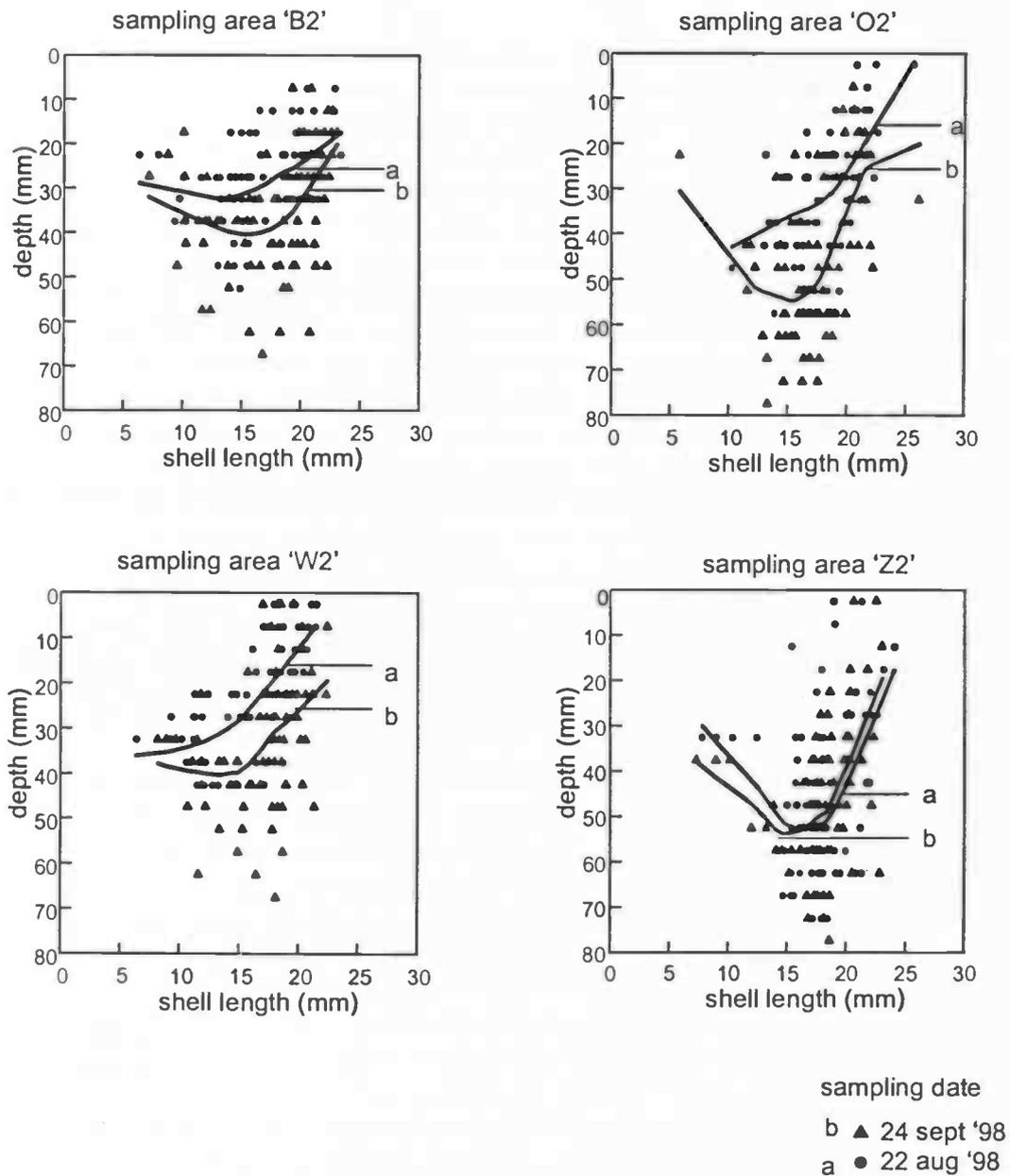
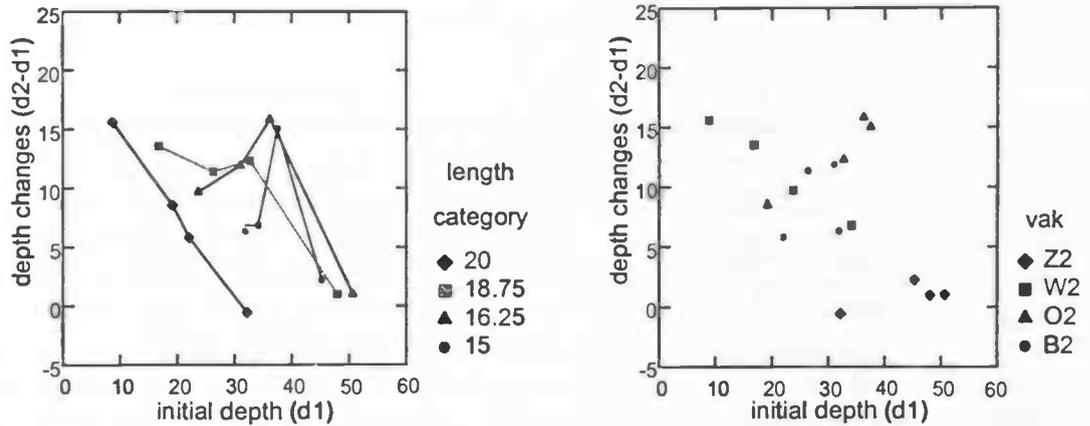


Figure 4-1: Depth distribution of the *Macoma* population at the four locations on the tidal flat. Two samples data, one on 22 August and the other on 24 September were taken during the season.

The second depth measurement differed significantly from the first depth measurement ( $P$ -value = 0.000;  $df = 15$ ), if data of all locations are taken together. Looking per location, at 3 of the 4 locations the clams bury deeper in the sediment the second measurement. Only at location 'Z2' clams do not bury significantly deeper (figure 4-1).



d1= sampling on 22 August '98  
 d2= sampling on 24 September '98

Figure 4-II: Depth changes between the two sampling data related to the initial depth per length class and the location

The initial depth and the interaction between initial depth and length class significantly help to explain the differences in depth, although only initial depth is highly significant (table 4-I and figure 4-II). The depth difference has a negative relation with initial depth. With increasing initial depth, the depth difference between final and initial depth is decreasing.

Table 4-I: Statistical output of the GLM test.

	Sum of Squares	Degrees of Freedom	P-value
N = 16 R <sup>2</sup> = 0.641			
Regression	292.270	4	0.016
-initial depth	275.839	1	0.001
-initial depth * length category	152.458	3	0.057
Error	164.003	11	-

## Discussion

Correspondingly to the findings of Reading and McGrorty (1978) and Zwarts and Wanink (1989) clams react on change in time by burying deeper at the end of the growth season. The clams of three of the four locations are burying deeper in the sediment at the end of the summer. Only the clams that are buried near the gully do not bury significantly deeper, although they seem to bury a tiny bit deeper. That clams bury deeper in the sediment at the end of the summer is caused by a change in food availability; the pelagic algae and benthic diatoms are becoming less (Cadée and Hegeman 1977), by which the trade-off between predation and starvation changes, causing the change in burying depth. However the change in burying depth is not for all clams equally, both initial depth and the interaction between initial depth and length class have their influence upon the depth changes between final and initial depth. The initial depth is negatively related with depth changes. The clams that are buried shallow high on the tidal flat have a large change between the initial and final depth, while the clams near the gully that are already living deep during the summer do not significantly change their burying depth. It could be that these clams are already safe from predation and therefore do not react. It is clear that because of the differences in reaction of the clams the differences in burying depth of *Macoma* populations between locations on the tidal flat diminished at the end of the season; the clams are more equally distributed.

The interaction between the initial depth and length class seems to have an effect upon the depth changes: the length classes seem to react differently at initial depth. The *Macoma* of length class 20 react in a clear negative relation, while the other length classes seem to have an optimum initial depth where they react strongest. More research needs to be done to confirm this and to understand this. Again the largest clams are buried shallowest.

## General discussion and conclusions

The main outcome of this research project is that predation risk of both Shore crabs and shorebirds seem to have an influence upon the burying depth of *Macoma*. Clams seem to bury deeper in the presence of a predator; they are probably safer from predation (Blundon and Kennedy 1982; Zwarts and Wanink 1989). However it is uncertain till what depth a crab can dig for its prey. This still needs to be investigated. If there is a trade-off, predation is certainly one of the important factors determining the burying depth of *Macoma*. When selection will favor deeper buried *Macoma*, they will possibly become less attractive as a prey, as they become more difficult to reach for the predators. With regard to evolution the predator has to react on this by either evolving better techniques to reach its prey or to switch to another more reachable prey.

However the results of this experiment in respect to the effect of predation presence upon the burying depth of *Macoma* are not significant. The second experiment is probably done too late in the season in the period that no or only a little difference occur in the burying depth of *Macoma* populations at different locations on the tidal flat. However with regard to that the first experiment is done at the right time in the season, but still the results are not significant. It could be that the presence of only one predator does not give a significant difference between the two treatments, but that more crabs present will have a stronger effect. With this experiment is only tested whether *Macoma* react on predator presence, but it would be more interesting to investigate what the relation is between the predation pressure and the burying depth. The relation can either be linear (with increasing crab density the clams are burying deeper) or with a threshold (a certain density of crabs will trigger the change in burying depth). Coherent with this is the question how *Macoma* perceives its predators; is this with chemical cues, mechanical cues, with a combination of both of with something different? And whether this technique to perceive predators can make the clams detect the density of the crabs?

The origin of a clam turns out to be an important factor that determines the burying depth of *Macoma*. *Macoma* originating from the gully, where crab is more abundant, bury deeper than those originating from high on the tidal flat where the crab density is low, irrespectively of where they are transplanted both high and low on the tidal flat. It would be interesting to look if this difference in burying depth can be inherited by their offspring.

As *Macoma* are able to react on change in density, change in season and predator presence, they seem to have phenotypic plasticity. However the fact that (in the experiment with crab as predator) the origin of a clam has an effect upon the burying depth, seems as a fact that they are not boundlessly plastic, but are plastic to a certain extent. By investigating whether these clams bury at a different depth at another location than their original location, it is possible to conclude something about the effect of environment on the burying depth. However in this research project it is not possible to conclude something about the effect of environmental factors upon the burying depth of *Macoma*, as no information is available. During the first experiment with the

Shore crab as predator the data of the experimental location near the gully are not available as most of the enclosures are torn loose. During the second experiment there is too little variation in burying depth to be explained by any factor. Therefore it would be interesting to test the effect of location upon the burying depth of *Macoma* again, as it is certain now that the two locations are different in environmental factors, immersion time and sediment. The sediment of the location near the gully has a lower silt/sand ratio, by which suspension feeding will be favorable here (Steur *et al.* 1996). According to Zwartz (1986) and De Goeij and Luttkhuizen (1998) this way of foraging is less profitable than deposit feeding and therefore this location will be less preferable. However the *Macoma* living there are able to survive. The longer immersion time, and therefore forage time, could mean that the less profitable way of foraging is sufficient enough to meet the metabolic requirements. The results of the experiment with the crab as predators seem to support this, as both clam populations, independent of their origin, seem to do better at the location near the. Whether this is a result of the longer immersion time or a differences in food abundance or food quality is a interesting feature to investigate. Even as the time the clams utilize to forage.

The clam population originating from high on the tidal flat transplanted to the location near the gully are in a better condition then those originating from the location near the gully. When corrected for condition the clams from high which are likely to deposit feed at their location of origin seem still to deposit feed at this location, while the clams originated from the gully are suspension feeders. This might suggest that the switch between the way of foraging is not that flexible; originally deposit feeders remain deposit feeders. In regard to the research suggestion about the forage time at both location, it will be interesting to investigate whether the clams of different origin at the same location have a different forage time.

According to the conditions of both clam populations at the location near the gully, this location will be much preferred. However the density of *Macoma* is here twice as low as at the location high on the tidal flat. Why do these clams prefer to live there? As it is not the food component, is it then the predation component of the trade-off that make them decide to live there? Crab density is highest near the gully and decreases with decreasing depth. Thus crab predation will be less high on the tidal flat. Therefore the choice of these clams to live this high on the tidal flat is understandable. However crabs are not the only predators, there are also avian predators, which begin to forage high on the tidal flat and follow the tidal movement. Could it be that the predation pressure by crabs and shorebirds are not equally weighted? That the 'right' location for *Macoma* is more depending on the absence of crabs than shorebirds?

To understand the whole system around *Macoma* many questions has to be solved, as is clear from the suggestion about the working system described here. To summarize these questions:

- what is the relationship between the mortality of *Macoma* and the location on the tidal flat
- what is the relationship between the mortality of *Macoma* and the burying depth of *Macoma*
- what is the quantity of the predation pressure for different predators

- what is the quantity and quality the food resources at both locations
- what is the forage time of *Macoma*

The density of clams has an influence upon burying depth of *Macoma*. In a low density of clams the clams are buried deeper than in a medium or high density of clams. In the experiment with the Shore crab and the shorebirds, the sediment in the cages were not made free of other bivalves due to the large amount of work it would be. Thereby I unconsciously made the assumption that the densities in all cages were the same. However since the density of *Macoma* at the location high on the tidal flat is more than twice the density of *Macoma* near the gully, this would not be correct. It would therefore be interesting to test whether the effect of predation risk upon the burying depth is related to the density of the clams.

The time of the year is of importance to the burying depth of *Macoma*. Depending on the initial depth the *Macoma* bury a little or a great part deeper at the end of the summer. The shallow living *Macoma* bury much deeper than the *Macoma* that were already deep. Thus the differences in burying depth of *Macoma* of different locations on the tidal flat diminish at the end of the summer. This could also be the reason why no difference occurred in burying depth distribution of the second experiment with the crab as predator: those *Macoma* were already buried deep.

There is another question that will be interesting to solve: Why do large *Macoma* live buried shallow in the sediment? This is the outcome of all experiments and field observations on the burying depth of *Macoma*. Are they limited by their constraints (e.g. oxygen or food demand) or do they suffer less predation risk, and therefore dare to expose themselves better? Experiments manipulating oxygen or food, or predator presence might give answer to this.

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## Acknowledgement

### Fieldwork

Two months living primitively on a boat in a container of approximately ten square metre to live in (work, eat and sleep) with at the most three other people (Pim Edelaar, Fiona Smith and a volunteer), the tidalflat with its macrobenthos and shorebirds as work-ground and Harlingen as a backyard, is one great adventure I went through this summer at Ballastplaat. I like to thank all the people that made this possible. Specially the people who 'supplied' us with new volunteers (KNRM and water police), the fresh vegetables and bread that were always welcome and the beloved mail and newspaper (Ane Dekinga and the crew of the *Navicula Tony*, Johan and Kees and Jan van Dijk and Dirk Kuiper of the *Phoca*). Then the people of Griend for their company over the *Motorola*, for their function as oracle and their talk every morning during heavy-weather-periods. The people on *Richel* for their bird catching; I tried to see a colour banded Knot this summer, but I did not succeed unfortunately. My first one I saw during bird catching on Griend this winter. Specially the volunteers Arnoud van Peteren, Jeroen Reneerkens I like to thank for their company and Eelco Boss for his great help. And not to forget Theunis Piersma for his help with the experimental design and then my regular companions during these two months Pim Edelaar and Fiona Smith for their company and support.

### Lab work and analyse

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### Other activities

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## Appendix I: Measuring method of the burying depth of a *Macoma* population

To know more about the depth distribution of a *Macoma* population the burying depth of a part of the population is measured; the depth of more than 50 individuals to get a more reliable measurement.

The depth measurement is determined by scraping off thin vertical slices of a sediment core with aid of a knife or ruler, till a *Macoma* is encountered. The burying depth is measured to the nearest 0.5 centimetre with a ruler from the posterior end of a clam to the sediment surface.

From all the clams the shell length and age is determined, to be able to look at the burying depth of an individual of a certain size and age.

■ 1997  
□ 1996

