Oysters on the Dyke:

Could commercial Pacific oyster aquaculture be possible on dykes?

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Abstract

Experiments were conducted to investigate whether conditions on the toe protection (kreukelberm) of the dykes in the Oosterschelde would be sufficient for the potentially commercial level growth and survival of the Pacific oyster (*Magallana gigas*). The primary environmental influences on the growth and survival of *M. gigas* in the Oosterschelde were identified through literature research as inundation time, food availability and level of hydrodynamics (wave exposure).

The combined effect of different food supply and inundation time were tested in a laboratory experiment where oysters were exposed to inundation times of 62%, 82% and 100% inundation and water of different ratios of organic (phytoplankton) and inorganic (silt) material (10000 cells/ml algae, (*Rhodomonas slinas)* with 0mg, 100mg or 200mg silt). Although no statistically significant results were found, the results suggest that oyster growth was greatest in longer inundation time with higher organic:inorganic material in the water.

A field experiment was set up to test the effects of inundation time (50% or 70% and hydrodynamics (low and high) on the growth and survival of the oysters. Unfortunately the field experiment was much afflicted by damage and washing away of equipment as well as theft of the oysters. An experiment at Schelphoek, on the Oosterschelde did remain long enough to gather sufficient data for analysis. Growth and survival varied between treatments with no obvious optimal level of inundation or hydrodynamics that could be applied as a general observation.

The results suggest that growing oysters on the kreukelberm is possible as results show that the oysters can survive and grow, but it depends on whether one considers the product worth the investment necessary to make it a successful venture.

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Introduction

The potential for growing oysters on the toe protection (hereafter referred to as 'kreukelberm') of the dykes was investigated as part of the RAAK PRO Building for Nature project. If utilising the dykes for aquaculture purposes is possible, the available space for aquaculture in the Ooster- and Westerschelde would increase significantly. Furthermore, as the aim of the Building with Nature project is to increase the natural values of the otherwise barren dyke banks, farming oysters, which are ecosystem engineers, on the dykes could also increase the biodiversity of the area.

Environmental factors important to Magallana gigas*.*

Shellfish aquaculture is a major industry in the Netherlands, and is particularly successful due to the suitable habitats found in the delta waters. Whether the farming of oysters is possible on the banks of the dykes is dependent on the environment in these areas being suitable for oysters. Several environmental factors are important to the survival and growth rate of *M. gigas*, particularly in the challenging intertidal zone. According to the literature temperature, salinity, food supply, exposure and hydrodynamics, are likely to play a large role both alone and in interaction with each other, in determining the shell length and flesh content of oysters.

Temperature

Temperature has long been known to have a significant influence on the survival and growth rate of oysters. Increasing temperature increases the rate of active metabolic processes (Newell, et al., 1967) and therefore growth rate increases with temperature until the temperature exceeds the upper limit for survival in the species (Pratt, et al., 1956).

Growth rate in oysters is well documented to be highest in the summer months and lowest in the winter months (Dame, 1972; Walne, 1972; Paynter, et al., 1990; Wang, et al., 2008; Gangnery, et al., 2003; Fredriksson, et al., 2010; Levinton, et al., 2013) Conversely, Ingle et al. (1952) found the similar growth patterns in oysters settling in the spring as those settling in the autumn. Malouf and Breese (1977) suggested that the growth rate of juvenile *M. gigas* fluctuate independently of temperature and are instead related to food supply. However, they did suggest that with sufficient food supply, optimal growth rate can be achieved at temperatures around 20°C, while His et al. (1989) reported that optimal growth in *M. gigas* was achieved at 30°C with a salinity of 30‰, and Zhang et al. (2006) suggested the optimal temperature range for maintaining lysosomal membrane integrity in the haemolymph of *M. gigas* was 13-17°C. Malham et al. (2009) reported that *M. gigas* are immuno-compromised and therefore less resistant to inorganic nutrients at 21°C compared with 12 °C. Garnier et al. (2007) also reported that in temperatures above 19 °C mass mortalities may be linked to pathogenic bacteria.

Chávez-Villalba et al (2010) suggested that high variations in temperature can impose stress on molluscs and reported large die-offs in *M. gigas* following sharp declines in temperature. Similarly, Malham et al. (2009) suggested that a mass mortality of *M. gigas* in the summer of 2003 in Ireland resulted from particularly high temperatures as well as high levels of nutrients in the water. Li et al. (2007) hypothesised that temperature fluctuations in the form of heat shocks of 37 °C and 44 °C can decrease the immune response and therefore thermotolerance of *M. gigas* particularly of postspawning individuals leading to summer mortalities.

Growth of oysters on the kreukelberm is likely to be highest during the warmer summer months, when increased temperatures lead to increased metabolic activity. Temperatures on the kreukelberm are likely to fluctuate significantly with the exposure time and shallow depth, however these fluctuations are unlikely to regularly exceed the upper limit for survival of the oysters. However if temperatures exceed a sustained level of around 19 °C (which might be possible on a dyke, in direct sunlight) it is possible that the oysters may be subject to increased levels of diseases or pathogens.

Salinity

Salinity is a major environmental factor influencing growth rate in oysters (Brown, et al., 1988; Nell, et al., 1988; Gangnery, et al., 2003; Chávez-Villalba, et al., 2010). Wang et al. (2008) suggested that oyster growth rates are significantly related to salinity, while growth peaks are related to high temperature and food supply. While salinity influences growth rate in oysters, it may have much less influence on the quality of the eventual oyster meat. Brown and Hartwick (1988) found oysters from areas with low salinities (<20 ppt) has slower growth rates, but equivalent dry meat weight:shell weight ratios compared with oysters from areas of high salinity and food supply with higher growth rates. Ingle et al. (1952) found remarkably rapid growth rates *C. virginica* in rapidly fluctuating salinities, despite their low glycogen content and presumably low vigor.

Wang et al. (2008) suggested that variations in salinity, such as changes in freshwater inflows significantly influence oyster growth rate, but that growth rate is dependent on the range of variation in salinity. They reported that the salinity range for optimal growth also varied with location in Apallachicola Bay, Florida, USA, with the optimal range at Cat Point being between 20-25 ppt and at Dry Bar between 17-26 ppt. Nell and Holliday (1988) reported that the optimum growth rate for *M. gigas* was achieved at 15–30 and 15–45 ‰, for 1.1 mg spat and 0.68 g spat respectively and that salinity had no effect on spat survival.

Salinity is unlikely to vary greatly for the oysters on the kreukelberm as the salinity in the Oosterschelde and Westerschelde remains relatively stable (Smaal & Nienhuis, 1992). Furthermore, the successful aquaculture industry in the Oosterschelde and Westerschelde indicate that the salinity is suitable for oyster farming.

Food supply

Food supply (food quantity as well as food quality) is generally regarded as the main environmental factor influencing oyster growth rate (Malouf, et al., 1977; Chávez-Villalba, et al., 2010). However food supply for oysters can vary according to factors such as season, phytoplankton composition (Hyun, et al., 2001), salinity (Wang, et al., 2008) and flow rate (Pogoda, et al., 2011).

Phytoplankton composition is a determining factor in food quality which influences oyster growth rate. Different species of algae afford differing nutritional values for the oysters, and combinations of different species can have synergic effects on food values. Epifanio (1979) tested different combinations of up to four different algae species on the growth rate of *C. virginica*. He found growth rates in the oysters varied markedly according to the different combinations. Among the monocultures tested, the highest growth rate was achieved using the algae species *Thalassiosira pseudonana* or *Isochrysis galbana*, but an even greater growth rate was achieved when the two algae were combined.

Salinity influences not only the oyster itself, but also the algae used as its food supply. Areas with fluctuating salinities such as those with freshwater inflows influence the algae species able to exist, and therefore influence what food is available to the local oysters (Wang, et al., 2008).

Flow rate also influences food supply for oysters as the movement of the water is the mechanism that transports the algae to the sessile oyster. Therefore, food availability and consequently filtration rates increase with flow rate (Walne, 1972). Flow rate is of such importance to the food supply for oysters that high hydrodynamics in offshore locations can compensate for the lower plankton concentration compared with coastal areas (Pogoda, et al., 2011).

Hydrodynamics are also responsible for the turbidity and suspension of sediment in the water. The ratio of organic:inorganic material in the water is the main determinant of food quality. The more suspended sediment there is present in the water, the more diluted the phytoplankton is and the harder the oyster has to work to filter the useful, organic material from the water. Barille et al. (1997) found that increased inorganic material in the water has a negative effect on the growth of oysters.

Considering the successful aquaculture industry in the Oosterschelde, and assuming the carrying capacity of the area has not been met, the food supply should be sufficient for oysters farmed on the kreukelberm, especially in areas with high flow rates.

Inundation time

Elevation, and the consequent inundation time is a significant predictor of many biological patterns (Bishop, et al., 2006). Exposure to air is a significant physical stress for marine organisms, which then have deal with periods of potential desiccation and starvation (Bishop, et al., 2006). The intertidal area is a high variable environment ranging from times of complete inundation to total exposure. inundation time is therefore an influential factor on the growth and survival of oysters. Ingle et al. (1952) found significantly higher growth rates in oysters originating from periodically exposed areas when they were transferred to locations where they were continuously submerged, compared with oysters that were not transferred. Zhang et al. (2006) found that from three hours of exposure the integrity of the lysosomal membranes in oysters were affected, decreasing the fitness of the oysters.

Walles et al. (2015) suggested that the optimum inundation time for *M. gigas* is 80-70%. This was taken into account when placing the experimental treatments of oysters on the kreukelberm with an attempt to place all oysters at roughly the appropriate elevation for 50% and 70% exposure time at all locations.

Hydrodynamics

While hydrodynamics plays an important indirect role in oyster growth food supply (Pogoda, et al., 2011) and in dispersal and settlement of larvae (Turner, et al., 1994), it can also directly influence the growth of juvenile and adult oysters. Walne (1972) found that *M. gigas* kept in high flow environments had greater average weight of organic matter and increased heart rate compared with oysters kept in low flow environments. Furthermore, he found that increasing the flow rate increased growth rate in the oysters. Lenihan et al. (1996) found that oyster growth increased monotonically with flow velocity and its effect was independent of food supply. However, Grizzle et al. (1992) reported an increase growth rate in *C. virginica* with increased flow speed up to a maximal growth rate at 1 cm s^{-1} beyond which the growth rate decreased. They suggested that this maximum growth rate was due to the maximum inhalant pumping speed of the oysters.

Interactive effect

None of the environmental factors described above occur independently of each other, in fact there is often a cumulative effect of more than one environmental factor influencing the growth and survival of oysters. Chávez-Villalba et al. (2010) described the complex interactions of various environmental factors including, but not limited to food, temperature and salinity and there effect on the condition index of *M. gigas* along with oyster densities. Malham et al. (2009) demonstrated that summer mortalities in Ireland and Wales result from the combined influences of water quality and increased temperatures. Gangnery et al. (2003) presented a growth model for *M. gigas* based on temperature, salinity and particulate organic matter in the Thau Lagoon, France and found that season had the most impact on growth rate in different locations due to temperature. Walne (1972) described the influences of animal size, water flow, temperature and food supply on oyster growth rate and found that water current was a more important influence on oyster growth rate than had previously been recognised.

Dykes in the Dutch delta

Dykes are hard constructions intended primarily for coastal defence against rising tides and wave action. The 'kreukelberm', or 'toe' of the dyke lies at the bottom of the dyke where it meets the sediment, and consists of hard, usually rocky substrate and is intended to absorb wave energy and protect the dyke from erosion (Figure 1). The necessity for dykes to be constructed from such rigid and infallible materials conveys that dykes experience high hydrodynamics as well as physical stresses characteristic of intertidal habitats such as extreme temperatures, potential desiccation during low tide, and changes in salinity due to rainwater. All of these factors make the dykes particularly challenging environments for organisms to colonise and occupy.

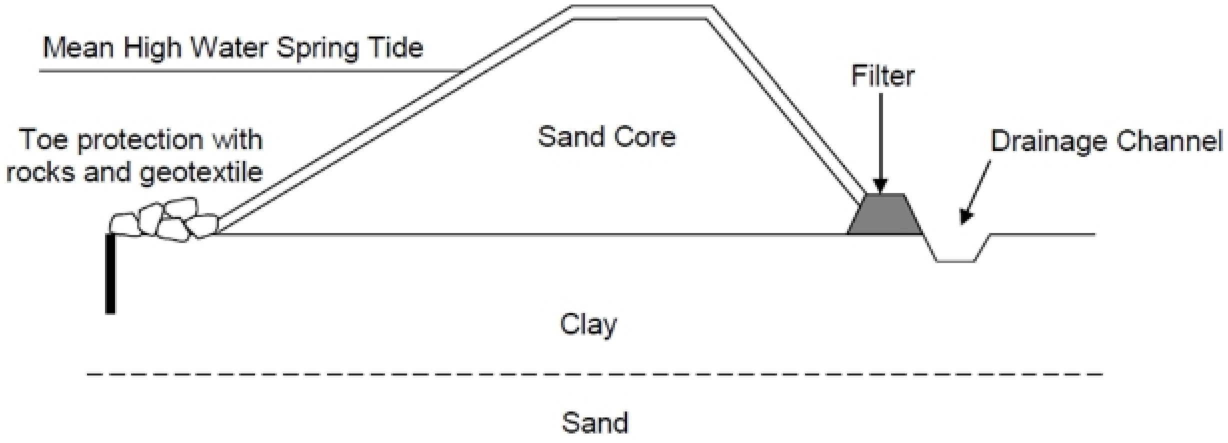

Figure 1. Schematic of a typical dyke in the Dutch delta. Toe protection with rocks and geotextile as seen in the picture refers to the 'kreukelberm' zone. Reference: Source: Linham and Nicholls, 2010.

Magallana gigas

The Pacific oyster, *Magallana gigas* is generally very tolerant of wave action due to their thick and calcified shells, and to desiccation due to their ability to maintain moisture by closing their valves when exposed to air during low tide. Oysters are known to decrease wave action and form buffer zones for hydrodynamics (Scyphers, et al., 2011). Furthermore oysters are ecosystem engineers and provide heterogeneous habitats with increased water retention for various other organisms that would otherwise be unable to survive in the harsh environment. If these qualities of oysters could be used to both protect the dyke against wave action, and increase the biodiversity of the dyke, they would be serving both a coastal defence and an ecological purpose. If these oysters could also be harvested in commercially viable condition then they would also serve an economic purpose.

The present research investigated the effect of wave action, exposure time, and food quality on the growth rate, survival and quality (flesh content) of the Pacific oyster, *Magallana gigas*. This research was conducted to build applicable knowledge and experience for exploring the possibility of using the kreukelberm as an oyster aquaculture area.

Methods

The research was conducted in two parts, a laboratory experiment and a field experiment. The laboratory experiment was designed to test the effect of different ratios of organic:inorganic material in the water, different inundation times and the interactive effect of both factors. The field experiment was designed to test a more realistic situation where the effect of different inundation time and wave exposure on the growth of the oysters could be compared.

The laboratory experiment began on 3 May 2016 and continued for two weeks. Field reared juvenile oysters of ca. 40 gram each were obtained from 'de Roem van Yerseke' and stored at the SEALab at HZ University of Applied Sciences.

Laboratory experiment: food quality and inundation time

A custom experimental system was designed and constructed so that groups of oysters could be exposed to different levels of organic:inorganic material in the water and different inundation times. Three large reservoirs (120 x 100 x 76 cm) were filled with 430 L saltwater to which 10000 cells/ml algae (*Rhodomonas slainas*) was added. Different volumes of silt in the form of bentonite clay (table) was then added to each reservoir (Table 2). Bentonite clay is a 100% soft natural clay mineral from volcano ash and has a grainsize of <30 µm. The bentonite clay was sieved with a mesh width of 38 µm before adding it to the experimental set-up. The clay was also burned in an oven at a temperature of 560 ℃ for four hours to ensure it was 100 % inorganic matter.

To maintain realistic concentrations of particulate matter. samples taken from the Eastern Scheldt at Kats (51°34'18.343''N, 3° 53' 38.744''E) and analysed for their organic:inorganic suspended material. The concentrations for the laboratory experiment were based on these samples (Table 1) .

Table 1: Calculations on inorganic fractions needed in the experimental set-up.

Sample	1	2	3
Dry weight silt (mg/L)	286,7	206,2	205,2
Ash free dry weight silt (mg/L)	73,1	60,1	54
Inorganic concentration silt (mg/L)	213,6	146,1	151,2

The algae and silt were kept in suspension in the reservoir water with aeration tubes and water circulation pumps. The reservoirs were elevated and three tubes were connected to each reservoir so that gravity would allow the water to flow from the reservoir into a container below in which 10 oysters were placed. The tubes connected to the reservoir at different heights of the tubes so that as the reservoir water level drained the highest tube would run dry first, followed by the middle and then the lowest tube. This ensured that the different containers received water for different periods of time, thus simulating different inundation periods. The inundation periods chosen were 15, 19 and 24 hours inundation, in percentages, 62%, 82% and 100% of the time under water (Table 2).

The containers in which the oysters were placed were designed to maintain a continuous flow of water over the oysters with water from the tube, and complete drainage once the tube ran dry. The tubes were all controlled further with valves so that fine scale adjustments to maintain a water flow rate of 0.25 ml/min could be made manually. Two sets of this system were set up and run at the same time for increased sample size (Figure 2 and Figure 3).

Prior to the running of the experiment oysters were selected randomly and measured for length, width, height and wet weight to produce a 'Health Index' of each individual oyster. Furthermore the ash free dry weight of the gills and palps was also measured separately so that the gill:palp ratio could be calculated as a measure of adaptation to different levels of food availability. A standard protocol was followed to determining the dryweight (Appendix 1) and AFDW (Appendix 2). These parameters were measured again after 14 days in the system to give the 'Health Index' and gill:palp ratio for each individual.

The Health Index of each individual oyster was calculated as:

$$HI = (Afdw(g) / ww(g)) \times 100$$

Where HI is the health index, afdw is the ash free dry weight of the meat (g) and ww is the wet weight of the meat.

The gill:palp ratio (AFDW stands for: ash free dry weight) was calculated as:

$$\frac{AFDW\ gills}{AFDW\ palps}$$

Every day for the two week duration of the experiment the main reservoirs were cleaned and filled up to 430 L with saltwater containing the appropriate ratios of algae and silt. *Rodomona salinas* was harvested fresh on a daily basis from the algae raceways in the SEALab.

Table 2: Ratio of organic:inorganic material in the experimental system (mg/l silt + 10000 algae/ml). Inundation times in the containers are I: 15 hours inundation, II: 19 hours inundation and III: 24 hours inundation. In percentages, 38%, 19% and 0%.

Reservoir	**mg silt (+ algae)**	**Containers and inundation time (hours/24 h)**		
		I (15 h)	**II (19 h)**	**III (24 h)**
A	100mg/l	A1	A2	A3
B	200mg/l	B1	B2	B3
C	0 mg/l (control)	C1	C2	C3
F	100mg/l	F1	F2	F3
E	200mg/l	E1	E2	E3
D	0 mg/l (control)	D1	D2	D3

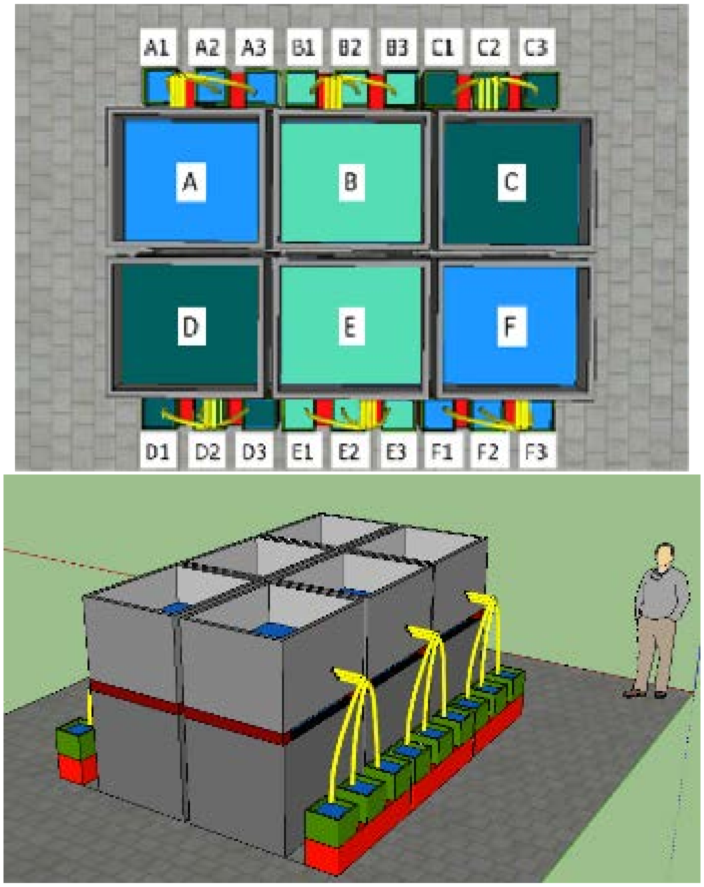

Figure 2. Schematic experimental design from above (top) and side (bottom). The system consists of 2x three reservoirs of water (A, B, C and D, E, F) containing different ratios of organic:inorganic material (colours indicate each ratio). Water from each main reservoir flows into three containers with 10 oysters (labelled as 1,2 and 3 with the letter of the reservoir they are connected to) through tubes that run dry at different times to simulate different inundation periods.

Figure 3. Actual experiment as set up in the SeaLab.

Field experiment

<u>2015</u>

Locations

To test the effect of inundation time and wave energy on the growth and survival of *Magallana gigas* nine locations were selected (four in the Westerschelde and five in the Oosterschelde) to place oysters and monitor their growth. The locations in the Oosterschelde were: Buiten Neeltje Jans, Binnen Neeltje Jans, Zeelandbrug, Kattendijke and Nieuw Strijen. The locations in the Westerschelde were Vlissingen, Ellewoutsdijk, Eversdijk and Rilland (Figure 4). These locations were specifically selected for two reasons, 1. because it would be possible to place oysters on the kreukelberm at 70 % inundation, and 2. Because they each had contrasting hydrodynamics (data collection for which was planned, but due to the early abortion of the experiment was not conducted) and would therefore be interesting to compare.

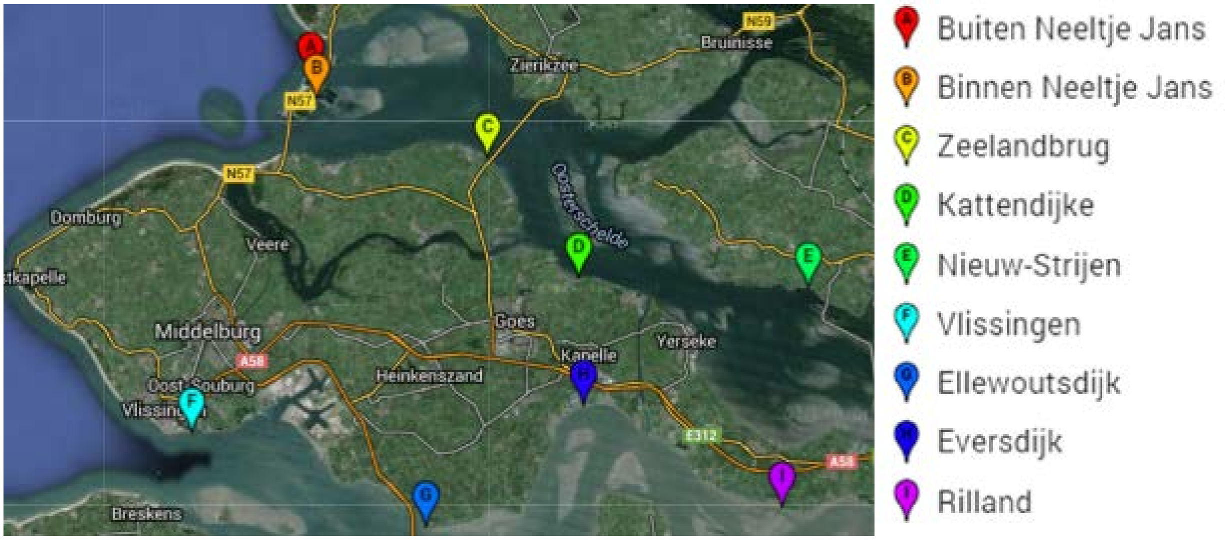

Figure 4. The nine locations (four in the Westerschelde and five in the Oosterschelde) originally selected for the field experiment (Source: Google Maps).

Experimental design

In March 2015, field reared juvenile *Magallana gigas* (50 – 100 mm shell length) were obtained from 'de Roem van Yerseke' and measured for length, width and height. Of these oysters, 30 were randomly selected and placed in a small plastic mesh bag. These small sacks were placed inside large plastic mesh sacks (50 x 100 cm with mesh diameter of 4 mm) along with approximately 8 kg of oysters. In this way the same selection of oysters inside the small mesh bag could be monitored for growth at both the beginning and the end of the experiment, but they would experience the movement and scouring of a much larger and fuller oyster sack. This scouring is important to remove the sharp edges of the oyster shells, thereby producing a commercially attractive (cup shaped) product.

At each location three oyster sacks were attached to a 1 x 2 m steel mesh sheet with cable ties, and placed on the kreukelberm. At each corner the steel sheets were attached with cable ties onto a steel anchor point set in a concrete block (each weighing around 27 kg). These blocks fit in between the rocks on the kreukelberm to help secure the mattress from water induced movement. On each set of oyster sacks a sign was attached to dissuade potential thieves (Figure 6).

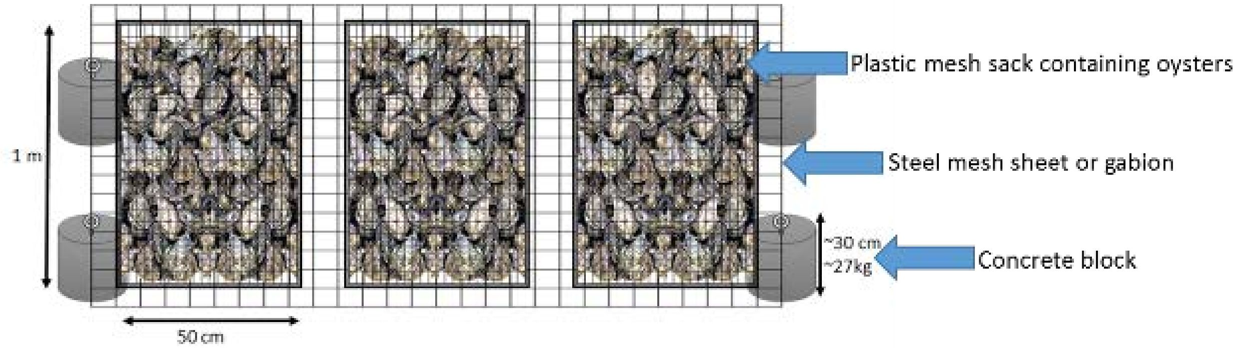

Figure 5. Schematic design of the oyster sacks attached to steel mesh sheets weighted at the corners with concrete blocks.

Figure 6. Oyster sacks (with small oyster bag inside) attached to the steel sheet, weighted by concrete blocks and placed on the kreukelberm (left) and signage intended to dissuade potential oyster thieves placed with each set of oyster sacks (right).

The intention for this field experiment was to monitor the growth of the oysters in the small mesh bags with each oyster sack one of three replicates for each location over a one year period. Data on wave action and phytoplankton density from Rijkswaterstaat and NIOZ would be used to define the hydrobiological factors of each location. Oyster growth between locations, and therefore different levels of hydrodynamics would then be compared and information about the factors most conducive to growing successful, commercial quality oysters on the kreukelberm would be presented.

Unfortunately the signage was not enough to dissuade potential thieves and, particularly at locations close to recreational camping areas like Nieuw Strijen, the oysters had been taken within a month. To increase security cages, or gabions, made of the same steel mesh as the mattresses were constructed to replace the mesh sheets with lids that could lock with high grade marine pad locks. New oysters were ordered, measured and placed in oyster sacks. These were then placed in the new gabions which were set on the kreukelberm and weighted with the same concrete blocks to prevent them washing away.

Figure 7. Steel gabion with oyster sacks in locations where increased security for the oysters was necessary.

Unfortunately the padlocked steel gabions were evidently not secure enough to dissuade or prevent thievery. At Ellewoutsdijk and Neeltje Jans Buiten all oyster sacks as well as the steel gabion had entirely disappeared leaving only the concrete blocks strewn in the kreukelberm (Figure 8). At Rilland the entire gabion, including concrete blocks mysteriously remained undamaged, but had been moved to the top of the dyke next to the bicycle path with the oyster sacks removed. At Nieuwe Strijen the rings of the anchor points in the concrete blocks had been deliberately bent open and the entire gabion removed.

In other cases it appears that the concrete blocks were not sufficient to anchor the gabion to the kreukelberm. At Zeelandbrug the gabion had come loose from the concrete blocks because the anchor points had been bent open, and was lodged, with oysters, higher up on the dyke.

By September of 2015 the only oysters left in place in the Oosterschelde were at Vlissingen and Kattendijke, and in the Westerschelde, Eversdijk and Neeltje Jans Binnen. Due to the limited possible comparisons with so few locations and the unsuccessful investment in increased security, this experiment necessarily aborted before sufficient monitoring data could be collected.

Figure 8. All that remains of the field experiment in some locations were the concrete blocks strewn in the kreukelberm. Here the concrete blocks at Neeltje Jans Buiten.

2016

Locations

Following the lessons learned during the first failed attempt at the field experiment the experiment was modified for a second and final attempt. Two locations were chosen based on their lower accessibility to the public as well as the ability to place oysters at two contrasting heights and levels of hydrodynamics. In comparing two levels of hydrodynamics in a single location also limits the variability in food availability in different locations. The breakwaters at Schelphoek and at Goese Sas were chosen as locations on which to place a new series of oyster sacks. These areas are both accessible for researchers, but are not frequented by the public.

Schelphoek is not particularly accessible to the public as the breakwater is often closed, and is rarely visited on foot. The area is visible from a boat, but not from land. The road on the breakwater at Goese Sas is closed to the public with a locked chain link gate for which only the council has the key. The breakwater is closed to the public because it is a resting place for birds at high tide. The area is, however easily visible from a boat and at low tide it is possible to walk beyond the gate on the mudflat.

Figure 9. Locations for the second attempt at the field experiment. Rectangles indicate approximately where the oysters were placed, red: high hydrodynamic area, blue: low hydrodynamic area.

Experimental design

100 field reared juvenile oysters (50-100 mm shell length) were measured and weighed as before and placed in each of an oyster sack. At the end of April 2016 three oyster sacks were placed at each of two different heights on the dyke where the inundation time was 70% and 50%. The appropriate heights where the gabions were placed were calculated from astronomic predictions and measured using a DGPS. For de Schelphoek the gabions are placed at -0,06m NAP and -0,76m NAP and at the Goese Sas at -0,08m NAP and -0,81m NAP.

To further investigate the effect of hydrodynamics on the growth and survival of the oysters, as well as the interactive effect of hydrodynamics and inundation time, oysters were not only placed at two different heights, but also on different sides of the breakwater where they would experience contrasting hydrodynamics. At Schelphoek the oysters were placed on the lee side of the breakwater for a low hydrodynamic environment, and on the seaward side of the breakwater for a higher hydrodynamic environment. At Goese Sas the low hydrodynamic area was the inner side of the breakwater facing the mouth of the canal, and the high hydrodynamic area was on the very point at the end of the breakwater.

At Schelphoek the oyster sacks were again placed in gabions as before, but this time with four marine padlocks to prevent the lid being bent open (Figure 10). At Goese Sas the oysters were attached to mesh sheets as described in the original experiment to because security of the area was considered sufficient enough to make the gabions an unnecessary financial investment (Figure 11).

Figure 10. Oyster sacks were placed in steel gabions at two heights and at two levels of hydrodynamics at De Schelphoek. Oyster sacks as placed at both heights (low for 70% inundation time and high for 50% inundation time) on the low hydrodynamic side of the breakwater (left), and on the high hydrodynamic side of the breakwater (right).

Figure 11. Oyster sacks were placed at two heights and at two levels of hydrodynamics at Goese Sas. Oyster sacks as attached to the steel sheet (left), placed low on the kreukelberm (70% inundation time) on the low hydrodynamic side of the breakwater (middle), and high on the kreukelberm (50% inundation time) on the high hydrodynamic side of the breakwater (right).

The steps taken to increase security of the experiment were somewhat successful. The oysters remained in both locations long enough to monitor fortnightly for two months. After that period the oysters at Schelphoek were left until mid February 2017 and those at Goese Sas until mid March 2017 so that the oysters had a chance to grow over a longer period of time. Unfortunately during this time the oysters in the high hydrodynamic area of Goese Sas from both inundation levels had disappeared while the mesh sheet and concrete blocks remained in place (Figure 12). The assumption is that the oysters were appropriated by unknown members of the public. Therefore the data from that treatment does not continue beyond the initial two months.

Figure 12. While the mesh sheet and concrete blocks remained in place at the high hydrodynamic areas at Goese Sas, the sacks with oysters had disappeared.

Monitoring

Wave loggers were used at both locations to quantitatively verify that the nominated ‘high’ and ‘low’ hydrodynamic areas experienced sufficiently contrasting wave action to make an experimental comparison worthwhile. To quantify the hydrodynamics of each location The Wave Gauge Blue wave loggers were placed at each location on both the low and high hydrodynamic areas. These wave loggers are submersible self-logging, self-powered pressure sensors and are borrowed from the NIOZ institute in Yerseke. They were attached to the gabion/mesh sheet with cable ties and left to record wave data five times per second for seven minutes every 15 minutes for two weeks (Figure 13).

Figure 13. Wave gauge meter attached with cable ties in one of the gabions at Schelphoek.

The oysters were measured three times during the summer of 2016. The oyster sacks were removed from the gabions and opened carefully. Each individual sack was emptied into a photo tray (Figure 14) and the empty sack was weighed with an electronic balance. The number of live and dead oysters was recorded for each sack. The live oysters were then weighed separately before being returned to the oyster sack along with the empty shells of the dead oysters to maintain the scouring effect. The sack was sealed with a cable tie and put back into place in the cages. The average weight per living oyster could hereby be calculated and extrapolated to estimate growth rate.

The growth rate of the experimental period was calculated as:

$$(W_{end}-W_{start})/t$$

where W is an individual oyster weight in grams and t as the experimental period in days. The health index of the oysters at the different heights and hydrodynamics were compared statistically.

Figure 14. Oyster sacks were emptied into a photo tray, where they were counted, weighed and the number of dead individuals recorded.

Condition Index

As opposed to those at Goese Sas, all treatments of the experiment at Schelphoek remained until the end of the experiment in February 2017. Therefore only the oysters from Schelphoek were also analysed for their condition index. Condition Index is a ratio of the proportion of meat to shell in shellfish and is used to determine the commercial quality of the product (Lawrence & Scott, 1982). A higher condition index means more meat per individual and therefore a more desirable product.

Condition index (CI) is calculated as:

$$CI = AFDW/SCV \times 100$$

Where AFDW is the Ash Free Dry Weight of the meat and SCV is the Shell Cavity Volume. A standard protocol was followed to determining the dryweight (Appendix 1) and AFDW (Appendix 2). Once calculated, the CI was compared between the same inundation time with different water dynamics, and for different inundation times with the same water dynamics with a student’s t-test.

Results

Organic:inorganic material and inundation time

The experimental system was successful in providing a simulated situation of varying water quality and inundation period, which could be compared in terms of their effect on the growth and Health index of oysters. However, monitoring the lab experiment was considerably labour intensive. Water, sediment and algae were added daily to the reservoirs and all hoses were continuously monitored to maintain a consistent flow during the two week period. The sediment in the water resulted in regular blockages within the hoses and taps which had to be cleaned often. Although measures were taken to shade the system from direct sunlight and keep the water at a consistent temperature (around 12°C), there was a difference in temperature of up to 2°C between the containers with oysters. Nevertheless, the system did provide a simulated situation of different water quality.

Mortality

Mortality in the laboratory experiment varied between treatments, but there was no clear pattern suggesting mortality was correlated to the conditions within the treatment. Mortality was particularly high (15 of the original 20 individuals dying before the experiment was completed) in the treatment with 100 mg/L silt and 15 h inundation and in the treatment with 200 mg/L silt and 24 h inundation. Six individuals (30 %) died in the treatment with 0 mg/L silt and 15 h inundation, while five (25 %) died in the treatment with 100 mg/L silt and 19 h inundation and two individuals died in the treatments with 200 mg/L silt and 15 h inundation and 0 mg/L silt and 24h inundation (Figure 15).

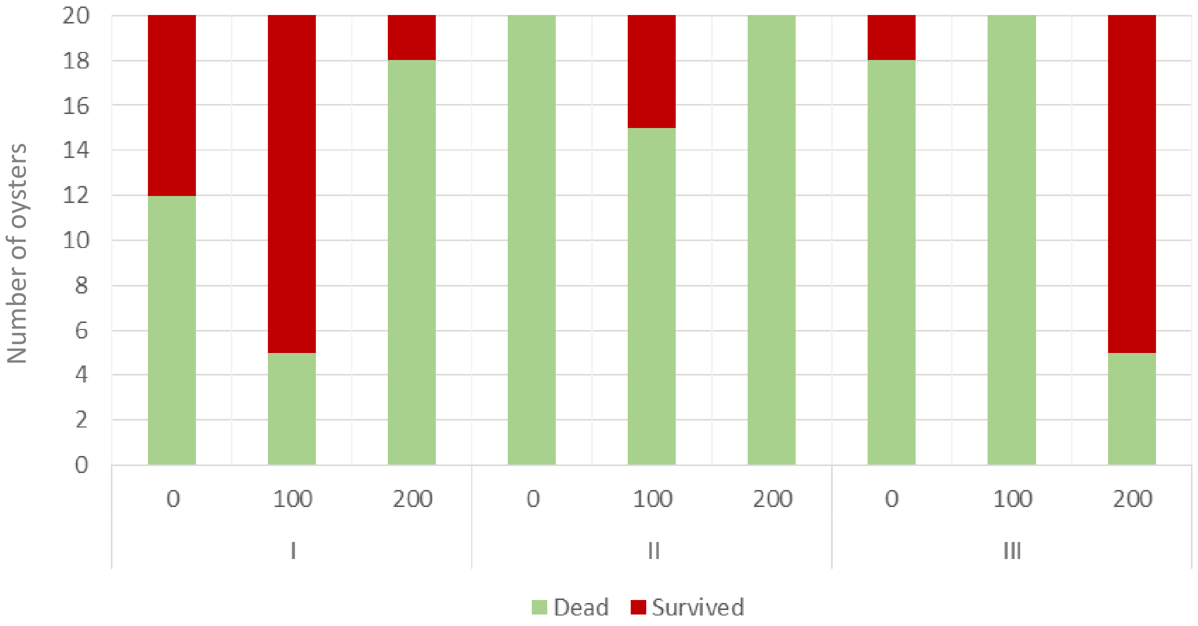

Figure 15. Mortality of oysters during the two week laboratory experiment where they were exposed to water with different organic:inonorganic ratios (0, 100 and 200 mg/L silt with 10000 algae/ml), and inundation times (I = 15h inundation, II = 19 h inundation and III = 24 h inundation. 0 = 0g ml silt/L).

Growth

There was high variation in the growth of the oysters during the two week laboratory experiment. In general, within the same inundation period oyster growth tended to decrease with increasing amounts of silt in the water. However variation was so high that there was no significant difference between the treatments ($F_{2,124} = 1.312$, $P > 0.05$) (Figure 16).

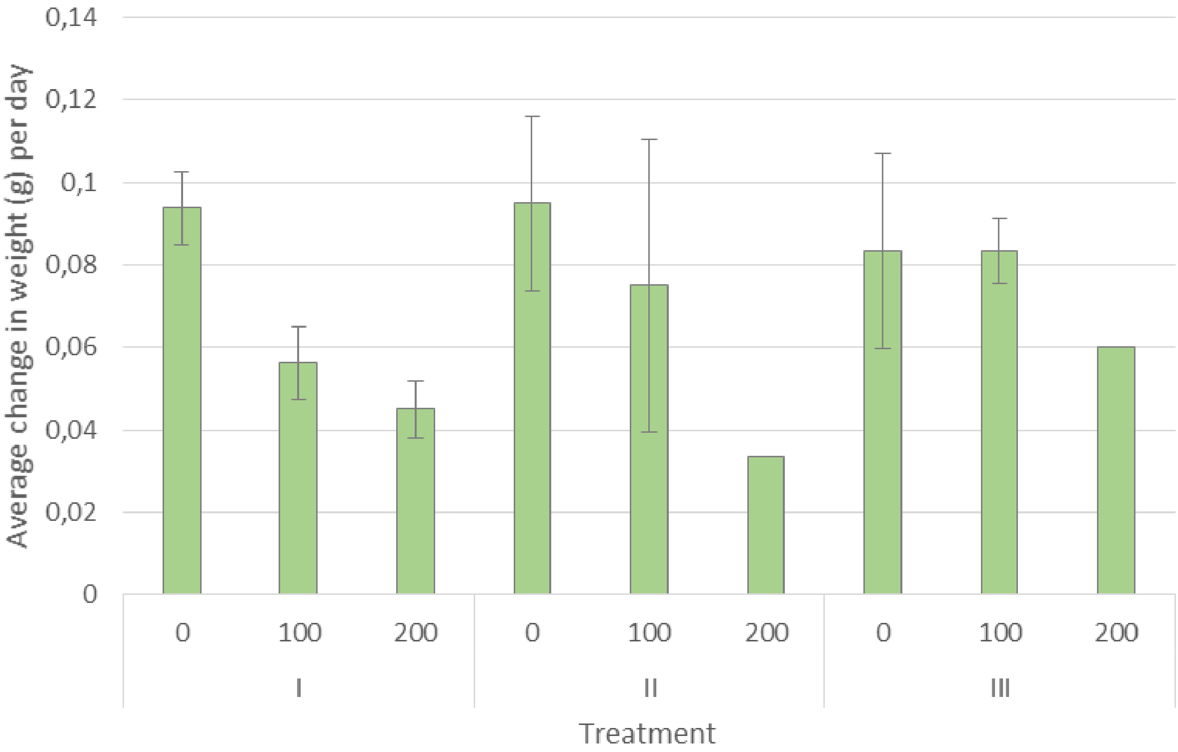

Figure 16. Average growth (change in total fresh weight (g)) per day in water with different organic:inonorganic ratios (0, 100 and 200 mg/L silt with 10000 algae/ml), and inundation times (I = 15h inundation, II = 19 h inundation and III = 24 h inundation. 0 = 0g ml silt/L).

The Gill:Palp ratio was on average lower in all treatments compared with the T0 sample. Within the same inundation time average gill:palp ratio decrease with decreasing ratio of organic:inorganic material. However variation was so high that there was no significant difference between the treatments ($F_{2,124}$ = 0.069, $P > 0.05$) (Figure 17).

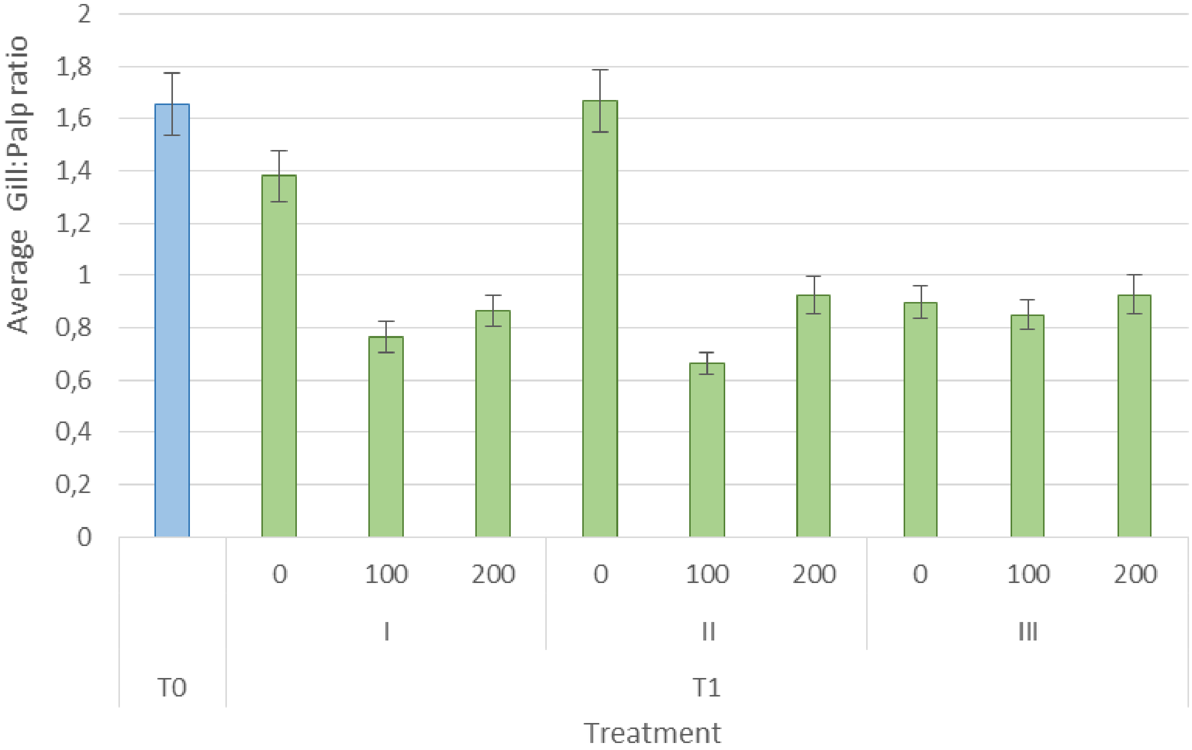

Figure 17. Average Gill: Palp ratio of oysters in water with different organic:inonorganic ratios (0, 100 and 200 mg/L silt with 10000 algae/ml), and inundation times (I = 15h inundation, II = 19 h inundation and III = 24 h inundation. 0 = 0g ml silt/L).

The average AFDW was lower in all treatments compared with the T0 sample except those with 0 mg silt. However variation was so high that there was no significant difference between the treatments ($F_{2,173}$ = 0.915, P > 0.05) (Figure 17).

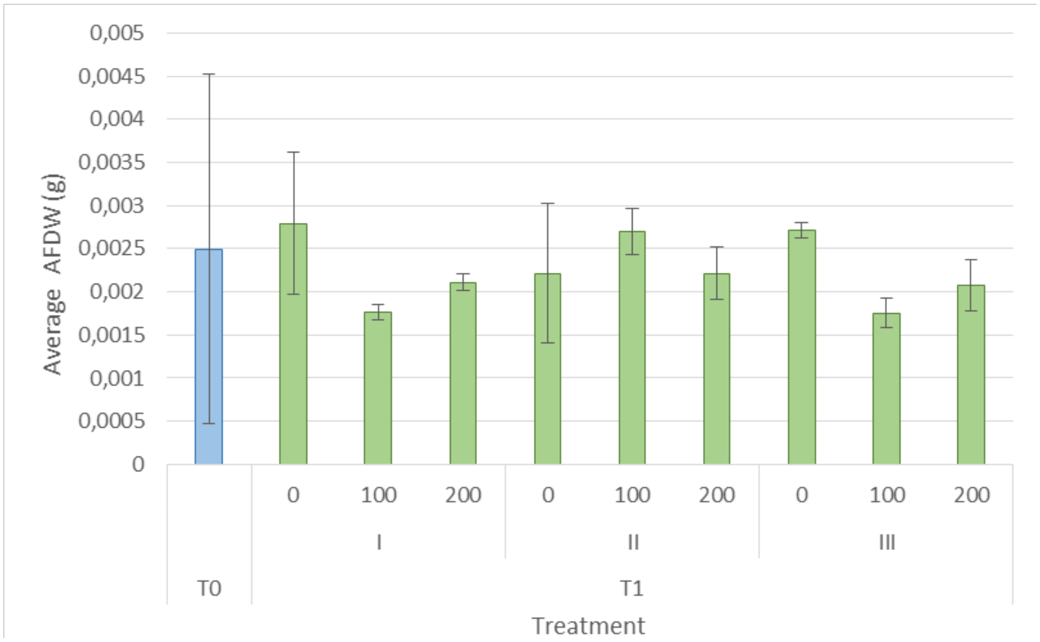

Figure 18. Average AFDW of oysters in water with different organic:inonorganic ratios (0, 100 and 200 mg/L silt with 10000 algae/ml), and inundation times (I = 15h inundation, II = 19 h inundation and III = 24 h inundation. 0 = 0g ml silt/L).

There was no significant effect of inundation time on the gill:palp ratio. At all inundation times the gill to palp ratio was highest for oysters with no organic material in the water (Figure 19).

The interactive effect of the ratio of organic:inorganic material and inundation time on showed a decrease in health index with an increase in of organic:inorganic material, except for the oysters with an inundation time of 19 h. The oysters that were constantly under water (24 h inundation) had a lower health index than oysters that were not constantly under water (15 h and 19h inundation) except where the oysters also had a high proportion of silt (200 mg/L) in the water (Figure 20).

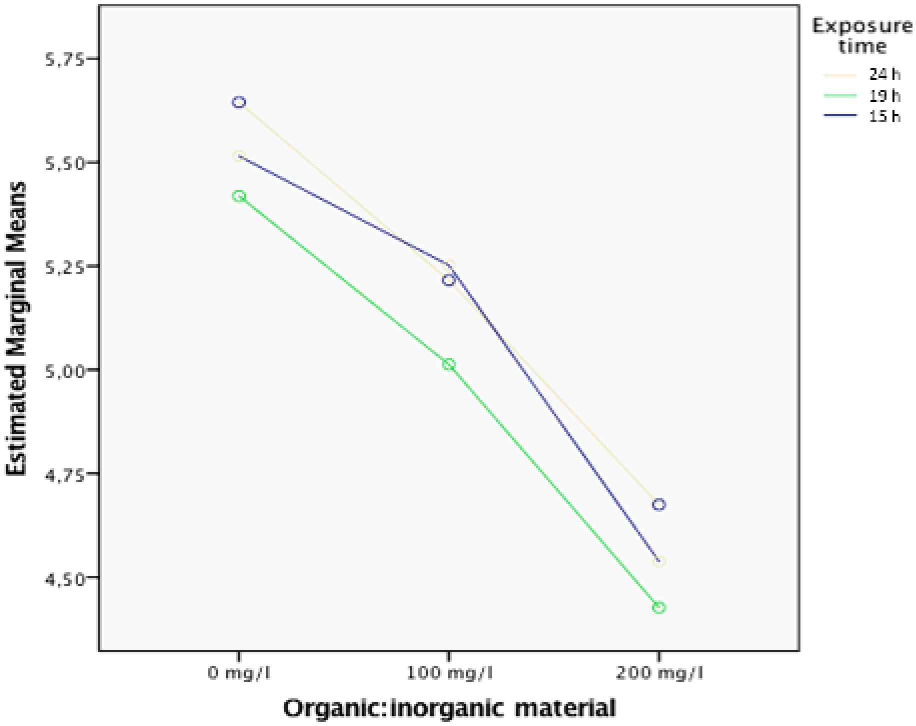

Figure 19. The interactive effect between inundation time and organic:inorganic material (x-axis) on the gill:palp ratio of oysters (y-axis shows the gill:palp ratio estimated marginal means). Gill:palp ratio was highest for oysters with no organic material in the water for all three inundation times.

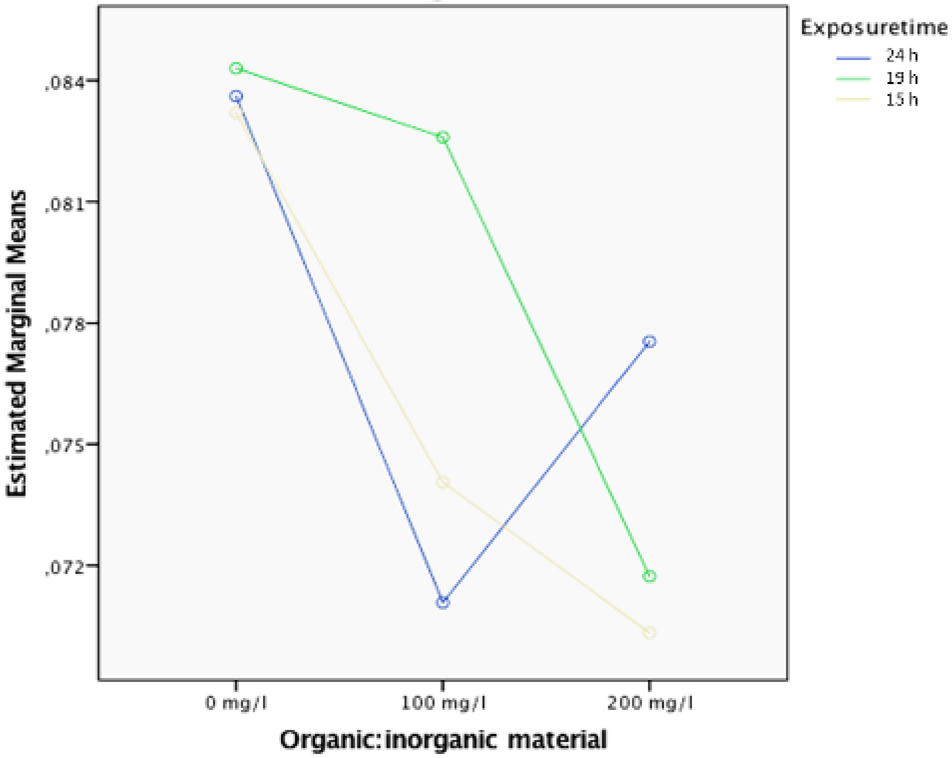

Figure 20. The interactive effect between inundation time and organic:inorganic material (x-axis) on the health index of oysters (y-axis shows the health index estimated marginal means). Health index was highest for oysters with no organic material in the water for all three inundation times

Hydrodynamics and inundation time

At both Schelphoek and Goese Sas the wave action varied over the two week measurement period. While there was overlap in the maximum and minimum wave pressure recorded between the nominated 'high' and 'low' hydrodynamic areas (likely due to high or low wind levels), there was a definite and consistent difference in average wave pressure. The average wave pressure for both hydrodynamic levels was greater at Schelphoek than at Goese Sas, while the difference in average wave pressure between the 'high' and 'low' hydrodynamic areas was greater at Goese Sas than at Schelphoek (Figure 21). These data support the choice of the nominated 'high' and 'low' hydrodynamic areas for the experiment and verify that comparisons made between the hydrodynamic levels are worthwhile.

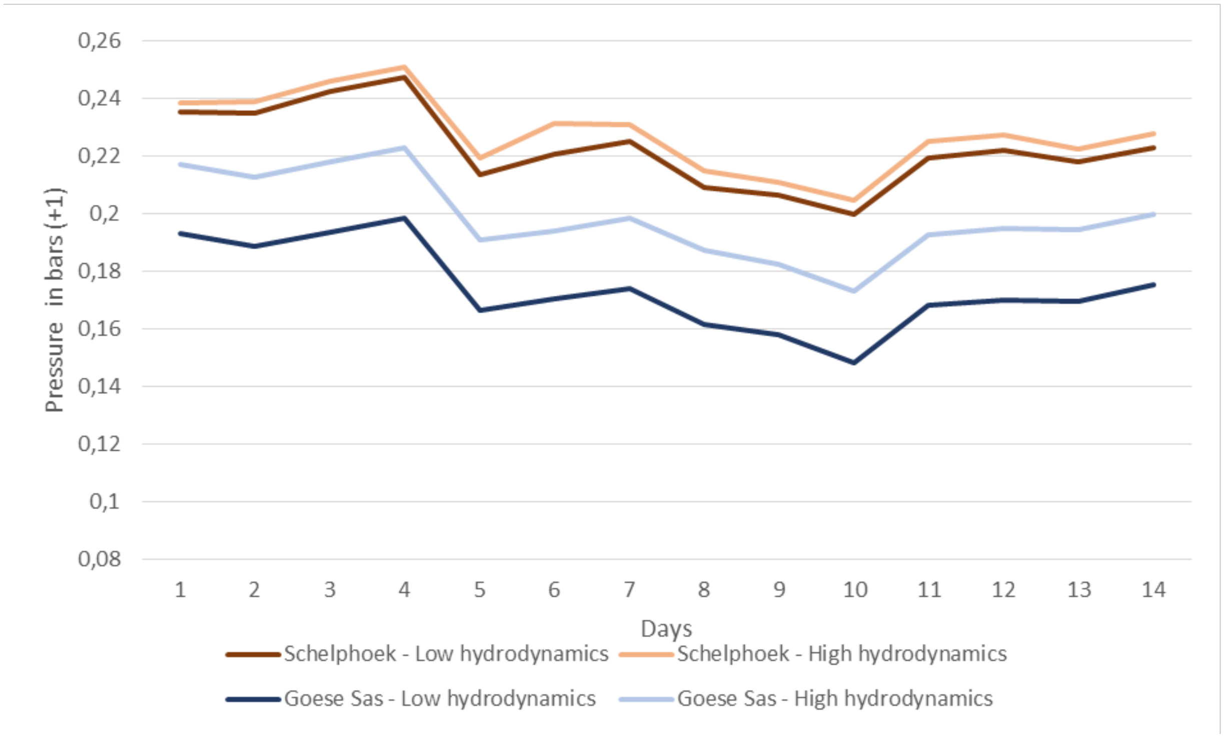

Figure 21. Average water pressure (in bars) at the 'low' and 'high'hydrodynamic locations at Schelphoek and Goese Sas where the growth of oysters was monitored.

Survivorship

Mortality was highest (survivorship lowest) in both locations in the low hydrodynamic area at 50% inundation with average survivorship dropping to 93% at Goese Sas and 88% at Schelphoek during the three week sampling period.

After 10 months survival at Goese Sas was higher in the low hydrodynamic side for 50% inundation (average of 86% survival) compared with 70% inundation (average of 76% survival) (Figure 22). At Schelphoek survival was highest in the low hydrodynamic area with 70% inundation time (average of 79% survival) and lowest in the low hydrodynamics and 50% inundation (average of 68%) while in the high hydrodynamics an average of 70% of oysters survived at both inundation times (Figure 23).

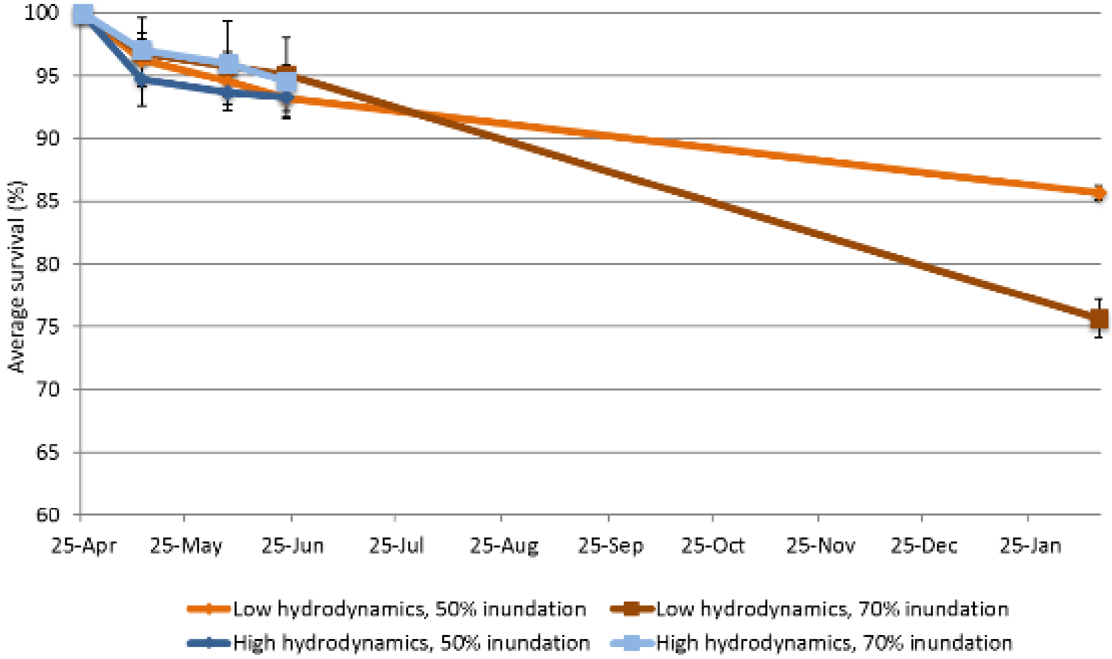

Figure 22. Average survival at Goese Sas of oysters in 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

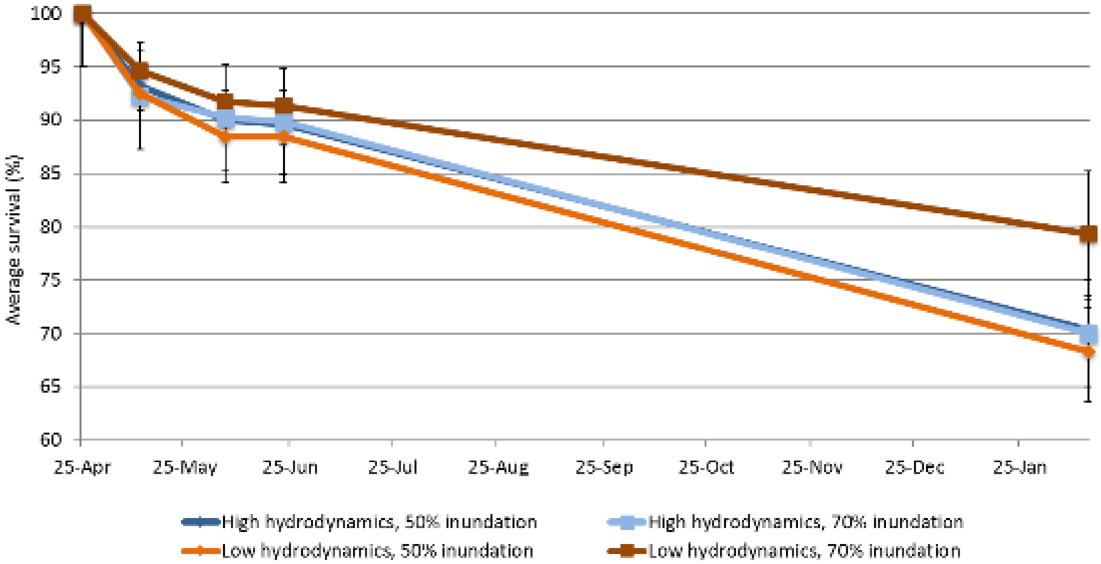

Figure 23. Average survival at Schelphoek of oysters in 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

It is important to compare the experimental data with that of other studies. Hiele and Hartog (unpublished data) monitored the growth and mortality of oysters in hanging baskets on the mud flat nearby the Goese Sas location of the present study monthly between June 2014 and April 2016. They compared oysters at two heights experiencing around 89% and 65% inundation time. Their experiment consisted of three replicate baskets each containing 5000 individuals weighing on average 137 g each. According to their data an initial high mortality was observed in both inundation periods in the first months of the experiment after which the survival rate remained relatively constant and similar for both inundation times.

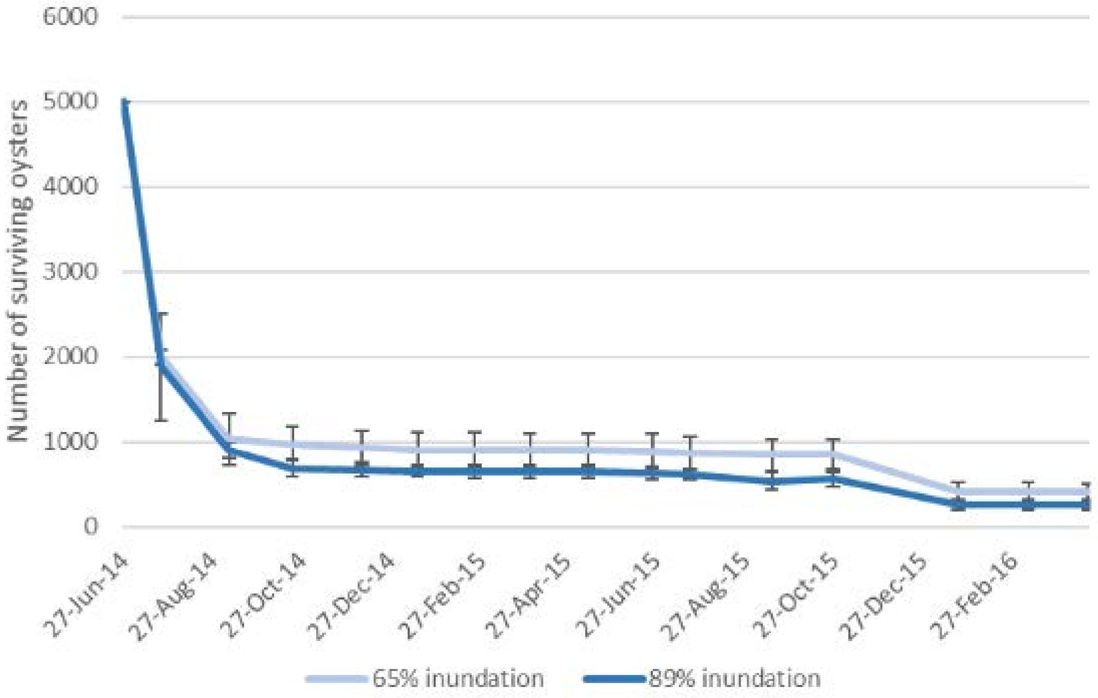

Figure 24. Survival of oysters grown in hanging baskets on the mud flat near Goese Sas at 65% anf 89% inundation time. (from Hiele and Hartog (unpublished data).

Growth

After the initial two months of monitoring the oysters from all treatments at both locations appeared to show an almost identical and very slight increase in wet weight. After 10 months, however, there appeared to be more difference in wet weight between treatments.

At Goese Sas the oysters that had remained after 10 months in the field in the low hydrodynamics location appeared to deviate in average weight with those in the low hydrodynamics showing an average weight of 81 g in 70% inundation and 59 g in 50% inundation (Figure 25).

At Schelphoek oyster wet weight as calculated in the field in the high hydrodynamic location with 50% inundation showed the greatest average weight per individual compared with the other treatments which ranged between 57 g in low hydrodynamics and 70% inundation and 61 g in high hydrodynamics and 50% inundation (Figure 26),

Hiele and Hartog (unpublished data) found that growth in both inundation times appeared identical for the two months before growth in 89% inundation increased at a faster rate than in 65% inundation. (Figure 27).

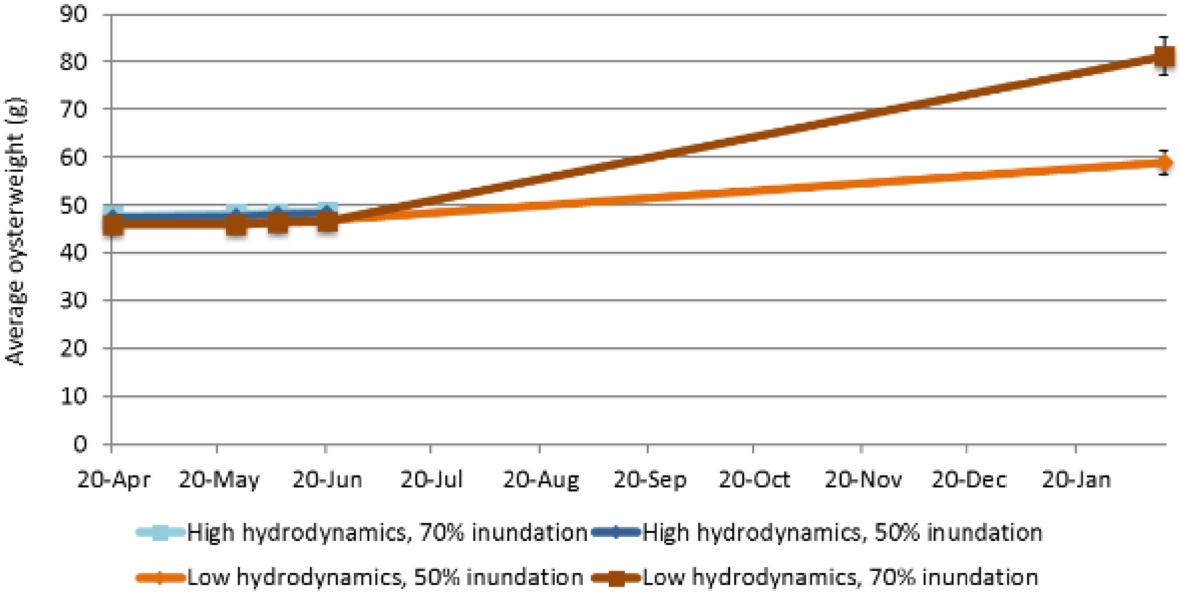

Figure 25. Average fresh weight (shell and meat) per individual oyster (extrapolated in the field) at Goese Sas over time in 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

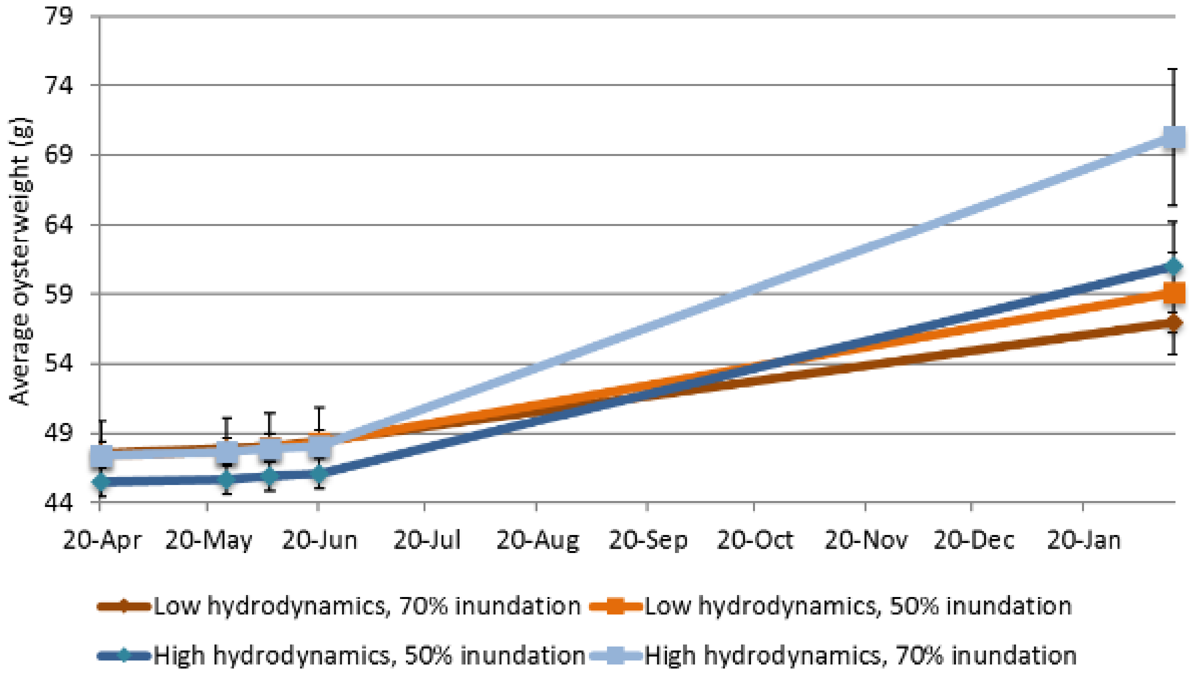

Figure 26. Average fresh weight per individual oyster (extrapolated in the field) at Schelphoek over time in 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

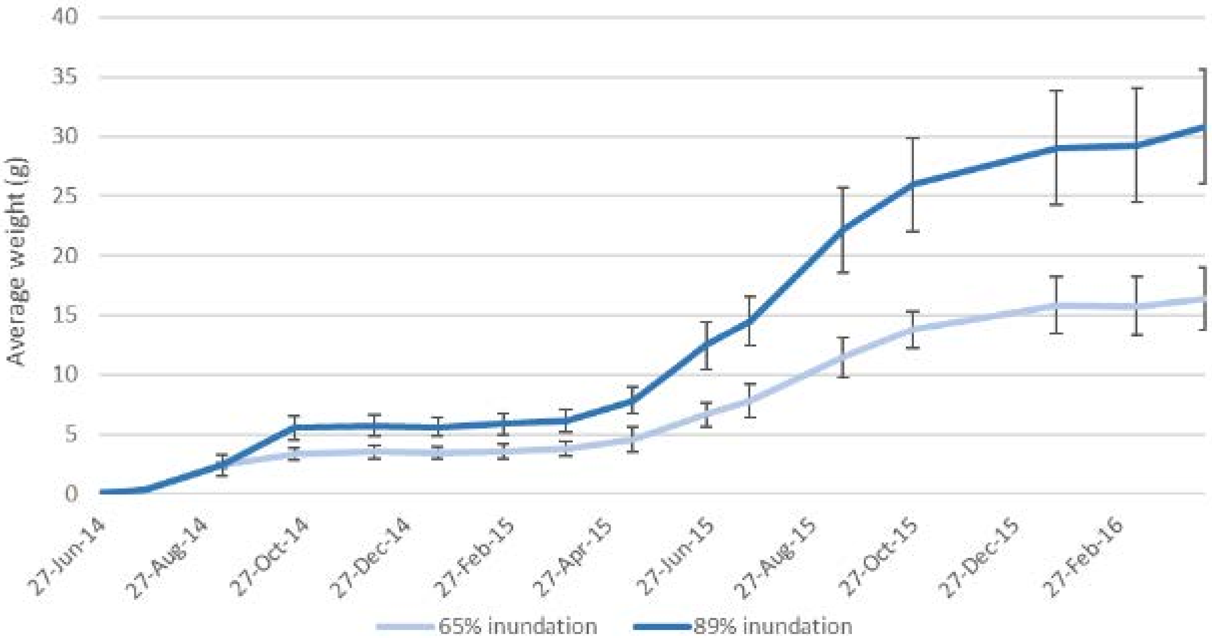

Figure 27. Average increase in fresh weight per individual oyster grown in hanging baskets on the mud flat near Goese Sas at two different heights/inundation periods. (from Hiele and Hartog (unpublished data)).

When comparing the final wet weight of the oysters (shell and meat) measured per individual in the lab (rather than extrapolated in the field) it appears that at Schelphoek oysters in low hydrodynamics and 70% inundation had slightly higher average wet weight per individual (Figure 28) compared with the field measurements of the weight of the living oysters divided by the number of living oysters. The lab results, however showed no statistically significant different between treatments in a Student's t-test (Table 3).

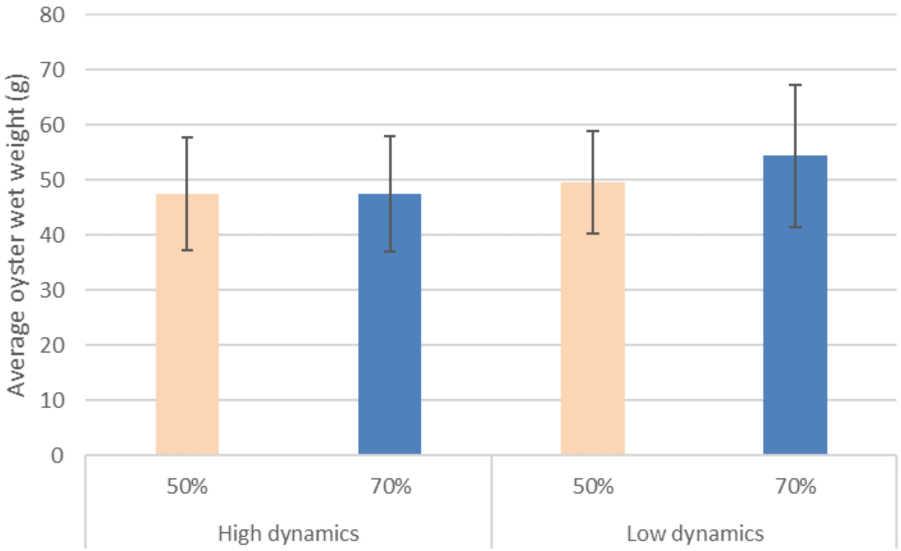

Figure 28. Average fresh weight per individual oyster (measured per individual as opposed to extrapolated) at Schelphoek 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

Table 3. Results of the student's t-test analysis of the wet weight of the oysters from the four treatments at Schelphoek after 10 months in the field. No significant different was found between the treatments.

Comparison	t-value	P-value
50% high vs 50% low dynamics	0.32242	> 0.05
70% high vs 70% low dynamics	0.54557	> 0.05
high dynamics 70% vs 50%	113.521	> 0.05
low dynamics 70% vs 50%	.488485	> 0.05

The average mm² as calculated by length x width x height of each individual oyster shell showed that the oysters in low hydrodynamics and 70% inundation were larger on average than those in the other treatments with an average of 1177 mm² (Figure 29). According to the students t-test, the only statistically significant difference in average mm² of oysters was that oysters in 70% inundation were larger in the low hydrodynamics compared with the high hydrodynamics (Table 4).

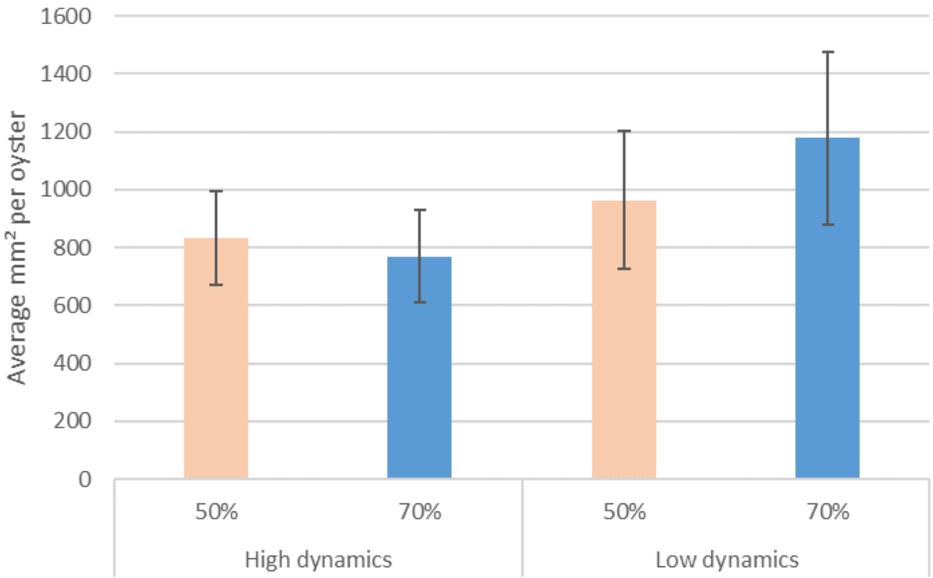

Figure 29. Average mm² per individual oyster (calculated in the lab as length x width x height) at Schelphoek 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

Table 4. Results of the student's t-test analysis of Average mm² per individual oyster from the four treatments at Schelphoek after 10 months in the field (significant p-value is highlighted) .

Comparison	t-value	P-value
50% high vs 50% low dynamics	101.733	> .05
70% high vs 70% low dynamics	.045163	< 0.05
high dynamics 70% vs 50%	0.27281	> .05
low dynamics 70% vs 50%	0.72048	> .05

The wet weight of the oysters (shell and meat) was divided by the mm² to give an indication of the amount of flesh of the oyster. Although by using this calculation it is not possible to determine what proportion of the weight is comprised of meat, shell, water or something else, it can nevertheless provide a rough indication of oyster quality.

While the average weight/mm² was highest in high hydrodynamics and 70% inundation and lowest in the low dynamics and 70% inundation (Figure 30), none of the treatments shows a statistically significant difference in average weight/mm² per oyster using a Student's t-test (Table 5).

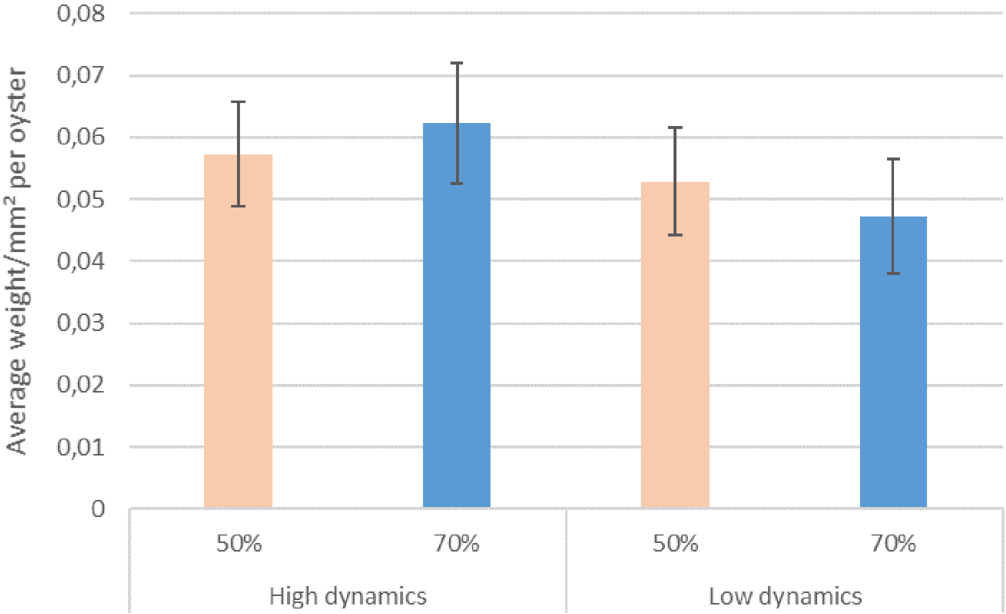

Figure 30. Average weight/mm² per individual oyster at Schelphoek 'low' and 'high' hydrodynamic locations and at 70% and 50% inundation time.

Table 5. Results of the student's t-test analysis of the weight/mm² per individual oyster from the four treatments at Schelphoek after 10 months in the field. No significant different was found between the treatments.

Comparison	t-value	P-value
50% high vs 50% low dynamics	-0.65219	> .05
70% high vs 70% low dynamics	-0.72171	> .05
high dynamics 70% vs 50%	113.521	> .05
low dynamics 70% vs 50%	-0.22973	> .05

Condition Index

There was much variation in CI of the oysters from all four treatments at Schelphoek after 10 months in the field (Figure 31). Although it appears that oysters experiencing 50% inundation and high water dynamics had the highest CI of all the treatments, the only statistically significant difference was that the 50% inundation with high dynamics had a higher CI than those in the low dynamics treatment (Table 4).

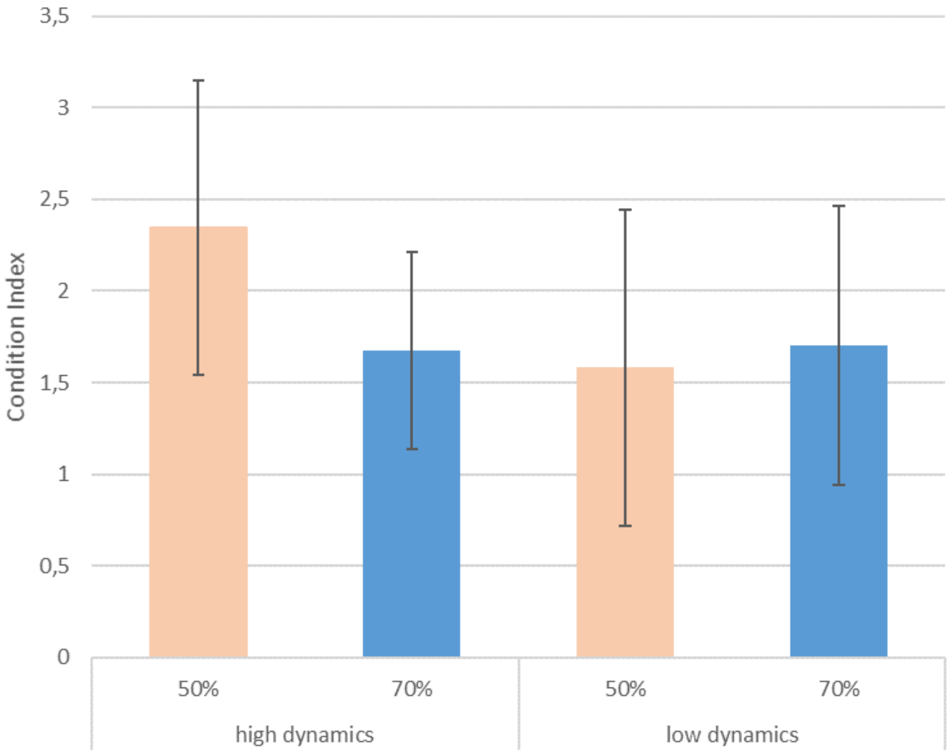

Figure 31. Condition Index of the oysters from the four treatments at Schelphoek after 10 months in the field.

Table 6. Results of the student's t-test analysis of the Condition Index of the oysters from the four treatments at Schelphoek after 10 months in the field (significant p-value is highlighted) .

Comparison	t-value	P-value
50% high vs 50% low dynamics	179.886	< 0.05
70% high vs 70% low dynamics	-0.44036	> 0.05
high dynamics 70% vs 50%	113.521	> 0.05
low dynamics 70% vs 50%	-112.764	> 0.05

Discussion

The results indicate a general high variation in ability of *Magallana gigas* to adapt to different environmental situations. In the laboratory experiment there was little obvious link between the high mortality observed in some treatments compared to others. While the different ratios of organic:inorganic material in the water and inundation time were expected determinants of growth and survival in *M. gigas*, this was not the case in the laboratory experiment. While some trend was observed where growth, gill:palp ratio and health index was higher when the oysters were submerged for longer and had less silt in the water, the variation between individual oysters prevented any statistically significant relationships between these factors. Furthermore, the gill:palp ratio and health index of the oysters after the experiment were not significantly different from the T0 measurements. This suggests that, for the oysters that survived, the organic:inorganic material in the water and the inundation time experienced by the oysters during the experiment were within the natural range of conditions to which the oysters can easily adapt without great detriment to their condition.

The laboratory experiment was conducted during a particularly warm period in May. Efforts were made to prevent excessive temperature discrepancy between the treatments with the use of shading and the physical orientation of the different treatments within the experiment. While this effort was generally successful, there was up to a 2 °C difference between treatments in the sun, and those in the shade. Whether this variation in temperature was enough to influence the results is difficult to establish due to the other variables present.

Nevertheless, despite the high variation, the results suggest that oysters in higher inundation time and a higher proportion of organic material show signs of more successful growth and condition. This suggests that successful aquaculture of *M. gigas* on the kreukelberm may be possible where the kreukelberm has long inundation periods and in locations with high phytoplankton levels and low levels of suspended sediment.

It is fair to say that the field experiment was an afflicted one. The original experimental design was aborted due to loss of equipment as a consequence of both natural processes (in terms of washing away), and anthropological ones (in terms of simple thievery). The second attempt at the field experiment was somewhat more successful in the sense that data was collected, albeit for a short period of time in some cases. The locked breakwater on which the experiment was placed at Goese Sas was not as secure as anticipated, at least in the high hydrodynamic area where the oysters were visible from the water and were eventually removed. While steps could be taken to prevent the oysters washing away, this experience serves as an indication that attempting to grow a commercial product in a publically accessible area must involve some form of security against opportunistic members of the public with a taste for shellfish.

As expected, oysters died in the field during the monitoring period. After two months of monitoring survival was close to 90% of the original number in both Goese Sas and Schelphoek. While the results of the present study come from a much smaller scale experiment, both this study and the experiment by Hiele and Hartog (unpublished data) show a mortality rate particularly early in the experiment. This early high mortality is typical for *M. gigas* (Troost, 2010) and indicate the necessity for long-term, larger scale experiments to be able to make reliable conclusions and comparisons of mortality in different environments. After 10 months survival ranged between 68% and 85%. The oysters grown in baskets nearby Goese Sas in the study by Hiele and Hartog (unpublished data) showed a plateau in survival following the initial steep decline. Whether this was also the case in the present study is difficult to determine considering the larger size of the oysters at the beginning of the experiment and the fewer measurements taken compared with their study.

Growth of the oysters in the field appeared to showed a similar trend for all treatments in the first two months of the experiment. The slope of the growth lines for all treatments at both locations barely deviated from each other in those initial measurements, but by the final measurement there was some deviation albeit not statistically significant. The growth observed shows that the in the early stages of the experiment the oysters had adapted to the environment in which they were placed and were able to grow. The fact that the growth rates appeared almost identical in the first months suggest that the environments for all treatments were within a similar range of stress or tolerance and none were considerably more optimal than the others. Hiele and Hartog (unpublished data) found that growth in both inundation times appeared identical for the two months before growth in 89% inundation increased at a faster rate than in 65% inundation. This suggests that the two months of regular monitoring in the present study was not sufficient to observe a notable difference in growth rate between the treatments.

The final growth measurements of the oysters at Schelphoek in February 2017 suggest that growth rate also deviated between the treatments following the initial measurements. The average wet weight per individual oyster after 10 months differed between the two methods used. The field method, for which the oysters were not individually measured showed higher average wet weight than those individually weighed in the lab. Although none of the differences in wet weight between treatments were statistically significant, it should be noted that while the field measurement technique is simple and easily achievable in the field, it is not particularly accurate when comparing it to the laboratory technique.

Calculating the average mm² of the oysters showed that oysters in 70% inundation grew larger shells in the low hydrodynamics compared with the high hydrodynamics, whereas there was no significant difference in average weight/mm². This result is not consistent with the results of Walne (1972) who found increased hydrodynamics lead to increased growth rate and in the current experiment the high hydrodynamics were unlikely to surpass the 1 cm s^{-1} suggested by Grizzle et al. (1992) as the water velocity threshold after which growth rate decreased. There are no obvious explanations for the present results.

While the condition index analysis showed little difference between treatments, there was a significant difference in CI between high and low water dynamics at 50% inundation. High water dynamics result in more water washing over the oysters in a given period of time. The more water an oyster comes into contact with, the more phytoplankton they will be able to consume (Pogoda, et al., 2011). Furthermore, the water that flows past the breakwater at Schelphoek is likely to be much more productive on the ‘high water dynamics’ side of the breakwater compared with inside the breakwater. The area outside the breakwater at Schelphoek is relatively productive and sustains various fisheries including mussel farms and lobster fisheries, while the inside of the breakwater primarily exists as a nature compensation area. Therefore, at least at Schelphoek, in the locations tested, at 50% inundation time, oysters grow to a higher quality outside of the breakwater in high water dynamics compared with inside the breakwater in low hydrodynamics.

The question of whether commercial aquaculture of *M. gigas* on the kreukelberm of dykes is possible very much depends on the amount of investment one is willing to make. If dykes with kreukelberms at heights where inundation periods were 70% or higher, could be found in locations with enough food availability in the water, and hydrodynamics to make it available to the oysters, and if a system was developed with which oysters could be efficiently placed without being washed away or stolen, then aquaculture of *M. gigas* on the kreukelberm could be possible. However, the investment necessary to meet all of the above criteria along with the potentially limited growth rate and condition of oysters grown on the kreukelberm make this a less than efficient option for aquaculturalists.

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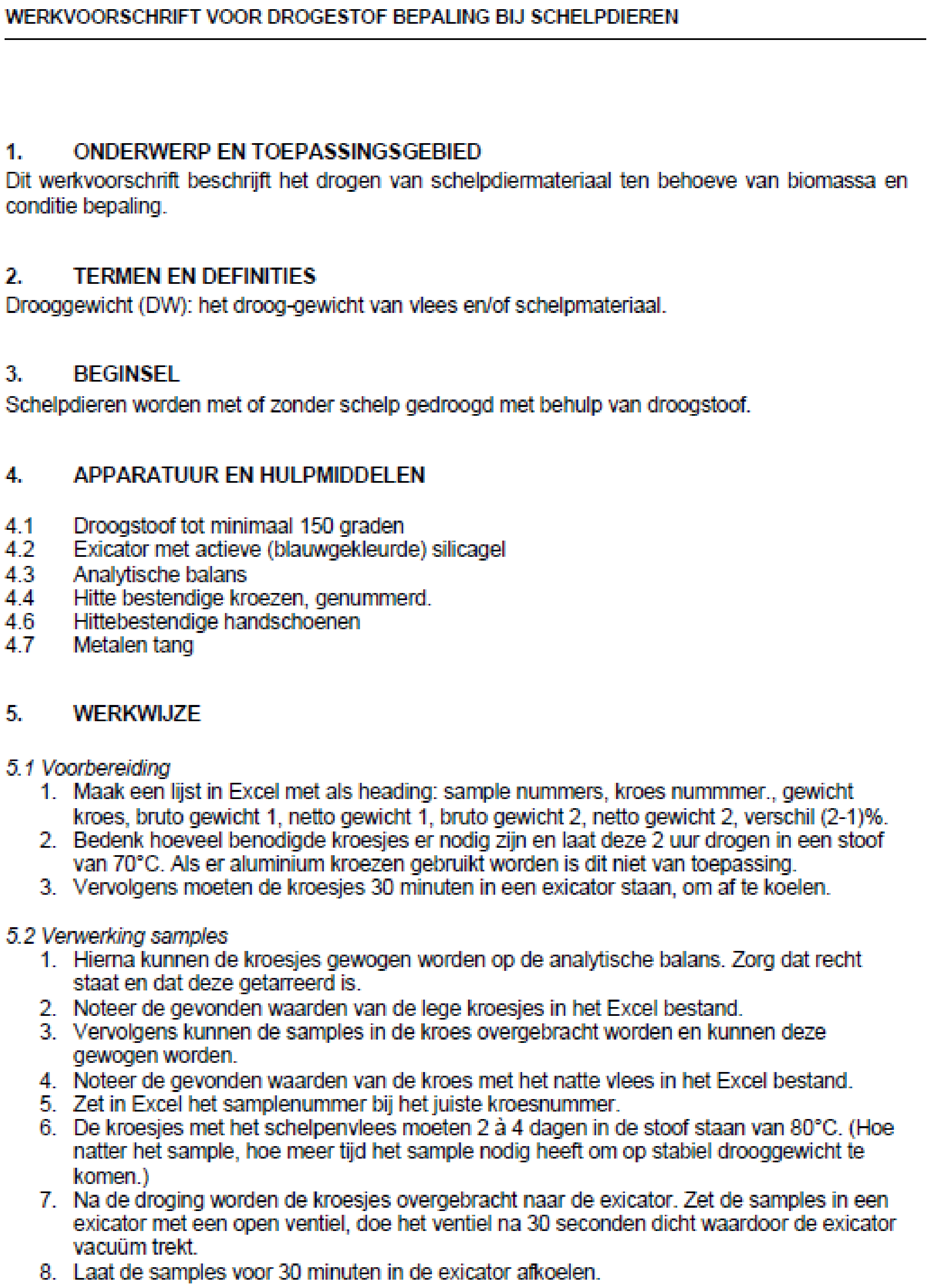
Appendices

Appendix 1. Protocol for determining the Dry Weight of bivalves



WERKVOORSCHRIFT VOOR DROGESTOF BEPALING BIJ SCHELPDIEREN

1. ONDERWERP EN TOEPASSINGSGEBIED

Dit werkvoorschrift beschrijft het drogen van schelpdiermateriaal ten behoeve van biomassa en conditie bepaling.

2. TERMEN EN DEFINITIES

Drooggewicht (DW): het droog-gewicht van vlees en/of schelpmateriaal.

3. BEGINSEL

Schelpdieren worden met of zonder schelp gedroogd met behulp van droogstoof.

4. APPARATUUR EN HULPMIDDELEN

4.1 Droogstoof tot minimaal 150 graden
4.2 Exicator met actieve (blauwgekleurde) silicagel
4.3 Analytische balans
4.4 Hitte bestendige kroezen, genummerd.
4.6 Hittebestendige handschoenen
4.7 Metalen tang

5. WERKWIJZE

5.1 Voorbereiding

1. Maak een lijst in Excel met als heading: sample nummers, kroes nummmer., gewicht kroes, bruto gewicht 1, netto gewicht 1, bruto gewicht 2, netto gewicht 2, verschil (2-1)%.
2. Bedenk hoeveel benodigde kroesjes er nodig zijn en laat deze 2 uur drogen in een stoof van 70°C. Als er aluminium kroezen gebruikt worden is dit niet van toepassing.
3. Vervolgens moeten de kroesjes 30 minuten in een exicator staan, om af te koelen.

5.2 Verwerking samples

1. Hierna kunnen de kroesjes gewogen worden op de analytische balans. Zorg dat recht staat en dat deze getarreerd is.
2. Noteer de gevonden waarden van de lege kroesjes in het Excel bestand.
3. Vervolgens kunnen de samples in de kroes overgebracht worden en kunnen deze gewogen worden.
4. Noteer de gevonden waarden van de kroes met het natte vlees in het Excel bestand.
5. Zet in Excel het samplenummer bij het juiste kroesnummer.
6. De kroesjes met het schelpenvlees moeten 2 à 4 dagen in de stoof staan van 80°C. (Hoe natter het sample, hoe meer tijd het sample nodig heeft om op stabiel drooggewicht te komen.)
7. Na de droging worden de kroesjes overgebracht naar de exicator. Zet de samples in een exicator met een open ventiel, doe het ventiel na 30 seconden dicht waardoor de exicator vacuüm trekt.
8. Laat de samples voor 30 minuten in de exicator afkoelen.

5.3 Weging van de samples

1. Vervolgens kan het drooggewicht bepaald worden met behulp van de analytische balans.
2. Noteer de gevonden waarden van de kroes met het sample in het Excel bestand.
3. Bereken het gewicht van het droge sample mbv:
   Droog gewicht kroes met droge sample – gewicht kroes = gewicht droge sample
4. Zet vervolgens de kroesjes terug in de stoof op 80°C voor 2 uur.
5. Verplaats na 2 uur de kroesjes naar de exicator en laat ze daar voor 30 minuten afkoelen. Zorg ervoor dat de exicator vacuüm trekt.
6. Weeg daarna de kroesjes met het sample voor de tweede keer. Bereken het netto gewicht van het sample. Het verschil tussen meting 1 en 2 kan nu als percentage uitgedrukt worden. (zie punt 6)
7. Omdat schelpdieren vaak in grotere hoeveelheden verast worden is het toegestaan een verschil te hebben met een afwijking van 1%. Als d afwijking meer is dan deze 1% wordt het protocol vanaf 5.3.5 herhaald. Dit wordt net zolang herhaalt tot het percentage lager is dan 1% .(Bij kleinere samples kan het toegestane verschil tot 0.1% teruggebracht worden.)

6. Berekeningen.

6.1 Netto droog gewicht

Bruto drooggewicht – gewicht kroes

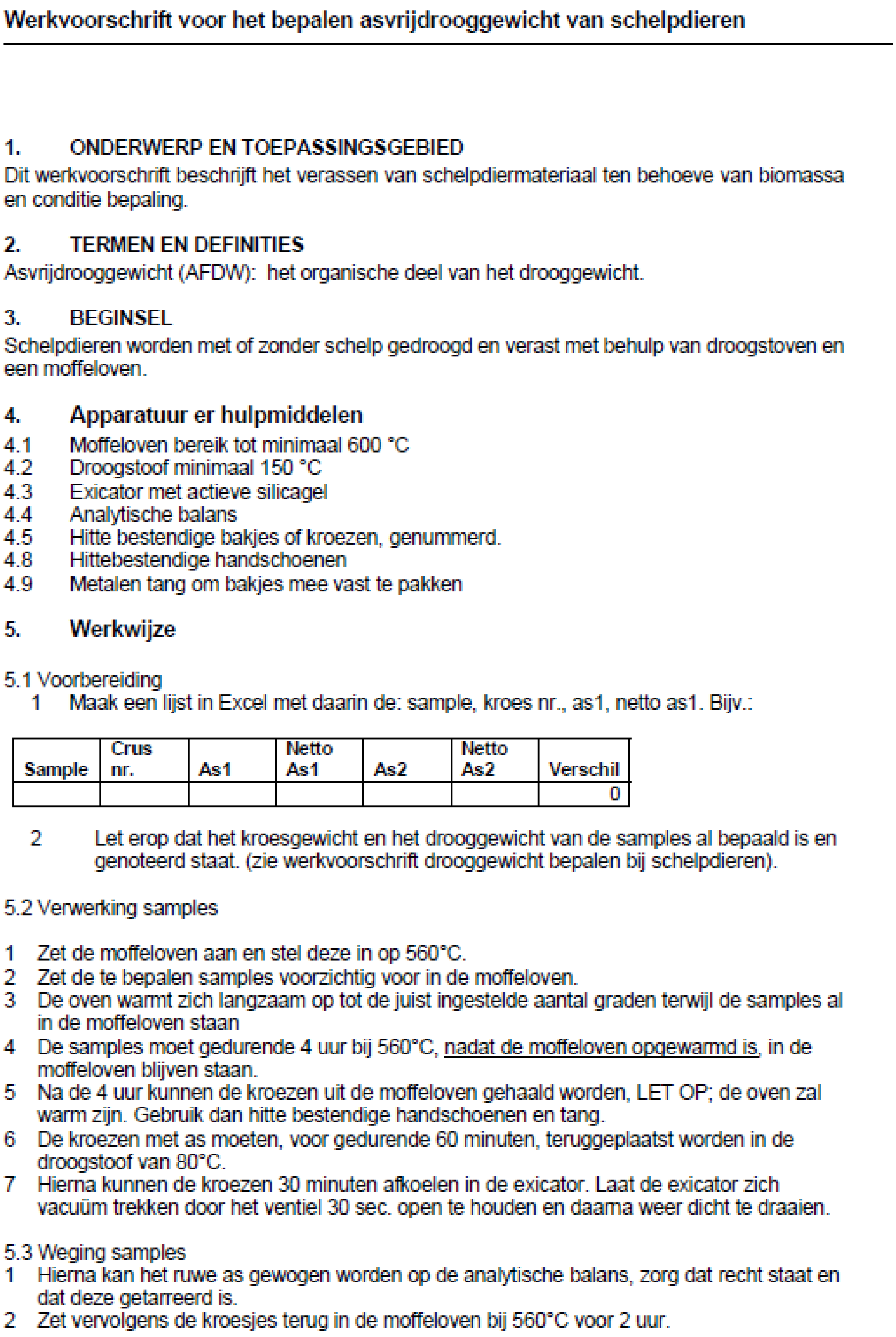
6.2 Percentuele afwijking

((netto gewicht 1 – netto gewicht 2) * 100)/ netto gewicht 1

6.3 voorbeeld spreadsheet

	A	B	C	D	E	F	G	H
1	Sample nummer	kroes nummer	gewicht kroes (g)	bruto gewicht 1 (g)	Netto gewicht 1 (g)	bruto gewicht 2 (g)	Netto gewicht 2 (g)	Verschil 2-1 (%)
2	meep 8	5	20.01	31.22	=D2-C2	30.11	[illegible]	=((E2-G2)*100)/E2

Appendix 2. Protocol for determining the Ash Free Dry Weight of bivalves



Werkvoorschrift voor het bepalen asvrijdrooggewicht van schelpdieren

1. ONDERWERP EN TOEPASSINGSGEBIED

Dit werkvoorschrift beschrijft het verassen van schelpdiermateriaal ten behoeve van biomassa en conditie bepaling.

2. TERMEN EN DEFINITIES

Asvrijdrooggewicht (AFDW): het organische deel van het drooggewicht.

3. BEGINSEL

Schelpdieren worden met of zonder schelp gedroogd en verast met behulp van droogstoven en een moffeloven.

4. Apparatuur er hulpmiddelen

4.1 Moffeloven bereik tot minimaal 600 °C
4.2 Droogstoof minimaal 150 °C
4.3 Exicator met actieve silicagel
4.4 Analytische balans
4.5 Hitte bestendige bakjes of kroezen, genummerd.
4.8 Hittebestendige handschoenen
4.9 Metalen tang om bakjes mee vast te pakken

5. Werkwijze

5.1 Voorbereiding

1 Maak een lijst in Excel met daarin de: sample, kroes nr., as1, netto as1. Bijv.:

Sample	Crus nr.	As1	Netto As1	As2	Netto As2	Verschil
						0

2 Let erop dat het kroesgewicht en het drooggewicht van de samples al bepaald is en genoteerd staat. (zie werkvoorschrift drooggewicht bepalen bij schelpdieren).

5.2 Verwerking samples

1 Zet de moffeloven aan en stel deze in op 560°C.
2 Zet de te bepalen samples voorzichtig voor in de moffeloven.
3 De oven warmt zich langzaam op tot de juist ingestelde aantal graden terwijl de samples al in de moffeloven staan
4 De samples moet gedurende 4 uur bij 560°C, nadat de moffeloven opgewarmd is, in de moffeloven blijven staan.
5 Na de 4 uur kunnen de kroezen uit de moffeloven gehaald worden, LET OP; de oven zal warm zijn. Gebruik dan hitte bestendige handschoenen en tang.
6 De kroezen met as moeten, voor gedurende 60 minuten, teruggeplaatst worden in de droogstoof van 80°C.
7 Hierna kunnen de kroezen 30 minuten afkoelen in de exicator. Laat de exicator zich vacuüm trekken door het ventiel 30 sec. open te houden en daarna weer dicht te draaien.

5.3 Weging samples

1 Hierna kan het ruwe as gewogen worden op de analytische balans, zorg dat recht staat en dat deze getarreerd is.
2 Zet vervolgens de kroesjes terug in de moffeloven bij 560°C voor 2 uur.

3. De kroezen met as moeten, voor gedurende 60 minuten, teruggeplaatst worden in de droogstoof van 80°C.
4. Hierna worden de kroesjes naar de exicator geplaatst waar ze voor 30 minuten afkoelen. Zorg ervoor dat de exicator vacuüm trekt.
5. Weeg daarna de kroesjes met het sample voor de tweede keer. Bereken het netto gewicht van het sample.
6. Zet de gevonden waarden in het Excel bestand en bereken het netto verschil uit in procenten.

(((Netto as1 – Netto as2)*100)/ Netto as1) = verschil tussen as1 en as2 in %

7. Is er een afwijking gevonden die groter is dan 1% dan moet de kroes nog 2 uur in de oven verhit worden op 560°C. LET OP; de oven zal warm zijn. Gebruik dan hitte bestendige handschoenen en tang.
8. De kroes gaat daarna 60 minuten in de stoof en vervolgens 30 minuten in de exicator.
9. Hierna kan het ruw as gewicht berekend worden.
10. De stappen vanaf 5.2 worden herhaalt tot de afwijking minder dan 1% is.

6. Berekeningen

6.1 Netto asvrijdrooggewicht:
Bruto drooggewicht – gewicht kroes

6.2 Percentage afwijking:
(((Netto as1 – Netto as2)*100)/ Netto as1) = verschil tussen as1 en as2 in %

6.3 Asgewicht
Asvrijdrooggewicht - Drooggewicht

6.4 Voorbeeld spreadsheet:

Sample	kroes (=nr)	Gewicht kroes (g)	Gewicht kroes + asgewicht Sample 1 (g)	Netto asgewicht Sample 1 (g)	Gewicht kroes + asgewicht Sample 2 (g)	Netto asgewicht Sample2 (g)	Verschil (%)	Asvrij droog gewicht	Asgewicht
bv: meep 8	5	20.01	31.22	=(31.22-20.01)	30.11	=(30.11-20.01)	=(((11.21-10.10)*100)/11.21)	10.1	=(20.01-10.10)

7. BIJLAGEN EN VERWIJZINGEN

Literatuur	Titel
Drooggewicht	WERKVOORSCHRIFT VOOR DROGESTOF BEPALING BIJ SCHELPDIEREN