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## Growth of *Crassostrea gigas* and *Mytilus edulis* in the Oosterschelde estuary in relation to local food availability

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Intern Rapport nr. 06.005  
10 April 2006



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Internal report  
Number: 06-005

Growth of *Crassostrea gigas* and *Mytilus edulis* in the  
Oosterschelde estuary in relation to local food availability

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## **Abstract**

Disappointing growth of cultured mussels and oysters in previous years resulted in a reduced yield for mussel and oyster farmers. This is accounted to the increasing amount of wild Pacific oysters (*Crassostrea gigas*) and the reduction of primary production of phytoplankton. The aim of this study was measuring growth of sub littoral *C.gigas* and *M.edulis* in the four compartments of Oosterschelde estuary, in relation to local food availability. Oysters and mussels in cages were put out in four areas of the Oosterschelde estuary. Growth was followed over two periods (1: June- July 2: July- August). Growth of *C.gigas* was higher in period 2 compared to period 1. This is ascribed to spawning of the oysters in period 1. Growth of *M.edulis* was higher in period 1 compared to period 2. It is assumed that mussels have lost energy through spawning in period 2, shell length increased while flesh weight barely increased. In period 1 no relation was found between growth and food availability and growth was apparently not limited by food availability. In period 2, growth increased with an increasing concentration of chlorophyll-a and growth was limited by food availability. In the North compartment of the Oosterschelde estuary local circumstances limited growth in relation to food availability. High mortality and limited growth in relation to food availability are attributed to a combination of reduced salinity, low currents velocity and the possibility of unfavourable algae.

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## 1. Introduction

There are signs that the carrying capacity for shellfish of Oosterschelde estuary is exceeded. In previous years the growth of cultured mussels and oyster was disappointing resulting in a reduced yield for mussel and oyster farmers. This is accounted to the amount of wild Pacific oysters (*Crassostrea gigas*) and the reduction of primary production of phytoplankton (Kater, 2003).

The carrying capacity for an ecosystem is defined as the maximum standing stock that can be supported by a given ecosystem for a given time (Smaal 1998).

When growth is reduced by low food availability, food limitation takes place and filter feeding species can decrease in numbers or even disappear, which will affect the Oosterschelde food web. The combination of filtration time, turnover of phytoplankton and retention time of the water in the four compartments of the Oosterschelde estuary shows that the chance on food competition is the highest in the northern and eastern compartments (fig.1). *C.gigas* seems to handle these circumstances better than the *M.edulis*, which can lead to a replacement of other shellfish at low food availability such as the cockle and the mussel (Geurts van Kessel, 2003).

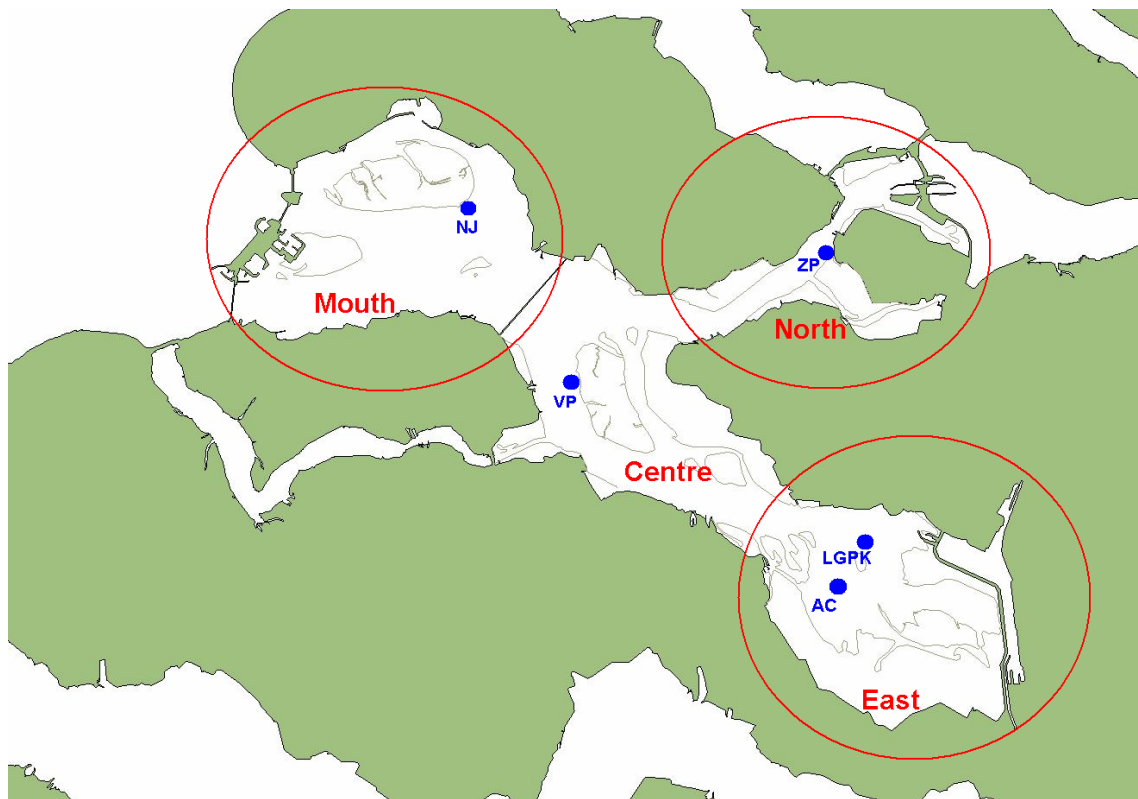


Figure 1 Measurement locations Oosterschelde estuary

The Centre for Shellfish research (CSO), located in Yerseke, specializes in the research of ecological durable exploitation of shellfish cultivated areas in de Dutch coast waters.

A research topic is the (changing) carrying capacity of the Oosterschelde. An important research part is the influence of *C.gigas* to the changing carrying capacity for mussels (*Mytilus edulis*).

The aim of this study is to follow growth of sub littoral *C.gigas* and *M.edulis* in the four compartments of Oosterschelde estuary, in relation to local food availability. The main research question was: In how far growth of *C.gigas* and *M.edulis* limited in the different compartments of Oosterschelde estuary because of food availability?

The main question is divided into sub questions:

- *Is growth of C.gigas and M.edulis different between the compartments of the Oosterschelde estuary?*
- *Is there a relationship between food availability and growth of C.gigas and M.edulis?*

### 1.1. Oosterschelde estuary

In the Oosterschelde estuary the most common shellfish are blue mussels, cockles (*Cerastoderma edule*) and Pacific Oysters. Originally the flat oyster *Ostrea edulis* was cultivated in the Oosterschelde estuary.

In the severe winter of 1962-1963 the amount of cultured *O. edulis* was reduced from 12 to 4 million oysters. After this high mortality, the oyster farmers imported French flat oysters and searched for alternatives. They introduced *Crassostrea gigas* from British Columbia in 1964 in the Oosterschelde estuary. The *C. gigas* cultivation would have a temporarily character until the dam was built to protect the land from floods (Drinkwaard, 1999). The Oosterschelde estuary would then be a fresh water system in which *C. gigas* would not be able to live. But the plans were changed at the end of the seventies. They built a Storm Surge Barrier, which left the estuary open and stayed a saltwater tide system. *O. edulis* died away because of a parasite *Bonamia ostreae* imported from France and *C. gigas* was not infected. (Nienhuis & Smaal, 1994)

The assumption that *C. gigas* could not reproduce in our cold waters appeared to be incorrect. In the summer of 1976 the temperature reached above 20 degrees for a period of fifties days at the benefit of *C. gigas*. That year was the first breakout of the oyster larvae (Drinkwaard, 1999). There was a good development and settling of the larvae on rocky shores. At the end of that year the import of *C. gigas* became forbidden. In 1982, another breakout of larvae followed and *Crassostrea gigas* was settled permanent in the Oosterschelde estuary (Kater & Baars, 2003).

Over time littoral *C. gigas* strongly developed in the Oosterschelde estuary from a total cover of 15 hectare in 1980 to 800 hectares in 2005 (fig.2). The sub littoral area covered by *C. gigas* is not quantified in detail. The development is believed to be the same as littoral *C. gigas*. (Kater & Baars 2002, unpublished data RIVO-CSO)

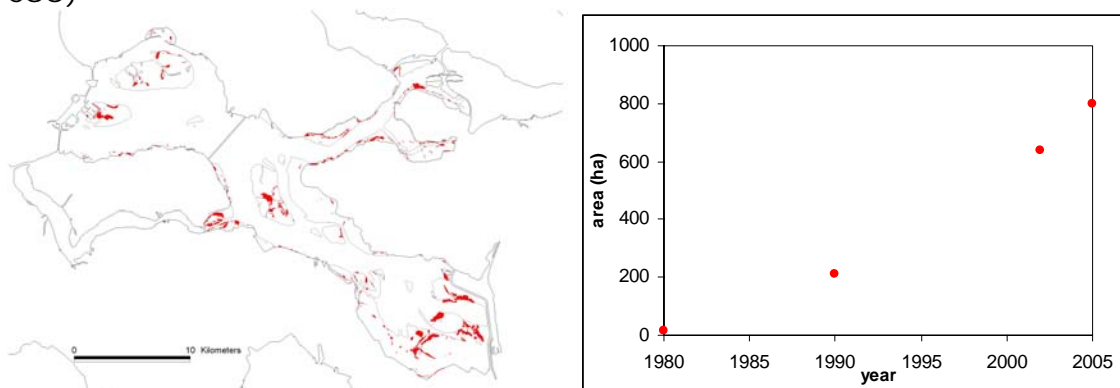


Figure 2: Littoral area cover of *C. gigas* in Oosterschelde estuary 2005 graph: Littoral areas cover *C. gigas* from 1980 until 2005



## 1.2. Commercial bivalves

### 1.2.1. *Crassostrea gigas* (Pacific oyster)

The scientific name of the Japanese Oyster is *Crassostrea gigas* and is classified as described in figure 3 (Thunberg, 1793). The natural habitat of *C. gigas* is an open shore ecosystem, rocky coasts. Oysters are often found on hard surfaces like rocks, other shellfish, but they also appear in sandy and muddy bottoms where small shells or stones serve as substrate. Oysters live in deep littoral and sub littoral areas. The maximum depth differs between locations (Reise 1998, Dupuy 1999).

The oyster can grow to 30 cm in length and can reach an age of 20 years. In adults the bottom shell is curved and often deeper than the top shell. The surfaces of the thick shells are very irregular, rough with sharp edges (Reise, 1998). Suitable water temperatures for *C. gigas* are between 11 and 34 °C. Lethal temperatures are below -4°C and above 43°C (Mann, 1998).


Phylum	<i>Mollusca</i>	
Class	<i>Pelecypoda</i> or <i>Bivalvia</i>	
Order	<i>Lamellibranchia</i>	
Family	<i>Filibranchia</i>	
Genus	<i>Crassostrea</i>	
Species	<i>Gigas</i>	

Figure 3 Classification *C. gigas*. Photo Jasse Snee

*C. gigas* has a growth cycle of a year in our climate zone from April until October with a maximum in June (Walne & Mann 1975, Walne & Spencer, 1975). From November until March *C. gigas* does not grow and could lose weight (Walne & Mann, 1975).

A permanent population of *C. gigas* can affect the ecology of an area. *C. gigas* settles sub littoral and littoral and can disrupt or eliminate the habitat of other endemic species. Natural enemies are starfish, crabs, lobsters and birds (Shatkin, 1997). In the Oosterschelde estuary the predation on *C. gigas* is low; because shape and size of *C. gigas* are not always attractive they prefer small oysters or other shellfish (Kater, 2003 review). *Polydora* worms are parasites of shellfish and are often seen in the Oosterschelde estuary (Engelsma & Haenen, 2002). More than 30 *Polydora* worms can be found in one oyster, but the parasite does not influence the mortality of *C. gigas* (Caceres-Martinez 1998). Sea squirts are also found in the Oosterschelde estuary, especially the colonial Tunicate *Didemnum lahillei* can overgrow the whole oyster, leading to suffocation of the oyster (De Kluijver & Dubbeldam).

### 1.2.2. *Mytilus edulis* (blue mussel)

The scientific name of the blue mussel is *Mytilus edulis* and is classified as described in figure 4. *M. edulis* is widely distributed throughout the cooler waters of both the northern and southern hemispheres. *M. edulis* has abilities to withstand wide fluctuations in salinity, desiccation, temperature and oxygen tension. (Dame, 1996) Mussels have two shell valves similar in size and are roughly triangular in shape. (Gosling 2002)

In the intertidal zone the blue mussel has a blue black and heavy shell, while in the sub littoral region, where mussels continuously are submerged, the shell is thin and brown with dark brown to purple radial markings (Sidall 1980 in Gosling 2002).


Phylum	<i>Mollusca</i>	
Class	<i>Pelecypoda</i> or <i>Bivalvia</i>	
Order	<i>Lamellibranchia</i>	
Family	<i>Filibranchia</i>	
Genus	<i>Mytilus</i>	
Species	<i>edulis</i>	

Figure 4: Classification of Medullas. Photo: Alfred Wegener Institut

Growth rate of *M.edulis* varies according to size, age and environmental conditions. Even mussels with similar size and age grown under identical conditions can exhibit widely different rates and it is known that growth variation is partially determined by genotype (Dame, 1996). The shell growth is rapid during the spring and summer and slows down over the winter, while flesh weight is subjected to seasonal fluctuations associated with the reproductive cycle (Kautsky 1982 in Gosling 2002).

#### 1.2.3. Bivalve feeding & life cycle

*C.gigas* and *M.edulis* use the gills for feeding. This method of feeding is called suspension or filter feeding because the gills with their differential ciliary tracts remove suspended particles from the water pumped through the mantle cavity. The gills divide the mantle cavity into inhalant and exhalent chambers. The water that enters through the inhalant opening or siphon is driven from the inhalant to the exhalent chamber by cilia on the gills and mantle surface, and exits by the exhalent opening or siphon. Both openings have a muscular velum, the inner fold of the mantle, which regulates water flow through the mantle cavity (Gosling, 2002).

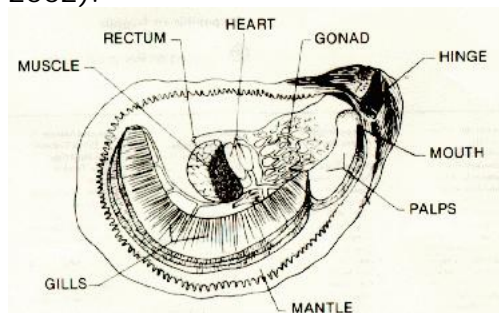


Figure 5: Anatomy oyster.

Bivalve filter feeders feed on particulate organic material from the water column. Phytoplankton in the water is one of the main food sources, together with flagellates and ciliates (Dupuy 1999). The uptake of food is determined by the filtration rate and the efficiency of the gills to retain particles (Riisgrd 1988). With the labial palps (fig.5) the oyster can distinguish living phytoplankton from dead material (Pastoureaud 1996) and so minimize the inorganic fraction. Oysters show clear periods of food uptake and periods of food digestion (Gerdes 1983).

The sex of the *C.gigas* and *M.edulis* is separated, dioecious, but changeable (Reise 1998, Gosling 2002). Larvae of the oyster develop as a male and change during their life in a female (Guo 1998, Gosling 2002). The moment of sex change is determined by genetic processes, barely by environment processes (Baghurst & Mitchell 2002, Gosling 2002).

When a young *C.gigas* reaches sexual maturity gonad are made up and covers the digestive outer gland. In ripe *C.gigas* gonads may be up to 6-8 mm thick and can make up a third of the total flesh weight (Walne 1974 in Gosling 2002).

*C.gigas* spawns from the first birth year. The fertilisation takes place externally. Spawning on the northern hemisphere is around July and August (Reise 1998). In June and September spawning can also take place (Arakawa 1990). The optimum spawning temperature lies around 20-25 °C (Mann 1991).

In ripe *M.edulis* the mantle containing the gametes is typically orange in females and cream-white in males (Gosling 2002) in the Oosterschelde estuary these colour differences are not clearly visible. The fertilisation takes place externally. Partial spawning occurs in the spring and a second period of gametogenesis takes place over the summer months, which culminates in spawning in early autumn. (Gosling 2002)

Spawning occurs when the water temperature exceeds 10°C (de Vooy, 1996).

Fertilized eggs develop in larvae in one day. At the end of the larval stage the larvae move to the bottom, group and search for suitable habitat. Larvae are plank tonic and settlement of the larvae of both species takes place 15 to 30 days after fertilisation (Reise 1998).

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## 2. Materials & Method

### 2.1. Experimental design

The growth of sub littoral oysters and mussels were measured monthly, period 1 and 2, (Table 1) on five locations spread over the four compartments in the Oosterschelde estuary. These locations were located in the mouth (NJ), in the centre (VP), in the northern sector (ZP) and two in the eastern sector of the Oosterschelde estuary. In the eastern sector, one of the locations lies on an oyster culture lot (AC) and one location lies outside the lot (LGPK) (fig.1).

Location	Period 1	Period 2
AC	June 14 <sup>th</sup> -July 28 <sup>th</sup>	August 9 <sup>th</sup> -July 6 <sup>th</sup>
LGPK	June 8 <sup>th</sup> -July 20 <sup>th</sup>	July 20 <sup>th</sup> -August 23 <sup>rd</sup>
NJ	June 8 <sup>th</sup> -July 20 <sup>th</sup>	July 20 <sup>th</sup> -August 23 <sup>rd</sup>
VP	June 8 <sup>th</sup> -July 20 <sup>th</sup>	July 20 <sup>th</sup> -August 23 <sup>rd</sup>
ZP	June 8 <sup>th</sup> -July 20 <sup>th</sup>	July 20 <sup>th</sup> -August 23 <sup>rd</sup>

Table 1: Exact date of experimental periods per location

The oysters used in the experiment were selected on wet weight (10-50 gram) and originate from an oyster culture lot in the Oosterschelde (oyster lot A. Cornelissen). The mussels used in the experiment were selected on shell length (4 cm with) and originate from the Wadden Sea (mussel lot Meep 10). Other shellfish, dirt and sea squirts were removed from the oysters and mussels before using them in the experiment.

The Oosterschelde estuary has strong currents and a lot of floating algae (fig. 6) and to prevent no growth data by losses of cages they were put out in fourfold per location.



Figure 6: Cage with algae

The first cages were put out in the Oosterschelde estuary in June 8th and June 14th. In July 20th and July 28th cages were collected (period 1) and at the same time new cages were put out replacing the old cages. Cages for location AC in period 2 were put out August 9th. In August 23rd and September 6th the cages of period 2 were collected.

The cages are connected with a heavy weight and are standing on the bottom of the Oosterschelde estuary (fig.7a). With a buoy, the cages were visible from the surface of the water.

During the two periods 800 oysters were put out in mesh cages (fig. 7 b& c). Each cage contained eight mesh bags with five compartments (fig. 6d) to follow individual growth each compartment contained one oyster. Each cage contained 80 oysters divided over two layers (fig. 7a). In the experiment mussels were also added to the cages in 2nd layer. In the first period a total of 200, each cage containing 10 mussels. In the second period a total of 500 mussels, each cage containing 25 mussels. The growth of mussels was followed as a group, they were not individually marked.

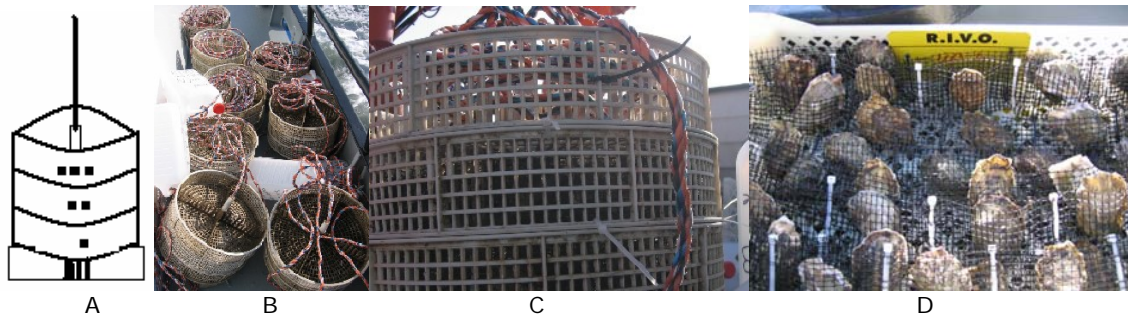


Figure 7: a) Set-up cage, oysters in layer 1 & 2, mussels in layer 2, b) the cages, c) close mesh cage, d) hand made mesh bags with each five compartments

## 2.2. Growth measurement

Growth of the oysters was measured over period 1 and 2 at four compartments of the Oosterschelde estuary. With weight measurements at the beginning and biometric measurements at the end of a period, growth of the oysters and mussels was determined. To estimate the start weight of flesh for the oysters and mussels that were put out, a subgroup of 100 oysters and 100 mussels were taken from each batch.

For location AC in period 2 a separate batch was used, because the batch in period 2 for the other locations was not sufficient enough to use for AC. Of the subgroups biometric variables were measured. The results were used to calculate the start weight of flesh of the ones that were put out for the experiment.

Before measuring the shellfish were taken to the lab and placed in Oosterschelde water to recover and to start filtering again. After at least half an hour the Wet Weight (WW) was measured and the shellfish were temporally put in a fridge or a freezer before further analyses. The Wet Weight (WW) of shellfish includes the shell and the flesh of the fresh oyster or mussel. Because most of the weight is the shell, what gives a good biomass overview in the field, but it is not representative for the biological active part of the shellfish. The dry weight (DW) and the Ash Free Dry Weight (AFDW) of the flesh are more accurate representatives for the biological active part. DW is the weight of the flesh after two days drying in a oven at a temperature of 70°C. AFDW is the loss in weight, the organic part of flesh, after incinerate the flesh at a temperature 540 degrees for 2 hours for mussels and 4 hours for oysters. Sand, pieces shell or other inorganic parts inside the fish were excluded by the AFDW procedure. Between the DW and AFDW a linear relation is found. The most accurate representative AFDW will be used to determine growth of flesh of the shellfish.



The Shell Wet Weight (SWW) includes only the wet shell of the oyster. The SWW was used to measure growth of the oyster shell. For the mussel the Length of the shell was measured for shell growth.

The daily growth is expressed in Relative Growth Rate (RGR). RGR is calculated with the following allometric equation (Gosling 2002):

$$\text{RGR} = \ln(w1/w0)/t$$

*t* = number days

*W0* = start weight of WW, SWW/length, AFDW

*W1* = end weight of WW SWW/length, AFDW

July and August are two months in the experimental period where spawning of the oysters could occur. Ripe gonads can be checked by rubbing softly over the flesh of an oyster after opening the oyster. Ripe gonads are visible as a milky substance. When an oyster spawns the gametes are discharged into sea and a milky substance is no longer apparent. In the first growth period no ripe gonads were observed before and after the growth experiment. In the lab before the second period started, spawning was observed in the bucket, a milky substance was visible in the water. It started before any measurements were done. Through spawning oysters loose weight and it is better to measure only growth without the influences of spawning on growth. The oysters were put in a seawater tank for two days with food, air bubbles and at a temperature of 25 (C to induce spawning. Only a part of the oysters did spawn, because the whole group was still needed for the experiment, both oysters that had spawned and oysters that had not were used for the experiment, period 2. For the mussels no ripe gonads were visually observed during the experimental periods.

### **2.3. Environmental parameters**

In the periods 1 and 2, data of the amount of chlorophyll-a and water temperature were collected. Chlorophyll-a is a good representative for food availability. The amount of chlorophyll-a was measured at all the compartments of the Oosterschelde estuary. The relative growth rate of five locations were plotted against the averages of chlorophyll-a over period 1 and 2. The water temperatures were collected from Waterbase ([www.waterbase.nl](http://www.waterbase.nl), a website of Rijkswaterstaat) for the location ZP, LGPK, and NJ. Location AC lies in the same compartment as LGPK. No data were available for location VP, the middle of the Oosterschelde estuary. With these environmental parameters growth and physical condition of the oysters and mussels may be explained.

### **2.4. Statistical analyses**

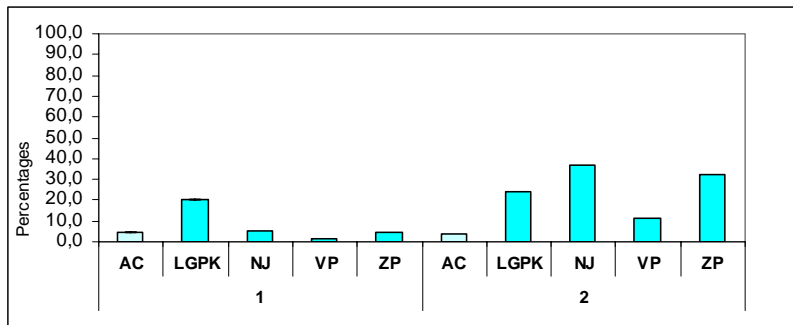
All statistical analyses were performed with the computer software SYSTAT 9.0. The compatibility between the subgroups and the experimental periods were tested with an ANOVA. For *C. gigas* relationships between WW and AFDW were tested with linear regression. For *C. gigas* and *M. edulis* growth differences between locations and for each location between periods were tested with a one-way ANOVA and a POST HOC, Tukeys test. The outcome of a test was considered significant when the probability value was smaller or equal to 0.05.

### 3. Results

#### 3.1. Mortality

In the experiment in period 1 and 2 cages were lost by the combination of floating algae getting stuck in the lines, creating more drag on the cages, and strong currents moving the cages along the bottom. In period 1 one cage at location LGPK and one at location ZP were lost. In period 2 one cage at location AC was lost. The mortality data is based on the dead oysters and mussels found in collected cages. Natural enemies of the shellfish, like small crabs and starfish were apparent in cages at all locations. The predation is thought to be the same at all the locations. Visually the same amount of predators was observed and mussels were more predated than oysters.

**A**



**B**

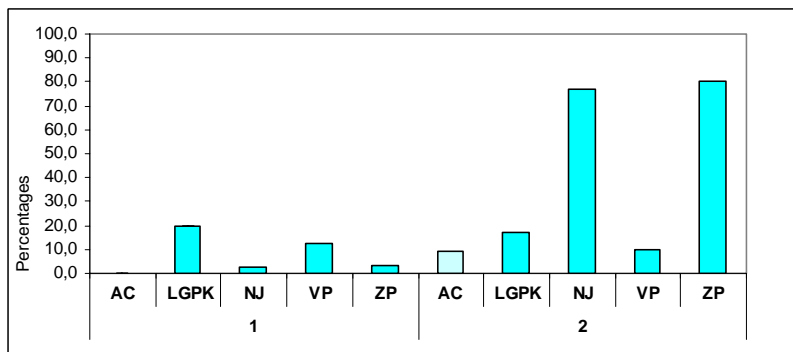


Figure 8: a) mortality *C.gigas* in period 1 and 2 at different locations, b) mortality *M.edulis* in period 1 and 2 at different locations.

*C.gigas* (fig. 8a) showed roughly the same mortality in period 1 and 2 for LGPK (20.8 and 23.8%). For period 1 LGPK have had the highest mortality. In period 2 NJ (36.9%) and ZP (32.5%) were the peaks.

For *M.edulis* (fig. 8b) the highest mortality was observed at location LGPK (20.0%) and VP (12.5%) in period 1. For *M.edulis* in period 2 locations NJ (77.0%) and ZP (80.0%) clearly showed highest mortality.

The location AC had a low mortality rate in both periods. AC is located in the same compartment in the Oosterschelde estuary as LGPK, but located inside a culture lot of an oyster farmer. A culture lot can give more shelter for the cages and could be a reason for the low mortality at location AC.

*C.gigas* and *M.edulis* (fig.8a&b.) show a higher mortality at almost all the locations in the second period compared to the first period. For both shellfish the locations NJ and ZP in the second period have experienced the highest mortality.

### 3.2. Growth of *C.gigas*

To estimate the initial weight of the flesh for oysters before they were put out in the Oosterschelde estuary, a subgroup of 100 oysters were taken from each batch. During evaluation of the data the initial Wet Weight (WW) of the oysters for the experimental period and for the subgroup were compared. It showed (fig.9) a significantly different WW for subgroup 1 ( $p < 0.05$ ) that would be used to calculate the initial AFDW weight period 1 and for subgroup 2 ( $p < 0.05$ ) that would be used to calculate the initial AFDW for period 2. Only subgroup 2 for location AC had no significant difference ( $p = 0.783$ ) between the start WW's.

The batch of *C.gigas* that was used for subgroup 2 and the experimental period 2 contained a low amount of oysters to select from. This can explain the highly significant difference between the subgroup 2 and period 2. The other batches contained a higher amount of oysters and therefore it was easier to select on the same weight. Still the other subgroups and periods were apparently not completely randomly selected.

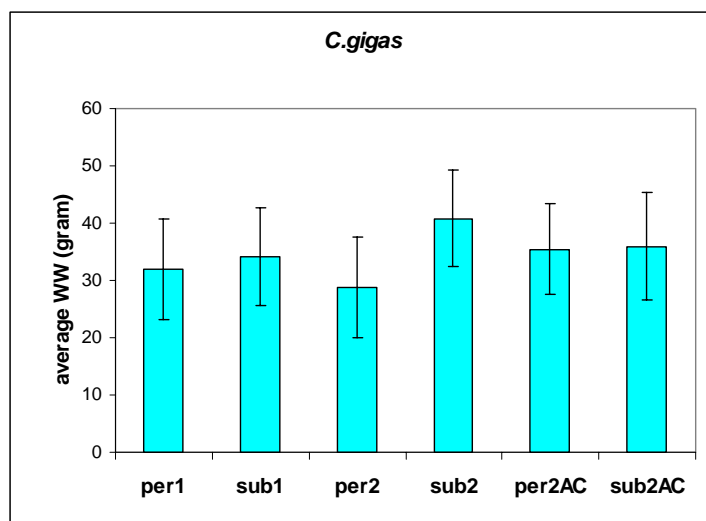


Figure 9: *C.gigas* compared initial WWs of subgroups and experimental period groups. Per1=period 1, per2= period 2 (locations LGPK, NJ, VP, ZP), per2AC=period 2 location AC, sub1, sub2, sub2AC= subgroups used for further calculations of experimental periods.

The averages of WW are different between some periods and subgroups, but the range of wet weights are the same. The relationship between AFDW and WW is linear. The initial AFDWs of the oysters for the different periods were calculated with the equations explaining the linear regression lines in the following graphs (fig.10). For subgroup 2AC the oysters were lean in AFDW compared to the other subgroups 1 and 2.



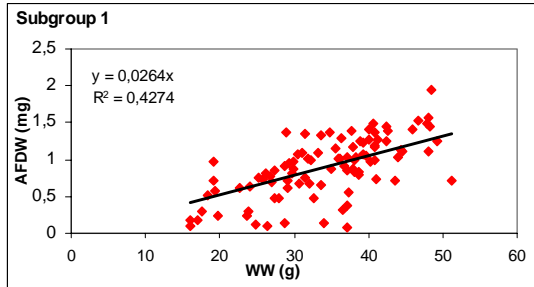
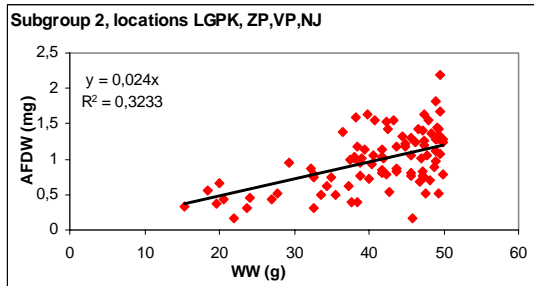
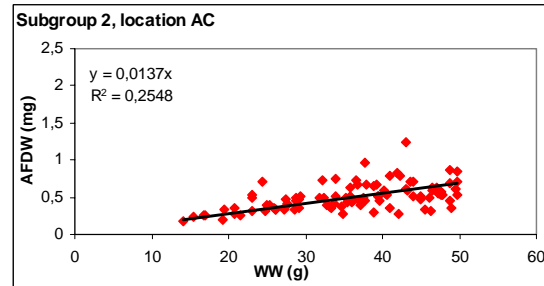
**A****B****C**

Figure 10: Oyster subgroups used for calculations for the initial AFDW weight in the periods. a) Subgroup 1 used for calculations in period 1 ( $p > 0.05$ ), b) subgroup 2 used for calculations in period 2 for location LGPK, VP, ZP and NJ ( $p > 0.05$ ), c) subgroup 2 used for calculations in period 2 for location AC ( $p < 0.05$ .)

The growth of the oysters was measured over period 1 and 2 at the four compartments in the Oosterschelde estuary. The graphs (fig.11) show the percentile relative growth per day for WW and AFDW. Relative growth per day for Shell Wet Weight (SWW) was also calculated and showed the same pattern as the WW growth.

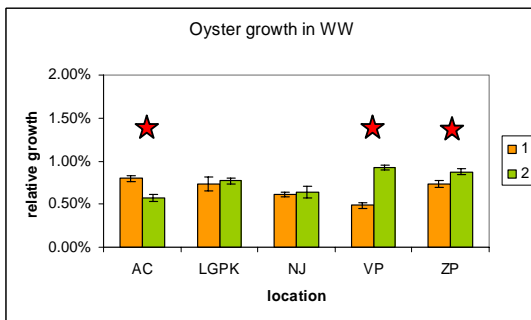
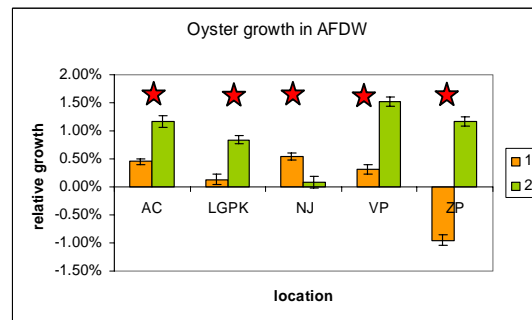
**A****B**

Figure 11: *C.gigas* relative growth per day in weight at different locations in period 1 and period. a) Growth in Wet Weight (WW), b) Growth in ash free dry weight (AFDW) significant difference in growth between period 1 and 2 ( $p > 0.05$ )

Location AC (fig.11a) had a higher relative growth in WW per day in period 1 compared to period 2. The other locations had a lower growth in period 1 compared to period 2. In period 1 the relative WW growth per day was highest at location AC (0.80%) and was a significantly ( $p < 0.05$ ) higher compared to NJ and VP. The relative WW growth per day was lowest at location VP (0.49%) and was significantly lower compared to ZP and LGPK. In period 2 it was the opposite. The growth per day was highest at location VP (0.92%) and was significantly ( $p < 0.05$ ) higher compared to AC and NJ.

The WW growth was lowest at location AC (0.57%) and was significantly lower compared to LGPK and ZP. Location ZP had significantly ( $p < 0.05$ ) higher growth compared to location NJ.

Location NJ (fig. 11b) had a higher relative AFDW growth per day in period 1 compared to period 2. Other locations show the opposite and had a lower relative AFDW growth in period 1 compared to period 2. In period 1 growth was highest at location NJ (0.54%) and was significantly ( $< 0.05$ ) higher compared to ZP. The growth was lowest at location ZP (-0.9%) and was significantly lower compared to all other locations. Location AC had significantly higher growth compared to location LGPK. The flesh weight (AFDW) at ZP decreased extremely during period1.

In period 2, AFDW growth per day was highest at location VP (1.53%) and was significantly higher compared to locations LGPK and NJ. The growth in AFDW was lowest at location NJ (0.09%) and was significantly lower compared to all other locations. Location ZP (1.17%) was significantly ( $p < 0.05$ ) higher in growth compared to location AC (1.16%).

Comparing the growth data of WW en AFDW contrasting patterns is observed at location AC, NJ and ZP.

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### 3.3. Growth of *M.edulis*

To estimate the initial weight of the flesh for mussels before they were put out in the Oosterschelde estuary, a subgroup of 100 *M.edulis* were taken from each batch. During evaluation of the data the initial Length of the mussels for the experimental period and for the subgroup were compared. It showed (fig.12) a significantly difference in Length for subgroup 1 ( $p < 0.050$ ) that would be used to calculate the initial AFDW for period 1. For subgroup 2 ( $p = 0.957$ ) that would be used to calculate the initial AFDW for period 2 and for subgroup 2AC ( $p = 0.211$ ) that would be used to calculate the initial AFDW for period 2AC had no significantly difference between the initial Lengths.

The batch of *M.edulis* that was used for subgroup 1 and the experimental period 1 contained a low amount of mussels to select from. This can explain the highly significant difference between subgroup 1 and period 1. The other batches contained a higher amount of mussels and were better to select the same size. Still subgroup 1 was apparently not completely randomly selected.

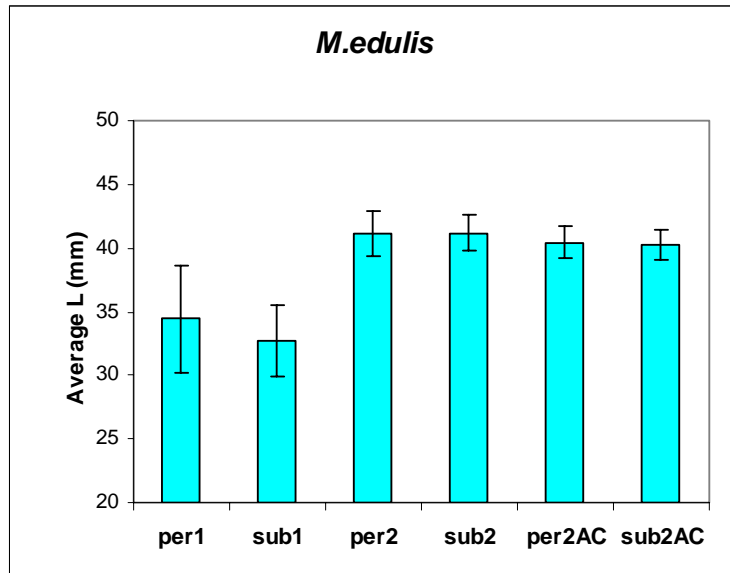


Figure 12: *M.edulis* compared initial WWs of subgroups and experimental period groups. Per1=period 1, per2= period 2 locations LGPK, NJ, VP, ZP, per2AC=period 2 location AC, sub1, sub2, sub2AC= subgroups used for further calculations of experimental periods.

The averages of Lengths are different between some periods and subgroups, but the range of wet weights are the same. The relationship between AFDW and Length is allometric. The selection of mussels took place on 4cm length and therefore a too small range is used to see a clear allometric relation in the graphs (fig.13). The initial AFDWs of the mussels for the different periods were calculated with the equations in the following graphs. For subgroup 2AC the oysters were lean in AFDW compared to the other subgroups.

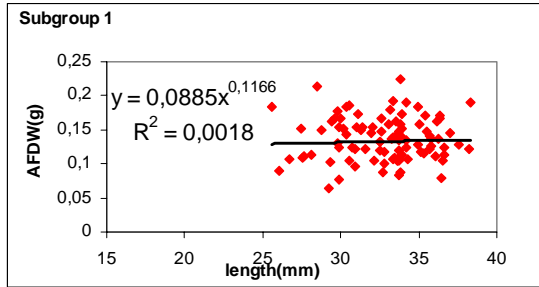
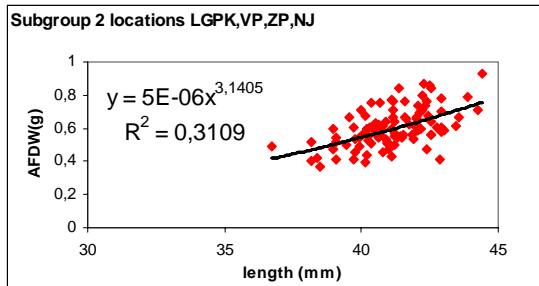
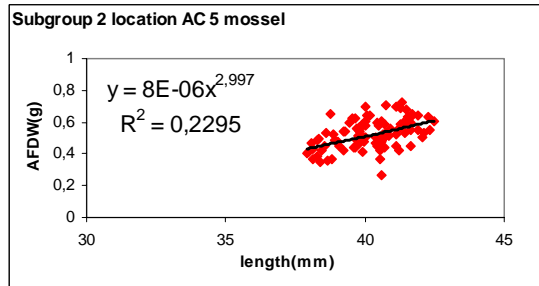
**A****B****C**

Figure 13: Mussel subgroups used for calculations for the initial AFDW weight in the periods. a) Subgroup 1 used for calculations in period 1 for all locations, b) Subgroup 2 used for calculations in period 2 for location LGPK, VP, ZP and NJ), c) subgroup 2 used for calculations in period 2 for location AC

The growth of the mussels was measured over period 1 and 2 at the four compartments in the Oosterschelde estuary. The graphs (fig.14) show the percentile relative growth per day for Length and AFDW. Relative growth per day for WW was also calculated and showed the same pattern as the Length growth. The Length is not influenced by water uptake of the mussels and is the best representative to show.

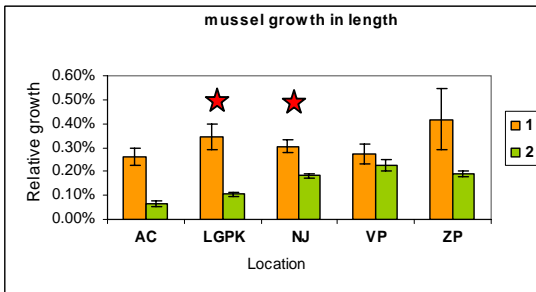
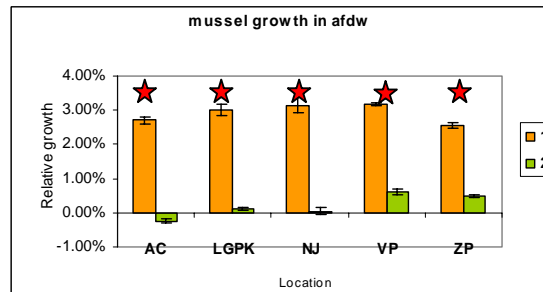
**A****B**

Figure 14: *M. edulis* growth in weight and length at different locations in period 1 and 2. a) Relative growth per day in Length, b) relative growth per day in Ash Free Dry Weight (AFDW) ★ Significant difference in growth between period 1 and 2 ( $p > 0.05$ )

The relative growth in Length (fig.14a) was higher in the first period compared to the second period for all the locations. The growth in period 1 was higher at location ZP (0.42%) and the growth was highest at location VP (0.26%). Also *C. gigas* had the lowest growth at location VP in period 1. For period 2 the Length growth was lowest at location VP (0.23%) and the growth was lowest at location AC (0.07%). For *C. gigas* the highest and lowest growth was also observed at locations VP and AC.

The relative growth in AFDW (fig.14b) was higher in period 1 compared to period 2. Period 1 showed relative growth above 2% per day for all locations. Highest growth in period 1 was observed for locations NJ (2.93%) and VP (2.95%) and the lowest growth was observed at location ZP (2.43%). *C.gigas* also showed the highest AFDW growth at location NJ and the lowest at location ZP. *C.gigas* decreased per day in AFDW at location ZP, but *M.edulis* showed growth above 2% per day. For the second period the highest growth was observed at location VP (0.76%). *C.gigas* also showed the highest growth at location VP. The lowest growth was observed at location AC (-0.21%) and was significantly lower compared to location VP. The flesh weight at AC decreased during period 2. AC is located on an oyster lot and an explanation could be food competition with oysters. *C.gigas* showed no lowest growth or decrease in flesh weight at location AC in period 2.

Comparing the growth data of Length and AFDW the same patterns are found between the two periods; period 1 a higher growth rate at all locations. The relative Length growth varies between locations, but relative flesh growth showed no real differences in growth between the locations.

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### 3.4. Environmental parameters

#### 3.4.1 Food availability

The amount of chlorophyll-a was measured at all the compartments of the Oosterschelde estuary. The relative growth rate of five locations for both species is plotted against the averages of chlorophyll-a over period 1 and 2 (fig.15).

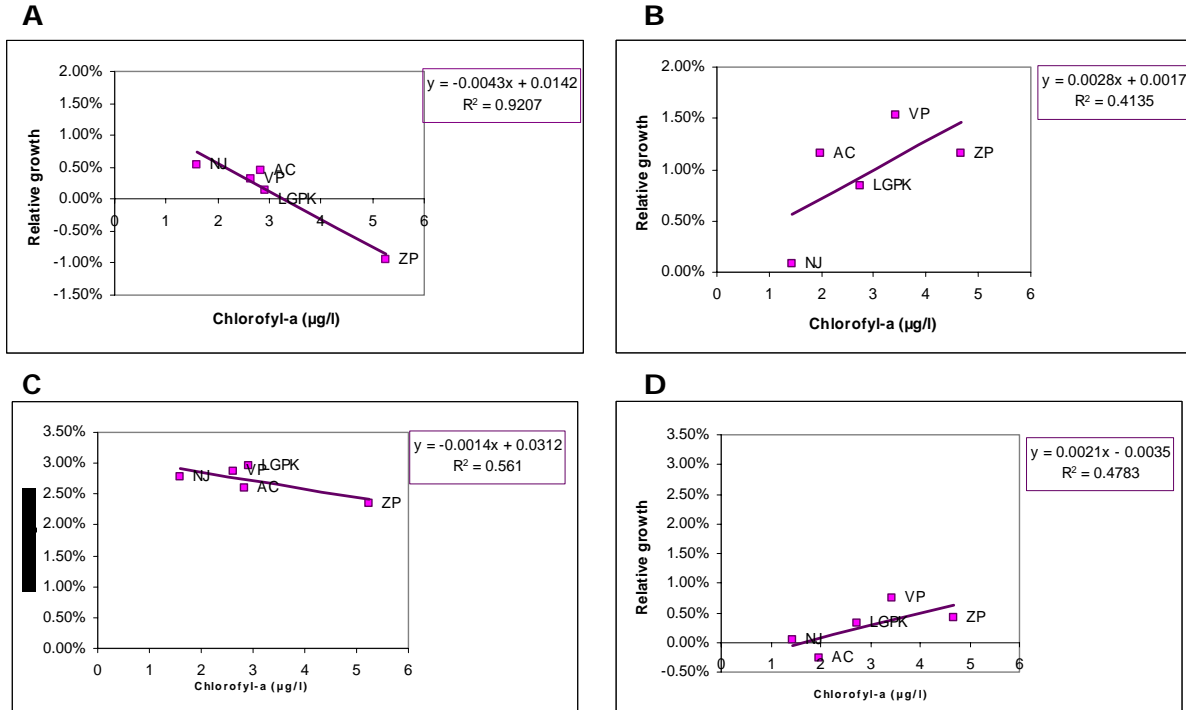


Figure 15: Food availability at different locations A) *C. gigas* Relative AFDW growth in period 1 against chlorophyll-a B) *C. gigas* Relative AFDW growth in period 2 against chlorophyll-a C) *M. edulis* Relative AFDW growth in period 1 against chlorophyll-a D) *M. edulis* Relative AFDW growth in period 2 against chlorophyll-a.

For *C. gigas* and *M. edulis* in period 1 (fig. 15A&C) food limitation did not take place. The highest amount of chlorophyll-a was available at location ZP, but lowest growth. The lowest amount of chlorophyll-a was at location NJ. Other influences than food availability played a role at location ZP, affecting growth of *C. gigas* more than growth of *M. edulis*.

In period 2 (fig. 15B&D) location ZP had the highest amount chlorophyll-a and location NJ the lowest. For *C. gigas* and *M. edulis* in period 2 the graphs show that growth increased with increasing chlorophyll-a. Other influences than food availability alone played a role at location ZP. Comparing relative growth of *M. edulis* in relation to food availability, in period 1 and 2, shows that other influences affected growth more in period 2.

By comparing the graphs of *C. gigas* with the graphs of *M. edulis* it shows that *C. gigas* had a higher growth at AC than at LGPK and the opposite was observed for *M. edulis*. Location AC and LGPK are located in the same compartment in the Oosterschelde estuary, only AC is located in an oyster lot and LGPK outside the oyster lots.

### 3.4.2 Water Temperature

Water temperatures (fig.16) were collected for three of the four compartments of the Oosterschelde estuary. The middle (centre) was not included; no data for location VP were available.

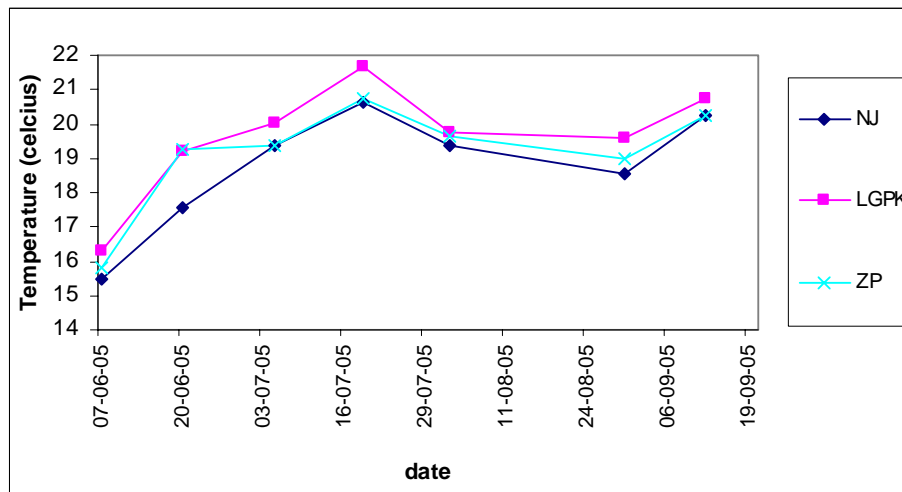


Figure16: Water temperatures over experimental periods June until beginning September. Period 2

The figure shows that spawning of the oysters could have occurred between July 3rd and August 11th. It is at the end of period 1 and at the halfway period 2. This means that spawning could have happened.

## 4. Discussion

### Method

The experiments have been carried out successful despite the following errors: During the two experimental periods, 3 cages were lost from sites LGPK, ZP, AC by the combination of floating algae getting stuck in the lines, creating more drag on the cages, and strong currents moving the cages along the bottom. The cages were put out in the Oosterschelde estuary in fourfold to prevent loss of all data of a location; hence enough cages were left to carry out the experiments.

The initial selections of the oysters and mussels for some of the subgroups were not representative for the animals that were used in the cages. Some subgroups appeared to be not completely randomly selected. For oysters, wet weight growth in the experimental periods 1 and 2 were underestimated because the animals in the subgroup appeared to be smaller than the animals put out in the field. For mussels, length growth of experimental period 1 was overestimated because the animals in the subgroup appeared to be bigger than the animals put out in the field. As a consequence growth rates have been slightly higher than measured in period 1 and 2 for *C.gigas* and slightly lower in period 1 for *M.edulis*.

### Mortality

*C.gigas* and *M.edulis* showed a higher mortality at almost all the locations in the second period compared to the first period. For both shellfish the locations NJ and ZP have experienced the highest mortality.

ZP is located close to the lock and drains fresh water into the Oosterschelde estuary. These freshwater draining results in lower salinity at ZP, but also in an input of nutrients that can cause extra blooms of algal species (Geurts van Kessel & Kater 2003). Unfavourable species, like blue-green algae may have deleterious effects on the shellfish.

Since the building of the Storm Surge Barrier, the North compartment where ZP was located, showed the highest decrease in current velocity (Geurts van Kessel & Kater, 2003). The possibility exists that the current speed is too low to balance the food uptake and supply in that area. The lower salinity, unfavourable blooms of algae and decrease in current velocity could have affected the growth and condition of the shellfish causing a high mortality. Before the start of experiment 2 a part of the oysters had already spawned in the lab. It seems that mussels spawned during period 2. After spawning, shellfish are weak and need food to retain strength (Smaal & Vonck, 1997). Location NJ had low food availability causing unfavourable conditions for the shellfish. This could be an explanation for the observed relative high mortality at NJ in period 2. A phenomenon often seen for *C.gigas*, but not yet explained, is summer mortality. This unexplained mortality during summer could also have played a role and must be taken into account.

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**Growth of *C.gigas***

Flesh weight of shellfish is subjected to seasonal fluctuations and associated with the reproductive cycle (Kautsky 1982 in Gosling 2002). For subgroup 2AC the oysters had a low flesh weight (AFDW) compared to the other subgroups and this shows no deviated growth after the experimental period compared to the other location. *C.gigas* had a better growth rate per day in WW and AFDW in period 2 compared to period 1. In period 1 the food availability did not limit growth. The highest amount of chlorophyll-a was available at location ZP, but the oysters at ZP showed lowest growth rates. The decrease in AFDW showed that the condition of the oysters was weak at ZP. At the end of the first period the temperature went up and reached above 20C. For spawning of the oysters, water temperatures of above 16-18(C are needed (Kater 2003). Spawning of the oysters at the end of the first period could explain the lower growth and together with changes in salinity and a possible bloom of other algae species it could explain the extreme decrease in AFDW at location ZP.

In period 2, growth increased with an increasing concentration of chlorophyll-a. Between locations AC and LGPK, both located in the East compartment, no significant differences was observed. Location ZP showed deviated growth in relation to food availability.

**Growth of *M.edulis***

*M.edulis* had a better growth rate in Length and AFDW in period 1 compared to period 2. In period 1 the food availability did not limit growth. The relative growth rate per day was higher than 2% for all locations.

In period 2, growth increased with an increasing concentration of chlorophyll-a. In relation to food availability a much lower growth was observed compared to period 1. It is assumed that mussels have spawned in period 2. The shell length increased while flesh weight barely did increase. For mussels a second period of gametogenesis takes place over the summer months, which can culminate in spawning in early autumn. (Gosling 2002). For *M.edulis* also location ZP showed deviated growth in relation to food availability.

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## **5. Conclusion**

Growth of *C.gigas* was higher in period 2 (July- August) compared to period 1 (June- July). This is ascribed to spawning of the oysters in period 1. Growth of *M.edulis* was higher in period 1 compared to period 2. It is assumed that mussels have lost energy through spawning in period 2, shell length increased while flesh weight barely increased.

In period 1, no relation was found between growth and food availability and growth was apparently not limited by food availability. In period 2, growth increased with an increasing concentration of chlorophyll-a and growth was limited by food availability.

In the North compartment of the Oosterschelde estuary local circumstances limited growth in relation to food availability. High mortality and limited growth in relation to food availability are attributed to a combination of reduced salinity, low currents velocity and possibility of unfavourable algae.

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## Acknowledgements

First at all, I would like to say special thanks to Karin Troost for her support and endless stimulations, to Emiel Brummelhuis for helping me always with the practical work and for the patience in answering questions. I want to thank Aad Smaal for the valuable experience. Thanks to all RIVO-employees for the nice time I have had in Yerseke.

I also want thank A. Cornelissen for providing the oysters, and the crew of M.S. de Valk for helping me in the field and watching over my cages on weekly basis. Final special thanks to my good friends Eric, Wilfred, Bart, Paul, Pim, Theo, Marc, Kristen, Jurrit, Jessica en Erik who helped me with making hundreds of mesh bags!

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