Cultivation of the Pacific oyster Crassostrea gigas in a FLUPSY

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6-3-2014, Vlissingen

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Report type:	Internship Bachelor Water Management
Date:	6-3-2014
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PREFACE

The internship report that is lying in front of you is the result of half a year research performed for the Roem van Yerseke at the HZ University of Applied Sciences, Research group Aquaculture in Delta Areas. As part of the RAAK Pro project of the Research group Aquaculture in Delta Areas, research on the Floating Upweller System (FLUPSY) of the Roem van Yerseke was performed to optimize the system and product, the Pacific oyster.

Looking back at the research, it proved to be very instructive. The research period has gone through ups and downs. The setting-up of the experimental proved to be more difficult than initially thought. But by perseverance the research was carried out in a successful manner. This period has learned me a lot on how to perform a scientific experiment, my scientific writing skills, and analytical skills and last but not least my statistical skills have improved substantial.

I would like to express my special thanks to my supervisors Jorik Creemers and Jacob Capelle for helping me to determine the right way forward of the experiment, giving constructive criticism, and helping analyzing my results and making the report to what it is now.

A second word of thanks goes to Frank Peene and Johan de Bat from the Roem van Yerseke. I would like to thank them for helping me, advising and entrusting me with working on their FLUPSY and supporting the experiment.

Third I would like to thank friend and colleague Friso Snijder for assisting multiple times in cleaning and setting up the experiment. Furthermore his sense of humor and swimming instructions has kept my spirit high.

Fourth I would like to thank Eva Hartog in assisting me with the initial contact with the Roem van Yerseke and helping me get the project. Furthermore the interviews for the RAAK Pro project were informative and have given me a look in the aquaculture world.

A fifth word of thanks goes to Mitra Vaskoska for helping analyzing the Chlorophyll-α samples.

Sixth I would like to thank Emiel Brummelhuis for helping me out analyzing the data collected by the C3 datalogger.

Last but not least I would like to thank Luc Smit from the Yacht Harbor at Wolphaartsdijk where the FLUPSY is located for advice and a friendly chat every time.

SUMMARY

The Roem van Yerseke breeds its oyster spat (*Crassostrea gigas*) indoors, which is called the hatchery phase. Spat is kept in an upwelling system, fed with cultivated micro algae. Once the spat reaches the T2 phase (± 3mm) the spat is moved outdoors, where the nursery phase begins and the oysters are kept till they are large enough to be placed in baskets or other cultivation methods where they grow till consumption size. The T2 spat is placed in a Floating Upweller System (FLUPSY) which is located in open water. In the FLUPSY natural occurring food (micro algae) is pumped through the spat, which distributes food in an efficient way over the oyster spat. Oyster spat is placed in silos, where the water flow comes in underneath, through a mesh on which the spat is rested. The water flow distributes the food efficiently and removes the faeces and other solids. An additional benefit from the upwelling water flow is that the spat abrades one another (the spat is kept in a slight suspension), which results in a cup shaped shell with a higher flesh weight compared to oyster not kept in a FLUPSY. Another additional benefit is that the constant abrading prevents biofouling from occurring on the shells, resulting in a clean product. These additional benefits result in a more valuable product.
There were however some problems, once the spat grew the water flow was no longer sufficient to keep them in suspension. The cup shaped shell could thus no longer be guaranteed, faeces were no longer efficiently removed, and biofouling started to grow on the oysters. On behalf of the Roem van Yerseke research at the HZ University of Applied Sciences was carried out. The research focused on finding the optimal flow rate for different sizes of oyster spat (T2, T4 and T6) for obtaining a cup shaped shell with an optimal biofouling removal.
The research question is: *Does an increasing flow rate in the silos of the FLUPSY result in a more cup shaped shell of the oyster spat (T2, T4 and T6) and does a higher flow rate reduces the biofouling on the shells of the Pacific oyster (Crassostrea gigas)?*

Over the course from the 5th of November till the 3rd of December a field experiment was set up in the FLUPSY. The first approach of the experiment was to figure out how to keep the different sizes oyster spat in suspension. It became clear that a flow rate difference was present in the FLUPSY; flow rate was higher close to the outflow source (paddlewheel) and indicatively lower at the very end. Based on these findings the largest spat (T6) was placed close to paddlewheel, the smallest spat (T2) at the very end of the FLUPSY and the medium sized T4 spat in-between. The water flow through the silo and layer of oyster spat showed to be not evenly spread, causing the entire water flow passing through a weak spot of the oyster layer. By doing this only a small percentage of the spat was kept in suspension. This problem was tackled by placing a pipe over the point of outflow in the silos. This pipe contained several small holes through which the water was sucked out into the central out flowing trough of the FLUPSY. The application of these pipes made sure the water flow through the silo and oyster spat was more evenly distributed, causing all the spat in a silo to be in suspension.

Available oyster spat was divided in size classes; T2, T4 and T6 spat. For the T2 spat two different treatments were performed, one being the T2(S) treatment in which the pipes were equipped in the silos (suspension was maintained), the other being the old situation as it was, being without a pipe attached in the silo. The treatments without a pipe attached in the silos served as a control.
T4 spat was subjected to three different treatments; the first being the old situation T4(HS), again without the addition of a pipe and thus not being in suspension. The second being the treatment in which the spat was brought into suspension (T4(S)) with the aid of the pipes. The third being the treatment in which the flow rate was increased as the spat grew toward the T6 stage (T4(T6S)).
T6 spat was subjected to 4 different treatments. T6(HS) being the treatment in which the old situation, thus no suspension, is created. Here again the spat is brought in suspension, T6(S). The third treatment placed the T6 spat in a location in which T4 spat was brought in suspension (T6(T4S)), this treatment was intended to show was the result was of this sub-optimal flow rate. The fourth placement concerned placing the spat in a silo in which the flow rate was increased as the spat grew to the T8 phase (T6(T8S)).
All mentioned treatments were performed in duplicate. A sample of 40 oysters was taken out of all silos weekly.

Environmental parameters were measured weekly by hand (pH, salinity, temperature, dissolved oxygen and chlorophyll-α). Beside the manual measurements, a C3 datalogger was installed to measure salinity, dissolved oxygen and chlorophyll-α continuously.

Results show that the variation in flow rate has had no significant impact on the oyster spat. An increase or decrease in flow rate has had no measurable positive influence on the improvement of the cup shape of the oyster spat. Neither was an increased prevention of biofouling measured.
Furthermore an ANOVA single factor based on the length measurement showed that the variation between groups of one size class was too big to assume a homogeneous distribution at the start of the experiment.

Based on the results of this experiment a variation in flow rate does not appear to have an effect on the improving of a cup shaped shell, nor was a higher prevention of biofouling measured. The results show that the initial flow rate as set by the Roem van Yerseke (HS flow rate indication) was optimal regarding the obtaining of a cup shaped shell and the maximum biofouling prevention. It must however be empathized that the experiment was carried out in late fall 2013, outside the growing season and already less available micro algae. However the addition of a pipe to the outflow of the silos did reduce the amount of silt collecting in the silos (not measured by visually noticeable) which leads to less required cleaning maintenance. Furthermore the addition of the pipes in the silos keeps the oyster spat in better suspension (more movement) which reduces the possibility of oyster remaining stagnant and growing attached to others. Another useful finding of this experiment was the divisions made for size classes to specific locations in the silos. The combination of these locations and the application of the pipes results in cleaner silos and are currently implemented.

SAMENVATTING

De Roem van Yerseke kweek oesterbroed (Crassostrea Gigas) indoor in the hatchery (hatchery fase). Oesterbroed wordt in een upwelling system gehouden, waar het gevoerd wordt met microalgen. Wanneer het oesterbroed een grootte van ±3 mm heeft bereikt (aangeduid met T2 fase) wordt het broed buiten geplaats in verschillende kweekmethodes tot de consumptie grootte bereikt wordt, de zogenaamde nursery fase. Het T2 broed wordt in een Floating Upweller System (FLUPSY) geplaatst, een systeem dat het voedsel efficiënt verdeeld over het aanwezige oesterbroed (Crassostrea gigas). Het oesterbroed wordt in silo's geplaatst, waar de waterstroom langs onder, door een maas waarop het broed rust stroomt. De waterstroom verdeeld het natuurlijk voorkomende voedsel (microalgen) efficient en verwijderd de faeces en andere vaste stoffen. Een bijkomend voordeel van de upwelling waterstroom is dat het broed in een suspensie komt en constant tegen elkaar aan schuurt, wat resulteert in een betere cup gevormde schelp, wat resulteert in een hoger visgewicht vergeleken met oesters die niet zijn opgekweekt in een FLUPSY. Een ander bijkomend voordeel is dat het constante schuren aangroei voorkomt op de schelpen, wat resulteert in een schoon product. Deze bijkomende voordelen maken dat de waarde van het product stijgt.
Er deden zich echter problemen voor, naarmate het broed groeit werd de snelheid van de waterstroom te laag om het broed in suspensie te houden. Hierdoor kon de garantie van het verkrijgen van een cup gevormde schelp niet langer gegarandeerd worden, faeces werd minder effectief verwijderd en aangroei begon te verschijnen. Namens de Roem van Yerseke werd er aan de HZ University of Applied Sciences onderzoek uitgevoerd. Het onderzoek richt zich op het vinden van een optimale behandeling in stroomsnelheid in de silo's voor verschillende groottes oesterbroed (T2, T4 en T6) om een cup gevormde schelp te verkrijgen en een optimale aangroei preventie te hebben.
De onderzoeksvraag is: Resulteert een toenemende stroomsnelheid in de silo's van de FLUPSY in een meer cup gevormde schelp van het oesterbroed (T2, T4 en T6) en resulteert deze hogere stroomsnelheid ook in preventie van aangroei op de Japanse oester (*Crassostrea gigas*)?

Gedurende de periode van 5 november tot de 3e van december heeft er een veldproef gelopen naar de FLUPSY. De eerste aanpak van het experiment was om te kijken hoe verschillende groottes oesterbroed in suspensie gehouden kunnen worden. Het werd duidelijk dat de stroomsnelheid in de FLUPSY niet overal gelijk was, wat betekende dat het grootste broed (T6) dicht bij het punt van uitstroom gehangen moest worden (schoepenrad), het kleinste broed (T2) aan de andere kant van de FLUPSY en het middel broed T4 hier tussen in. De waterstroom door de silo en door de laag oesterbroed bleek niet gelijkmatig verdeeld te zijn, wat resulteerde in dat de gehele waterstroom door een kleine opening stroomde in het zwakste punt binnen in de laag oesterbroed. Doordat dit gebeurde kwam er slechts een klein deel van het oesterbroed in suspensie, het merendeel bleef stil op de bodem liggen. Dit probleem werd opgelost door een opzetstuk te plaatsen op het punt van uitstromen binnenin de silo's. Deze opzetstukken bevatten meerdere gaten waardoor de waterstroom gaat en de silo verlaat. Deze applicatie zorgde ervoor dat de waterstroom meer evenredig verdeeld werd door de laag oesterbroed, wat er voor zorgde dat al het broed in de silo in suspensie kwam.

Beschikbaar broed werd verdeeld in klassen naar grootte: T2, T4 en T6 broed. Het T2 broed werd aan twee verschillende behandeling onderworpen, een daarvan is de T2(S) behandeling waarbij de opzetstukken in de silo's werden geplaatst (suspensie staat), de andere de oude situatie, dus zonder een opzetstuk in de silo's. De behandelingen zonder een opzetstuk dienden als een controle (de oude situatie). Het T4 broed werd aan drie verschillende behandelingen onderworpen: de eerste is de oude situatie T4(HS), wederom zonder een opzetstuk. De tweede behandeling is T4(S) waarbij het broed doormiddel van de locatie in de FLUPSY en het opzetstuk in suspensie werd gebracht. De derde handeling is een behandeling waarbij de stroomsnelheid geleidelijk werd opgevoerd naarmate het broed groeit naar de T6 fase toe (T4(T6S)).
T6 broed werd aan vier verschillende behandelingen onderworpen. T6(HS) is de eerste, waarbij de oude situatie wederom werd nagebootst. Bij T6(S) werd het broed wederom in suspensie gebracht. De derde behandeling is het situeren van het T6 broed op een locatie waar T4 broed normaal gesproken in suspensie is; T6(T4S). Deze behandeling diende om de effecten van een sub-optimale stroomsnelheid te laten zien. De vierde behandeling plaatst het broed wederom in een silo waar de stroomsnelheid wordt opgevoerd naarmate het broed groeit naar de T8 fase (T6(T8S)).

Alle boven genoemde behandelingen werden in duplo uitgevoerd. Wekelijks werd er een monstername van 4 individuen genomen per silo.
Omgevings parameters werden wekelijks met de hand gemeten (pH, saliniteit, temperatuur, zuurstof gehalte en chlorofiel-α). Naast de metingen met de hand werd er een C3 datalogger opgehangen die de saliniteit, zuurstifgehalte en chlorofiel-α continu registreerde.

De resultaten lieten zien dat een variatie in stroomsnelheid geen significante effecten had op het oesterbroed. Een toename of afname in stroomsnelheid had geen meetbare positieve effecten op de verbetering van de cup vorm van het oesterbroed. Tevens werd er geen positief effect op de preventie van aangroei gemeten. Een ANOVA single factor analyse gebaseerd op de lengte metingen toonde aan dat de variatie binnen een grootte klasse te groot was om een homogene verdeling aan de start van het experiment aan te kunnen nemen.

Gebaseerd op de resultaten van die onderzoek bleek dat een variatie in de stroomsnelheid geen effect bleek te hebben op een verbetering van de cup vorm van het schelp, noch had het een positief effect op de preventie van aangroei. De resultaten laten zien dat de initiële start situatie zoals ingesteld door de Roem van Yerseke (aangeduid met HS) vrijwel optimaal was gezien het verbeteren van de cup vorm of aangroei preventie. De plaatsing van een opzetstuk in de silo's heeft echter wel de hoeveelheid slib in de silo's verminderd (gebaseerd op visuele waarnemeningen) wat resulteert in minder onderhoud. Verder resulteert het gebruik van de opzetstukken in meer beweging binnen het broed in een silo, wat de kans verkleint op het onderling vergroeien van oesterbroed. Een andere bruikbare uitkomst van het onderzoek was de positionering van de silo's binnen in de FLUPSY naar de grootte van het broed. Een combinatie van deze drie toepassingen resulteert in schonere silo's en worden nu geïmplementeerd.

CONTENTS

1. INTRODUCTION

1.1. MOTIVATION AND OBJECTIVE

The southwest delta of the Netherlands is an area which has been altered by man for centuries. The delta is known for its fertile soils and its rich fishing grounds. The fishing industry of fish and shellfish has been an important economical factor for this region of the Netherlands for decades. However it becomes harder for the sector to remain economically viable. Increasing competition, declining margins and increasing spatial limitations require for innovations of the sector to ensure its existence (Aquaculture, 2012).
Within the project RAAK PRO *Zilte Productie* (English: Salty Production) the HZ University of Applied Sciences works closely together with IMARES-Wageningen UR, Dalhousie University (Canada) and shellfish cultivation entrepreneurs in the Southwest Delta area of the Netherlands for a period of 4 years. The goal of this consortium is to gain more knowledge in shellfish cultivation techniques, optimizing these techniques and work closely together with the entrepreneurs to stay innovating, achieving the fullest possible understanding in the relation between environmental factors (temporal variation), controllable variables and harvest (Aquaculture, 2012). This results in the research question for the RAAK PRO Zilte Productie:

What is the effect of the controllable variables on the seaside embankment on- and off bottom shellfish cultivation in relation with the temporal variation in environmental factors in the Southwest Delta area?

Remote data collecting devices or data loggers will be purchased and placed on the cultivation locations of the affiliated shellfish cultivation entrepreneurs. This data collection on the local environmental factors (food concentration, flow rates and temperature) and several controllable variables (starting size of the cultivated species and frequency of decimating of the stock) will give more sense on the local circumstances. All this data will be used to make a model, which will act as action plan for the shellfish cultivators. This action plan can be used to make decisions on the controllable variables to reach eventually a maximal harvest of the product.

One of the shellfish production locations is the yacht harbor of Wolphaartsdijk where the Roem van Yerseke placed a FLUPSY. This production system is different than the oyster grow-out plots, and specifically aims for the nursery phase of oyster production. FLUPSY stands for FLoating UPweller SYstem and is a fairly new technique which acts as a nursery system for shellfish. The Roem van Yerseke is breeding with oysters in their indoor hatchery (hatchery phase). At a certain point the oysters cultivated here are becoming too big, and need to be relocated outdoors. But the oysters are not big enough to be located in the wild and let them remain there till they reach consumption size. Therefore the oysters spat is placed outdoors in a FLUPSY, the so called nursery phase. In this system the oysters will remain till they are big enough to be relocated in either oyster baskets, or sewn onto a plot on the bottom.
The FLUPSY is easily accessible (for it is positioned in a yacht harbor), the circumstances are somewhat more controlled and there were some concrete questions.

The FLUPSY is designed in such a way to make sure that an optimal food supply is guaranteed and the feces and other suspended solids are removed automatically. This is done by creating a flow rate through the containers (silos) in which the spat is placed. This flow rate is keeping the spat in a suspension state, meaning the spat is not remained still in the silos. An additional benefit of this suspension state is that the spat is constantly bumping into each other. By this bumping into each other, the growth edges are abraded from the shells, which results in a more

cup shaped shell, which also results in a more smooth shaped shell compared to the ridged and course shape of a wild bred oyster. This cup shape results in a higher flesh weight, and thus a more desired product. Furthermore the bumping into each other removes biofouling which gets attached to the shells. The FLUPSY shows to be a promising nursery technique, with a relative high survival rate compared to other nursery systems and with several already mentioned advantages.

The question from the Roem van Yerseke was how they could optimize this system, for the exact flow rate through the silos remained unknown. Furthermore they wanted to know what flow rate was required for different sizes of oyster spat, and if the advantages of the FLUPSY could be increased. In short the question was how the FLUPSY nursery system and the product could be optimized.

1.2. MAIN QUESTION AND HYPOTHESIS

The Roem van Yerseke has been using the FLUPSY for several years now, and just as many variants of the FLUPSY. The system shows to be very promising, yet some questions remain on how to optimize the FLUPSY. The main question as proposed by the Roem van Yerseke is if a variation in the flow rate has a positive effect on the shape of the oyster spat, the removal or prevention of biofouling and on the growth (rate) of the oyster spat.

The research question and the (null) hypotheses are as follows:

> *Does an increasing flow rate in the silos of the FLUPSY result in a more cup shaped shell of the oyster spat (T2, T4 and T6) and does a higher flow rate reduces the biofouling on the shells of the Pacific oyster (Crassostrea gigas)?*

Null hypothesis (H_0): A variation in the flow rate does not result in a more cup shaped shell, optimal growth and maximal prevention of biofouling accumulation on the shells of the Pacific oyster spat. A variation in the flow rate to a state in which the oyster spat is fluidized (suspension state) and is upscalled synchronized to the growth does not result in a positive effect on the shape, growth and biofouling prevention on the shells of the oyster spat.

Alternative Hypothesis (H_A): A variation in the flow rate does result in a more cup shaped shell, optimal growth and a maximum prevention of biofouling accumulation on the shells of the Pacific oyster spat. The best positive effects are achieved for treatments (variation in flow rate) in which the spat is fluidized (suspension state) and is upscalled synchronized to the growth.

SUB-QUESTIONS

1. What is the optimal treatment for different sizes of oyster spat (T2, T4 and T6), to keep them in suspension and prevent them from flushing out of the system or remain still on the bottom?
2. What is the optimal treatment for different sizes of oyster spat, to make sure the spat is kept in suspension which is required to abrade the growth edges (desired cup shape) and prevent biofouling on the shells?

2. BACKGROUND

2.1. THE PACIFIC OYSTER (CRASSOSTREA GIGAS)

The Pacific oyster (*Crassostrea gigas*) is a bivalve of the family Ostreoida (Figure 1). The Pacific oyster is a native species in the Pacific and Asia, but has become an introduced species in larger parts of the world such as Europe, North America, Australia and New Zealand. The Pacific oyster was introduced in the Eastern Scheldt in 1964 by shellfish farmers after a great mortality occurred of the native oyster species *Ostrea edulis* in the harsh winter of 1962-1963 (K.Troost, 2009). Since the introduction, the Pacific oyster has established itself successfully in the Dutch seawaters, despite the initial expectations that it would not be able to reproduce itself in the Dutch waters due a too low water temperature for the spat to survive.

Figure 1. On the left showing the Pacific oyster *Crassostrea gigas*. On the right a natural oyster reef, a common site in the Dutch estuary of the Eastern Scheldt (Idscaro) (Visualphotos.com).

The Pacific oyster prefers hard substrates to attach to, this can be rocks, debris or shells. It is however also found on soft substrate like mud and sandy bottoms. In July and August the oysters release their gametes into the water. For the next three weeks the larvae live in their pelagic, after which they settle onto a substrate. The left valve will cement to the substrate, which is of great influence to the eventual shape of the oyster. The shell can grow to a maximum length of 30 cm. The Pacific oyster feeds by filtering the water for planktonic organisms as well as detritus (K.Troost, 2009).
The species is found in depths up till 40 m below the surface. The optimum salinity for the Pacific oyster is between 20 and 25 parts per thousand (ppt), but they are known to tolerate salinities as low as 10 ppt and as high as 35 ppt.

Nowadays the Pacific oyster has become the major cultivated oyster species in the Netherlands and the whole world, with an annual production of 4.38 million tons in 2003 (Nations). The Pacific oyster is cultivated in bottom culture, off-bottom culture, suspended culture and floating culture (Nations). In 2008 the fists contamination with the oyster herpes virus was noticed in Europe, and first noticed in 2010 in the Netherlands. This virus causes substantial mortality amongst young larvae. It is known that the virus is active in water above 14°C, below this temperature the virus remains inactive. Furthermore

a significant decrease in growth is noticed with adults once infected with the virus. Till now no working measures are known to prevent the virus from infecting the oysters. However the oyster companies are experimenting with virus-resistance families, selected for their high survival rates once infected, or by picking wild oysters which seemed to be resistance to the virus.

2.2. ROEM VAN YERSEKE

The Roem van Yerseke (RvY) is one of the major suppliers of seafood on the market. The focus of the RvY is the fresh seafood market, such as mussels (*Mytilus edulis*), oysters (*Crassostrea gigas* and *Ostrea edulis*), the common cockle (*Cerastoderma edule*) and the pullet carpet shell (*T.phillipinarum and T.decussatus*) (Yerseke). Both of the oyster species are the more highly priced products (*Ostrea edulis* the highest, yet less requested).

The Roem van Yerseke reproduces its oyster broodstock (*Crassostrea gigas*) in the hatchery, which was established in late 2005 (Yerseke). The broodstock is given a temperature shock which causes them to spawn spontaneously. The fertilized eggs are placed in an upweller with filtered (1µm) and UV-treated seawater. Once hatched, the larvae are kept in this upwelling system and fed with self cultivated algae at a stocking density of approximately 10 million larvae per upwelling tank (Yerseke) (Peene & de Bat, 2013). This phase will take 2 weeks, dependant on the water temperature. Once larvae end their pelagic life phase and are ready to begin their benthic phase (post larvae), they are once again placed in an upwelling system. This ensures an optimal growth and survival rate. Furthermore the desired shape is reached in such a system (Yerseke). By now the oyster spat has reached a size of minimal 2 mm and are referred to as T2 spat (for they are selected on size by sieving them on a sieve with a mesh size of 2 mm) (Table 1).

Table 1. Sorting and corresponding length and weight for the Pacific oyster (*Crassostrea gigas*) (Yerseke).

Sorting	Length (mm)	Weight (mg)
T2	3-4	5-10
T4	6-8	30-50
T6	8-12	80-120

The water temperature in the upwelling systems is approximately 28°C for both the larvae and post larvae (Peene & de Bat, 2013). This temperature ensured an optimal growth for the larvae, which shortened the time of them being in the upwelling systems. There were however some problems with keeping them at this temperature; the first being that the spat needed to be acclimatized before it could be placed outside in the nursery. Gradually the temperature would be lowered to the same temperature of the water in the outside pools or to the temperature of the FLUPSY or other outdoor locations where they were sown. The second problem is posed by the oyster herpes virus, which increased the mortality and significantly lowered the growth rate of the surviving spat. The virus shows however to be non-active at a temperature below 16°C. Therefore the temperature was lowered in the upwelling system to around 14°C, to make sure the virus would not infect the oyster larvae. Another benefit from this lower temperature in the hatchery was that it is no longer necessary to acclimatize the spat to an outdoor water temperature (for this is mostly around 14°C at the times the oyster spat is placed outdoor). Thus a temperature of 14°C has the advantage of preventing infection with the oyster herpes virus, does not acquire an acclimatizing period and still gives a sufficient growth rate.

2.3. THE LOCATION

The FLUPSY is located in the yacht harbor of the sailing school ''De Viking'' at Wolphaartsdijk. An agreement was made between the Roem van Yerseke and the yacht harbor master Luc Smit to place the FLUPSY in the yacht harbor of "De Viking'' in Wolphaartsdijk (Figure 2). During the preparations of the experiment the FLUPSY was moored close to the quay, which meant it was possible to stand in the water next to the FLUPSY. At the end of October the water level is lowered in Lake Veere from 1m NAP to approximately -0.3m NAP (Rijkswaterstaat). Therefore the initial mooring position next to the quay was no longer sufficient, for the water level would be too low. The FLUPSY was moved and moored a couple of meters away from the quay, providing a deeper water level and spacing between the bottom and the underside of the FLUPSY. At this mooring site it was no longer possible to stand in the water next to the FLUPSY (Figure 3).

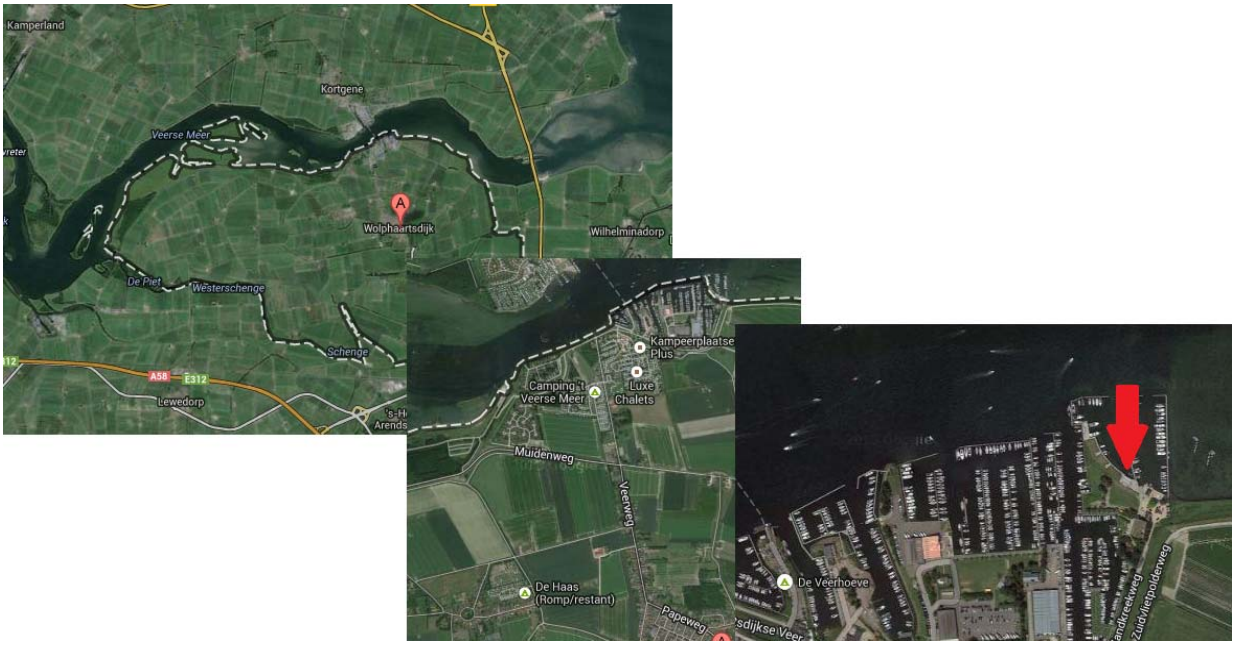


Figure 2. Maps showing the location of the FLUPSY (red arrow) in the yacht harbor of sailing school ''De Viking'' at Wolphaartsdijk.

Figure 3. Left the initial docking site of the FLUPSY next to the quay where it was possible to stand in the water next to the FLUPSY. Right is the new location shown of the FLUPSY, where it was moved a couple of meters to the right from the initial location. The deeper water where it was now laying proved to be better in many aspects.

2.4. THE FLUPSY

The Roem van Yerseke places the oyster spat after the hatchery in an outdoor nursery. They are experimenting with a floating upwelling system (FLUPSY) in the yacht harbor of Wolphaartsdijk. Here the oyster spat is placed in silos once they reach the T2 stage (3-4 mm) and kept here till they have reached the T6 stage (8-12 mm). The limited time they spend in the FLUPSY is determined by the fact that once the spat has reached the T6 stage, the current is no longer suffice in the system. At this size the spat has become too large and too heavy to keep them in a proper suspension, which causes a lower growth rate, more biofouling and the desired shape can no longer be guaranteed.

The FLUPSY (Figure 4) is in fact a raft with two rows of silos attached to it. The silos in the FLUPSY are PVC pipes with a diameter of 0.45m. At one end the silos are covered with a fine steel mesh of 1 mm mesh size. This size ensures that the spat does not get trapped diagonally in the mesh, which would lead them to grow stuck in it. Furthermore it has experimentally showed that the 1 mm mesh size is easy to clean, and does not get clogged easily. The silos are 51.5 cm high. In the silo is a large hole in the side, which acts as a flow out. The top of the silos are emerged, which means the actual water column in the silo is approximately 32.8 cm where the water flows out through the outflow (water level exactly till the top of the outflow pipe). The outflows of the silos collect in a central trough. In total there are 26 silos attached to the FLUPSY.

Figure 4. The FLUPSY located in the yacht harbor of Wolphaartsdijk.

In between the two rows of silos is the trough situated, to which a paddle wheel (powered by an electrical motor) is mounted at one end. The central trough is completely closed off from the surrounding water, the only inflowing water being from the silos. The paddle wheel acts to remove the water in the through, which is the actual outflow of all the silos. The turning speed and the depth of the paddles in the water of the paddle wheel regulate the flow rate in the through. Since the mesh in the silos is permeable and the water level in the through is a little lower than the water level in the silos, a pressure head is formed. This way the rotation of the paddle wheel causes a continuous water flow through the system. This flow rate ensures a food rich inflow into the silos, and the current removes all the feces and other suspended solids in the water. A beneficial advantage of the suspension state is that the growth edges are being abraded, which results in a more cup shaped shell. The forming on this cup shaped shell results in a higher flesh weight of the oyster. Furthermore the abrading prevents the accumulation of biofouling on the shell more efficiently.

Under optimal growing conditions of the oyster spat (parameters are optimal) the growth from one phase till the next can be realized in weeks (within the growth season). This means regular check on the growth is required. If the spat reaches a next phase (which is initially determined by sights), the spat is put on a sieve (the mesh size of this determines the actual sorting indication). The spat which hasn't reached the sorting indication (e.g. T6

indicates a mesh size of 6 mm) falls through and stays in the silos till it grows into the next phase. The spat which has outgrown the sieve is placed into a next silo. See table 2 for the sorting's and the corresponding length, weight and stocking density.

Table 2. The stocking density per phase in one silo of the FLUPSY (Peene & de Bat, 2013).

Sorting	Length (mm)	Weight (mg)	Stocking density (per silo)
T2	3-4	5-10	300.000
T4	6-8	30-50	200.000
T6	8-12	80-120	100.000

2.5. THE DESIRED SHAPE OF THE OYSTER

A Pacific oyster which can be found on an oyster reef, a coastal defense structure or on the beach has an inconstant shape. The left shell can have many outgrowths or extremities. The shell is laminated which causes the shell to be solid, yet very rough. The right, or lower shell, is smaller and fits inside the left shell, acting as a lid. Due to the irregular growth pattern of an oyster, a majority of the energy budget can be invested in the growth of the shell (especially the outgrowths) without causing the internal volume to grow. This can result in a large oyster, which contains little flesh inside. The desire of the consumer is to have a nice looking oyster, which is a less roughly shaped shell with a higher flesh content. By placing the young oyster spat in a FLUPSY this less irregular shape is created and maintained. The oysters spat is kept in a slight suspension, causing the oyster to swirl around and bump into each other. This constant contact on virtually the entire shell, will cause the so called growth edges (which causes the laminated shape) to abrade. This abrading makes sure any outgrowths or extremities are removed and will not get a chance to grow. Therefore the growth of the oyster is more regulated to be invested in the length, width and height of the oyster rather than the outgrowths. This causes the internal volume to be increased and more energy is invested in the growth of the flesh instead of the shell.
If this abrading and creating a more consumer desired shape is obtained in its early life phase, the oyster will maintain this shape throughout its entire life. Eventually this will lead to a nicer shaped oyster according to the consumer's desire. Thus a sort of ''upbringing'' of the oysters spat in a FLUPSY will pay off in a more desired shape throughout the rest of its life, even if the period of the oyster in the FLUPSY is only a couple of millimeters of growth (Figure 5).

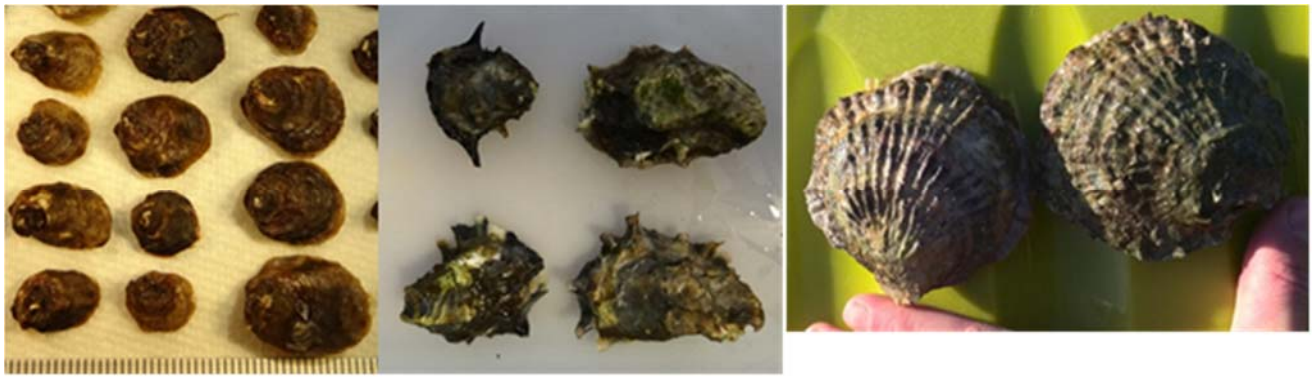


Figure 5. On the left Pacific oysters (*Crassostrea gigas*) are shown which were placed in the FLUPSY for several months (T6 stage). In the middle Pacific oysters are shown which were grown on substrate, and are between 3 to 4 cm long. On the right the more gentle shaped (less irregular shaped and more laminated) *Ostrea edulis* or European oyster is shown. It clearly

shows that the Pacific oysters cultivated in the FLUPSY have a more gentle form, less irregular than the ones cultivated on substrate. The oysters grown in the FLUPSY will eventually shape wise more or less resemble the more laminated *Ostrea edulis*.

3. METHOD AND MATERIALS

3.1. PREPARATIONS

Before the experiment was started the flow rate had to be determined that would get the oysters in suspension. Before the experiment the oysters in the silos were not in suspension, they formed a layer on the bottom of the silos except the T2 spat, which was in suspension.

No size selection was achieved for a long time, meaning that different sizes of spat were still mixed in the silos. Therefore, the first step was to sieve the silos by hand, to divide the oysters to the spat sizes.
Two sieves were used, one with a 2mm mesh, and one with a 6mm mesh. Since the diagonal length of the 2mm mesh is ≈2.8mm, all the spat which fell through was T2 spat (the spat was named after the mesh size they would remain on top of)(Figure 6). Everything that was left on the mesh was once again sieved with a 6mm sieve (diagonally 8.4mm). All the spat which fell through the 6mm sieve is indicated as T4 spat (between 2.8 and 8.4mm), the spat which was left on the sieve was indicated as T6 spat (>8.4mm) (Table 3).

Figure 6. The sieving as done at the FLYPSY by using a sieve with a mesh size which corresponds with the spat indication and size.

Initial stocking density of one silo would be according to the data given by the RvY. As a trial, initial stocking densities of different sizes of oyster spat were randomly assigned to the silos on the FLUPSY. The randomization was be done by an online randomization tool.

Table 3. Stocking density in individuals and in kilograms for the different spat sizes per silo.

Sorting	Length (mm)	Weight (mg)	Stocking density (per silo in individuals)	Stocking density (per silo in kg)
T2	≤ 2.8	5-10	300.000	5.2
T4	2.8-8.4	30-50	200.000	6.8
T6	≥ 8.4	80-120	100.000	11.6

Results of the trial showed that when oyster spat of different sizes were assigned at random in the silos, it was nearly impossible to bring them in suspension at such high stocking densities.
It showed that flow rate close to the paddle wheel in the central trough was higher than it was at the end of the trough. Furthermore the problem with keeping all sizes of oyster spat in suspension was that it seemed to have a

sort of critical value. At this critical value (flow rate) the spat would get in suspension, and a flow rate below this critical value would not get them in suspension at all.
Based on these findings it became clear that the stocking density in the silos should be lower than initially thought (Table3).

Besides the critical value, the flow through the silo and the oyster spat seemed to be a turbulent flow (visual observation). This means the flow was not equally distributed over the whole surface in the silo. The water flow will always seek the path of least resistance, which meant that in a spot in the layer of oyster spat where the resistance was lower, almost the entire water flow would go through this spot. The holes were a product of the current, a weak spot in the spat layer eventually formed a hole (observation: once a finger was placed in the oyster layer, this weak spot became a hole). Only the spat surrounding this weak point was in slight suspension, and the rest of the spat remained stagnant in the silo. This problem was tackled by placing a longer pipe on the outflow opening. This pipe was made of a Ø11 cm PVC pipe with a length of approximately 40 cm. The pipes were placed on the outflow with a transfer piece (double Lijmmof pijp 110 mm). The pipes were closed off at the end with a lid (Speciedeksel 110mm). In the pipes 10 holes were drilled, 5 at each side, so the holes were opposite of each other. The holes were 35mm in diameter.
Once the pipes were placed on the outflows of the silos, the water was no longer able to leave the silo at one location on one side of the silo. With the pipes attached the outgoing water was more equally distributed and the flow was more laminar (visual observation). The forming of weak spots in the oyster spat layer was no longer observed, and instead the entire layer of oyster spat was brought into suspension.

DETERMINING THE EXPERIMENTAL SET-UP

The trial showed it was no longer possible to distribute the different spat sizes randomly over the FLUPSY, for the

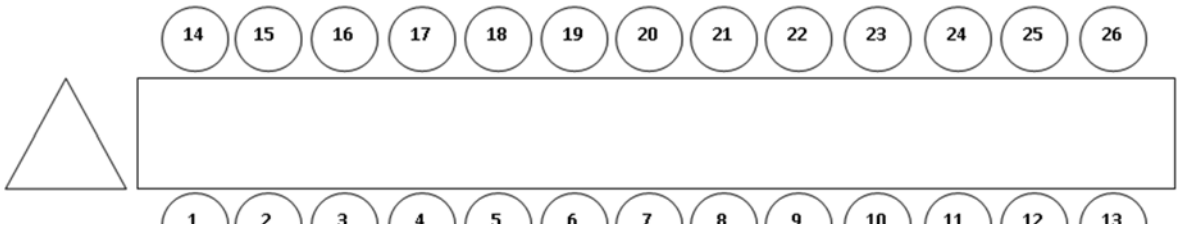


Figure 7. Schematic overview of the FLUPSY. The triangle represents the paddle wheel. Based on the findings of the flow rate by experience, the choice was made to place the larger spat (T6) closer to the paddle wheel where the flow rate is higher (approximately silo 14 till 17 and 1 till 4. The medium sized T4 spat was placed in the middle of the FLUPSY, 18 till 20 and 5 till 7. The smallest sized spat, T2, required the lowest flow rate and was therefore placed at the end.

flow rate close to the paddle wheel is higher than at the very end. Therefore the choice was made to place the larger spat (T6) as close as possible to the paddle wheel, the medium sized T4 in the middle and the smallest spat (T2) at the other end, furthest from the paddle wheel (Figure 7).

Since the accumulation of biofouling and the deterioration of the cup shape with the oyster spat begin to occur once they reach the T4 stage, the research focuses on the T4 and T6 spat. The experiment will be set up for three different sizes of oyster spat, T2, T4 and T6. Although there were little to no considerable signs of biofouling or a deterioration of the cup shape at T2 spat, they were included in the experiment to find after what period their problems started to occur (how quick they reached the T4 stage). The T2 spat will be kept at a constant flow rate (PF "present flow rate") throughout the entire experiment. This way the growth will be monitored in the situation as it is now (being without the addition of the pipes on the outflow), the spat will be kept at this similar flow rate even once it reaches T4 and T6 size (hopefully at the end T8) and therefore act as a control. A T4 spat batch will be

deployed as well at the present flow rate (HS) till it reaches T6. And a T6 spat batch will be deployed at the PF till it reaches T8 (T6(T8S)).

One T4 spat batch will be deployed in a silo set to the present flow rate (HS) and kept at this flow rate throughout the experiment (T4(HS)). Another T4 spat batch will be kept at a flow rate which will be visually assessed and determined to be suffice in keeping the T4 spat in suspension (T4(S)). Yet another T4 spat batch will be placed in a silo where the flow rate will be increased to a flow rate at which T6 spat is in suspension. The flow rate increasing up till that of T6 suspension will be done over a period of 4 weeks, meaning per week the flow rate will be increased by 25% of the flow rate difference between T4 and T6.

One batch of T6 spat will be deployed in a silo set to the present flow rate (HS) and kept at this flow rate throughout the experiment. Another T6 spat batch will be kept at a flow rate which will be visually assessed and determined to be suffice in keeping the T6 spat in suspension (T6(S)). Yet another T6 spat batch will be placed in a silo where the flow rate will increased to a flow rate at which T8 spat is in suspension (T6(T8S)). The flow rate increasing up till that of T6 suspension will be done over a period of 4 weeks, meaning per week the flow rate will be increased by 25% of the flow rate difference between T6 and T8.

Figure 8 and Table 4 give an overview of all treatments (8 in total) and how oyster spat is assigned to treatment. Each treatment consisted of 2 experimental units (duplicate). In total 16 experimental units were placed. Figure 9 and Table 5 gives a schematic overview of the FLUPSY and the silos with the treatments of it.

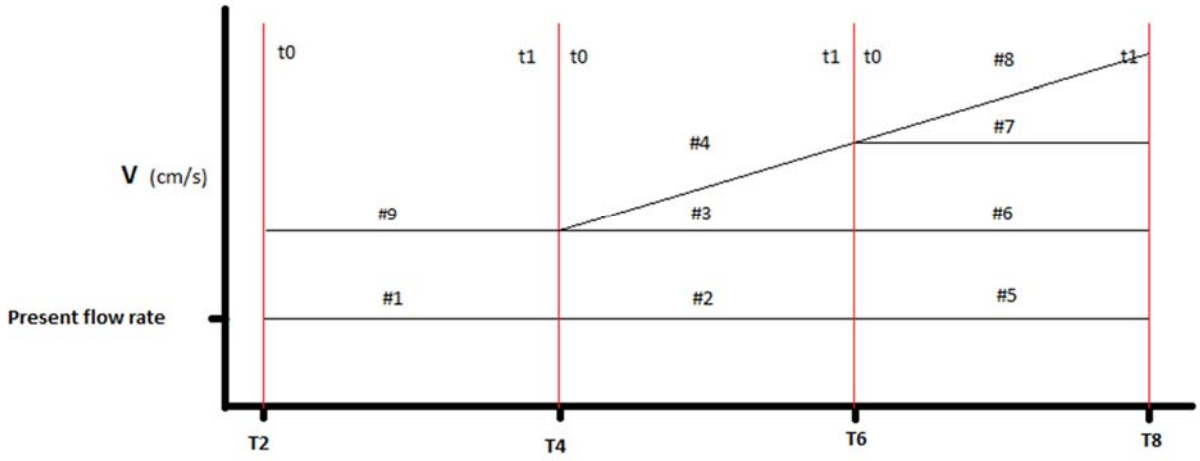


Figure 8. Systematic overview of the experimental set-up (Creemers & Capelle, 2013).

Table 4. Overview of the different spat batches and the experiments to which they are subjected (Creemers & Capelle, 2013), with an explanation of the flow rate to which they are subjected.

Treatment / Spat class	X(HS)	T2(S)	T4(S)	T4(T6S)	T6(S)	T6(T4S)	T6(T8S)
T2	1	4					
T4	2		5	6			
T6	3				7	8	9

Flow rate (indication)	Explanation
X(HS)	Old (current) situation, without the attachment of a pipe on the outflow
T2(S)	Flow rate at which T2 spat is fluidized
T4(S)	Flow rate at which T4 spat is fluidized
T4(T6S)	T4(S) gradually increased till T6(S)
T6(S)	Flow rate at which T6 spat is fluidized
T6(T4S)	T6 spat kept at a flow rate at which T4 spat is fluidized
T6(T8S)	T6(S) gradually increased till T8(S)

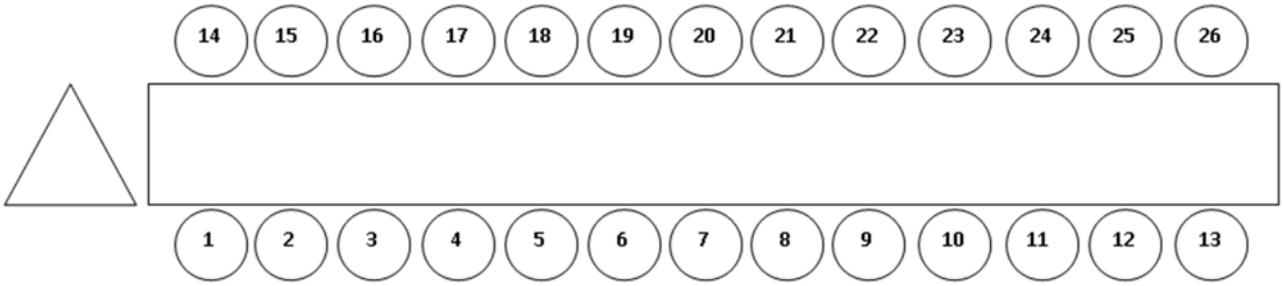


Figure 9. Schematic overview of the numbering of the silos. The triangle represents the paddle wheel.

Table 5. Overview of the treatments for each silo in the FLUPSY.

1	Not in use	14	Not in use
2	$T6_{S}$	15	$T6_{T8S}$
3	$T6_{T8S}$	16	$T6_{S}$
4	$T4_{T6S}$	17	$T6_{T4S}$
5	$T6_{T4S}$	18	$T4_{T6S}$
6	$T4_{S}$	19	$T4_{S}$
7	$T2_{buis = suspension}$	20	$T2_{buis = suspension}$
8		21	
9		22	
10	$T6_{HS}$	23	$T6_{HS}$
11	$T4_{HS}$	24	$T4_{HS}$
12	$T2_{open = HS}$	25	$T2_{open = HS}$
13	Not in use	26	Not in use

SETTING UP THE EXPERIMENT

Once the experimental set-up was determined (locations of the silos and the corresponding treatments) the oyster spat could be placed. Silos were filled with the available spat. Silos filled with T2 spat all contained the same biomass, idem dito for T4 and T6 spat.

In total the experiment consisted out of 8 silos with T6 spat, 4 with T4 spat and 4 with T2 spat. Due to a lack of sufficient spat, it was not possible to put the same biomass in each silo for different sizes of spat, disposing of leftover spat was not possible (biomass between T2, T4 and T6 treatments differed). The spat which was present and sieved to its corresponding size was therefore distributed over the amount of silos required for the experiment. This was only the case for the T6 and T4 spat, the T2 spat was available in larger amounts, for it could be resupplied directly from the hatchery. Table 6 gives an overview of eventual stocking density per silo for the corresponding oyster spat size.

The spat was equally divided over the number of required, thus all silos for a size class (e.g. all T6 treatment silos contained the same amount of biomass) were equal to one another.

Table 6. Overview of the actual stocking density for each spat size in the experiment, expressed in kilograms and individuals.

Spat size	Stocking density per silo in individuals	Stocking density per silo in kg
T2	± 300.000	± 5 kg
T4	± 50.000	± 3 kg
T6	± 15.000 - 20.000	± 2 kg

DETERMINING THE SAMPLE SIZE

Before the sieving to different spat sizes was done, a large sample was taken from one of the silos which was not sieved, thus all the different spat sizes were present in the silo. These oysters were placed in a plastic bottle and brought to the Ecolab. Here the length and the weight of 127 oysters were determined.
Based on the distribution in the sample size of 127 oysters, the actual sampling size was determined by a power calculation for a one sample t-test, using R (Team, 2013). The initial data showed that the size distribution was in such a way that with a power of 0.8 and a significance level of 0.05, it would require a sample size of 150-200 individuals to find a 10% growth difference. Removing larger oyster spat (≥T8) increases power to such an extent that 30-40 specimens were necessary to find a difference in growth of at least 10%. Thus the sampling size of 40 individuals per silo was chosen, assuming that oyster spat larger than T8 would not be present in the silos and samples.

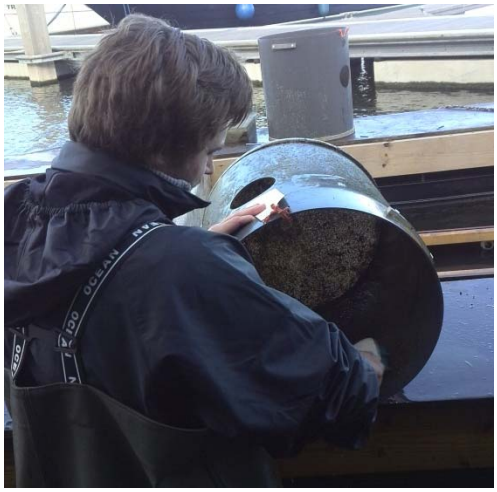
3.2. FIELDWORK

SAMPLING DATES

The experiment ran for 4 weeks, there were 5 sampling moments, including the t=0 reference (Table 7).

Table 7. Dates of the sampling moments.

Measuring point	Date
t=0	5-11-2013
t=1	12-11-2013
t=2	19-11-2013
t=3	26-11-2013
t=4	3-12-2013



CLEANING THE SILOS

Cleaning of the silos in the FLUPSY is required at least once a week (during the period which the experiment ran, in the summer with more algae in the water more than once a week is required). Regular cleaning of the silos (the mesh and the walls) is required to remove the faeces and silt which collects in the silos, causing the water flow to diminish and will eventually clog completely. The fouling of the silos will cause the growth of the oyster to be sub-optimal. Silt collected on the mesh and the oysters was removed by disconnecting the silo from the central through, taking it almost completely out of the water (leaving the oysters under water) and moving the silo quickly up and down into the water. This needed to be done in a careful way, for the small oysters are very fragile and heavily shaking might not only cause physical damage, but might stress the oyster as well. Once shaken, the silt is flushed out, clearly visible in the surrounding water which would turn murky for a short period of time.

The mesh was cleaned with a brush. The silos were lifted out of the water, supporting it on the side of the central through. With the brush silt and biofouling on the mesh could easily be brushed off, leaving a clearly visible clean mesh behind (Figure 10).
The outflow (Ø 11cm) of the silo into the central through is vulnerable for clogging with biofouling as well. Especially macro

Figure 10. Cleaning of the silo with the brush, before the experiment started the inside of the silo was cleaned as well. The partially clogged and dirty mesh in the bottom of the silo is clearly shown.

algae do collect here. These algae and other fouling were removed with the brush as well. All of the cleaning methods were performed on a weekly basis.

SAMPLING AND MEASURING PARAMETERS

Sampling occurred every Tuesday for the experimental period of 5 weeks. Before taking the samples, silos were cleaned with a brush and by shaking (see *cleaning the silos*). Furthermore the gentle shaking in the water made sure the content was evenly distributed.

A random number of oyster samples were collected and were placed in a small plastic bottle, closed with a lid and stored in the fridge. We made sure no extra water was collected in the bottle: without water and cooled, samples will last longer. The plastic bottles were marked according to sampling moment, silo number and silo treatment. In the lab 40 oysters were randomly selected for further analysis.

After the samples were taken, environmental parameters were measured, in the water surrounding the silos at the exact same place after every sampling moment.
Besides the measurement of parameters with a weekly interval, a datalogger (C3) was installed to measure continuously. The location to measure the environmental parameters was above the place where the C3 was placed. The environmental parameters which were measured by hand were: *pH*, *salinity*, *temperature*, *dissolved oxygen* and *chlorophyll-α*. The pH, salinity and dissolved oxygen were measured with a corresponding meter from the HZ. Over the sampling period the same meters were used. The temperature was measured on all three the meters and the average of these three values was taken.
A water sample of approximately 3l was taken to measure the chlorophyll-α level. These samples were processed shortly after collecting. One liter of water sample was filtrated over a Whattman filter and placed in an aluminum cup, folded, marked and placed in the freezer (-20°C), for further analysis.

MEASURING THE FLOW RATE IN THE SILOS

Since it was unclear what the expected flow rate would be in the FLUPSY it was unclear what measuring device could be used. Literature is ambiguous about the flow rate in an upwelling system.

Initially flow rate was measured with an *OTT flow meter* (Figure 11), with the aid of several screws. Each screw had its own optimal range in which it could adequately measure flow rate. It was however not clear which screw could be used; flow rate in the silos was unknown, furthermore it was not clear which screw could measure flow rate in the occurring range.

First measurements on the flow in the silos was done with the #3-22966 screw, a test with this screw showed that the number of rotations per second was >1.92.

The number of rotations was measured by using a *Hydrometrie Z30 Quarts Time* measuring apparatus. This analog counter could be set to a specific interval (time period) during which it measured the number of rotations made by the screw attached. If the number or rotations made by the screw was larger than the screw was able to measure, an error would be indicated by the rotation measuring counter.

Figure 11. A picture of the OTT hydrometer, with a screw attached to it. The screw was connected to the analog rotation counter by a contact wire.

Since it was not known which screw could be used, several were tested, to see whether the counter would give an error or not. Tests showed that the #3-22966 screw could measure in the occuring flow rate range. Number of rotations per minute were measured and noted for every silo, in order to calculate actual flow rate during measurement.

Each screw has its own formulas, by which the number of rotations can be calculated to the actual flow rate. Multiple formulas for each screw apply; the one used depends on the range of rotations per minute.

Since the rotations per second were more than 1.92, the following formula was used to calculate the actual flow rate: $n>1.92 \quad v = 0.2595n + 0.006$

Flow rate in the silos during measurements ranged from 0.85 m s^{-1} to 1.34 m s^{-1}. A flow rate of this magnitude would have flushed the oyster spat out of the silos and create a very powerful and fast current in the central through. Since this was not the case this measurement was discarded. Unfortunately calculations were done when the oyster spat was removed from the silos to be placed in baskets.

3.3. DATA ANALYSIS

SAMPLING SIZE, WET-WEIGHT, PHOTOGRAPHING AND STORAGE

Once the samples were taken, oysters were transported back to the Ecolab of the HZ University of Applied Sciences in the sampling jars, where the samples were processed.

At random 40 oysters were taken and placed on a paper towel. Extremities were removed from the sample and replaced by normal individuals. Extremities include odd growths (deformed shells), extreme sizes (larger or smaller than the rest of the sample) or oysters which were grown to one another. The placing on the paper towel gave a clear contrast which made selecting easier, and excessive water was absorbed.

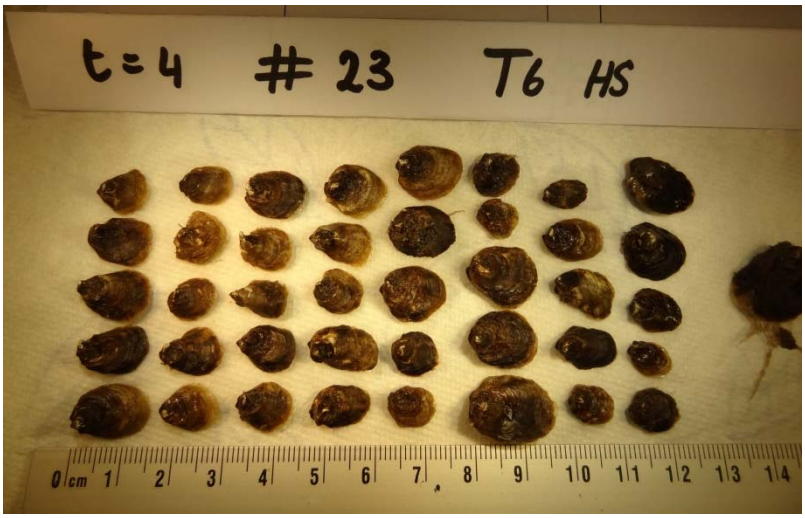


Figure 12. A photograph of the oyster which will act as the actual sample (of 40 individuals) laid out in a matrix of 8 x 5. These photographs will serve to determine the shape and amount of biofouling of the oyster spat.

The oysters were placed in a matrix of 8x5, with a marker indicating the silo number, silo treatment, spat size and the sampling date. A photograph was taken of the oyster-matrix, for further analysis on the shell shape and biofouling (Figure 12).
After making sure the excess water was removed from the shell, the 40 oysters were placed in an aluminum cup and weighed for the wet-weight of the sample (tare weight).

The removed flesh from the oysters was stored in a freezer.
After weighing the oyster in the samples for wet-weight, samples (as one batch) were placed on a porcelain cup and placed in a microwave for 20 seconds at 800W[1]. When the oysters are heated in a microwave, oysters would die and relax their adductor muscle. The aluminum cups were marked on the bottom with the sampling date, silo number and silo treatment and folded (preventing the oyster from falling out of the aluminum cups).

Of each measuring point the T4 and T6 samples were placed in a plastic freezing bag separately, which was marked with the containing spat size and measuring point before closed and placed in the freezer.

[1] This treatment was performed on T4 and T6 oyster spat, not T2 oyster spat, for their flesh weight would be so little, it would be nearly impossible to subtract the flesh out of them. Furthermore the research question and hypothesis states that the T2 spat does not suffer any harmful effects. Therefore the T2 spat was only weighed and later on measured.

REMOVING FLESH

In order to determine the flesh weight of the samples the flesh was extracted out of the shells. The samples were taken out of the freezer to let them thaw. Flesh was removed from the shells by using pincers and a scalpel. The flesh was placed in a pre-weighed porcelain cup. Great care was taken to remove all the flesh out of the shells, including the muscles and possible gills which were dried onto the shells. In most cases the shells were opened by the microwave treatment, but in some cases the shells would still be closed. If the oysters were still closed, opening them was done by placing the tip of the scalpel in the hinge of the oyster, pressing it carefully in and giving a short twist of 90°, thus opening them in the same way as a consumption oyster would be opened.
The combined flesh of one sample was weighed, and by subtracting the pre-weighed porcelain cup flesh weight was calculated (note that the microwave removed a percentage of the water in the flesh). After having removed the flesh from the shells (thus after the oyster were placed in the microwave) the combined amount of flesh from one sample was weighed in the porcelain cups. Measuring at this point did not act to gather data, but rather as a safety procedure to check if no mistakes were made during the collecting.
In some cases after opening the shells it showed that the specimen was dead. The shells would often be filled with smaller other shells or with silt. The number of dead and the weight of the dead shells were noted for calculating the Condition index.

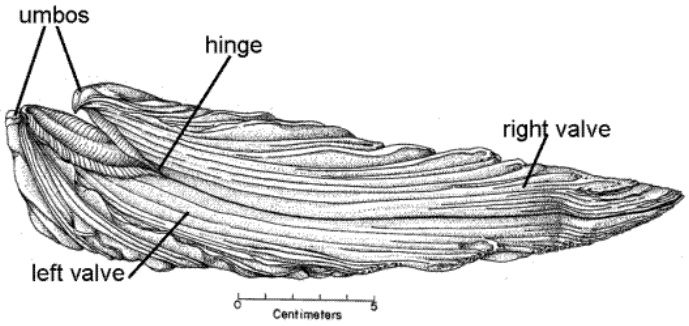


Figure 13. Schematic overview of the external anatomy of a Pacific oyster, showing the hinge, right and left valve. The left valve is the larger of the two and is the valve that is cemented to the substratum. The left valve is the deeper of the two. The right valve is flat and smaller that the left, acting as a cover over the cup of the left valve. (Ellenbaas, 2012)

By removing the flesh the right shell would often fall or break off, especially if the shell did not open in the microwave and needed to be opened by hand (Figure 13). But great care was taken to prevent any damage occurring on the left shell. The left and right shells would be placed back into the marked aluminum cups and folded shut. After processing an entire spate size (e.g. T4 or T6), the closed (but not airtight) aluminum cups were placed back into the plastic freezer bags and closed again (not completely to let the remaining moisture escape the bag). The plastic bags were stored in plastic trays which were stacked onto each other in order to prevent possible damage to the fragile shells.

DRY TISSUE WEIGHT

The porcelain cups were placed in a drying oven at 80°C for a period of 24h to remove the water from the samples (Mercado-Silva N. , 2005)

After drying for 24h at 80°C the porcelain cups were taken out of the oven and placed in a desiccator. This was done for in order to weigh the substance the porcelain cups and the content must be of the same temperature as

at which the analytical balance is kept. The cooling of the cups and content attracts water, which is prevented by the silica gel lumps inside the desiccator[2].

DRY SHELL WEIGHT

The aluminum cups containing the empty oyster shells from the samples were folded to protect the fragile shells. The folded cups were however not airtight, which allowed air to escape and make sure the shells were completely dried at room temperature. Shell weight was used for determining the condition index.

CONDITION INDEX

The condition index (CI) is a tool used in the bivalve aquaculture to gain more insight in the effect of environmental factors on the flesh, or meat quality (Mercado-Silva, 2005). Multiple methods exist for determining the condition index, condition index chosen for this experiment is described by Rainer & Mann 1992, as CI = [dry tissue weight (g)*100/dry shell weight]. Other methods often used shell volume instead of dry shell weight. Due to the small size of the oysters shell weight was preferred.

The condition index was assessed per silo, thus per experimental unit. Condition index was determined for total sample and divided by number of oysters in the sample to account for dead oysters, represented by empty shells.

[2] Note that the desiccator was used, not the vacuum desiccator, due to practical reasons.

MEASURING LENGTH AND WIDTH

For all the oysters in each sample length and width was measured. Height could not be measured, due to the fact that the right valve often broke off with the flesh removal. Only the oysters which were alive before the microwave treatment were measured, dead oysters were excluded from analysis. The oysters were measured from the dorsal-ventral axis (length) and the anterior-posterior axis (width) of the left shell (Figure 14).

The measuring was performed by using an automatic caliper, the Mitutoyo Absolute IP67, which converted the measured data into Microsoft Excel (Figure 15). The accuracy of the automatic caliper is approximately 0.001in. The average length and width was calculated for each silo at the 5 measuring points.

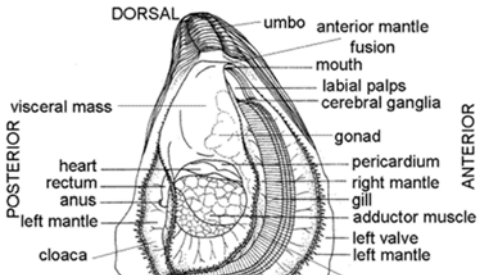


Figure 14. Cross section of the left shell of a Pacific oyster, indicating the dorsal-ventral axis and the anterior-posterior axis (Ellenbaas, 2012).

Figure 11. The Mitutoyo Absolute IP67 automatic caliper used to measure the length and width of the oysters and transport the data automatically in Microsoft Excel. (Source picture: http://ecatalog.mitutoyo.com/ABSOLUTE-Coolant-Proof-Caliper-Series-500-with-DustWater-Protection-Conforming-to-IP67-Level-C1384.aspx)

C3 SUBMERSIBLE FLUOROMETER

Besides measuring the parameters manually once a week, a C3 Submersible Fluorometer manufactured by Turner Designs was used. The C3 is a datalogger which is equipped with several optical sensors, by which it performs measurement at beforehand, programmed intervals. This C3 Submersible Fluorometer was equipped with sensors to measure the temperature, salinity and the chlorophyll-α content of the water. By using the C3 these parameters were constantly measured for the experimental period. This was done to see if any irregularities occurred, especially in the food supply.

ANALYZING THE SHAPE OF THE OYSTER SPAT

There is very little literature available about the shape of an oyster which was nursed in a FLUPSY. In general there was very little literature about an artificial produced shape for oysters. Since the oyster spat which was nursed in a floating upwelling system had a very distinct shape and does not resemble the ridged and coarse shape of a wild oyster a new method had to be developed. After consultations with the Roem van Yerseke, it became clear what their view was on an optimal shape with the highest market value. Based on these indications a comparison could be made for the oysters in the FLUPSY in classes.

A key was made based on the photographs taken of the samples each week of each treatment (see Appendix). This key consists out of five classes for the shape. The highest class, five, is the most favorable or desired shape as indicated by the Roem van Yerseke. Class one, being the lowest, was only assigned to shells which showed deformities. Table 8 shows the classes and their description.

Table 8. The division description of shape classes for the Pacific oyster. Each class is related to a description and an explanation, which were assigned to the photo indices of the oyster samples. A more detailed class description with photograph examples and the key can be found in Appendix x.

Class:	Description:	Explanation:
1	Very unfavorable	General shape contains a lot of extremities or outgrows. The shell is not symmetrical at all. The overall shape resembles that of a wild oyster (looks like *the FLUPSY had no effect on the contour of the shell*). Or other *deformities* are present.
2	Unfavorable	The shell is not symmetrical. There are clear outgrowths/extremities. It looks like the period spend in the FLUPSY has had hardly any impact on the shells contours. The shell contains upstanding rims or wrinkles. The total outgrowths/extremities, gaps or upstanding rims together is four or more.
3	Neutral	The shell is more or less elongated, no longer oval. Or the shell may contain several outgrowths/extremities. The overall shape is a bit more rigged. The shell may contain upstanding rims (wrinkles). The total outgrowths/extremities, gaps or upstanding rims together is three.
4	Favorable	The shell shape is almost completely oval. There are only *one of two outgrowths, extremities or gaps along the valve edge*. The shell is however not perfectly symmetrical. The shell is not perfectly symmetrical. The top left valve is still smooth, thus no rimples or upstanding bands are present.
5	Very favorable (desired shape)	Shape is oval (rounded), *no extremities, outgrowths or gaps occur along the valve edges*. Shell is formed in a gentle way, laminated. Overall shape resembles that of a European flat oyster. The shell is symmetrical. The period spend in the FLUPSY has had a clear influence on the shape. The shell is smooth, without any rimples or curves (upstanding bands) on the top shell (left valve).

GROWTH RATE

Growth rate was calculated based on the length measurements of the oyster spat. The average length of one sample was taken. Since the treatments were not altered up till the end of week three (thus including the measurements of week three) the growth here was determined for this period in mm per day. Growth rate was calculated as the length taken at week 3 minus the average length of week 0 divided by the number of days in between (21days). Furthermore the growth rate was calculated once between measuring week 3 and 4 in which flow rate for treatments T6(T8S) and T4(T6S) was increased. The average length difference between these measuring moments was taken and divided by the number of days in between, being 7.

ANALYZING THE BIOFOULING OF THE OYSTER SPAT

Since there was very little literature available on how to determine the amount of biofouling on an oyster shell, a new method had to be developed. After consultation with my supervisors, the choice was made to develop a new tool based on photos of the oysters. A large number of photographs which were taken in advance were analyzed to get an idea of what kind of variation in biofouling occurred. After having done this, a tool was made as well. This tool, with a photo index, consisted out of three groups. In this case the choice was made to add a higher number or value to the oyster shells which had no biofouling attached to it. Thus a shell with a severe amount of biofouling was given a low number or value. By doing this the total score would give a logical and easy indication of the amount of biofouling which was present in a sample. A high value was an indication of a good scoring sample, meaning there was little biofouling present. A low value was an indication of a poor scoring sample, meaning there was more biofouling present. Three classes of biofouling was made (Table 9)

Table 9. The division description of biofouling classes for the Pacific oyster. Each class is related to a description and an explanation, which were assigned to the photo indices of the oyster samples. A more detailed class description with photograph examples and the key can be found in Appendix.

Score	Description	Explanation
3	No biofouling	There is no biofouling on the shell present. The shell is completely clean.
2	A little biofouling	There are a few strands of macroalgae present (one or two). There are however no patches of macroalgae present.
1	Severe biofouling	Biofouling is present in a severe form, meaning long strands of macroalgae or patches of macroalgae on the shell.

STATISTICAL ANALYSIS OF THE DATA

Testing the hypothesis: flow rate in the silos has no effect on the shape and amount of biofouling of the oyster spat.

Treatment:

Treatment	Description
#1	HS (Current State) the control group
#2	S (Suspension) by use of the pipe
#3	↑S (Up calling flow rate of the suspension pipes) = T6(T8S) & T4(T6S)
#4	↓S (Spat in a suboptimal suspension state) = T6(T4S)

Spat size	Treatment given
T2	#1 & 2
T4	#1 & 2 & 3
T6	#1 & 2 & 3 & 4

4. RESULTS

4.1. SHAPE

Figure 12 shows that average shape of the T6(S) duplicate showed a slight increase over time, from an average total score of 134 at t=0 to an average total score of 144 at t=4. The average shape increase of T6(T8S) was from 132.5 to 135 (1.89%) over the whole run of the experiment. The treatment T6(T4S) flow rate had an increase of 10.61% (from 132 at t=0 to 146 at t=4). The T6(HS), or the treatment that has had no changes during the experiment and remained the same as the RvY had it set, showed an increase of 13.51% (from 129.5 to 147).

Figure 17 shows that the highest increase for shape for the T4 spat was seen in the T4(S) spat (17.19%), with a score of 128 at t=0 and 150 at t=4. The treatment with an increasing flow rate, T4(T6S), scored considerably lower, with an average increase of 13.41% (123 at t=0 and 139.5 at t=4). An increase of 9.34% for the T4(HS) treatment was measured (increase from 129.5 to 140.5).

Figure 18 shows that the T2 oyster spat had the highest increase for the shape in T2(HS) treatment, with an average increase of 10.37% (135 at t=0 to 149 at t=4). The T2(S) treatment scored considerably lower, with an average increase of a mere 2.93% (136.5 to 104.5 at t=4).

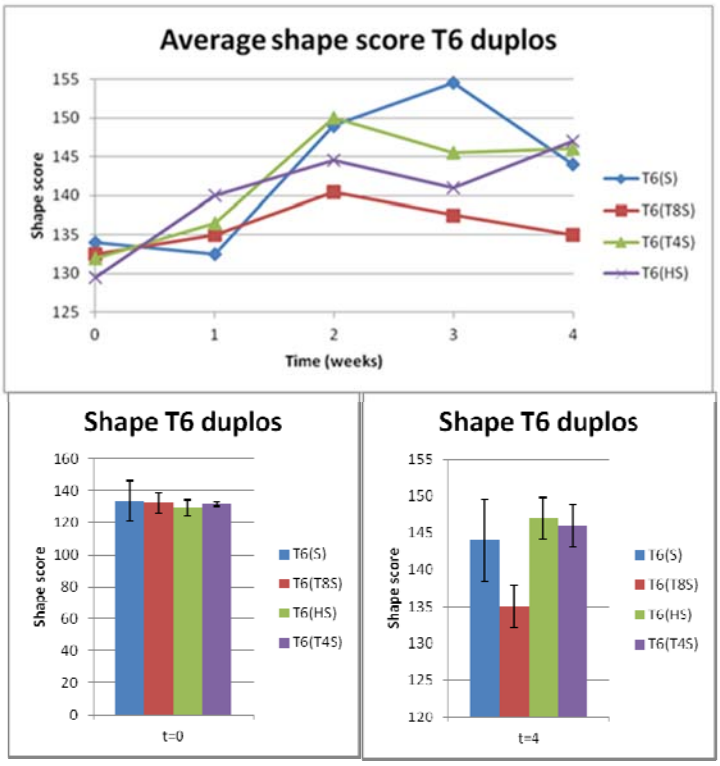


Figure 12. The shape score for all the T6 spat and their corresponding treatments. The average score of the sum for the duplicates was taken. Standard deviation is shown between t=0 and t=4.

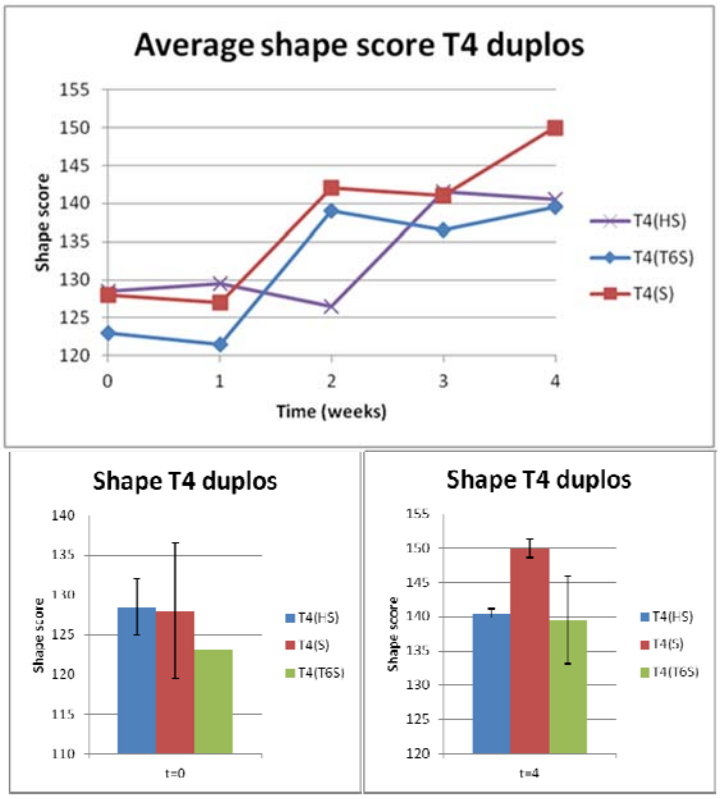


Figure 13. The shape score of the average for the T4 spat and the corresponding treatments. Standard deviation is shown between t=0 and t=4.

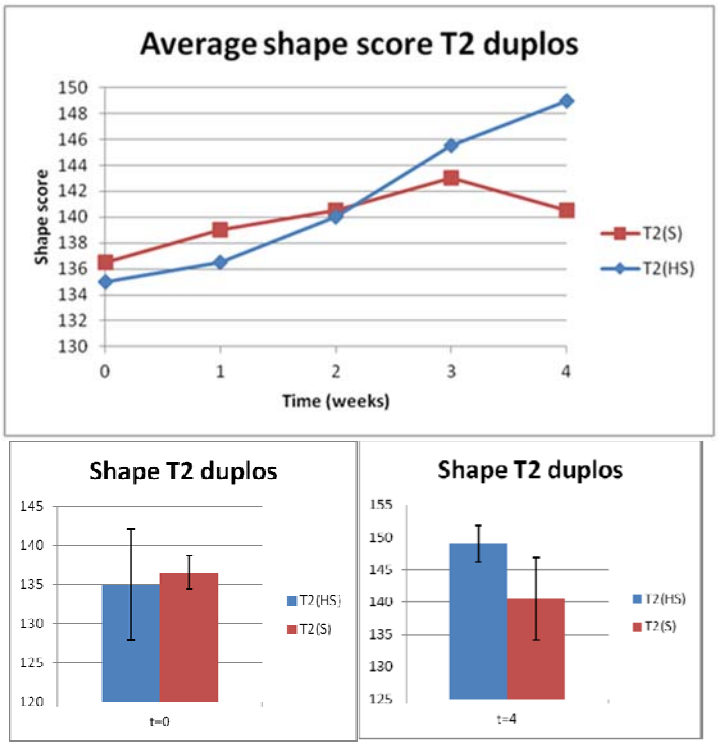


Figure14. The shape score of the averages of the duplicates for the T2 spat and the corresponding two treatments; T2(S) and T2(HS). Standard deviation is shown between t=0 and t=4.

The biggest increase in shape was observed in the T4(S) treatment, with an increase of 17.19% of the duplicates. The smallest increase was observed in the T6(T8S) treatment (1.89%).

In Figure 19, the results for the shape is presented ordered from low to high on the x-axis. The score is set on the y-axis and the number of treatments (9 duplicates, thus 18 in total) is set on the x-axis. The shape was determined by the index made and the corresponding scores (Appendix). The sum of the scores was used for each sample per measuring point. Colors represent the duplicates.
In case the hypothesis would be true, thus that the variation in flow rate within a silo would result in a better shape, the treatments in which the flow rate was higher than the current situation would score higher over time, and be represented in the upper right corner. The expectation was that all treatments were randomly scattered through this plot at measuring point t=0. As the experiment runs, colors and thus treatments were expected to clusters according to the treatments. A division between treatments and the converging of duplicates was expected.

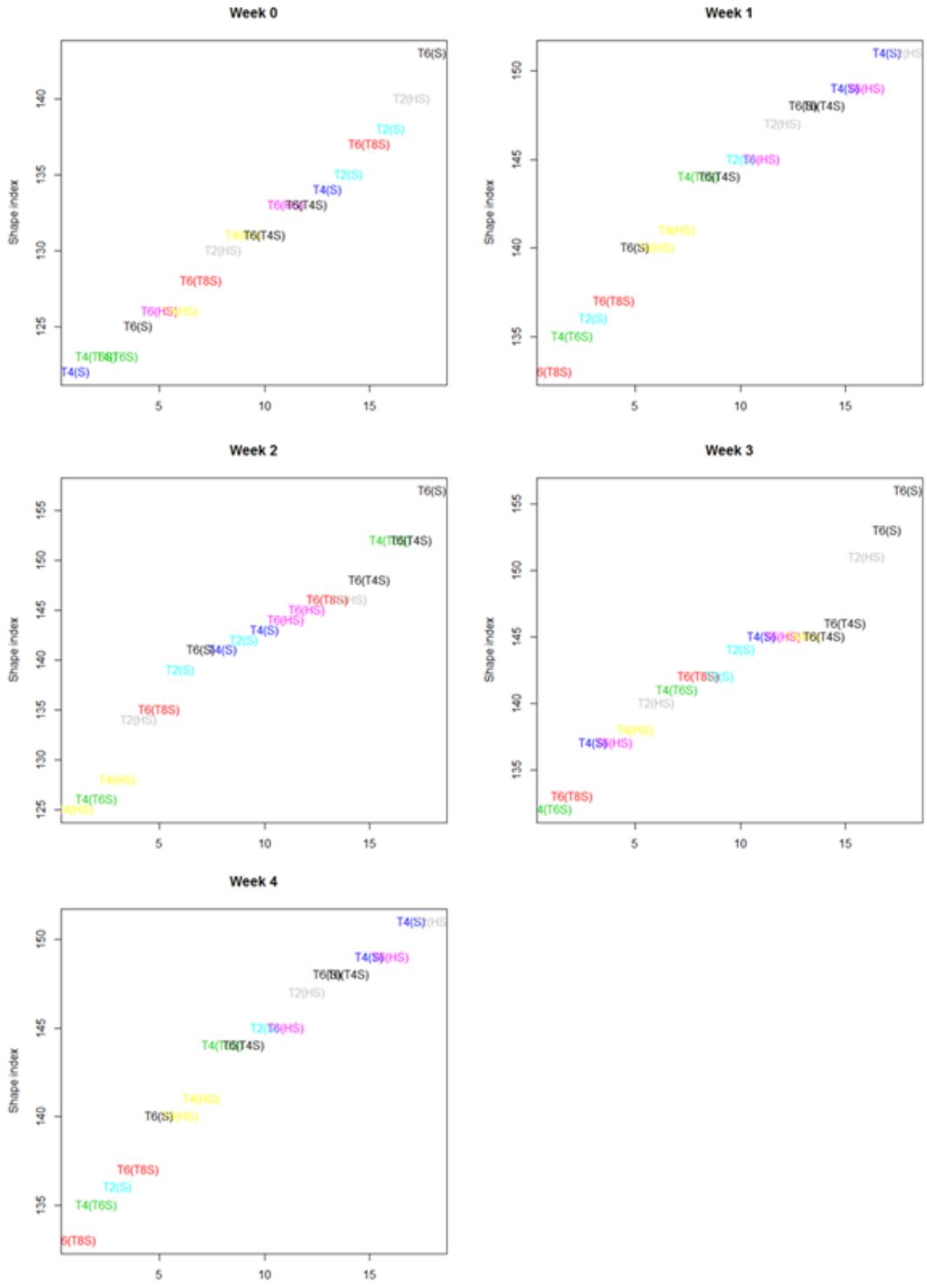


Figure 15. A plot which present the results of the shape scores per treatment, per week. In this graph the averages were not taken, thus each sample (silo) is presented separate. The y-axis present the shape index scores (see shape index) and has no unit. The x-axis does not have a unit either. The graphs show the total scores of each sample, presented in an order from a low score in the lower left corner, to a high score in the upper right corner. These graphs give a clear and quick glance on the shape indices score of all the treatments and how they compare to other treatments.

Figure19 shows that in week 0 the distribution of the shape index per silo is scattered. Difference in treatment was supposed not to have influence on shape, concluding distribution was too large at t=0. In week 1 both of the T4(S) treatments scores very high, although the week before one of the treatments scored the lowest. Furthermore the rest of the duplicate treatments are quite far apart in week 1. The same results are obtained in week 2, 3 and 4. The duplicates often have scores which are quite far apart. These results show that the results of the shape index are not consistent and seem to be at random.

4.2. BIOFOULING

The majority of the biofouling which occurred on the oyster spat shells, were present on the T4 and T6 spat. A high biofouling score represents a low amount of biofouling on the shells. If no biofouling was present on the shells, the maximum score was 3x40 = 120. A trend can be seen in the results for both T4 and the T6 treatments, with in all an increase in total biofouling on the shells, thus a lower score.

Figure 20 shows the amount of biofouling for all the T6 spat and the corresponding treatments. All treatments show an increase in biofouling on the shells (represented by a lower end score than the starting score). The T6(HS) duplicate treatments showed the least increase in biofouling, from a total average score of 108 at t=0 to 105.5 at t=4. The suspension state of T6 (T6(S)) showed an increase of biofouling from 106.5 at t=0 to 103 at t=4. The treatments T6(T8S) and T6(T4S) showed the highest increase in biofouling with 106.5 to 92 at t=4 and 116 to 108 at t= respectively.

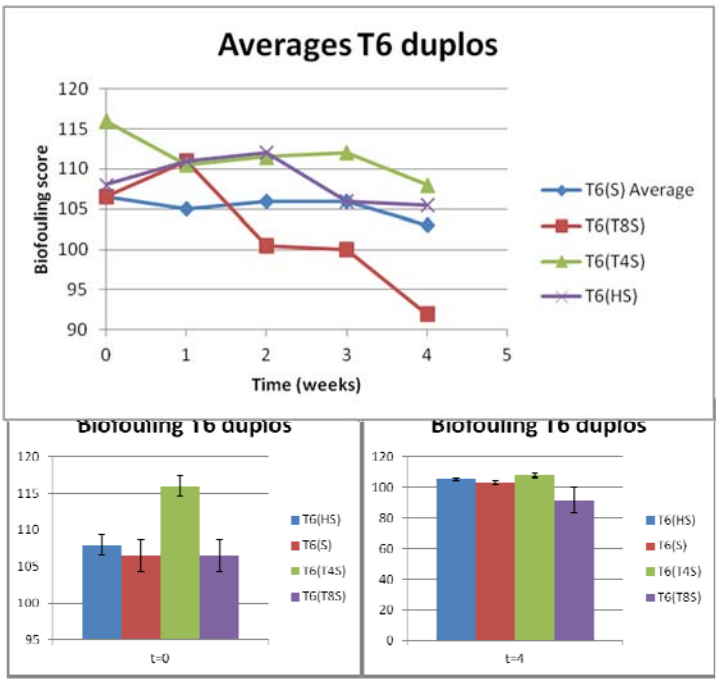


Figure 6. Graph showing the biofouling score for the average of the T6 duplicates. A high score is an indication of a low amount of biofouling present, thus a low score an indication of a higher amount of biofouling on the shells. All the treatments for the T6 spat show in increase in biofouling on the shells over the run of the experiment. The T6(T8S) treatment showed the highest increase in biofouling of all treatments. Standard deviation is shown between t=0 and t=4.

For the T4 spat and the corresponding treatments, the highest increase in biofouling was observed in the T4(S) treatment with an increase from 118 a t=0 to 113 at t=4. The T4(T6S) treatment showed an increase from 112.5 to 108.5. The lowest increased in biofouling was observed in the T4(HS) treatment, with an decreasing score from 113.5 at t=0 to 112.5 at t=4 (increase of 0.88%)(Figure 21).

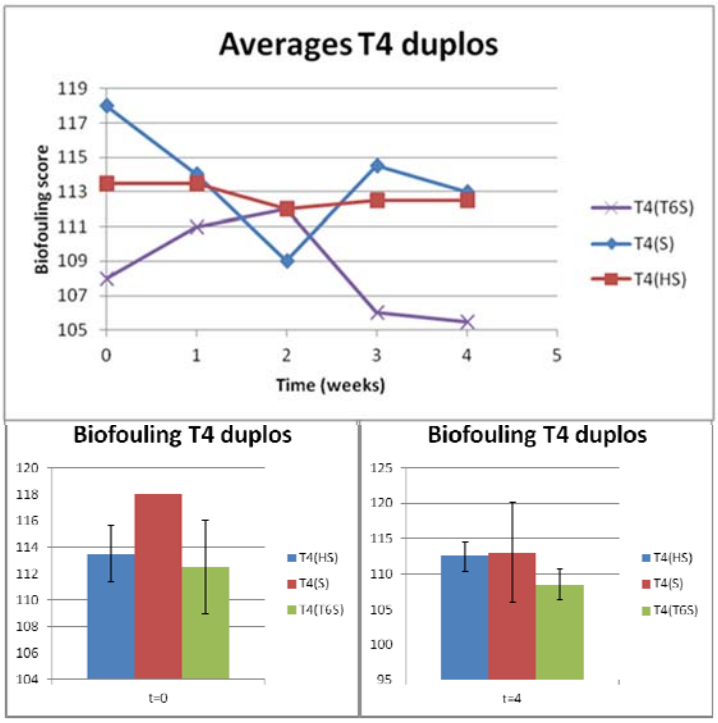


Figure 21. The increase of biofouling on the treatments with T4 spat in them. The average of the duplicates was taken and shown in the graph. All treatments showed an increase in the amount of biofouling, with 3.56%, 4.24% and 0.88% for T4(T6S), T4(S) and T4(HS) respectively. Standard deviation is shown between t=0 and t=4.

The major part of the T2 spat showed little to no biofouling. The samples taken from both one T2(S) and one T2(HS) showed no biofouling at all. At measuring point t=1 in one of the T2(HS) treatments (silo #12) one oyster with score 2 ''a little biofouling'' was found, resulting in the score of 120.
In the duplicate of the T2(S), at measuring moment t=3 there was one oyster found with biofouling (the score 1 was given) which explains the drop at this moment in the graph. The T2(S) duplicate had two oyster with biofouling at measuring moment t=3, which resulted in a total score of 117 and the drop in the graph (one oyster with score 2 and the other with score 1) (Figure 22).

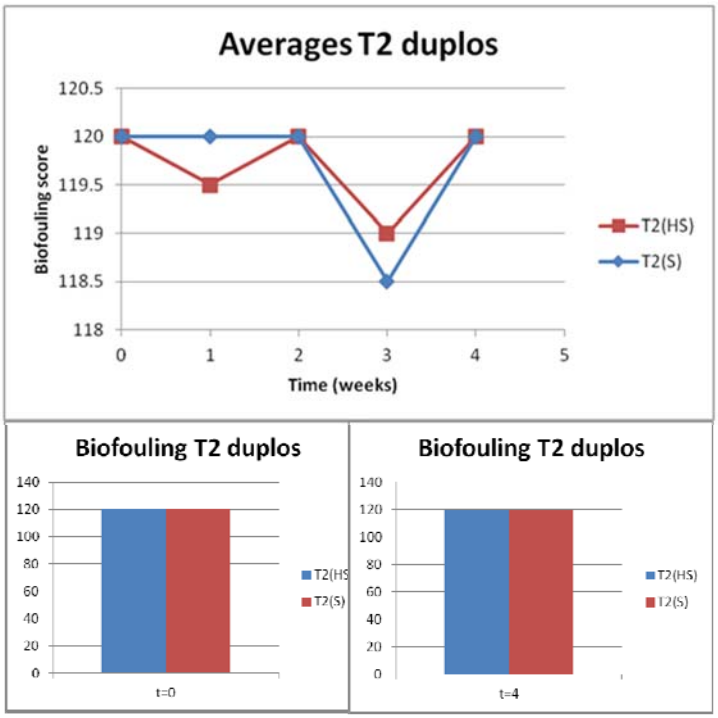


Figure 22. The biofouling score for the T2 duplicate treatments is shown in the graph. The majority of the T2 spat remained clean and free of biofouling for the entire run of the experiment. In one T2(HS) silo an oyster with biofouling was found at measuring moment t=1. In one of the duplicates for both T2(HS) and T2(S) biofouling was found on oyster spat at measuring point t=3, but on t=4 none was found in the samples. Very little biofouling was found on the T2 spat, with only 4 oyster shells found with biofouling for the whole run of the experiment, out of a total sampling amount of 800 T2 oysters. Standard deviation is shown between t=0 and t=4.

Figure 23 shows the biofouling scores per measuring moment in one graph. All the treatments are shown separate per measuring moment, thus the average of the duplicates is not shown. As is the case for the other biofouling graphs, the higher the score, the lower the amount of biofouling that was found on the oyster shells. At week 0, the biofouling scores for all treatments were all quite close together. Colors represent duplicates. Just as for the shape index a certain pattern was expected to occur according to the treatments. Again at t=0 the treatments would be more random divided. As the experiment progresses, the clustering of duplicates was expected. Furthermore treatments with a high flow rate were expected to give the best results and clusters in the top right corner.

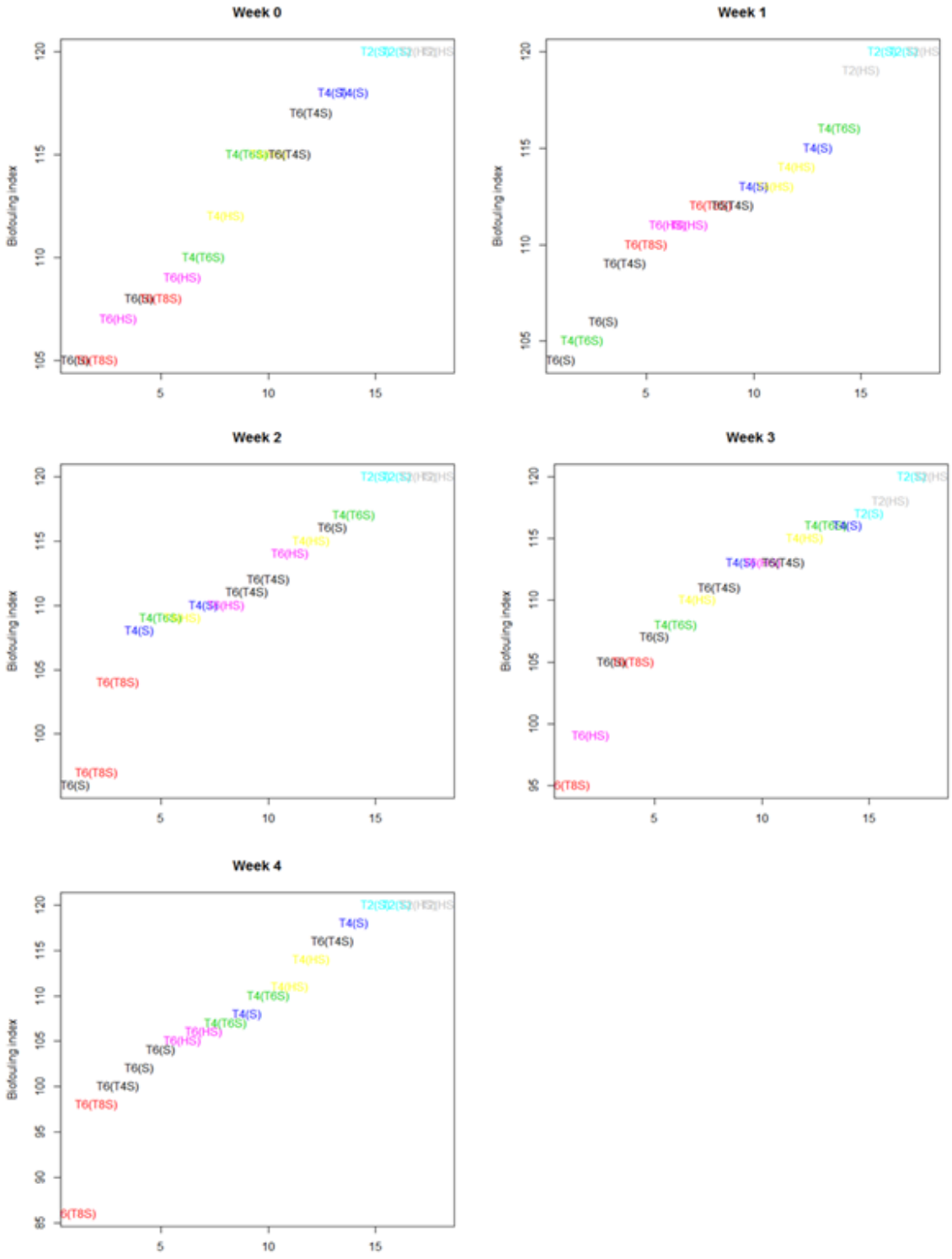


Figure 23. A plot which represent the biofouling scores per treatment, per week (measuring point). In this graph the averages were not taken, thus each sample (silo) is presented separate. The y-axis present the biofouling index scores (see biofouling

index) and has no unit. The x-axis does not have a unit either. The graphs show the total scores of each sample, presented in an order from a low score in the lower left corner, to a high score in the upper right corner. These graphs give a clear and quick glance on the biofouling indices score of all the treatments and how they compare to other treatments.

4.3. LENGTH AND GROWTH RATE OF THE OYSTERS

Within all the treatments of the T6 spat, only the T6(S) and the T6(T8S) showed an increase in length. The treatments T6(T4S) and T6(HS) showed an average decrease in length over the entire run of the experiment (11.67mm to 9.85mm and 10.71mm to 9.66mm respectively)(Figure 24). This trend occurred because the average length of the samples became smaller (smaller spat in the following measurements).

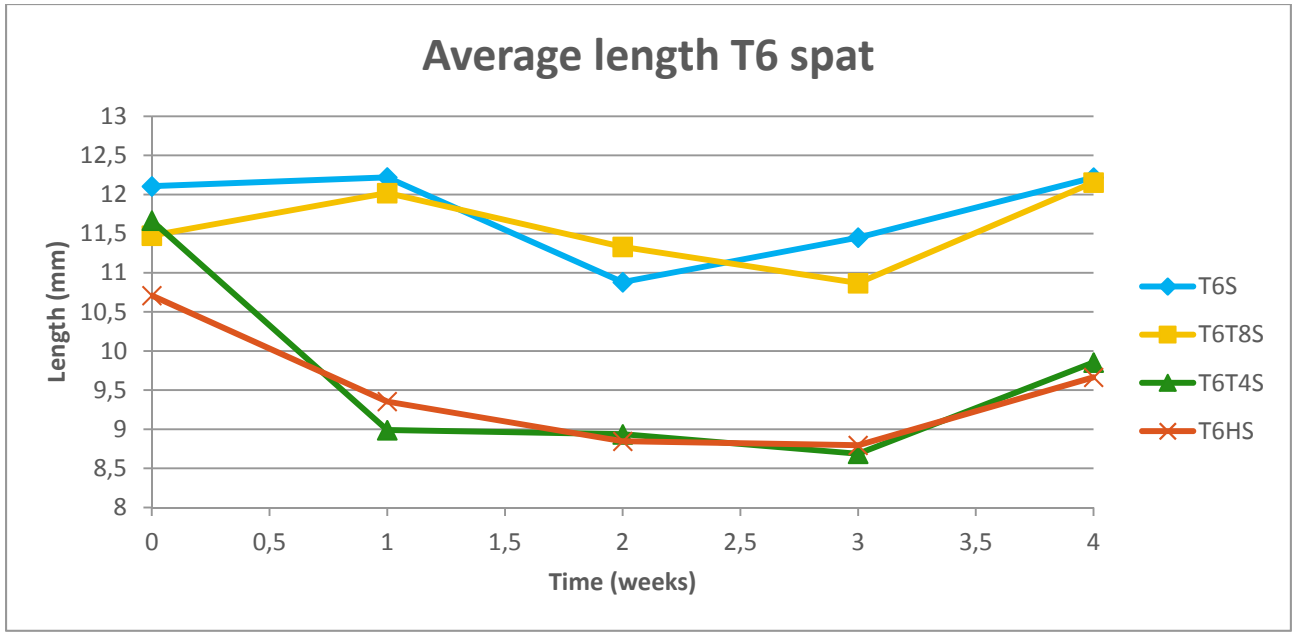


Figure 24. Graph showing the average length of each sample (the average of the duplicates was taken) per measuring point. Notice the drop in the average length for all the T6 spat treatments. The average length of the oyster spat in treatments T6(T4S) and T6(HS) at the end of the experiment is lower than it was at the start of the experiment.

All the T4 spat treatments showed an average decrease in measured length between following measurements. T4(T6S) showed a decrease of 8.16mm at t=0 to an average of 7.47mm at t=4. The T4(S) treatment showed an average decrease from 7.96mm (t=0) to 7.46mm (t=4). The T4(HS) showed an average decrease from 7.87 at t=0 to 7.45 at t=4 (Figure 25).

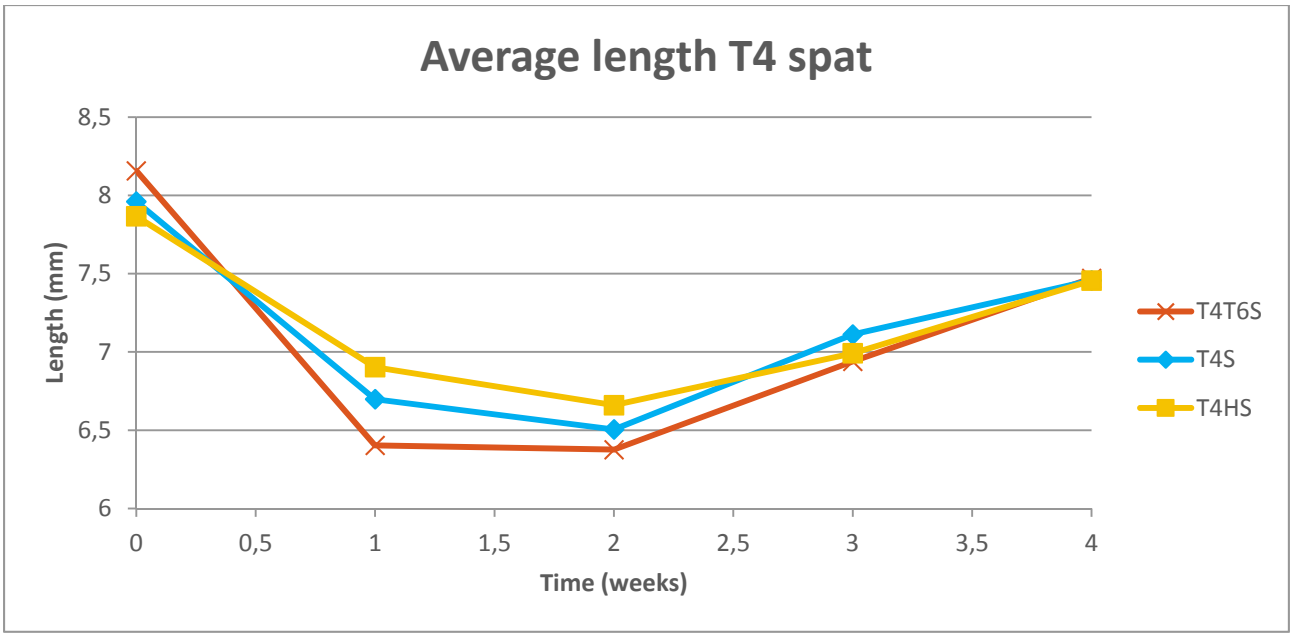


Figure 25. The average lengths of the duplicates for the T4 spat treatments are shown. Again all treatments show a drop in the average length.

Just as the T6 spat and T4 spat treatments, the average length of the T2 spat treatments showed a reduction in average length as well. The T2(S) showed the least decrease in length, from 4.41mm to 4.17mm (from t=0 to t=4). The T2(HS) showed the highest average decrease of length between successive measurements, from 4.88 at t=0 to 3.92 at t=4 (Figure 26).

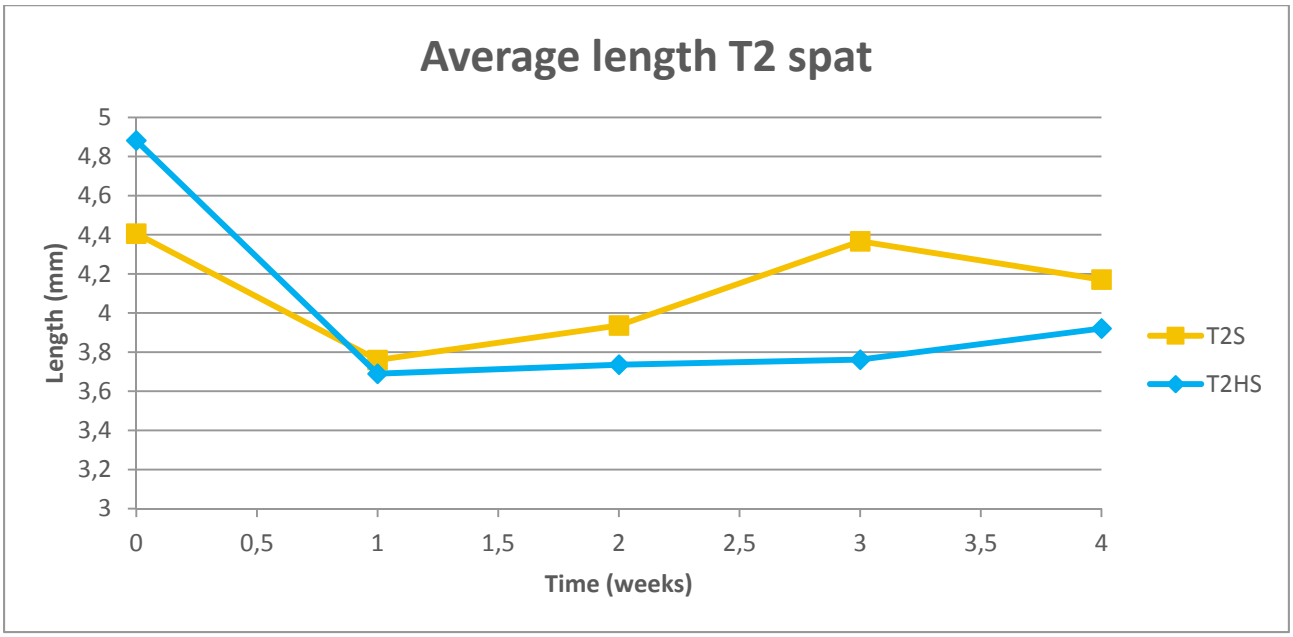


Figure 26. The average length of the duplicates for the T2 spat treatments is shown. Just as was the case for the T6 and T4 treatments, the average length here is lower at the end of the experiment than it was at the start.

Based on the length results at t=0 for all the T6 spat, it is safe to say the variance in length of oyster spat was too large to measure impact of the treatments on the spat. At t=0 the P-value between all the T6 groups had a value of 0.009 (9.017E-03), thus rejecting the null hypothesis initial variation was small (*Shapiro-Wilk normality test*).

Up till t=3 these treatments did not differ from each other, for the increasing of the flow rate had not been applied up till this point. After the measurements performed on t=3, it was seen that the flow rate had to be increased in the silos for the treatments T6(T8S) and T4(T6S). This was decided seen the fact that the oyster spat seemed to be lying stagnant in the silo based on sight. After confirming the sighting and assumption by feeling with the hand for resistance, it became clear the flow rate had to be increased.
At measuring point 4 (t=4) the first signs of a smaller variance between the treatments within oyster spat classes is seen, except for the T2 spat. The variation between groups for the T6 spat at t=4 was calculated to be 0.17 (1.74E-01), 0.20 (2.04E-01) for the T4 spat treatments and 2.27E-07 for the T2 spat treatments.

This trend for low P-values between the groups of one oyster spat class, indicates that the zero hypothesis is rejected (the zero hypothesis being that the variance between the groups should be small, since the silos were filled with spat from one class, which was essentially one large group which was thought to be uniform). This rejection indicates that the assumption made that of the distribution within and amongst the groups being equal, was false.

The growth rate was calculated based on the average length of the sampled oyster of each silo. Since some treatment had an increase in the flow rate after t=3 (thus between t=3 and t=4), the growth rate was calculated for the period up till this change (measuring point 0 to 3). Furthermore entire growth rate for the entire run of the experiment was calculated as well. To see what the effect of the increasing in the flow rate was for the treatment which actually underwent an increase, the growth rate which occurred in the last week (between week 3 and 4) was calculated as well
(Table 10).

Table 10. The table shows the growth rate of each individual silo over the entire run of the experiment. The growth rate was once determined for the first three weeks and once for the entire run of the experiment (four weeks). To show what the effects of the variation in flow rate were, and specifically for treatments T6(T8S) and T4(T6S), the growth rate was determined for the period between week 3 and 4 as well. Based on these figures, the effect of the upscalling of the flow rate can be compared to the other situations.

Growth rate (mm·day^{-1})									
	Week	0	1	2	3	4	GR.p.day (3 weeks)	GR.p.day (4 weeks)	GR.p. day in week 4
Treatment	Silo No.								
#12 T2(HS)	12	5.327	5.381	4.97825	4.88725	5.48	-0.020940476	0.005464286	0.084678571
#25 T2(HS)	25	4.43575	4.236	4.56475	4.67075	4.657	0.011190476	0.007901786	-0.001964286
#7 T2(S)	7	4.59725	5.23925	4.672	5.42325	5.44575	0.039333333	0.030303571	0.003214286
#20 T2(S)	20	4.2145	4.5245	5.4315	5.9075	5.099	0.080619048	0.031589286	-0.1155
#11 T4(HS)	11	7.523077	8.722632	8.313784	8.297368	9.365385	0.036871024	0.065796703	0.152573742
#24 T4(HS)	24	8.210294	8.436486	8.232581	9.039744	9.332	0.039497594	0.040060924	0.041750916
#6 T4(S)	6	8.399643	9.102727	8.491053	8.62975	9.23475	0.010957483	0.029825255	0.086428571
#19 T4(S)	19	7.521379	8.620625	7.709474	9.058421	9.390769	0.073192464	0.066763926	0.047478311
#4 T4(T6S)	4	8.384483	8.061071	8.048077	8.230345	9.732162	-0.007339901	0.048131407	0.214545334
#18 T4(T6S)	18	7.930323	8.081724	8.311389	9.175128	9.7245	0.059276458	0.064077765	0.078481685
#10 T6(HS)	10	10.81714	12.00275	11.14975	10.74425	11.657	-0.003471088	0.029994898	0.130392857
#23 T6(HS)	23	10.60133	11.14417	10.48194	11.04351	11.98974	0.021056199	0.04958584	0.135174761
#2 T6(S)	2	11.81921	12.07455	10.64895	11.67974	11.96	-0.006641283	0.005028195	0.04003663
#16 T6(S)	16	11.90333	12.36316	11.11162	11.21947	12.47385	-0.032564745	0.020375458	0.179196067
#5 T6(T4S)	5	12.20821	11.35568	10.50588	10.14514	11.728	-0.098241061	-0.017150183	0.226122449
#17 T6(T4S)	17	11.12444	11.005	10.95525	11.10625	12.45825	-0.000866402	0.047635913	0.193142857
#3 T6(T8S)	3	11.81921	12.46429	11.64237	10.93737	12.63771	-0.041992481	0.029232277	0.242906552
#15 T6(T8S)	15	11.13286	11.57447	11.02063	10.79939	11.67128	-0.0158792	0.019229461	0.124555445

4.4. ENVIRONMENTAL PARAMETERS

Salinity, pH, temperature, dissolved oxygen and chlorophyll-α were measured weekly manually. Besides the manual measuring, the C3 datalogger measured the temperature, chlorophyll-α continuously (Figure 27). Furthermore the C3 datalogger measured the Colored Dissolved Organic Matter (CDOM) continuously as well. All of the parameters are within the range of the oyster spat, and no strong fluctuation was measured.

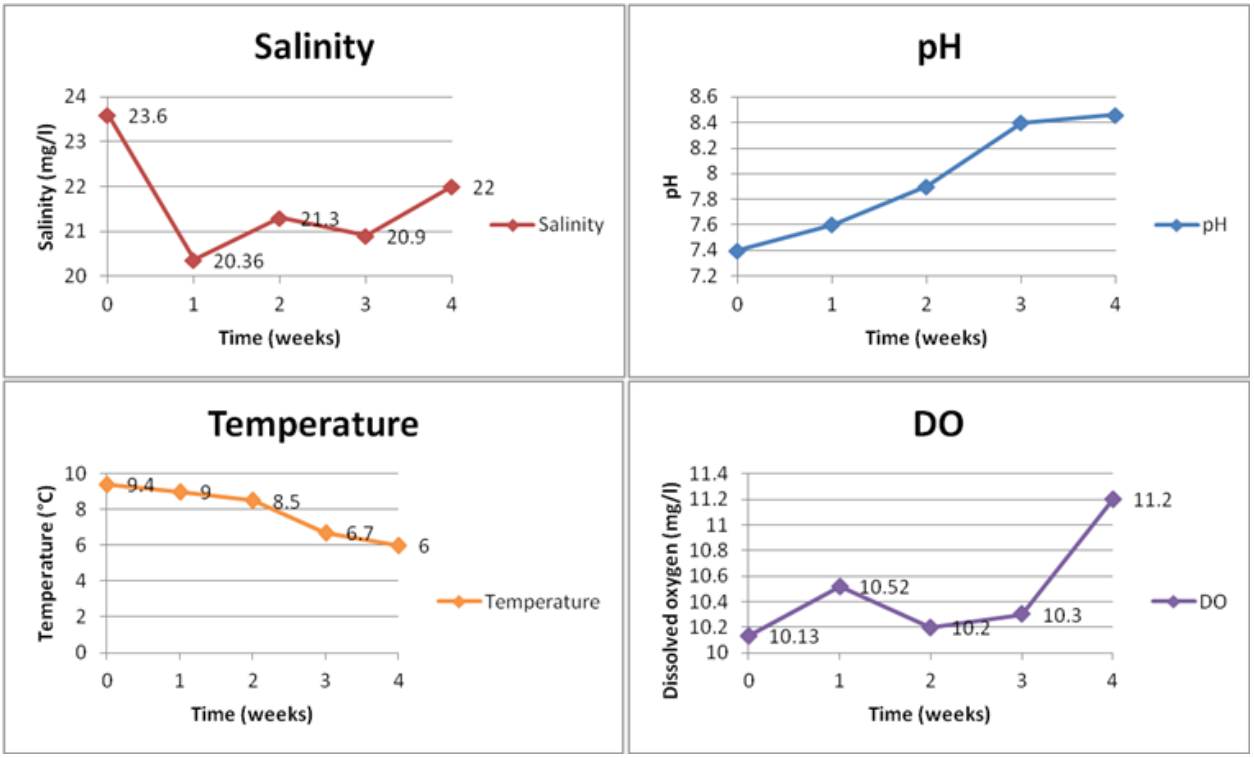


Figure 27. Salinity (mg/l), pH, temperature (°C) and dissolved oxygen (mg/l) measured over the course of the experiment, measured weekly manually.

The temperature measured by the C3 datalogger showed the same trend as measured manually (see Appendix 5). Therefore the temperature as measured by the datalogger was not included in the graphs and only the chlorophyll-α and CDOM readings are shown.

5. DISCUSSION

The results show that variation in the flow rate has had no measurable impact on the oyster spat. An increase or decrease in flow rate did not result in a positive influence on the cup shape of the oyster spat. Neither was there an increased prevention of biofouling on the oyster shells. The effects of the variation in flow rate on the growth (measured in length) could not be determined in a reliable way either, since the results show a negative growth rate.

5.1. GROWTH OF THE OYSTER SPAT

The length data collected prove to be not reliable since the variation in size of the oyster spat at the beginning of the experiment showed to be too big. The T6 spat showed a skewed distribution at measuring point t=0. At time t=0 all the T6 spat could be treated as if it was one population, for the samples in the silos came out of one large group.
The P-value between the all the T4 spat treatments (or silos) at t=0 was calculated to be $p<0.001$ (4.06E-03) based on the ANOVA single factor analysis. The P-value for the T2 spat treatments at t=0 had a value of 1.15E-09 based on the ANOVA single factor analysis. These low P-values for the measured spat per treatment and separated per spat class (T6, T4 or T2) indicate that the variance within the groups were too large at the start of the experiment.

A pairwise t-test with pooled SD using Bonferroni adjustment for all treatments caused no problem for T2 and T4 spat and corresponding treatments, comparing T6 spat resulted in P-values too high ($p>0.1$). Indicates the null hypothesis would be rejected.Therefore it is not possible to draw any statistical safe conclusions concerning the effects of the flow rate on the growth rate of the oyster spat for T6 spat.

At measuring point t=1 the ANOVA single factor tests between the spat classes results again in an extreme small P-value. At t=1 the P-value for all the T6 spat treatments was 1.70E-02. The T4 spat treatments resulted in a P-value of 6.52E-03 and for T2 it was 4.34E-09.

For the growth rate, almost all treatments showed a large increase in growth in week 4 (between measuring point 3 and 4). One of the duplicates of the T4(T6S) treatments did show the highest growth rate in week 4 (silo #4) of all the T4 spat treatments . The other duplicate, silo #18 showed to have the third highest growth rate, with only its duplicate and one T4(S) treatment having a higher. Based on these results it seems to confirm that an increase in the flow rate which was done perpendicular to the growth did indeed result in a higher growth rate. Compared to growth rates for the T4(T6S) in the previous three weeks, the increased flow rate seems to have stimulated to growth significant.
For the T6(T8S) treatments the same trend is seen as for the T4 spat which had an increased flow rate in week 4. Here again one duplicate obtained the highest growth rate in week 4 of all the T6 treatments (silo #3). Yet again the other had a quite lower growth rate, almost half of its duplicate (silo #15). Surprisingly one of the T6(T4S) treatments has a growth rate of 0.226 in week 4, its duplicate 0.193, compared to 0.243 of one of theT6(T8S) treatments. The treatments T6(T4S), which in fact is a suboptimal flow rate, showed to have a quite high growth rate in week 4 as well. This might indicate that the growth rate for all treatments increased in this week. Therefore the conclusion cannot be drawn that he increasing flow rate between week 3 and 4 for the T6(T8S) and T4(T6S) treatments had a significant positive effect on growth.

Samples were taken after the silos were detached and shaken in the water to remove silt and feces which accumulated on top and in between spat. Assumption was made this shaking would not result in the distribution of

spat according to size. There is however a possibility this might have occurred and affected the results to a certain degree.

5.2. BIOFOULING

The data collected for the shape and biofouling was based on count data rather than continuous data. All treatments for the T6 and the T4 spat did not show a positive effect of the variation in flow rate on the amount of biofouling present on the oyster shells over the run of the experiment (t=0 to t=4). Due to the set up of the biofouling score a high score indicates a clean sample, where less biofouling is present. Based on the results for all the treatments applied on the T6 spat, a deterioration of the biofouling was measured, thus an increase of biofouling. The T6(HS)treatments, in fact the old method without an added pipe to distribute the flow rate showed the least deterioration in biofouling (in total -2.37%). The T6(T8S) treatments which was expected to reduce the amount of biofouling the most, due to the increasing flow rate perpendicular to the growth, showed the highest deterioration in biofouling (-13.62%).
Furthermore the treatment T6(T4S) which based on the position in the FLUPSY was expected to have the highest increase of biofouling did not show to have the highest increase of biofouling. Of the new treatments of this experiment (thus apart from T6(HS)) it still scored better with -6.90% than T6(T8S) which had a deterioration of -13.62%.

Just as for the T6 spat, of all the applied treatments on the T4 spat, the old situation as it was made by the Roem van Yerseke scored the best. Namely the T4(HS) treatment showed to have a deterioration of -0.88% over the entire run of the experiment. Here again the treatment which was expected to have a positive effect on the amount of biofouling, thus a decrease in the amount of biofouling, scored even worse than the old situation T4(HS) with -3.56% compared to -0.88% respectively. The T4(S) treatment showed the worst deterioration in the biofouling score for the T4 spat treatments, thus the highest increase of biofouling (-4.24%). This implicates the old situation has the most positive effect on biofouling prevention compared to the suspended (HS) treatment.

For both the T2 treatment no significant difference was found in amount of biofouling removal by variation in the flow rate. One of the T2(S) treatments had two oysters which had biofouling on them (one scoring a 2 and the other 1), which explains the dip in the graph at t=3. For the T2(HS) treatments, in one of the duplicate a score of 119 was reached at t=1 and 118 at t=3. Both of these scores were obtained by one individual with biofouling on the shell (score 2 at t=1 and score 1 on t=3). Since most samples of the T2 spat (both T2(S) and T2(HS)) show no biofouling, the idea arises that biofouling occurs sporadically on T2 and the individuals found with biofouling are rare findings. This might be caused by the shape and structure of the shell, but requires further research to find the reason.

5.3. SHAPE

All treatments for all the different spat classes have shown an expected increase in the shape index due to growth.

For the T6 spat and the corresponding treatments in flow rate variation the highest increase in shape (and thus an improvement of the overall average shape) was measured for the T6(HS) treatment. Over the run of the experiment, the shape for this treatment showed an improvement of 13.51% compared to the start of the experiment (t=0 compared to t=4). Again, just as for the biofouling, this outcome rejects the initial idea, that the

increased flow rate would result in better shape. The idea was that a higher flow rate would results in more abrading of the shells to one another, which would result in a rounder shape with fewer ridges. For the T6 spat treatments the T6(T8S) treatment which had the highest flow rate and was expected to results in the highest scoring shape showed to score the poorest of all treatments for the T6 spat, with a increase of 1.89% from start to the end of the experiment. Furthermore the increase in shape score for the treatment T6(T4S) which was supposed to be a flow rate below the optimal flow rate, scored even better than the T6(T8S) treatment with an total increase of 10.61%. This treatment even scored higher than the suspension treatment T6(S) which resulted in a shape index increase of 7.46% over the run of the experiment. For the T6 treatments it can be concluded that a variation in flow rate does not result in a general better or more desired shape, since the initial treatment as carried out by the Roem van Yerseke scored the highest (T6(HS)).

For the T4 spat treatments, the flow rate at which the oyster spat was kept in suspension (T4(S)) gave the highest increase in shape, with an increase of 17.19% over the run of the experiment. Here again the treatment with a flow rate which increased perpendicular to the growth (increasing velocity in the silos once spat grew), and which was expected to give the best results, scored less than a presumed ''sub-optimal'' flow rate. T4(T6S) showed an increase of 13.41% on the shape index over the run of the experiment, less than suspension state (T4(S)). But for the T4 spat the initial situation or treatment as carried out by the Roem van Yerseke (T4(HS))scored the lowest of all treatment with a mere increase of 9.34% on the shape index.

For the T2 spat treatments, it was expected the increased flow rate which kept the spat in suspension, or better in suspension than the initial set up, again scored less. The T2(S) treatment resulted in an increase of 2.93% compared to the initial set-up T2(HS) which resulted in an increase of 10.37%. Based on these results it is safe to conclude the variation in flow rate did not led to an increase of a more desired shape.

Shape indices were made after close consultation with the RvY regarding their view of optimal shape and indices were checked by RvY before conducting measurements. Indices was made based on observations during the experiments, conducting the experiments in the growing season might results in need to alter the indices, perhaps even more classes.
Note that precise flow rates in the silos could not be measured. Assumption was made that flow rate between duplicates was exactly the same, due to location in the FLUPSY and adjustments with the outflow pipes. Furthermore the assumption was made flow rate differed between treatments in size classes. There is a possibility this assumption is false, and this uncertainty should be excluded by being able to measure the exact flow rate. Furthermore experiment and measurements were conducted late in the year, well after the growing season. Resulting in a lower water temperature, decreasing light intensity and thus less phytoplankton (chlorophyll-a) content in the water. If the experiment would have been conducted in the growing season, growth is presumed to be higher, as well as biofouling. Higher growth is assumed to result in better measurable differences in shape, but this as well as biofouling can only be excluded by performing the exact same experiment and measurements in the growing season.

5.4. CONCLUSIONS

Based on the results the research question and its corresponding hypotheses can be answered. Furthermore the sub-questions will be answered.

Research question:
Based on the results of the growth, growth rate, shape and biofouling the conclusion can be drawn that a variation in flow rate does not appear to have a positive effect on these variables. Comparing the flow variations with the initial state and set-up; none of the made flow variations show to have a significant positive effect on the measured variables. As explained in 5.1. Growth of the oyster spat, 5.2. Biofouling and 5.3. Shape, no significant positive effect was found based on the results of the variations in the flow rate.

Hypotheses:
Therefore the alternative hypothesis can be rejected and the null hypothesis is accepted. With the null hypothesis being: *A variation in the flow rate does not have an effect on the shape, optimal growth and maximal prevention of biofouling accumulation on the shells of the Pacific oyster spat. A variation in the flow rate to a state in which the oyster spat is fluidized (suspension state) and is upscalled synchronized to the growth does not result in a positive effect on the shape, growth and biofouling prevention on the shells of the oyster spat.*

Sub-questions:
Sub-question 1: What is the optimal treatment for different sizes of oyster spat (T2, T4 and T6), to keep them in suspension and prevent them from flushing out of the system or remain still on the bottom?

Although the results show that the difference in flow rate has had little to no profound effect on the shape or biofouling prevention, the set-up of the FLUPSY (locations of the silos) seemed to have some useful applications. The new locations of the silos in the FLUPSY based on the oyster spat size, seem to keep the silos itself clean in a better way.

Furthermore the application of the pipes which were attached to the point of outflow on the silos does keep the spat in suspension. The application of the outflow pipes makes sure that a more oyster spat is kept in suspension (more spat is kept slightly moving compare to the HS treatments which was the initial set-up. By doing this less silt and feces was accumulating in the silos itself. Therefore there is less labor required to keep the silos clean.

Sub-question 2: What is the optimal treatment for different sizes of oyster spat, to make sure the spat is kept in suspension which is required to abrade the growth edges (desired cup shape) and prevent biofouling on the shells?

The results show that the applied variations in flow rate have had no significant effect on the shape or biofouling prevention. Therefore the conclusion can be drawn that the old situation (indicated as HS) does not need to be adjusted. Nevertheless I suggest that the lay-out of the silos in the FLUPSY is kept according to Figure 29 and Table 11. This seen the fact the silos are kept more clean by applying this lay-out (see sub-question 1).

5.5. EXPERIMENTAL PROCEDURE

Silos were filled with available oyster spat, requiring more biomass of classes T4 and T6 was not possible. Furthermore removing excess biomass was not an option either. Therefore silos were filled with the exact same

amount of biomass for each size class (e.g. all T6 treatments contained the same amount of biomass per silo, same goes for T4 and T2 treatments). The biomass between classes differed, especially T4 treatment silos contained considerable less biomass compared to other size classes treatments. The fact not all the treatments consisted out the same biomass might have influenced the results, making comparison difficult. Only a comparison within a size class could be made, rather than the preferred possibility to compare different treatments.

Before the samples were taken, the silos were detached from the outflow pipe and the securing pipes. The silos were shaken (gentle up and down movements) in the water, to remove the feces and silt which might have accumulated on the spat layer. The assumption was made that this shaking would also mix the oyster spat, bottom and top layer mixing. Once lifted out of the water, a small slot was made through the entire spat layer, thus removing the spat from the mesh, creating a clear slot. On the sides of these slots the samples were taken. There is however a possibility that this shaking in the water might have created an accidental size distribution of spat sizes within one silo.

6. RECOMMENDATIONS

One of the problems which occurred during the experiment and which show in the results is the large variation in size of the T6 oyster spat. The T2 spat was sieved once, meaning the spat was placed on a sieve with a surface of 2 by 2mm. The same was done for the T6 spat (placed on a sieve of 6 by 6mm). The spat which remained on top of the T6 spat sieve, was indicated as T6 spat, thus spat which was larger than 8.5mm, everything that fell through fell on the T2 sieve (diagonally 2.8mm). The variation in the T6 was therefore very large, every oyster larger than 8.5mm was indicated as T6 spat, even if oyster larger than 10mm fell in this class. This means that the spat which fell through the T6 sieve and stayed on top of the T2 sieve was indicated as T4 spat. Therefore the T4 spat was sieved (or actually selected based on size) with an upper and lower boundary, contradictory to the other size classes. Due to this fact, the variation of the T4 spat was the smallest (between >2.8mm and <8.5mm). Since the T6 and the T2 spat was not sieved twice, the variation in this spat class was greater. My recommendation is to sieve all spat classes with an upper and lower boundary, which will result in a smaller variation which is more beneficial for the statistical analysis of the results.

The locations of the silos and the corresponding spat sizes are as follows: T6 spat should be placed close or right next to the paddlewheel, where the flow rate is somewhat higher and therefore better able to keep the larger spat in suspension. The T2 spat, which is the smallest, requires a lower flow rate; therefore they should be placed at the other end of the FLUPSY, thus furthest from the paddlewheel. The T4 spat should be placed in between the T6 and T2 spat, somewhere in the middle of the FLUPSY (Figure 29 & Table 11).

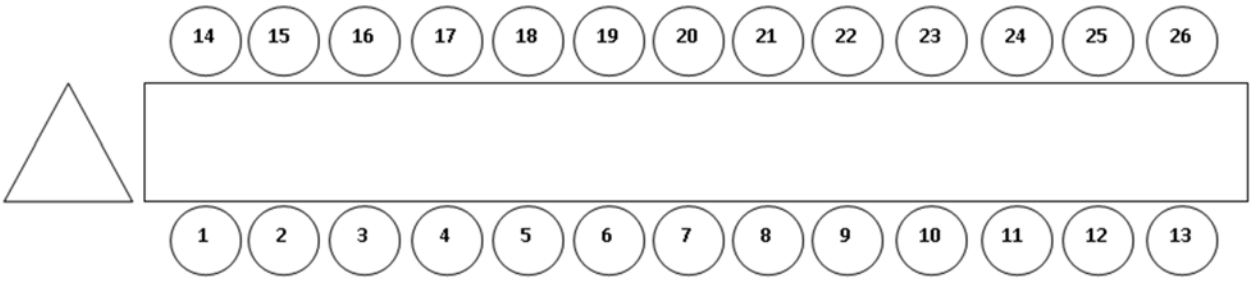


Figure 29. Lay-out of the FLUPSY with the silos numbered, the triangle represents the paddlewheel.

Table 11. Suggested location of oyster spat to the silos.

Spat size	*Silo number*
T6	14-15-16-17-1-2-3-4
T4	18-19-20-21-5-6-7-8
T2	22-23-24-25-26-9-10-11-12-13

Since the experiment was carried out in November, the growing season was not included in this experiment. In order to get more insight on the research question and the corresponding hypotheses and sub-questions, the research should be carried out once again in the middle of the growing season. Furthermore it would be even better to run the experiment for a longer period of time. This research was carried out over only four weeks, if the research would be carried out in the growing season and for a longer period of time (e.g. eight weeks) a more significant growth should be measured. If the experiment would be carried out in the growing season, for a longer period of time, perhaps different results for growth rate, shape and biofouling prevention will be found.

Since the experiment was carried out, outside of the growing season, the growth measured was very low, or slow. This meant the growth was such, that the increasing of the flow rate (treatment T6(T8S) and T4(T6S)) was not required until the end of measuring week 3. Up till this point the growth was slow, and the spat was still kept in suspension, only at this point it was noticed that the growth had caused the spat to remain still on the bottom of the silo. At this point the outflow pipes were adjusted (adding holes) to such a point the spat was kept in suspension again. This means the treatments with an increasing flow rate perpendicular to the growth was only carried out and measured for one week. Based on these results of one week, outside of the growing season, the conclusions might turn out very different if this experiment would be carried once again. The experiment should be carried out in the growing season, and with the treatments of an increased flow rate for a longer period of time. This way the results of these treatments might turn out very different, for a clear distinction between treatments intra size classes can perhaps be made.

During my experiment I encountered problems on how to measure the flow rate in the FLUPSY. The first problem being what equipment to use, the first equipment used was a hydro flow meter based on sound waves. It became clear the flow rate in the FLUPSY was too low for this apparatus to be measured. A second time the flow rate was measured using an OTT hydro flow meter, which measures the flow rate based on the number of rotations made by a screw. Several screws were available, each corresponding to a specific flow range in which it could measure. But it was not know which screw was able to measure the flow rate in the FLUPSY, since there were no indications on what the range in the system might be. The first measurements in the FLUPSY were performed using the #3-22966 screw. The measurements performed with this screw proved to be not reliable (>1m/s). A quick test was done on 6 silos with a different screw, #1-21648, which showed to give more plausible, for the flow rate was within a range of 0.2-0.3 m/s, which is significant lower. After these results showed the FLUPSY was already cleared of the largest spat (removed and placed in baskets). Therefore the flow rate could no longer be measured. My suggestion is to reinstall the FLUPSY according to the recommended lay-out (the location of the silos as described in method and materials) and fill them with the exact same amount of spat and measure, set the paddlewheel to the same settings and measure the flow rate again by using the #1-21648 screw on the OTT meter.

Regarding the sampling, in a continuation of this experiment or a similar experiment, sampling should be done without shaking first in the water column. This was done to remove silt and faeces from the spat, but might have created a size distribution within the silo. Samples should be taken directly after taking the silos out of the water, over the entire depth of the spat layer.

Another recommendation I propose is to attach the silos in a different way. The silos were attached by placing the hole in the side of the silo over the outflow pipe (short extending pipe). Furthermore another smaller hole was placed right above the outflow hole, where a small pipe which was attached to the central trough was inserted. In a right angle a small bleu strip was inserted. This bleu strip secured the silo to the pipes. On one occasion one of these bleu strips was thrown out due to waves, thus releasing the silo which had sunken to the bottom. Luckily the

silo sank straight down, remaining positioned with the opening upwards, thus minimizing the spat which might have been lost. No signs of losing a large portion of the spat were visible. Since large waves enter the harbor regularly, a different and more secure attaching mechanism should be made. This securing mechanism should be robust, yet easy to remove to be able to take the silos out for maintenance.

Although results show that a difference in flow rate compared to the initial set-up had no noticeable effect on growth rate, shape and biofouling prevention. Placement of outflow pipes did however seemed to have a positive effect on keeping oyster spat in suspension, which in turn resulted in less silt and feces accumulation in the silos. This results in less required labor to clean the silos.

Further trials of this experiment or similar ones should be carried out with exact same amount of biomass for silos and treatments. A biomass difference between size classes might have had an influence and makes the comparison of results harder.

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APPENDICES

1. MANUAL DETERMINATION OF CHLOROPHYLL-A LEVELS

FILTRATION OF THE WATER SAMPLE

Each week a water sample was taken at the FLUPSY after the oyster samples were taken. This was done to make sure the chlorohyll levels in the water would not drop during long storage.
Arrived at the HZ, the water samples were immediately filtered over a Whattman filter.

The Whattman filter was placed in the vacuum water filtration apparatus. The water sample was shaken, to make sure nothing was settled on the bottom. Since the cup of the vacuum filtration could only process 250 ml in one go, the process was repeated 4 times per sample. The water was poured in the storing cup, and the vacuum pump would be turned on. Once the storage compartment was empty, the sides were rinsed with demi-water, to make sure no chlorophyll would remain on the sides. This process was repeated 4 times, till 1l of sampling water was poured over the filter.

After filtration the filter could be removed gently and placed in the aluminum cups, which were marked on the bottom with the sampling date and the amount of water filtrated (although this was always 1l).

CHEMICAL ANALYSIS OF THE FILTERS

REQUIREMENTS:

- Glass bottles with a lid (two for each filter to be analyzed)
- 25 ml ethanol 95% technically denaturized with 5% methanol (per filter)
- Water bath at 75°C
- Ultrasonic bath
- Spectroscopic photometer
- Two cuvette for a spectroscopic photometer
- Bekerglass of ethanol to set the reference
- Demi water to rinse the cuvette
- A couple of transfer pipettes
- 0.1 M HCL

EXECUTION:

The filters will be placed in the glass bottles. The bottles were marked corresponding to the filter they contained. 25ml of ethanol 95% technically denaturized with 5% methanol was added in each glass bottle, and closed with the lid. The lid was however not turned to tight.
The glass bottles would then be placed in the water bath at 75°C for a period of 10 min. Since the ethanol would slightly evaporate, the lid was not turned to tight; making sure the vapors could escape. During the 10 minutes the glass bottles were swung a couple of times (gently) making sure the filter was kept submerged in the ethanol at all times.

After the 10 minutes in the water bath, the glass bottles were placed in a rack and placed in the ultrasonic bath for another period of 10 minutes. Once again the bottles were swung regularly but gentle during the period in the bath.

After the ultrasonic bath the content of the glass bottles were poured over in a new glass bottle, which again was marked with the sample number. The filter would remain in the first glass bottle and could remain there.

FIRST MEASUREMENT AT 665 AND 750NM

One cuvette was now filled with ethanol 95% technically denaturized with 5% methanol. This cuvette would be placed in the spectroscopic photometer. The photometer was set at a wavelength of 665 nm. The cuvette with ethanol 95% technically denaturized with 5% methanol was placed in and used to set the reference of the photometer (by pressing set ref.).

Now the other cuvette could be filled with the solution of the glass bottle with a transfer pipette. The cuvette was placed in the photometer and the value which was stated could be noted once it was stabilized. The cuvette could be taken out (the lid of the photometer closed) and the content poured out of it. The cuvette was rinsed with demi water by using a new transfer pipette. The cuvette was carefully dried by using a paper towel, making sure the fingers were placed on the sides which were matte.

The next sample could be placed in the cuvette by using a new of carefully rinsed (with demi water) transfer pipette and placed in the photometer. This process was repeated till all the samples were processed.

After all the samples were measured at 665nm, the spectroscopic photometer was set to a wavelength of 750 nm and by using the cuvette filled with ethanol set to reference once again. The samples were measured once again by using the same principal as for 665nm.

SECOND MEASUREMENT AT 665 AND 750NM

After the first measurement, 2 ml of 0.1 M HCL was added to all the glass bottles containing the samples. This was set to wait for a period of 15 minutes.

After the 15 min period all the samples were measured again at a wavelength of 665nm and 750nm. The same protocol as for the first measurement was used.

CALCULATING THE CHLOROPHYLL-A CONTENT

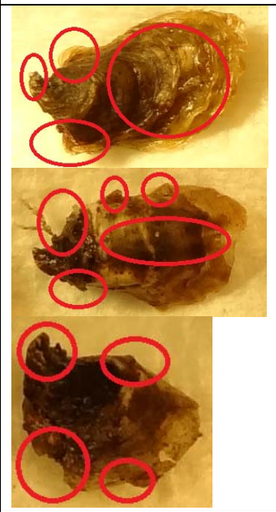
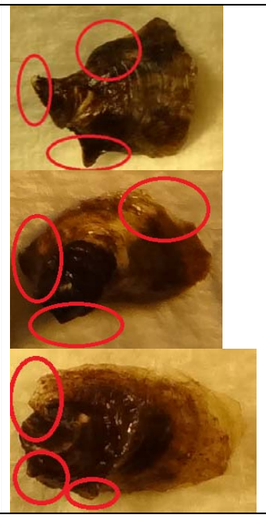
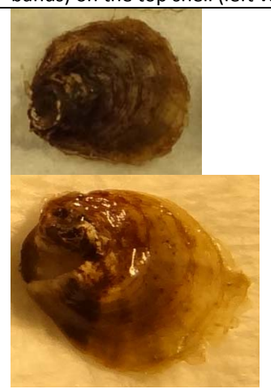
The gained results will look as follows:

Measurement without addition of HCl			
665nm (#1)		750nm (#2)	
t=0		t=0	
t=1		t=1	
t=2		t=2	
t=3		t=3	
t=4		t=4	
Measurement with addition of 2ml 0.1M HCl			
665nm (#3)		750nm (#4)	
t=0		t=0	
t=1		t=1	
t=2		t=2	
t=3		t=3	
t=4		t=4	

The following formula was used to calculate the chlorophyll-α content for each sample in µg/l:

$$Chlorophyll\ \alpha\ (\mu g/l) = 296 * \big((\#1 - \#2) - (\#3 - \#4)\big) * \frac{25}{1000} * 10$$

2. SHAPE INDEX PACIFIC OYSTER CRASSOSTREA GIGAS

Score	**1**	**2**	**3**	**4**	**5**
Description	Very unfavorable	Unfavorable	Neutral	Favorable	Very favorable (desired shape)
Explanation	General shape contains a lot of extremities or outgrows. The shell is not symmetrical at all. The overall shape resembles that of a wild oyster (looks like *the FLUPSY had no effect on the contour of the shell*). Or other *deformities* are present.	The shell is not symmetrical. There are clear outgrowths/extremities. It looks like the period spend in the FLUPSY has had hardly any impact on the shells contours. The shell contains upstanding rims or wrinkles. The total outgrowths/extremities, gaps or upstanding rims together is four or more.	The shell is more or less elongated, no longer oval. Or the shell may contain several outgrowths/extremities. The overall shape is a bit more rigged. The shell may contain upstanding rims (wrinkles). The total outgrowths/extremities, gaps or upstanding rims together is three.	The shell shape is almost completely oval. There are only *one of two outgrowths, extremities or gaps along the valve edge*. The shell is however not perfectly symmetrical. The shell is not perfectly symmetrical. The top left valve is still smooth, thus no rimples or upstanding bands are present.	Shape is oval (rounded), *no extremities, outgrowths or gaps occur along the valve edges*. Shell is formed in a gentle way, laminated. Overall shape resembles that of a European flat oyster. The shell is symmetrical. The period spend in the FLUPSY has had a clear influence on the shape. The shell is smooth, without any rimples or curves (upstanding bands) on the top shell (left valve).
Picture examples *(Note: the shape of the pictures are not stretched in any way)*					

Does the shell contain clear deformities?

Yes the shell has clear deformities

1

No

Is the shell oval shaped?

Yes, the shell is oval to round shaped

No, the shell is more elongated

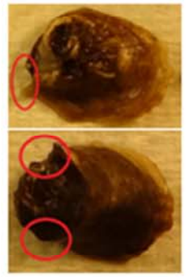
Does the shell have 1 or 2 outgrowths/extremities or gaps along the valve edge? Does the shell have rimples/wrinkles or upstanding rims?

No, the shell is almost perfectly symmetrical with an almost perfectly smooth (laminated) top surface and general contour

5

Yes, the shell is not perfectly symmetrical and has 1 or 2 outgrowths/extremities or gaps. The shell has no upstanding rim

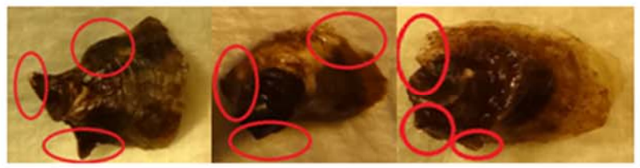
4



The shell is more or less oval but contains more than 2 outgrowths/extremities or gaps along the valve edge. Furthermore the shell may contain more than one upstanding rim

The shell is elongated and contains outgrowths/extremities and may contain an upstanding rim. The total outgrowths/extremities, gaps and upstanding rims together is 3.

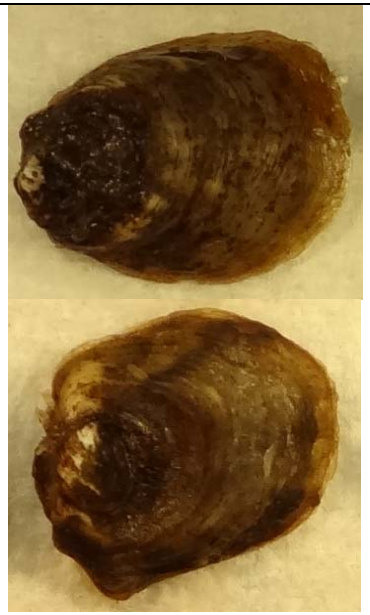
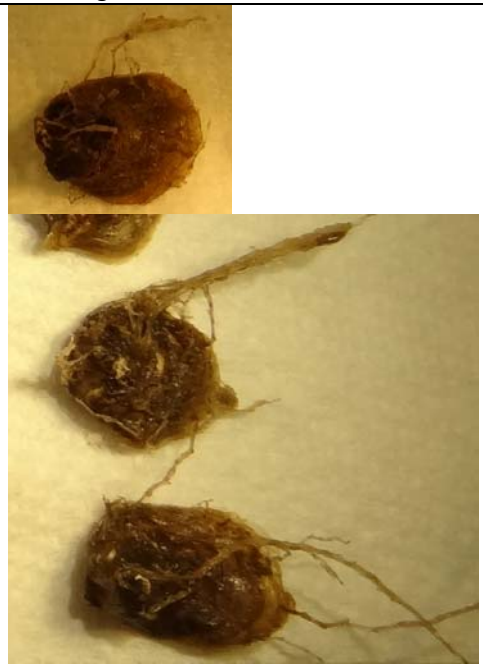
3



The shell is elongated and contains outgrowths/extremities and may contain an upstanding rim. The total outgrowths/extremities, gaps and upstanding rims together is 4 or more.

2

3. BIOFOULING INDEX PACIFIC OYSTER CRASSOSTREA GIGAS

Score	**3**	**2**	**1**
Description	No biofouling	A little biofouling	Severe biofouling
Explanation	There is no biofouling on the shell present. The shell is completely clean.	There are a few strands of macroalgae present (one or two). There are however no patches of macroalgae present.	Biofouling is present in a severe form, meaning long strands of macroalgae or patches of macroalgae on the shell.
Picture examples *(Note: the shape of the pictures are not stretched in any way)*			

4. CONDITION INDICES

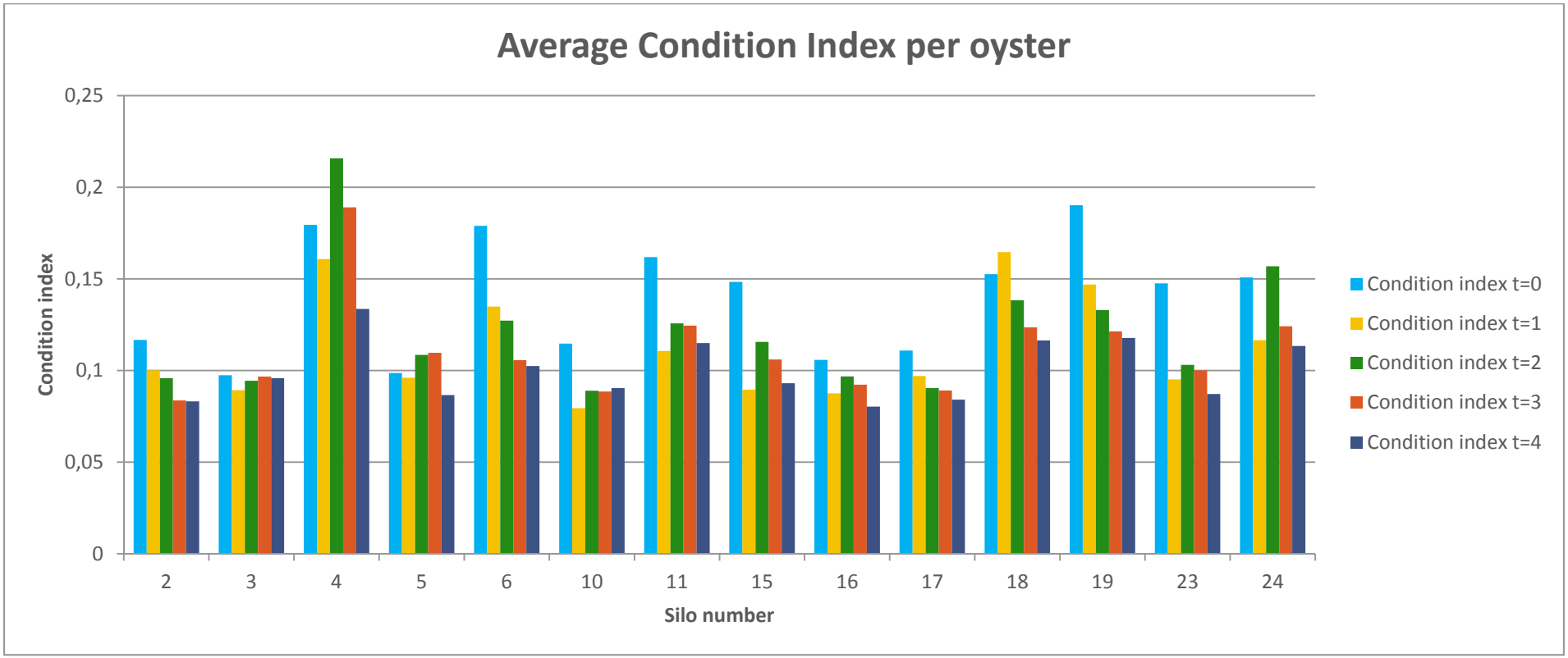


Figure 1. The average condition index was taken per oyster for each sample. The condition index was calculated as follows: CI= [dry tissue weight (g)*100/dry shell weight] (Mercado-Silva N. , 2005). The average condition index was taken, to take account for the dead oysters, thus not giving a false indication. Thus the average condition index was calculated for one oyster per sample.

5. C3 ENVIRONMENTAL PARAMETERS

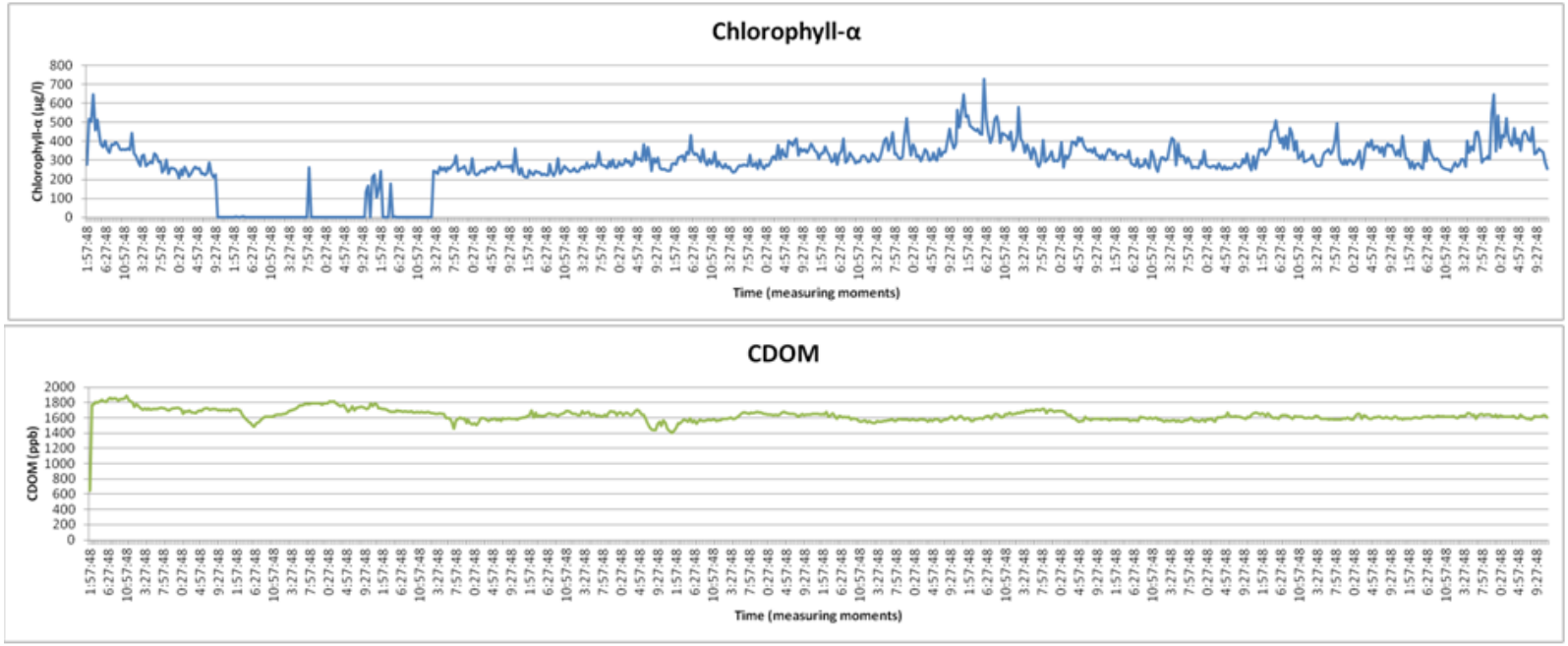


Figure 8. Environmental parameters measured by C3 datalogger. Chlorophyll-α (µg/l) and CDOM (ppb) were measured continuously over the entire duration of the experiment. The chlorophyll measurements show some drops, below the measuring range of the datalogger, thus indicated as 0 measurements. No explanation can be given for these readings compared to the other environmental parameters. The CDOM levels remained fairly constant throughout the experiment.