Oyster cultivation of Crassostrea gigas and Ostrea edulis in the Waterdunen oyster trench.

> By Lotte Sanders Submitted for the degree of Applied biology

# Oyster cultivation of *Crassostrea gigas* and *Ostrea edulis* in the Waterdunen oyster trench.

A Thesis

By Lotte Sanders Student number 3025804 Submitted for the degree of Applied biology. Graduation coach Wilfred Sewnandan

Photo cover: L. Sanders, 2021

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### Preface

Presented before you is the thesis "Oyster cultivation of *Crassostrea gigas* and *Ostrea edulis* in the Waterdunen oyster trench." written by Lotte Sanders. The research for this thesis focuses on the possibility and success of cultivating two different oyster species, namely *Crassostrea gigas* and *Ostrea edulis* in a new nature reserve called Waterdunen, located in the south of the Netherlands. This thesis was written as part of my graduation process for the applied biology course at the Aeres Hogeschool Almere.

In order to do my research, I did an internship at the research group "aquaculture in delta areas" of the University of Applied Sciences Vlissingen under the supervision of Jasper Houcke and Gabriëlle Verbeeke. Together with Gabriëlle and several other interns, we collected data every week, which I could then use to write my thesis. My supervisors were always ready to answer my many questions and helped me to critically look at the data.

I would hereby like to thank my supervisors for the guidance and the ever enjoyable and educational fieldwork. The support I received during the process was invaluable and I greatly enjoyed my time there. I would also like to thank the other interns I worked with during the fieldwork for their encouragement, support, and valuable advice.

Many thanks to my coach Wilfred Sewnandan for his guidance and feedback and for always being available for pressing questions. Finally, I would also like to thank my second assessor Annet Pauw and my replacement coach Sander Harting for their feedback and guidance during this process. I hope that you enjoy reading the contents of this thesis.

# **Summery English**

The cultivation of oysters is one of the oldest forms of aquaculture and accounts for more than 50% of the global seafood supply. With a growing world population, the demand will most likely increase. New and improved techniques will be necessary to keep up with the rising demands and threats that surround the cultivation of oysters. Oysters have been cultivated in the Netherlands since the mid-19<sup>th</sup> century using on-bottom techniques. Worldwide another technique is more regularly and successfully used, namely the off-bottom technique which makes sure the oysters are suspended in the water column. However, this technique is hardly being used in the Netherlands.

Located in the Waterdunen lies a so called "coastal laboratory" which will be used to test the possibility of cultivating oysters and saline crops in this area. An oyster trench was constructed and in December 2020 this oyster trench was taken into use to test the suitability of the oyster trench in the Waterdunen for various off-bottom techniques for the cultivation of *Crassostrea gigas* and the onbottom technique for the cultivation of *Ostrea edulis*. The cultivation of three age groups for the *Crassostrea gigas* (spat, half-grown and consumption size) and consumption size *Ostrea edulis* were tested over a period of 6 months from December 2020 until June 2021. Data was collected every three weeks for the spat and every 6 weeks for the half-grown and consumption size oysters. To decide on the successfulness of each system all age groups were analyzed. The lengths, weight, mortality, growth rate as well as the thickness and width of the spat was collected and analyzed. The total- fish- and shell weight as well as the condition index, mortality and growth rates for the half-grown and consumption size oysters were collected and analyzed. Lastly, data on chlorophyll-a, turbidity, salinity, and dissolved oxygen was collected on four different spots in the oyster trench to then compare with the optimal growing conditions of *Crassostrea gigas* and *Ostrea edulis*.

T40 system showed the least favorable results with low grow rates and high mortalities. T20 and the hanging baskets showed similar results, but the hanging baskets showed more favorable and consistent shapes. The cultivation of *Ostrea edulis* showed to be no success with almost no growth and high mortalities. The water parameters were almost all according to the cultivation requirements of both species. However, the temperature and salinity were found to be on the low side. With fluctuations this might be cause for concern. The turbidity was also on the high side for *Ostrea edulis*. Unfortunately, the species is very intolerant to suspended materials and this might have been the cause of the results.

The overall recommendation is to further investigate the use of hanging baskets for the cultivation of *Crassostrea gigas* in the oyster trench of Waterdunen since this system showed the most favorable results. The cultivation of *Ostrea edulis* was not successful and therefore not advisable. It is also advised to keep monitoring the water parameters seeing as it is a developing nature reserve which is subject to changes still.

### **Summery Dutch**

De kweek van oesters is een van de oudste vormen van aquacultuur en is goed voor meer dan 50% van het wereldwijde aanbod van schaal- en schelpdieren. Met een groeiende wereldbevolking zal de vraag naar alle waarschijnlijkheid toenemen. Nieuwe en verbeterde technieken zullen nodig zijn om aan de stijgende behoefte te voldoen en om de bedreigingen rond de oesterteelt in de hand te houden. Oesters worden in Nederland sinds het midden van de 19e eeuw gekweekt met behulp van on-bottom technieken. Wereldwijd wordt een andere techniek regelmatiger en met meer succes toegepast, namelijk de off-bottom techniek, die ervoor zorgt dat de oesters in de waterkolom hangen. Deze techniek wordt in Nederland echter nauwelijks toegepast.

Gevestigd in Waterdunen ligt een zogeheten "Kustlaboratorium" waarin de mogelijkheid van de teelt van oesters en zilte gewassen in dit gebied kan worden getest. Een oestergeul is aangelegd en in december 2020 is deze oestergeul in gebruik genomen om de geschiktheid van de oestergeul in de Waterdunen te testen voor verschillende off-bottom technieken voor de kweek van Crassostrea gigas en de on-bottom techniek voor de kweek van Ostrea edulis. De kweek van drie leeftijdsgroepen voor de Crassostrea gigas (broed, halfvolwassen en consumptie formaat) en consumptie formaat Ostrea edulis werden getest over een periode van 6 maanden, vanaf december 2020 tot juni 2021. De metingen vonden om de drie weken plaats voor het oesterbroed en om de zes weken voor de halfvolwassen en consumptie oester. Om een oordeel te vellen over het succes van de systemen werden alle leeftijdsgroepen geanalyseerd. Gegevens over de lengte, het gewicht, de sterfte, de groeisnelheid en de dikte en breedte van het broed werd verzameld en geanalyseerd. Het totale- vis en schelp gewicht, de conditie-index, de mortaliteit en de groeisnelheid van de halfvolwassen en de consumptie formaat oesters werden verzameld en geanalyseerd. Tenslotte werden gegevens over chlorofyl-a, troebelheid, zoutgehalte en opgeloste zuurstof verzameld op vier verschillende plaatsen in de oestergeul om deze vervolgens te vergelijken met de optimale groeiomstandigheden van Crassostrea gigas en Ostrea edulis.

Het T40-systeem gaf de minst gunstige resultaten met lage groeicijfers en hoge sterftecijfers. T20 en de hangende manden gaven vergelijkbare resultaten, maar de hangende manden gaven gunstigere en consistentere vormen. De teelt van *Ostrea edulis* was geen succes met bijna geen groei en hoge sterftecijfers. De waterparameters waren bijna allemaal in overeenstemming met de kweekvereisten van beide soorten. De temperatuur en de saliniteit bleken echter aan de lage kant te zijn. Bij schommelingen zou dit reden tot zorg kunnen zijn. De troebelheid was ook aan de hoge kant voor *Ostrea edulis*. Helaas is de soort zeer intolerant voor zwevend materiaal en dit kan de oorzaak zijn geweest voor de verkregen resultaten.

De algemene aanbeveling is om het gebruik van hangende manden voor de teelt van *Crassostrea gigas* in de oestergeul van Waterdunen verder te onderzoeken, aangezien dit systeem de gunstigste resultaten opleverde. De kweek van *Ostrea edulis* was niet succesvol en daarom niet aan te bevelen. Er wordt ook aangeraden de waterparameters te blijven controleren, aangezien het een natuurgebied in ontwikkeling is dat nog steeds aan veranderingen onderhevig is.

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## **1. Introduction**

The cultivation of oysters is one of the oldest forms of aquaculture and has in the last decades shown a rapid increase in production with oysters being the leading molluscan species to be cultivated (Botta *et al.*, 2020). Aquaculture accounts for more than 50% of the global seafood supply and with the predicted world population growth this will most likely increase (Botta *et al.*, 2020; Garlock *et al.*, 2019; Lacoste & Gaertner-Mazouni, 2015). An increase in production follows a demand for the optimization of existing techniques and the realization of new techniques to enable cultivation in different ways (Botta *et al.*, 2020).

In the mid-19th century, oyster farming began to take off in The Netherlands (Dijkema, 1997; FAO, n.d.-c). These aquacultural practices take place in two areas, namely the Lake Grevelingen and the Oosterschelde with the Oosterschelde being the largest production source (FAO, n.d.-c; Engelsma *et al.*, 2010). The shellfish sub-sector resulted in 2019 in around 3,000 tons oysters (of which the *Crassostrea gigas* accounted for the majority). In 2005 the total production of *Crassostrea gigas* had a farm gate value of 3.3 million euros. Most of the oysters grown in The Netherlands are exported to Belgium and France (Central Bureau for Statistics, 2021; FAO, n.d.-c).

The cultivation of oysters has been practiced worldwide for millennia, yet the production of oysters has never been sufficiently stable. In fact, this form of aquaculture experiences many problems involving viruses, parasites, and predation (Botta et al., 2020). A major part of these problems can be solved by using a different method of cultivation, namely off-bottom cultivation where the oysters are no longer in contact with the soil (Walton et al., 2013). Off-bottom oyster culture is a method in which the oysters are suspended within the water column. The oysters are put in either bags, cages or baskets and tied to structures at a certain height depending on the preferences and situation (Hensey, 2020; Davis et al., 2012). As a result, species which prey on oysters such as the predatory snails Ocinebrellus inornatus and the Urosalpinx cinerea have more difficulties in reaching the oysters. In addition, fewer mortalities are caused by suffocation in the soft sediment. Off-bottom also makes it possible to cultivate in places where this would normally not be possible (Walton et al., 2013). Furthermore, this technique provides more control over the growing conditions. For example, the density at which the oysters are grown and the depth at which the oysters are suspended (and thus the food supply) can be adjusted to the needs of the farmers (Hensey, 2020). In addition, the farmer has more control over the amount of biofouling that takes place, and the shape of the oysters is easily regulated (Davis et al., 2012).

Off-bottom techniques are used worldwide but have not been widely used in The Netherlands (Capelle *et al.*, 2020). To this day only on-bottom techniques have been used for the cultivation of oysters in The Netherlands (FAO, n.d.-c; Dijkema, 1997; Capelle *et al.*, 2020). Originally, cultivation in The Netherlands was limited to the native European flat oyster (*Ostrea edulis*) (Dijkema, 1997). However, the *Ostrea edulis* encountered several problems which resulted in a gradual decline in the production around the world, including in The Netherlands (Engelsma *et al.*, 2010; FAO, n.d.-c). Because of the difficulties with *Ostrea edulis*, oyster farmers started to focus on the cultivation of the Pacific oyster (*Crassostrea gigas*). This species has a greater tolerance for diseases and environmental changes (Comesaña *et al.*, 2012) and a faster growth rate, *Ostrea edulis* takes around 4-5 years to grow to market size whereas *Crassostrea gigas* takes about 2-3 years (Roberts, 2021; Goulletquer, 2021). This resulted in a decline in the cultivation of *Ostrea edulis* as shown in figure 1 and 2 (FAO, n.d.-a; FAO, n.d.-b; Smaal *et al.*, 2009; Wijsman *et al.*, 2008).

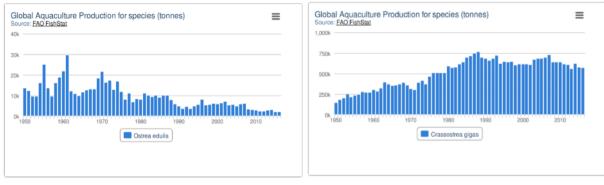


Figure 1 shows the Global aquacultural production of the *Ostrea edulis* over the years (FAO. 2019 -d).

Figure 2 shows the Global aquacultural production of the *Crassostrea gigas* over the years (FAO, 2019 -e).

Another development that has been emerging in recent years is that of in-land shellfish farming. This new development involves the creation of a basin, either directly linked to the sea or closed off, into which seawater is pumped and in which shellfish can be cultivated. This gives the farmer considerably more control over what happens in the farming process and eliminates many of the difficulties such as predation (Global Aquaculture Alliance, 2018). Since 2009, in-land shellfish production pilots have been conducted at several locations along the coast of The Netherlands. These pilots test the technical and economic feasibility of in-land shellfish production (Robert *et al.*, 2013).

The coastal areas of The Netherlands are increasingly affected by salinization, and this brings problems for various land uses such as agriculture (Oude Essink *et al.*, 2010). This is also the case for the coasts in the province Zeeland where many of the polders lie well below sea level. Due to the rise in sea level, the soil is influenced by salt water and salinization takes place. Keeping the saline groundwater artificially low is becoming progressively more difficult and expensive and thus requires a different approach (kustlaboratorium, n.d.). Situated in Zeeland lies Waterdunen, a new nature reserve created in a widened and strengthened coastal zone. The area, for which the construction was largely completed in 2017 was constructed following an idea to find a new purpose for land which because of the salinization could no longer be used for agriculture activities. The area has the character of an estuary where at high tide seawater flows into the system (Waterdunen, n.d.). In addition to having the purpose of a nature reserve and recreational area a part of it will also serve as a place to cultivate oysters and grow saline crops.

In the Waterdunen an area of approximately 30 hectares has been designated where shellfish can be cultivated in an oyster trench and saline crops can be grown experimentally, the so-called coastal laboratory. The coastal laboratory is an initiative of the foundation "Het Zeeuwse Landschap" and the research group "Aquaculture in Delta Areas" of the University of applied sciences Vlissingen. The Aquaculture in Delta Areas research group addresses practical problems by conducting research in cooperation with entrepreneurs. Several Zeeland entrepreneurs have expressed interest in growing oysters in the coastal laboratory of Waterdunen. One of these is the Dhooge oyster family business.

In December 2020, the oyster trench was put into use by the Aquaculture in Delta Areas research group to study various off-Bottom methods for growing *Crassostrea gigas* and the on-bottom technique to grow *Ostrea edulis*. This study will look at the potential of this oyster trench to cultivate *Crassostrea gigas* and *Ostrea edulis* using these techniques.

There remains yet much unknown about the characteristics of Waterdunen, mainly because the area is still in a transitional phase where the biotic and abiotic factors are subject to considerable changes. For this reason, in November 2020, measuring instruments were also placed in the water around the oyster trench to map the characteristics of the Waterdunen area (Verbeeke, Personal communication, 23 March 2021).

In addition, the cultivation of oysters in an oyster channel such as this is new. There is no literature and no practical examples available to compare with. Due to the complexity of the new and still developing system, the first year will focus on mapping the characteristics of the oyster channel and will look at the suitability of different methods. This report will focus on the effectiveness of off-bottom methods for the cultivation of *Crassostrea gigas* and the on-bottom method for the cultivation of *Ostrea edulis* in the oyster channel located in the Waterdunen. The main research question of this report is: How suitable is the oyster trench in the Waterdunen for the cultivation of *Crassostrea gigas* using off-bottom techniques and the cultivation of *Ostrea edulis* using the on-bottom technique?

Several sub-questions were formulated to answer the main research question:

- Which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the Waterdunen?
- To what extent is it possible to cultivate *Ostrea edulis* with the on-bottom method in the Waterdunen?
- To what extent do the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*?

The hypothesis for the research question is as follows. The unique and still changing characteristics of Waterdunen can be expected to have an impact on the development of the oysters. The tidal conditions will result in the oysters running dry for a few hours each day, how long the oysters run dry will depend on the method being used. This will result in a difference in food intake and the amount of biofouling between each culture method and this will be reflected in the results (Fitridge *et al.*, 2012). The hanging baskets bring the most benefits compared to the other methods and are thus expected to show the best results. One reason for this is that baskets are in constant motion and therefore have a continuous supply of food (Davis *et al.*, 2012). The temperature changes brought about by the changing tide will have an impact on the *Ostrea edulis* due to a low tolerance to changes. Therefore, it is expected that harsh winters and warm summers will cause high mortality numbers in *Ostrea edulis*. In addition, *Ostrea edulis* are likely to experience smothering in silt and possible predators because of the on-bottom techniques used.

### 2. Methodology

This chapter describes the research setup after which the methodology for each sub-question is described.

### 2.1. Research setup

The oyster trench is located in Waterdunen, a nature reserve located in the province Zeeland in The Netherlands. This nature reserve is bordered at the northern side by the Westerschelde and is from all other sides surrounded by agricultural fields. The oyster trench is located approximately south-east of Waterdunen and is realized between two creeks (indicated in figure 4 and figure 10). The oyster bed is surrounded by low erosion resistant embankments with a height of 1.5 m above sea level. The inside of these embankments are reinforced to prevent erosion. The tidal culvert provides Westerschelde water twice a day and on the east side of the oyster trench there are three

paddlewheels which provide a flow of salt Westerschelde water flowing west (figure 3). The bottom of the oyster channel lies at 0.5 meter below sea level and is formed by a stabilized sand bed (Provincie Zeeland, 2018).

Triploid Ostrea edulis and Triploid Crassostrea gigas oysters were used. The use of triploid oysters was adopted as they do not need to put energy into reproduction and thus use more energy for growth (Wadsworth *et al.*, 2019). This allows for a reduced time until consumption size has been reached and the possibility of year-round harvest (Botta *et al.*,



Figure 3 Paddlewheels on the east side of the oyster trench provide waterflow (L. Sanders, 2021).

2020). The different age class groups for both *Ostrea edulis* (consumption size) and *Crassostrea gigas* (spat, half-grown and consumption size) and the necessary materials such as tables, poles and fixing materials were provided by the Dhooge Oyster family business.



Figure 4 Oyster trench located in Waterdunen, The Netherlands (Sentinel-hub, 2021).

In the oyster trench four culture structures were laid out: on-bottom plots for the cultivation of *Ostrea edulis*, tables at 20cm and at 40cm height with bags and tables with hanging baskets for the cultivation of *Crassostrea gigas*. Each bag or basket was labeled for identification and was then randomly distributed throughout the trench. Figure 5 gives a schematic overview of the oyster trench and the placement of each label. Figure 6 shows a photo of the oyster trench and the placement of the structures.

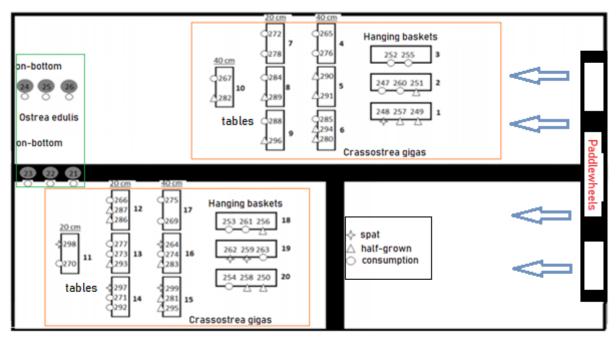


Figure 5 Schematic overview of the oyster trench in Waterdunen. Marked green are the on-bottom *Ostrea edulis* oysters and marked red are the off-bottom *Crassostrea gigas* oysters. The numbers indicate the labels (example: star 248 = label 248 spat) and the blue arrows indicate the flow direction (Verbeeke & Houcke, personal communication, 2020).

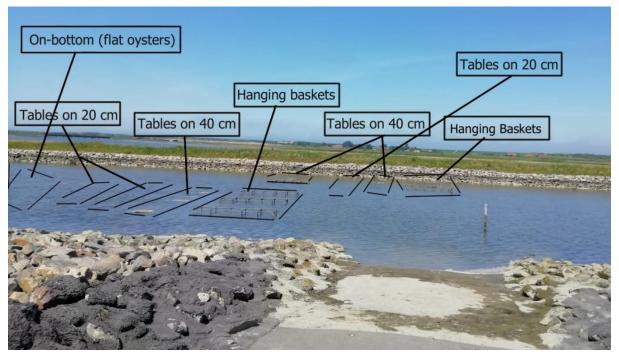


Figure 6 Photo of the oyster trench in the Waterdunen showing the layout of the structures, the paddlewheels are on the right side of this picture (L. Sanders, 2021).

The on-bottom technique involved randomly spreading the oysters on the substrate (figure 7), this technique was placed furthest of the paddlewheels on the left side of the oyster trench (figure 5 and 6). This technique corresponds to the situation of wild oysters where the oysters are in continuous contact with a substrate. During this research only Ostrea edulis were cultivated using this method.

The off-bottom techniques involved positioning the oysters above the substrate using two methods, namely the use of hanging baskets (Figure 8) and bags on tables (Figure 9). As can be seen in figure 5 and 6, these structures were placed in the middle and closest to the paddlewheels. The structure for the baskets was built so that the baskets hung about 20 cm from the substrate. The baskets were suspended in the water perpendicular to the watercourse. This positioning ensured that the baskets were consistently moved by the current. Tables came in two varieties, namely the tables at 20cm- and 40cm height measured from the substrate. The bags were attached to the tables by clamping the tips of the bags with hooks. The bags with spat contained a plastic construction inside the bag to prevent the spawn from being crushed and to allow enough space inside the bag to move.



substrate (Penncoveshellfish, n.d.).



Figure 7 Oysters directly on the Figure 8 Oyster baskets hanging from a construction (Auburn, 2012).



Figure 9 Oyster bags on racks (Boegger, n.d.).

# 2.2. Which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the Waterdunen?

To answer this sub-question measurements were taken over a period of six months. The baseline measurements (indicated at T0) took place in December 2020 and the last measurements took place in June 2021. The baseline measurement was done by measuring all the oysters in one age group together. Every system starts with the same baseline measurement.

Monitoring of the oyster spat took place every three weeks. This involved disconnecting the labels with spat from the systems and taking them to the laboratory. Each culture method was tested in triplicate, for each method 300 oysters were selected at random. The length per oyster was determined in centimeters and the total weight per bag or basket (indicated by a label) in grams. In addition, per label the mortality over the number of measured oysters was recorded. Additionally, the thickness and width of the spat was collected at the end of the research.

Monitoring of the half-grown and consumption size oysters took place every six weeks. A subsample was collected and taken to the laboratory. The total weight of each oyster was measured after which the oyster was cut open to measure the meat weight and shell weight. In addition, per bag or basket the mortality over the number of measured oysters was registered. During each monitoring event, all bags and baskets were checked for biofouling as well as fixture.

If there was too much biofouling this was then removed to ensure enough waterflow. If a fixture were, for example, broken this was then fixed to ensure no bags or baskets would be lost. Unusual details such as disconnected baskets were recorded.

The collected raw data was then processed in which outliers were extracted. The spat was corrected according to length and the half-grown and consumption size groups were corrected according to total weight- fish- and shell weight. For the half-grown and consumption size oysters, the condition index was determined by calculating the flesh weight relative to total weight. Lastly the growth rate compared to the start-weight and relative to the previous measurement were calculated.

The data was analyzed using IBM SPSS Statistics (version 25). First a test homogeneity of variance was carried out to determinate the possibility to use ANOVA. ANOVA was then used to test the growth in time by system and the differences between the systems. Line graphs and box plots were constructed to visualize the growth in time and the differences between the systems. When ANOVA was not possible the Games Howell test was carried out.

### 2.3. To what extent is it possible to cultivate *Ostrea edulis* with the onbottom method in the Waterdunen?

To answer this sub-question measurements were taken over a period of six months. The T0 took place in December 2020 and the last measurements took place in June 2021. Monitoring of the consumption size *Ostrea edulis* oysters took place every six weeks. A subsample was collected from each label and taken to the laboratory. The total weight of each oyster was measured after which the oyster was cut open to measure the meat weight and shell weight. In addition, per label the mortality over the number of measured oysters was registered.

The collected raw data was then processed in which outliers were extracted. The *Ostrea edulis* consumption size group was corrected according to total weight- fish- and shell weight. The condition index was then determined by calculating the flesh weight relative to total weight. Lastly the growth rate compared to the T0 and relative to the previous measurement were calculated.

The data was analyzed using IBM SPSS Statistics (version 25). First a test homogeneity of variance was carried out to determinate the possibility to use ANOVA. ANOVA was then used to test the growth in time. Line graphs and box plots were constructed to visualize the growth in time.

# 2.4. To what extent do the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*?

To answer this sub-question, data was collected by means of analysis- and measuring instruments that were placed in and around the oyster trench. Table 1 shows which instrument was used to collect data on which parameter.

In total 4 instruments were placed in and around the trench, figure 10 shows the location of these instruments. For the analysis, the oyster trench was divided into three monitoring areas. The inlet, the mid-area, and the creek end. Two instruments were placed at the inlet, one at the mid-area and one at the creek end. In each zone, water parameters were monitored at fixed times to assess the biotic and abiotic characteristics of the oyster channel.

The factors measured were temperature, oxygen, salinity, chlorophyll, turbidity. These factors were measured daily and once a month the meters were read out, cleansed and reinstalled (Verbeeke & Houcke, personal communication, 2020). Line graphs were constructed to visualize the collected data.

INSTRUMENT	USED FOR
HOBO conductivity logger 100 – 55000 uS/cm [U24002C]	Collecting data on the conductivity and temperature.
HOBO dissolved oxygen DO logger [U26001] with anti-	Collecting data on the dissolved oxygen and temperature.
fouling guard	
HOBO Waterproof shuttle [UDTW1]	Reading the collected data.
JFE Advantech Co., LTD - Infinity – CLW, model ACLW2-	Collecting data on chlorophyll and turbidity
USB-CE	

Table 1 Analysis and measuring instruments used for collecting data.

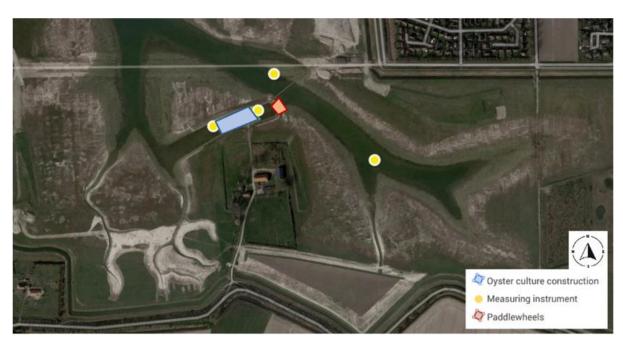


Figure 10 A map showing the locations of the measuring stations in the Waterdunen, The Netherlands (Google inc, n.d.).

A literature review was then conducted to determine the ideal water parameters of *Crassostrea gigas* and *Ostrea edulis*. The measured parameters and the data from the literature review were then compared to determine if the parameters met the requirements for growing *Crassostrea gigas* and *Ostrea edulis*. Table 2 gives an overview of the scientific databases and the keywords that were used.

SCIENTIFIC DATABASES	Google scholar			
	ResearchGate			
	Green-I			
	Springer			
	Science direct			
		SYNONYMS		
KEYWORDS	Temperature	Water temperature		
		Temperature tolerance		
		Thermal conditions		
	Oxygen	O <sup>2</sup> content		
		Oxygen concentrations		
		Dissolved oxygen		
	Salinity	Salt content		
		Salinities		
	Chlorophyll	Phytoplankton		
		Food supply		
		Algae		
	Turbidity			
	Crassostrea gigas	Pacific oyster,		
		Japanese oyster		
		Creuse oyster		
	Ostrea edulis	Flat oyster		
		European oyster		
		European flat oyster		
		Native oyster		
	Cultivation requirements	Breeding requirements		
		Grow conditions		
		Optimal farming conditions		
		Growth performance		
	Water parameters	Water conditions		

Table 2 Scientific databases, dates and keywords used for the literature review.

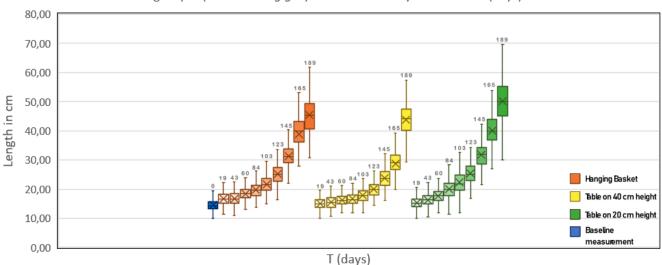
### 3. Results

In this chapter the results of research set-up are visualized and described. First the results from the off-bottom techniques for the *Crassostrea gigas* will be given, after which the results from the cultivation of *Ostrea edulis* using on-bottom techniques are described. Lastly the measured water parameters and the parameters which will be used to compare the collected data with are presented.

# 3.1. Which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the Waterdunen?

In this section the results of the research setup of the off-bottom cultivation of *Crassostrea gigas* are visualized and described. The results for each age group (spat, half-grown and consumption size) and for the different systems, namely the hanging baskets, oysters cultivated on tables at 20 cm height (T20) and oysters cultivated on tables at 40 cm height (T40) will be visualized using boxplots. The blue boxplot in each figure represents the baseline measurement done at the beginning of the experiment. The red boxplots represent the hanging baskets from the first measurement after the T0. The yellow boxplots represent the tables on 40 cm height from the first measurement after the T0. After which the results from the ANOVA tests or Games Howell will be described.

### 3.1.1. Spat *Crassostrea gigas*



Length spat (Crassostrea gigas) in the different systems after T (days).

Figure 11 Boxplots indicating the measured length of spat *Crassostrea gigas* in the different systems over a period of T days (every 3 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 11 shows the results of the *Crassostrea gigas* spat monitoring by means of boxplots. What is noticable is that T40 shows a different growth pattern conpared to the other systems. The difference between each measurent is smaller compared to the other systems. The difference in time for the T40 is only significant for the last two measurements (Appendix I table 5). The hanging baskets and T20 show a comparable growth pattern to each other. Both show a significant difference in time starting from the first measurement, with the exception of no difference between day 19 and day 43 for the hanging baskets (Appendix I table 5).



Figure 12 *Crassostrea gigas* Spat label 298 in bags at 20 cm depth in the oyster trench, Waterdunen. 29-1-2021, 30-3-2021 and 31-5-2021 (E. Merks, 2021; L. Sanders, 2021).

The difference between the systems is therefore also quite noticable. There is a significant difference from day 43 onwards between system T20 and T40 and from day 19 onwards between the hanging baskets and T40 (Appendix I table 4). The hanging baskets and the T20 are quite similar with only a significant difference on day 19, 60, 165 and 189 (Appendix I table 4).

Mortality rate spat (Crassostrea gigas) in the different systems on

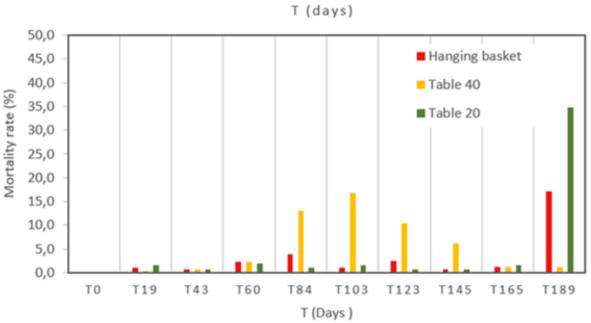


Figure 13 Bar graph indicating the mortality rate of spat (*Crassostrea gigas*) on each measurement day for each system in the oyster trench, Waterdunen.

Figure 13 and Appendix II table 24 show the measured mortality on each day. The mortality rate was quite similar for all systems until day 60. After day 84 the T40 started to show an increasing mortality rate which declined again on day 123. It is worth noting that during the whole period the mortality of the hanging baskets and the T20 were below 5% but during the last measurement, the mortality of the hanging baskets and the T20 were extremely high (17,1% for the hanging baskets and 34,8% for the T20).

Thickness in cm

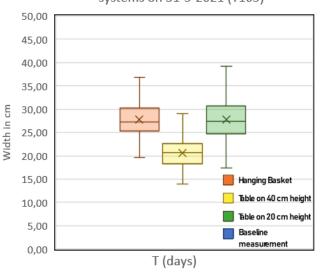




Figure 14 Boxplots indicating the measured width of spat *Crassostrea gigas* in the different systems over a period of T days (every 3 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Thickness spat (Crassostrea gigas) in the different systems on 31-5-2021 (T165)

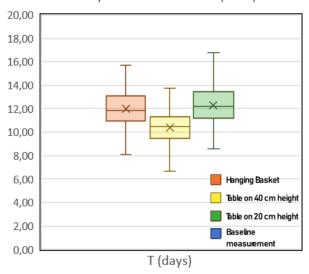


Figure 15 Boxplots indicating the measured thickness of spat *Crassostrea gigas* in the different systems over a period of T days (every 3 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 14 and figure 15 show the results of the monitoring done on day 165 (31<sup>st</sup> of May 2021) for *Crassostrea gigas* spat by means of boxplots. This monitoring respectively looked at the width and thickness of the spat measured on that day. What is noticeable is that for both the width and thickness of the spat from T40 showed lower numbers than of those of the other systems. For both parameters measured the spat from T20 showed the highest averages but the biggest spread between the minimum- and maximum thickness and width measured.

The differences in length, width and thickness can also be seen in figures 16 till 18. The oysters from the hanging baskets have varying shapes but are generally elongated and convex. The T40 oysters are fairly uniform in shape but are generally flatter than the other systems. The T20 oysters show a wide variety of shapes. Both very elongated as well as rounded and convex oysters were found. The differences between the systems are significant. All the significances are 0,000 except for the difference in width between T20 and hanging baskets with a 0,023 significance. The only exception is seen in the difference in thickness between the hanging baskets and the T20 with a significance of 0,986.

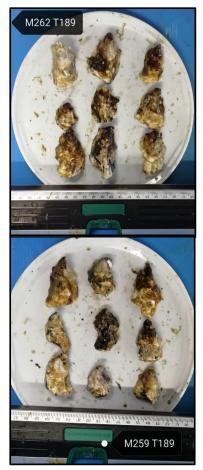


Figure 16 *Crassostrea gigas* spat label 262 (upper) and 259 (lower) from the hanging baskets on day 189 in the oyster trench, Waterdunen (L. Sanders, 2021).

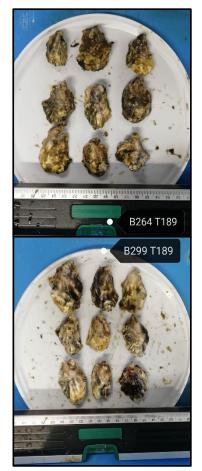


Figure 17 *Crassostrea gigas* spat label 264 (upper) and 299 (lower) from the T40 on day 189 in the oyster trench, Waterdunen (L. Sanders, 2021).

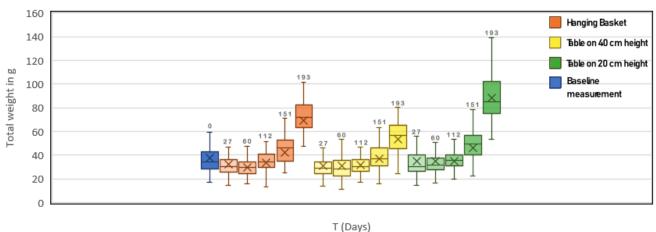


Figure 18 *Crassostrea gigas* spat label 297 (upper) and 2298 (lower) from the T20 on day 189 in the oyster trench, Waterdunen (L. Sanders, 2021).

#### 3.1.2. Half-grown size *Crassostrea gigas*

The half-grown oysters did not show a significant difference for any measured parameter. Thus, the ANOVA test could not be performed for this age group. The Games Howell test was chosen to be performed for the parameters that could not be tested with ANOVA.

Figure 19 shows the results of the *Crassostrea gigas* half grown monitoring of the total weight by means of boxplots. What is noticeable is that all systems show a decrease in total weight after the baseline measurement followed by an upwards growth (appendix II table 29). The differences in time are consistently significant from day 60 onwards for the hanging baskets and the T20. T40 only shows a significant difference after day 112 (appendix I table 7).

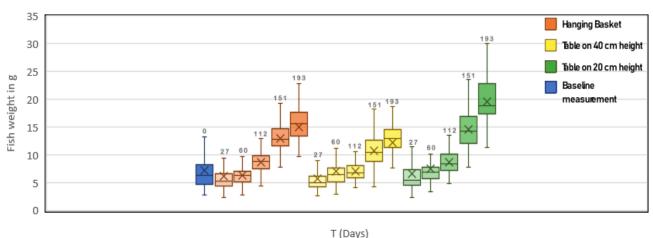


Total weight half-grown (Crassostrea gigas) in different systems after T (days)

Figure 19 Boxplots indicating the measured total weight of half-grown *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

There was no significant difference between any of the systems for the first three measurements. From day 151 onwards there was a difference between the T40 and the other systems (appendix I table 6). The shapes of the bloxplots however is slightly different between the systems. The spread between the minimum and maximum measured total weights shows the largest in oysters from T20 and smallest in oysters from the hanging baskets.

Figure 20 shows the results of the *Crassostrea gigas* half grown monitoring of the fish weight by means of boxplots. It is noticeable that all systems show a decrease in fish weight after the baseline measurement followed by an upwards growth from day 60 onwards (appendix II table 30). The hanging baskets show a significant difference between the measurement days starting from day 60. T40 shows an insignificant difference between day 0 and day 27 and between day 60 and day 112. After day 112 the differences are significant consistantly. T20 shows a consistant significant difference between day 27 and day 60 (appendix I table 9).



Fish weight half-grown (Crassostrea gigas) in different systems after T (days)

Figure 20 Boxplots indicating the measured fish weight of half-grown *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

For the first two measurements (day 27 and day 60) the differences between the systems are not significant. From day 113 onwards a significant difference can be seen between T40 and the other systems. The hanging baskets and the T20 only show a significant difference at the last measurement on day 193 (appendix I table 8). There are some noticable differences between the systems when looking at the growth speed and the difference between the minimum and maximum measured fish weights. The oysters from T20 show the largest growth speed between the monitoring days and shows the biggest difference between the minimum and maximum measured fish weights. The oysters from T40 shows the slowest growth speed between the monitoring days (appendix II table 26).

Figure 21 shows the results of the *Crassostrea gigas* half grown monitoring of the shell weight by means of boxplots. It is noticeable that all systems show a decrease in shell weight after the baseline measurement. The hanging baskets showed a decrease of 2,56 grams, T40 of 3,49 grams and T20 of 2,78 grams (appendix II table 31). This decrease is followed by an upwards growth but this decrease is not significant for the T20 and T40 (appendix I table 11). T20 and T40 show no significant difference in time till day 112. The hanging baskets show no significant difference from day 27 till day 112 (appendix I table 11).

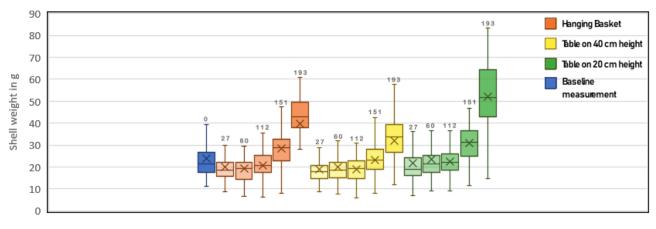






Figure 21 Boxplots indicating the measured shell weight of half-grown *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

There is no significant difference between the systems till after day 60. The T40 and T20 after the first to show differences between each other on day 112 after which on day 151 a difference is clear between the hanging baskets and T40. The hanging baskets and the T20 are quite similar in growth and only show a significant different on the last day (appendix I table 10). The spread between the minimum and maximum measured shell weights shows the largest in oysters from T20 and smallest in oysters from the hanging baskets.

Appendix I table 12 and 13 show the Games Howell output and appendix II table 28 shows the averages of the condition index for each system for the half-grown size oysters. The condition index of the hanging baskets increased significantly after day 27. The condition index of T40 first dropped by 0.5% (from 17.7 to 17.3) and then rose to 22.2%. Between day 60 and day 112, there was no significant growth for T40. T20 showed a constant significant growth throughout the whole monitoring period (appendix I table 13). At the last measurement there was a decrease in the condition index for each system (appendix II table 28).

Figure 22 and Appendix II table 27 show the measured mortality on each day for the half-grown size oysters. The hanging baskets showed quite a consistent mortality rate of around 9% with an high outlier on day 112 of 14,1% and a lower mortality on day 151 with 6,7%. T20 showed an increasing mortality rate till day 112 after which it dropped from 11,3% to 9,9% and then climbed to 14,4% again on the last day. The mortality was the highest with the oysters from T40 with the highest being 14,6% on day 60 and the lowest on day 151 with 12,8%.

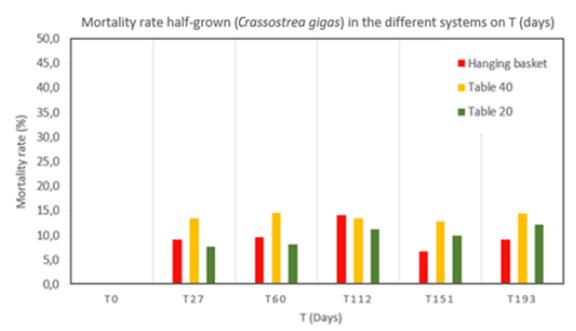
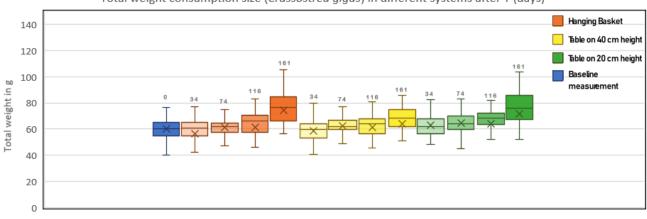


Figure 22 Bar graph indicating the mortality rate of half-grown (*Crassostrea gigas*) on each measurement day for each system in the oyster trench, Waterdunen.

#### 3.1.3. Consumption size *Crassostrea gigas*

The consumption size oysters only showed a significant difference for the parameter "fish weight", the ANOVA was used for this parameter. For the parameters "total weight", "shell weight", and "condition index" the ANOVA test could not be performed and therefore the Games Howell test was used.

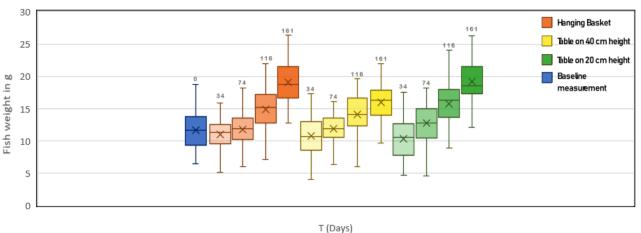


Total weight consumption size (Crassostrea gigas) in different systems after T (days)

T (Days)

Figure 23 Boxplots indicating the measured total weight of consumption size *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

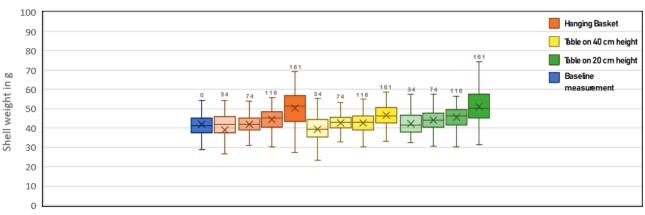
Figure 23 shows the results of the *Crassostrea gigas* consumption size monitoring of the total weight by means of boxplots. What is noticable is that all systems show no significant difference in time till the last measurement (appendix I table 15). This is also reflected in the growth rate, the growth rate stays underneath 0,10 for each system with only around 2 grams average of growth between day 34 and 74 (appendix II table 32 and 35). This is clearly visible in figure 23 where all the systems show a huge difference between day 116 and day 161 There is no significant difference between the systems till day 116. The monitoring on this day showed a significant difference between T20 and T40. As can be seen in figure 23, the T20 shows a clear average higher total weight starting from this monitoring day. The hanging baskets and T40 show a significant difference on the last monitoring day. The hanging baskets and T20 shows a very comparable growth pattern, on day 161 it has a significance of 1,000 (appendix I table 14).



Fish weight consumption size (Crassostrea gigas) in different systems after T (days)

Figure 24 Boxplots indicating the measured fish weight of consumption size *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 24 shows the results of the *Crassostrea gigas* consumption size monitoring of the fish weight by means of boxplots. This parameter is the only one that could be tested with ANOVA. As can be seen in figure 24 and in table 17, appendix I is that the difference in time is significant starting at day 34 for the hanging baskets and T20 and on day 74 for the T40. All systems do show a decline in the average measured fish weight after the T0 (appendix II table 36). The difference between the systems is not significant till day 74 for T40 and T20 and till day 116 for the hanging baskets and T40. The hanging baskets and the T20 showed no significant difference during the monitoring period (appendix I table 16, appendix II table 36).



Shell weight consumption size (Crassostrea gigas) in different systems after T (days)

T (Days)

Figure 25 Boxplots indicating the measured shell weight of consumption size *Crassostrea gigas* in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 25 shows the results of the *Crassostrea gigas* consumption size monitoring of the shell weight by means of boxplots. What immediately catches the eye is that there is little growth in shell weight in all systems. All systems show no significant difference in time till the last measurement on day 161 (appendix I table 19) and T40 even shows some decline in shell weight on several occasions (appendix II table 37). There is also little difference between the systems (appendix I table 18). There is no significant difference between the systems except for between T40 and T20 on the last measurement day (day 161). Appendix I table 20 and 21 show the Games Howell outputs and appendix II table 34 indicates the averages of the condition index for each system for the consumption size oysters. What is noticeable is that all systems decreased in condition index after the T0 and increased again after the first monitoring on day 34. The hanging baskets showed a significant increase in condition index in the last 2 measuring days. The T20 showed a significant increase on measuring day 74 and 116 and T40 showed a significant increase on day 116. There was no significant difference between the systems.

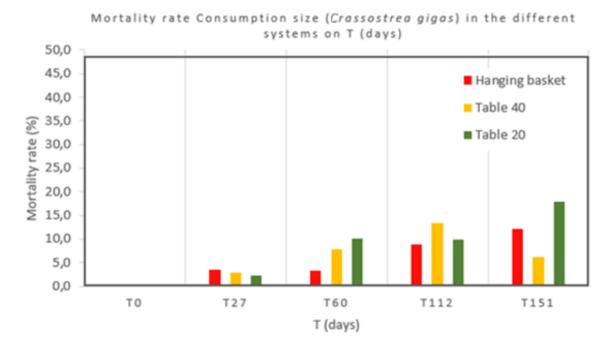
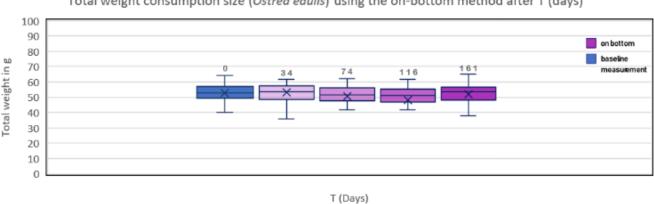


Figure 26 Bar graph indicating the mortality rate of consumption size (*Crassostrea gigas*) on each measurement day for each system in the oyster trench, Waterdunen.

Figure 26 and Appendix II table 33 show the measured mortality on each day for the consumption size oysters. The mortality rate of the consumption size oysters is quite irregular. The hanging baskets first showed a small decrease of 0,3% (from 3,6% on day 35 to 3,3% on day 74) after which it increased with 5,6% to 8,9% on day 116. During the last measurement the mortality was 12,2%. The T40 oysters showed an increase in mortality till day 116 (starting with 2,9% on day 34 and ending with 13,3% on day 116) after which it showed a decrease from 13,3% to 6,3%. Table 20 started with a mortality of 2,2% on day 34. On day 74 it showed an increase of 7,8% to 10% which stayed about the same on day 116 with a mortality of 9,9%. On day 161 there was again an increase of 8,1% to a 18% mortality rate.

#### To what extent is it possible to cultivate Ostrea edulis with the on-3.2. bottom method in the Waterdunen?

In this section the results of the on-bottom cultivation setup of Ostrea edulis are visualized and described. The results for each measured parameter will be visualized using boxplots. The blue boxplot in each figure represents the baseline measurement done at the beginning of the experiment. The purple boxplots represent the on-bottom results from the first measurement after the TO. After which the results from the ANOVA tests or Games Howell will be described.



Total weight consumption size (Ostrea edulis) using the on-bottom method after T (days)

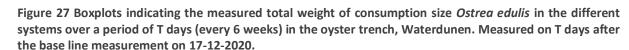
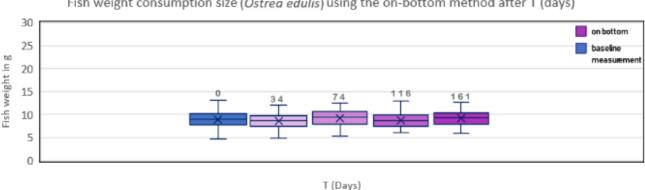


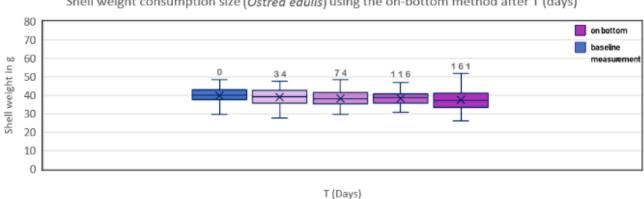
Figure 27 shows the results of the Ostrea edulis consumption size monitoring of the total weight by means of boxplots. What is noticable is that there is little to no change in the average total weights measured over the monitoring period. There is even some decline noticable on day 74 and 116 (appendix II table 41). The changes between the monitoring days were not significant (appendix I table 22 and 23).



Fish weight consumption size (Ostrea edulis) using the on-bottom method after T (days)

Figure 28 Boxplots indicating the measured fish weight of consumption size Ostrea edulis in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 28 shows the results of the Ostrea edulis consumption size monitoring of the fish weight by means of boxplots. What is noticable is that there is little to no change in the average total weights measured over the monitoring period. There is even some decline noticable on day 34 and 116 (appendix II table 42). The changes between the monitoring days were not significant (appendix I table 22).



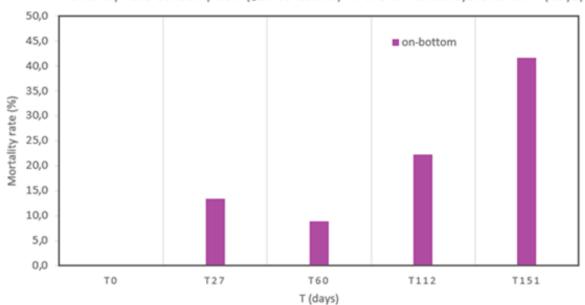
Shell weight consumption size (Ostrea edulis) using the on-bottom method after T (days)

Figure 29 Boxplots indicating the measured shell weight of consumption size Ostrea edulis in the different systems over a period of T days (every 6 weeks) in the oyster trench, Waterdunen. Measured on T days after the base line measurement on 17-12-2020.

Figure 29 shows the results of the Ostrea edulis consumption size monitoring of the shell weight by means of boxplots. What is noticable is that there is little to no change in the average total weights measured over the monitoring period. There is even some decline noticable starting on day 34 which continues to decline till the last measurement done on day 161 (appendix II table 43). The changes between the monitoring days were not significant (appendix I table 22).

Appendix I table 22 shows the ANOVA output and appendix II table 40 shows the averages of the condition index for each system for the consumption size *Ostrea edulis*. After the T0 there is a decline in the condition index (from 21,3% to 16,3%) after which it increases again. On day 116 there is once again a decline after which it rises again on the last day. Only between day 34 and 74 was there a significant difference in condition index. The overall growth rate of the *Ostrea edulis* showed to be negative on day 74 and day 116 and stayed below 0.05 for the remaining days.

Figure 30 and appendix II table 39 show the measured mortality on each day for the consumption size *Ostrea edulis*. After the T0 there is a mortality of 13,3% which declines to 8,9% on day 74. On day 116 the mortality has increased with 13,3% to 22,2% and at the last measurement this rate has almost doubled to a 41,6% mortality rate.

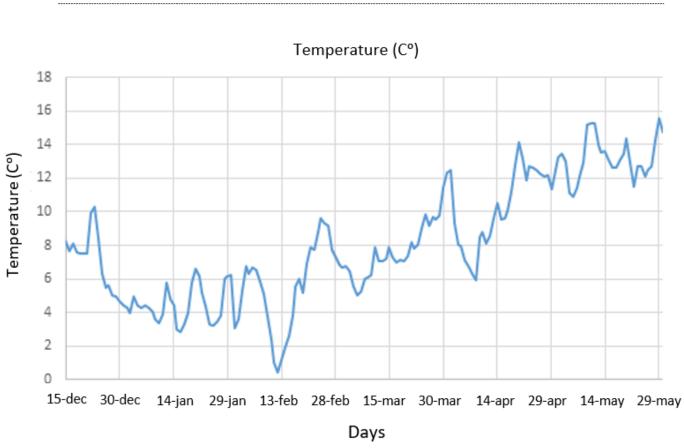


Mortality rate consumption (Ostrea edulis) in the different systems on T (days)

Figure 30 Bar graph indicating the mortality rate of consumption size (*Ostrea edulis*) on each measurement day in the oyster trench, Waterdunen.

# 3.3. To what extent do the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*?

In this section the results of the acquired water parameter data will be visualized and described using line graphs and the results of the literature study will be given in a table. The water parameters were measured on four different locations in the oyster trench. Averages were then taken because no big differences were found between the locations.



#### 3.3.1. Collected field data

Figure 31 Line graph indicating the temperature (in Celsius C°) measured from December 2020 till June 2021 in the oyster trench of the Waterdunen, The Netherlands.

Figure 31 shows the measured water temperatures from December 2020 till June 2021 for the oyster trench in the Waterdunen. The parameter temperature shows a lot of fluctuations over the course of the research. The temperature generally begins to rise around march but still shows high peaks and drops. The biggest drop is seen on 13 February where the temperature almost reaches 0 C°. In the winter months the temperature stays around 4-5 C° and nearing the summer it rises to between 12 and 16 C°.

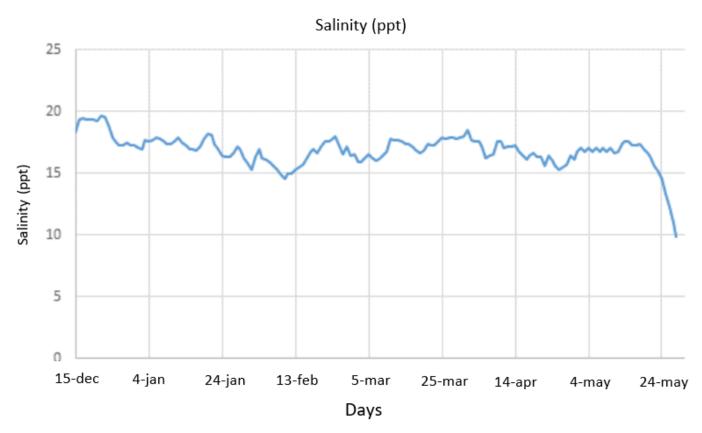


Figure 32 Line graph indicating the salinity (in ppt) measured from December 2020 till June 2021 in the oyster trench of the Waterdunen, The Netherlands.

Figure 32 shows the measured salinity from December 2020 till June 2021 for the oyster trench in the Waterdunen. The salinity shows a fluctuation between 15 and 20 ppt. Around 20 May there appears to be a major drop in salinity and around 4 June it has reached 10 ppt.

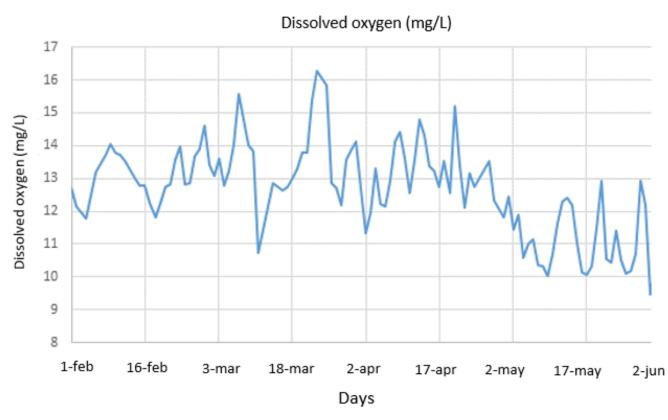
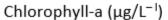
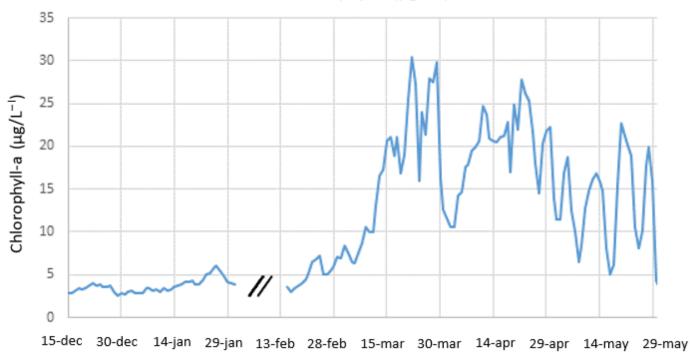


Figure 33 Line graph indicating the Dissolved oxygen concentration (in mg/L) measured from December 2020 till June 2021 in the oyster trench of the Waterdunen, The Netherlands.

Figure 33 shows the measured Dissolved oxygen from December 2020 till June 2021 for the oyster trench in the Waterdunen. The Dissolved oxygen fluctuates heavily over the course of the research. In the first few months it stays between 12 and 16 mg/L with a drop to 10,7 on 11 march. From May onwards the concentrations drop to fluctuate between 10 and 13 mg/L.





Days

Figure 34 Line graph indicating the Chlorophyll-a concentrations (in  $\mu g.L^{-1}$ ) measured from December 2020 till June 2021 in the oyster trench of the Waterdunen, The Netherlands.

Figure 34 shows the measured Chlorophyll-a from December 2020 till June 2021 for the oyster trench in the Waterdunen. From 15 December till 18 February the chlorophyll-a stays between 2.5 and 5.5  $\mu$ g.L<sup>-1</sup> after which it starts to increase. From then on large fluctuations are seen in the chlorophyll-a concentrations. Between 20 March and 20 April, the concentrations heavily fluctuate between 15 and 30  $\mu$ g.L<sup>-1</sup> with a big drop around the start of April. After 20 April the average concentration seems to drop to fluctuate between 5 and 25  $\mu$ g.L<sup>-1</sup>. From 27 January to 31 January no data is available.

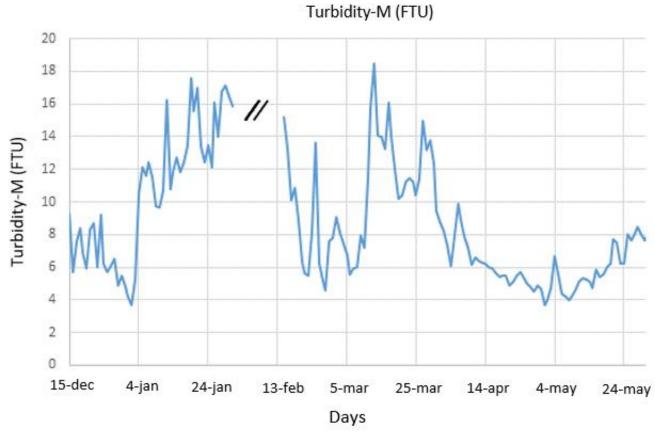


Figure 35 Line graph indicating the turbidity (in FTU) measured from December 2020 till June 2021 in the oyster trench of the Waterdunen, The Netherlands.

Figure 35 shows the measured turbidity from December 2020 till June 2021 for the oyster trench in the Waterdunen. The turbidity shows a lot of fluctuation in the first few months. It starts low between 4 and 10 FTU (or between 1,33 and 3,33 mg/L) till 2 January after which it increases quickly. It continues to fluctuate between 5 and 19 FTU (or between 1,66 and 6,33 mg/L) till around 12 April. From then on the fluctuations lessen and the turbidity stays lower between 3 and 9 FTU (or between 1 and 3 mg/L). From 27 January to 31 January no data is available.

### 3.3.2. Literature study: optimal parameters for *Crassostrea gigas* and *Ostrea edulis*.

Table 3 Results of a literature research on the optimal conditions for *Crassostrea gigas* and *Ostrea edulis* for the parameters temperature, Dissolved oxygen, Salinity, Chlorophyll-a, and Turbidity.

SPECIES		PARAMETER	SOURCE
CRASSOST	TREA GIGAS		
	Temperature	Range : 8.0 – 34.0 °C Optimal : 15.0 – 18.0 °C	Brown & Hartwick (1988)
		Range : 3.0 – 35.0 °C Optimal : 18.0 – 20.0 °C	Goulletquer (2021)
	Dissolved oyxgen	Not limiting at saturation levels above 70%.	Brown & Hartwick (1988)
		Above 4 mg/L dissolved oxygen. (+/- between 39% - 42% at optimal temperature) (below 4 mg/L = harmful)	Goulletquer (2021)
	Salinity	Range : 10.0-35.0+ ppt Optimal : 24.0+ ppt	Brown & Hartwick (1988)
		Range : 14.0-37.0 ‰ or ppt Optimal : 20.0 – 25.0 ‰ or ppt	Park <i>et al.</i> (1989).
	Chlorophyll-a	Range : 1.0-55.0 $\mu$ g.l <sup>-1</sup> chlorophyll $\alpha$ or mgl- <sup>1</sup> POM Optimal : 12.0+ $\mu$ g.l <sup>-1</sup> chlorophyll $\alpha$ or mgl- <sup>1</sup> POM	Brown & Hartwick (1988)
	Turbidity	Range : 0.0 – 100.0 mg.l <sup>-1</sup> PIM Optimal : 0.0 – 8.0 mg.l <sup>-1</sup> PIM	Brown & Hartwick (1988)
		Harmful above 25,3 mg.l <sup>-I</sup>	Goulletquer (2021)
OSTREA E			
	Temperature	Range : 2.0 - 30°C Optimal (for spawning) : 20.0 - 25.0°C	Barnabé (2018)
		Range : 1.5 - 30°C Optimal : 17.0 - 25.0°C	Hutchinson & Hawkins (1992)
	Dissolved oyxgen	Tolerant to periods of hypoxic conditions of < 2 mg O <sup>2</sup> /I (7-10 days at 18°C) but long periods cause mass mortality.	Lenihan (1999
	Salinity	Range : 15- 38‰ or ppt Optimal : ?	Barnabé (2018)
		Range : 17 - 40‰ or ppt Species is euryhaline so is able to withstand a wide range. Optimal : >23 ‰ or ppt	Hutchinson & Hawkins (1992)
	Chlorophyll-a	Range : ? $\mu$ g.l <sup>-1</sup> chlorophyll $\alpha$ or mgl- <sup>1</sup> Optimal : ? $\mu$ g.l <sup>-1</sup> chlorophyll $\alpha$ or mgl- <sup>1</sup>	
	Turbidity	Species is intolerant to suspended material. At 10mg/l filtration was completely inhibited and at 5mg/l considerably reduced.	Hutchinson & Hawkins (1992)

## 4. Discussion

The main objective of this thesis was to determine the effectiveness of off-bottom methods for the cultivation of *Crassostrea gigas* and the on-bottom method for the cultivation of *Ostrea edulis* in the oyster channel located in the Waterdunen. The collected data was used to answer the main research question, namely, "How suitable is the oyster trench in the Waterdunen for the cultivation of *Crassostrea gigas* using off-bottom techniques and the cultivation of *Ostrea edulis* using the on-bottom technique?". Several sub-questions were formalized in order to provide a well-rounded answer.

The first of which was to determine which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the Waterdunen. When comparing the systems across all age groups and measured parameters, it is immediately apparent that the T40 system shows the lowest overall results. The difference was especially evident with the spat monitoring. The oysters were significantly smaller than those of the other systems and the overall mortality rate was higher.

One factor that could explain the difference in results is the variations in immersion time. Both the hanging baskets and the T20 had a similar immersion time because of a similar depth. The T40 were placed 20cm higher and were thus exposed to the air for a longer period of time. Capelle *et al.* (2020) stated that immersion time had no effect on the difference in condition index because of the spawning activities in the summer. The food supply is highest in summer and therefore spawning occurs. However, Capelle's study used diploid oysters and this study used triploid oysters. Triploid oysters do not spend energy on spawning and can use the high food supply to invest in growth. It can therefore be assumed that the immersion time in this case does have an impact on the difference in condition index. The T20 and the hanging baskets are submerged for a longer period of time and thus have access to the food supply longer than the T40 which has a shorter submerged time. This was also hypothesized. The higher mortality could also be explained by the fluctuating temperatures during the monitoring period. Because the oysters of T40 had a low immersion time they would have been affected by the weather more so than the oysters from the other systems (Malham *et al.*, 2009).

An almost doubling of the mortality rate was registered at the last spat monitoring day. These numbers can be explained by the fact that the density in the baskets and bags had become too high. The bags and baskets were filled to the brim and some oysters had stunted growth because of the lack of room. The optimal density of the oysters want not taken into account for this research. T40 had the lowest density because of the smaller size of the oysters and shows a lower mortality rate on the last day. The high density could have had a negative impact on the oysters (Capelle *et al.*, 2020; Mizuta & Wikfors, 2019). For example, a limited flow results in less food availability for the oysters in the inner zone. The limitations for the inner zones would result in more deaths and without the aeration these dead oysters would result in faster microbial contamination (Mizuta & Wikfors, 2019; Supan, 2014).

The hanging baskets and the T20 showed overall similar results. The biggest differences were found in the width and thickness of the spat oysters measured. When comparing the hanging baskets and T20 spat based on length, width, and thickness, it is evident that the hanging baskets are more consistent in overall shape and size. This is evident in Figures 14 and 15 when the minimum- and maximum measured thickness and width of the hanging baskets are compared with those of the T20. The T20 shows a larger difference, indicating that oysters with a more diverse shape were found. The differences between the two systems are also significant, except for the thickness with a significance of 0.986. Although it is not significant, it is still a difference.

This can be attributed to the functioning of the systems and environmental conditions such as water currents. In this case there is a difference in how much the systems allow the oysters to move under the influence of the water current. The bags on tables (T20 and T40) are tied to the tables and do not move with the current. Instead, the water flows through the bags. The hanging baskets are positioned in such a way that the baskets move with the current. The oysters therefore move both through the water flowing through them and through the movement of the baskets. The oysters here are exposed to more disturbance and this causes the shell to be broken frequently. As was hypothesized, these oysters have relatively deeper than longer shells, a shape that is sought after by oyster farmers (Mizuta & Wikfors, 2019). Another difference between the systems was that of the labor intensity. It was found that during moderate or high tide it was quite challenging to collect the oysters from the tables on 20 cm depth. They were hard to spot and detach which made it a time consuming activity. The hanging baskets were much easier to detach during higher tides because of the way they are attached (as can be seen in figure 8 and 9).

The second sub-question was to determine the possibility to cultivate *Ostrea edulis* with the onbottom method in the Waterdunen. What was immediately apparent was that the *Ostrea edulis* showed little to no growth during the monitoring period. The growth rate as can be seen in table 38 was negative on day 74 and day 116 and climbing high mortality rates were registered over the course of the monitoring. A decline, in weight was registered in all parameters, with the biggest decline being in fish weight. This was however not a significant decline. A loss in fish weight can be explained by a change environmental conditions such as temperature, food availability and turbidity (Çelik *et al.*, 2015; Yildiz *et al.*, 2011; Newell *et al.*, 1977). If this was the case during this research is uncertain, it could be that the oysters were affected by the transportation from the oyster farm to the coast laboratory. The *Ostrea edulis* oysters were being grown using the on-bottom technique. The oysters were cultivated on sand, however over time silt build-up occurred. Grant *et al.* (1990) found that even small buildups of silt reduce the growth rate of *Ostrea edulis*. Since oysters are sessile and are unable to burrow up, respiration, feeding and the removal of waste become restricted if there is enough build-up of silt. The oysters then risk being suffocated. This can explain the high mortalities seen during later monitoring days

The third sub-question was to determine to what extend the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*. *Crassostrea gigas* and *Ostrea edulis* have a wide temperature range that they can withstand. The measured temperature is lower than that of the optimal temperature for both species, however it is still within the acceptable range. Longer periods of temperatures below 10C° might however negatively impact both *Crassostrea gigas* and *Ostrea edulis* (Goulletquer, 2021; Hutchinson & Hawkins, 1992). The measured salinity levels are on the lower side but still within the range of both species. The *Ostrea edulis* is an euryhaline species so lower levels of salinity for a longer period will not cause high mortalities as long as they are gradual. The *Crassostrea gigas* can endure a wide range of salinity levels, however the drop at the end of the measuring period might have a negative effect on this species if this continues for a longer period of time. Brown and Hartwick (1988) and Hutchinson & Hawkins (1992) found that temperature and salinity are co-dependent so that very high or low temperatures and low salinity can have a negative effect on the growth and even cause mortality in *Crassostrea gigas* and well as in *Ostrea edulis* depending on how long these conditions last. Both parameters were detected to be on the low side, so fluctuations could cause problems for both species. High temperature spikes in the summer and low temperatures in the winter can be a cause of concern.

The measured levels of dissolved oxygen fall well above the range for both species. No problems are to be expected for both species concerning this parameter. The chlorophyll-a levels are well within the range for the *Crassostrea gigas*. During the first few months the concentrations were not optimal (below 12  $\mu$ g.L<sup>-1</sup>) but still within range. The sudden spike in chlorophyll-a concentrations and the temperature rise could be the cause for the increased growth rates of all age groups. No information about the optimal chlorophyll-a concentrations could be found for the *Ostrea edulis*. The measured turbidity is within the range for the *Crassostrea gigas* however it is on the high side at the start of the research. *Ostrea edulis* is a species which is very intolerant to suspended materials and even low concentrations measured above 5mg/L could have had an impact on the mortality rate and growing rate of the *Ostrea edulis* because a lower amount of food can be filtrated when the turbidity rate gets too high. The data collected on the water parameters give a clear few of the current situation in the oyster trench and can also be used for other purposes, such as looking at the possibility of culturing other types of shellfish.

A factor that has potentially influenced the results of both the *Crassostrea gigas* and *Ostrea edulis* oysters is biofouling. As can be seen in appendix II picture 36 there was biofouling caused by barnacles. The consumption size and half-grown oysters were the most affected by the biofouling, the spat started showing biofouling during the last two measurements and the *Ostrea edulis* showed some biofouling but less than the *Crassostrea gigas*. Biofouling has varying negative effects on the oysters such as: food competition (which can lead to undersized shells and slender meat), water flow reduction and misshapen shells (Mizuta & Wikfors, 2019; Gosling, 2008). No data has been collected from this factor during the research and the effect this could potentially have on the results can therefore not be established.

It is possible that the results obtained are not entirely representative because of the way in which the baseline measurement was taken. For this study, a baseline measurement was taken of the entire age group, after which these oysters were divided among the different systems. The subsequent measurements are therefore only of a part of the baseline measurement per system and this may have given a distorted picture. Lastly, for this research no control groups were being used for either oyster species. However, a study by Li (2008) showed that several farm handling practices could have a significant effect on the condition of *Crassostrea gigas*. One can assume that this is also the case for *Ostrea edulis*. Thus, it is possible that the research practices have had an effect on the growth of the oysters but this cannot be ascertained from the data obtained.

The results of this research can not only be used to choose the best method suitable for the oyster trench in Waterdunen but can also contribute to follow-up studies. In addition, the cultivation of oysters with off-bottom methods is not only being tested in Waterdunen but also in other places in the Netherlands, the results of this study could also be meaningful for these studies. The results of the cultivation of *Ostrea edulis* in the oyster trench of Waterdunen gives a clear few on if it is possible to cultivate this species in Waterdunen and what could potentially be the cause of the unfavorable results. The data collected on the water parameters give a clear few of the current situation in the oyster trench and can also be used for other purposes, such as looking at the possibility of culturing other types of shellfish.

## 5. Conclusion

In order to assess whether it is possible to cultivate *Crassostrea gigas* and *Ostrea edulis* in the oyster trench in Waterdunen, a research was carried out. *Crassostrea gigas* was tested using three off-bottom methods and *Ostrea edulis* was tested with the on-bottom method. The main research question was "How suitable is the oyster trench in the Waterdunen for the cultivation of *Crassostrea gigas* using off-bottom techniques and the cultivation of *Ostrea edulis* using the on-bottom technique?".

To be able to provide a well-rounded answer several sub-questions were formulated:

- Which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the Waterdunen?
- To what extent is it possible to cultivate *Ostrea edulis* with the on-bottom method in the Waterdunen?
- To what extent do the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*?

The first sub-question was to determine which off-bottom technique is best suited for the cultivation of *Crassostrea gigas* in the oyster trench in Waterdunen. What was apparent from the results was that system T40 showed the least favorable results. The overall weight and condition index were the lowest and the mortality was, especially at the end, high. The hanging baskets and T20 showed similar results in multiple parameters. The biggest differences were noticeable in the shape of the oysters. When comparing the two systems it was apparent that the hanging baskets produced the oysters with the most desirable shape, namely round and thick. This together with the satisfactory results of the growth rate makes this system the best option for the oyster trench in Waterdunen.

The second sub-question was to determine the possibility to cultivate *Ostrea edulis* with the onbottom method in the Waterdunen. What was apparent from the results was that *Ostrea edulis* oysters showed little to no growth during the monitoring period. All parameters showed a decrease in weight after the first monitoring, followed by a slight increase. The growth rate during the monitoring period stayed close to 0. The condition index declined during the monitoring period, from an average of 21.3% on the T0 to an average of 17.5% on day 161. The mortality of *Ostrea edulis* was also quite high. During the last measurement, a mortality of 41.6% was measured. With these results it can be said that the cultivation of *Ostrea edulis* is not a success in the oyster channel of Waterdunen. The third sub question was to determine to what extent the water parameters of Waterdunen meet the cultivation requirements of *Crassostrea gigas* and *Ostrea edulis*. The collected data of the water parameters of Waterdunen were compared with the optimal parameters collected from a literature study. What was apparent was that the water parameters detected in the Waterdunen fluctuate a lot. All the parameters are within range for the cultivation of *Crassostrea gigas* however the parameters temperature and salinity are on the lower side. Large fluctuations could be harmful and reduce the growth. Information on the range and optimal chlorophyll-a concentrations for *Ostrea edulis* could not be found during the literature study. No conclusion could therefore be made on if the cultivation requirements for *Ostrea edulis* are met. *Ostrea edulis* is very intolerant to suspended materials, a small amount of turbidity can already cause filtration problems for this species. The turbidity found in Waterdunen is on the high side for this species and might have been a cause for the poor results found.

Through the answers on the sub-questions the main question can now be answered. Considering the observed water parameters and the results of the research, it can be said that the oyster bed in Waterdunen is suitable for the cultivation of *Crassostrea gigas* oysters, whereby the method using hanging baskets gives the best results. However, the fluctuating values of salinity and temperature need to be monitored, since large and especially long-term fluctuations can cause problems. The research showed that the cultivation of *Ostrea edulis* is not successful in the oyster trench of Waterdunen.

## 6. Recommendation

Based on the results, T20 and the hanging baskets give comparable results regarding growth rate, final weight, mortality, and condition index. However, since the aim is to grow oysters for consumption, not only the growth rate and final weight of the oysters is important, but also the final shape and taste of the oyster. Oyster breeders ultimately want a good quality oyster, this means an oyster with a high condition index and a desirable shape (Li, 2008). When looking at the shape of the oysters the hanging baskets give the best results. Another factor that plays an important role in the successfulness of a cultivation system is the labor intensity and economical successful of the systems. For example, it was noticed that the T20 tables are harder to maintain than the hanging baskets because of the lower position in the water. During moderate or high tide, it was quite challenging to collect the oysters from the lower tables. The hanging baskets showed to be easier to access even with high tide. It is therefore recommended to look further into the use of hanging baskets in Waterdunen instead of the use of tables.

There are different kind of hanging baskets that can be used for the cultivation of oysters. During this research only one type was being tested. Another type for example is the floating baskets. These differ from the hanging baskets by having floating devises attached to them. This system has the benefit of being near the surface and thus having more water movement which benefits the shape of the oysters. Because of the construction of the system the oysters stay underwater so the food supply is not limited by being close to the surface. Lastly the system is partly surfaced and by way of flipping the system routinely the biofouling can be controlled (Walton *et al.*, 2013; Davis *et al.*, 2012). For further research it can be worthwhile to look into the use of these different systems.

It is also recommended that for further research the use of control groups will be implemented. This way the effects of the farming and research practices can be monitored. Furthermore, the optimal density of the systems and when they should be thinned out will have to be investigated for each system to ensure a healthy system and optimal growth.

The cultivation of *Ostrea edulis* oysters were unsuccessful in the oyster trench of Waterdunen. It is a species which is vulnerable to changes and therefore harder to maintain. It is therefore not advisable to cultivate this species in the Waterdunen.

Waterdunen is a still developing nature reserve, this is also reflected in the fluctuation parameters. Until recently, the area was under the influence of fresh water and only in the last few years has the saline influence begun to surface. It will take a long time before the area is balanced and therefore the water values will not be stable for some time. It is impossible to say how this will ultimately affect the feasibility of oyster farming in the coastal laboratory. It may be that a certain group is doing well now but will behave differently in a few years' time. For this reason, it is important to continue to investigate these values.

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# Appendix

- Appendix I Statistical results.
- Appendix II Result tables: growth rate, mortality, total weight, fish weight, shell weight and condition index.
- Appendix III Biofouling example.

### Appendix I Statistical results.

Spat

	Lengh	t difference	e between :	systems
			System	
		Basket	Basket	T40
		T40	T20	T20
	то	0,981	0,979	1,000
	T19	0,000	0,000	0,995
P	T43	0,000	0,613	0,008
after TO	T60	0,000	0,039	0,000
	T84	0,000	0,989	0,000
days	T103	0,000	0,077	0,000
in d	T123	0,000	0,999	0,000
F	T145	0,000	1,000	0,000
	T165	0,000	0,000	0,000
	T189	0,000	0,000	0,000

Table 4 Statistical significance of *Crassostrea gigas* spat difference between the systems for the parameter "length". Red = no significance., green = significance.

		Lenght difference in time								
					Tin	days after	TO			
		то	T19	T43	T60	T84	T103	T123	T145	T165
		T19	T43	T60	T84	T103	T123	T145	T165	T189
E	Basket	ket 0,000 1,000 0,000 0,000 0,000 0,000 0,000 0,000						0,000	0,000	
Syster	T40	0,720	0,947	0,078	0,894	0,000	0,000	0,000	0,000	0,000
T20 . 0,075 . 0,002 . 0,000 . 0,000 . 0,000 . 0,000 . 0,000 . 0,000 . 0,000							0,000	0,000		

Table 5 Statistical significance of *Crassostrea gigas* spat difference in time for the parameter "length". Red = no significance., green = significance.

### Half grown

			Gam	es Howell
		Total w	eight diffe	rence betv
			System	
		Basket	Basket	T40
		T40	T20	T20
2	то	- 1	-	-
5	T27	1,000	0,994	0,882
fe	T60	0,943	1,000	0,794
a ke	T112	0,803	0,990	0,133
in days after	T151	0,000	0,461	0,000
Ē	T193	0,000	0,000	0,000

Table 6 Statistical significance of half-grown *Crassostrea gigas* difference between the systems for the parameter "total weight". Red = no significance., green = significance.

	Games Howell								
		То	tal weight	difference	in time				
			1	in days af	ter TO				
		TO	T27	T60	112	T151			
		T27	T60	T112	T151	T193			
Ε	Basket	0,001	1,000	0,017	0,000	0,000			
System	T40	0,013	1,000	0,215	0,000	0,000			
s,	T20	1,000	1,000	0,000	0,000	0,000			

Table 7 Statistical significance of half-grown Crassostrea gigas difference in timefor the parameter "total weight". Red = no significance., green = significance.

			Gam	es Howell			
		Fish wei	ight differe	ence betw			
			System				
		Basket	Basket	T40			
		T40	T20	T20			
2	то	-	-	-			
after TO	T27	0,970	1,000	0,554			
E.	T60	1,000	1,000	1,000			
in days	T112	0,000	1,000	0,000			
E.	T151	0,000	0,052	0,000			
F	T193	0,000	0,000	0,000			

Table 8 Statistical significance of half-grown *Crassostrea gigas* difference between the systems for the parameter "fish weight". Red = no significance., green = significance.

	Games Howell								
		Fis	h weight d	lifference i	in time				
			Т	in days aft	er TO				
		TO	T27	T60	112	T151			
		T27	T60	T112	T151	T193			
ε	Basket	0,062	0,106	0,000	0,000	0,000			
System	T40	0,992	0,000	0,212	0,000	0,000			
S	T20	0,000	0,266	0,000	0,000	0,000			

Table 9 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "fish weight". Red = no significance., green = significance.

			Game	es Howell				
		Shell we	ight differ	ence betw				
			System					
		Basket	Basket	T40				
		T40	T20	T20				
2	то	-	-	-				
after	T27	0,985	0,982	0,521				
툲	T60	1,000	0,955	0,592				
a ka	T112	0,815	0,815 0,919 0,038					
in days	T151	0,000	0,000 0,678 0,000					
⊢	T193	0,000	0,009	0,000				

Table 10 Statistical significance of half-grown *Crassostrea gigas* difference between the systems for the parameter "shell weight". Red = no significance., green = significance.

	Games Howell									
		She	ell weight (	difference	in time					
			Т	in days aft	ter T0					
		то	T27	T60	112	T151				
		T27	T60	T112	T151	T193				
В	Basket	0,001	0,001 0,957 0,628 0,000 0,0							
System	T40	0,051	0,946	0,999	0,002	0,000				
s	T20	0,961	0,988	0,548	0,000	0,000				

Table 11 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "shell weight". Red = no significance., green = significance.

			Games	Howell				
		Condition i	index differe	ence betwe				
			System					
		Basket	Basket	T40				
		T40	T20	T20				
2	то	-	-	-				
after	T27	0,982	1,000	0,844				
afi	T60	0,037	0,999	0,521				
avs	T112	0,000	0,000 0,133 0,000					
in days	T151	1,000	1,000 0,862 0,811					
F	T193	0,024	1,000	0,819				

Table 12 Statistical significance of half-grown *Crassostrea gigas* difference between the systems for the parameter "condition index". Red = no significance., green = significance.

		Games Howell								
		Cond	ition index d	lifference ir	n time					
			Tin	days after	то					
		то	T27	T60	112	T151				
		T27	T60	T112	T151	T193				
E	Basket	1,000	0,041	0,005	0,000					
Syster	T40	0,000	0,000	1,000	0,000	0,000				
ŝ	T20	0,000	0,000	0,000	0,000	0,000				

Table 13 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "condition index". Red = no significance., green = significance.

		Games Howell								
	٦	Fotal weight	difference b	etween syste	ms					
			System							
		Basket	Basket	T40						
<b>T</b> 0		T40	T20	T20						
e	то	-	-	-						
aftei	T34	1,000	0,954	0,447						
in days	T74	0,999	0,999 0,288 0,801							
	T116	0,472	0,997	0,021						
Ē	T161	0,000	1,000	0,000						

Table 14 Statistical significance of consumption size *Crassostrea gigas* difference between the systems for the parameter "total weight". Red = no significance., green = significance.

		Games Howell								
		Total w	eight differe	nce in time						
			T in day	rs after TO						
		то	T34	T74	T116					
		T34	T74	T116	T161					
8	Basket	1,000	1,000	0,099	0,000					
System	T40	1,000 0,408 0,989 0,00								
Ś	T20	0,914	0,960	0,278	0,000					

Table 15 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "total weight". Red = no significance., green = significance.

			Tukey											
		Fish weight difference between system												
			System											
		Basket	Basket	T40										
2		T40	T20	T20										
after TO	то	-	-	-										
	T34	1,000	0,986	0,999										
ays	T74	0,942	1,000	0,799										
in days	T116	0,920	0,856	0,038										
Ē	T161	0,000	1,000	0,000										

Table 16 Statistical significance of consumption size *Crassostrea gigas* difference between the systems for the parameter "fish weight". Red = no significance., green = significance.

		Tukey										
	Fish weight difference in time											
		T in days after TO										
	то	T34	T74	T116								
	T34	T74	T116	T161								
Basket	0,963	0,044	0,000	0,000								
T40	0,779	0,545	0,000	0,006								
T20	0,205	0,000	0,000	0,000								
	T40	T0 T34 Basket 0,963 T40 0,779	Fish weight difference        Tin days        T0      T34        T34      T74        Basket      0,963      0,044        T40      0,779      0,545	Fish weight difference in time        T in days after T0        T0      T34      T74        T34      T74      T116        Basket      0,963      0,044      0,000        T40      0,779      0,545      0,000								

Table 17 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "fish weight". Red = no significance., green = significance.

			Games Howel	I	
	9	Shell weight d	lifference bet	ween syster	ns
			System		
		Basket	Basket	T40	
2		T40	T20	T20	
after TO	то	-	-	-	
	T34	0,769	1,000	0,241	
ays	T74	1,000	0,578	0,859	
in days	T116	0,770	0,998	0,111	
F	T161	0,110	1,000	0,016	

Table 18 Statistical significance of consumption size *Crassostrea gigas* difference between the systems for the parameter "shell weight". Red = no significance., green = significance.

		(	Games Howel		
		Shell we	ight differenc	e in time	
			T in days	after T0	
		то	T34	T74	T116
		T34	T74	T116	T161
Ξ	Basket	1,000	1,000	0,247	0,000
System	T40	0,668	0,155	1,000	0,001
ŝ	T20	1,000	0,881	0,950	0,000

Table 19 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "shell weight". Red = no significance., green = significance.

			Games Howe	II	
	C	ondition inde	x difference b	etween syste	ems
			System		
		Basket	Basket	T40	
atter TO		T40	T20	T20	
	то	-	-	-	
	T34	1,000	0,733	0,265	
In days	T74	0,438	0,999	0,986	
	T116	0,999	1,000	0,931	
-	T161	0,131	1,000	0,233	

Table 20 Statistical significance of consumption size *Crassostrea gigas* difference between the systems for the parameter "condition index". Red = no significance., green = significance.

<u> </u>			Games Howe	1									
		Condition index difference in time											
			T in days after T0										
		то	T34	T74	T116								
		T34	T74	T116	T161								
ε	Basket	1,000	1,000	0,032	0,032								
Syste	T40	1,000	0,984	0,000	0,770								
S	T20	0,606	0,000	0,000	0,190								

Table 21 Statistical significance of half-grown *Crassostrea gigas* difference in time for the parameter "shell weight". Red = no significance., green = significance.

			Tukey										
		Flat oyste	rs difference i	n time									
	T in days after TO												
		то	T34	T74	T116								
		T34	T74	T116	T161								
er	Total	1,000	0,975	0,996	0,897								
ramete	Fish	0,826	0,085	0,396	0,690								
Ē	Shell	0,546	0,998	1,000	0,813								
2	Condition	0,688	0,028	0,574	0,920								

Table 22 Statistical significance of consumption size *Ostrea edulis* difference in time for the parameters "total- flesh- shell- weight and condition index". Red = no significance., green = significance.

# Appendix II Result tables: growth rate, mortality, total weight, fish weight, shell weight and condition index.

### Spat

Age class	System	TO	T19	T43	T60	T84	T103	T123	T145	T165	T189
Spat	Basket		0,09	0,01	0,10	0,04	0,11	0,17	0,27	0,43	0,23
	Table 40		0,03	0,02	0,05	0,02	0,07	0,11	0,17	0,28	0,60
	Table 20		0,05	0,04	0,09	0,10	0,12	0,15	0,27	0,43	0,41

Table 23 Growth rate of *Crassostrea gigas* spat for all systems compared to precious measurement shown in mm/day.

		Mortal	Mortality ( % open shells on total number measured)								
Age class	System	TO	T19	T43	T60	T84	T103	T123	T145	T165	T189
	Basket		1,0	0,7	2,3	4,0	1,1	2,6	0,7	1,3	17,1
Spat	Table 40		0,3	0,7	2,3	13,0	16,8	10,4	6,3	1,3	1,3
	Table 20		1,6	0,6	2,0	1,0	1,6	0,7	0,7	1,6	34,8

Table 24 Mortality rate of *Crassostrea gigas* spat for all systems shown in percentages (%).

		Average	e length								
Age class	System	TO	T19	T43	T60	T84	T103	T123	T145	T165	T189
Spat	Basket	14,05	15,64	16,83	18,47	19,66	21,84	25,15	31,37	39,90	45,34
	Table 40	14,05	15,48	15,42	16,16	16,61	17,80	19,98	23,80	29,36	43,79
	Table 20	14,05	15,56	16,29	17,69	20,01	22,35	25,45	31,68	40,36	50,10

Table 25 Average lengths of *Crassostrea gigas* spat for all systems shown in mm.

### Half-grown

	Growth rate	Growth rate compared to the previous measurement(in grams total weight/da										
Age class	System	TO	T27	T60	T112	T151	T193					
Half grown	Basket		-0,12	0,01	0,09	0,32	0,65					
	Table 40		-0,16	-0,03	0,09	0,19	0,43					
	Table 20		-0,14	0,03	0,12	0,38	0,88					

Table 26 Growth rate on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems compared to precious measurement shown in mm/day.

	Mortality (%						
Age class	System	TO	T27	T60	T112	T151	T193
Half grown	Basket		9,1	9,6	14,1	6,7	9,2
	Table 40		13,4	14,6	13,4	12,8	14,4
	Table 20		7,7	8,2	11,3	9,9	12,2

Table 27 Mortality rate measured on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems shown in percentages (%).

	Condition inde	x ( % fish w					
Age class	System	TO	T27	T60	T112	T151	T193
Half grown	Basket	17,69	17,43	20,88	25,50	27,74	21,65
	Table 40	17,69	17,30	22,17	22,18	27,59	23,21
	Table 20	17,69	17,90	21,35	24,26	29,24	22,16

Table 28 Condition index measured on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems shown in percentages (%).

	Total weight (g)						
Age class	System	TO	T27	T60	T112	T151	T193
Half grown	Basket	32,53	29,18	29,62	34,35	46,83	74,08
	Table 40	32,53	28,21	27,12	31,75	39,18	57,19
	Table 20	32,53	28,87	29,80	36,10	50,90	88,06

Table 29 Total weight measured on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems shown in grams (g).

	Fish weight (g)						
Age class	System	то	T27	T60	T112	T151	T193
Half grown	Basket	5,78	5,08	6,18	8,67	13,00	15,99
	Table 40	5,78	4,88	5,95	6,98	10,66	13,13
	Table 20	5,78	5,17	6,35	8,67	14,61	19,64

Table 30 Fish weight measured on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems shown in grams (g).

	Shell weight (g)						
Age class	System	TO	T27	T60	T112	T151	T193
Half grown	Basket	20,43	17,87	18,53	20,74	28,48	44,81
	Table 40	20,43	16,94	17,63	19,02	23,29	34,48
	Table 20	20,43	17,65	19,30	22,33	30,92	52,11

Table 31 Shell weight measured on T days after baseline measurement of half-grown *Crassostrea gigas* for all systems shown in grams (g).

### Consumption Crassostrea gigas

Age class	Growth rate of	Growth rate compared to the previous measurement (in grams total weight/day)											
	System	TO	T34	T74	T116	T161	μ						
Consumption	Basket		0,03	0,01	0,09	0,27	0,10						
	Table 40		-0,05	0,10	0,02	0,12	0,05						
	Table 20		0,07	0,05	0,08	0,22	0,11						

Table 32 Growth rate on T days after baseline measurement of consumption size *Crassostrea gigas* spat for all systems compared to precious measurement shown in mm/day.

Age class	Mortality (% open shells on total number measured)									
	System	TO	T34	T74	T116	T161				
Consumption	Basket		3,6	3,3	8,9	12,2				
	Table 40		2,9	7,8	13,3	6,3				
	Table 20		2,2	10,0	9,9	18,0				

Table 33 Mortality rate measured on T days after baseline measurement of consumption size *Crassostrea* gigas for all systems shown in percentages (%).

Age class	Condition inde					
	System	TO	T34	T74	T116	T161
Consumption	Basket	18,81	18,06	20,59	22,69	24,70
	Table 40	18,81	17,92	19,11	22,08	23,17
	Table 20	18,81	16,61	19,86	23,07	24,60

Table 34 Condition index measured on T days after baseline measurement of consumption size *Crassostrea* gigas for all systems shown in percentages (%).

	Total weight (g)					
Age class	System	то	T34	T74	T116	T161
Consumption	Basket	60,32	61,36	61,66	65,39	77,53
	Table 40	60,32	58,63	63,02	63,66	68,94
	Table 20	60,32	62,65	64,75	68,15	78,10

Table 35 Total weight measured on T days after baseline measurement of consumption size *Crassostrea* gigas for all systems shown in grams (g).

	Fish weight (g)					
Age class	System	TO	T34	T74	T116	T161
Consumption	Basket	11,29	11,01	12,70	14,88	19,12
	Table 40	11,29	10,59	11,94	14,07	16,00
	Table 20	11,29	10,42	12,79	15,76	19,22

Table 36 Fish weight measured on T days after baseline measurement of consumption size *Crassostrea gigas* for all systems shown in grams (g).

	Shell weight (g)					
Age class	System	TO	T34	T74	T116	T161
Consumption	Basket	41,04	42,17	42,29	44,43	50,34
	Table 40	41,04	39,86	42,89	42,75	46,60
	Table 20	41,04	42,40	43,96	45,36	51,06

Table 37 Shell weight measured on T days after baseline measurement of consumption size *Crassostrea* gigas for all systems shown in grams (g).

### Consumption Ostrea edulis

	Growth rate compared to the previous measurement(in grams total weight/day)								
Age class	System	T0	T34	T74	T116	T161	μ		
Consumption	on-bottom		0,01	-0,01	-0,01	0,02	0,00		

Table 38 Growth rate on T days after baseline measurement of consumption size *Ostrea edulis* compared to precious measurement shown in mm/day.

Mortality (% o	pen shells	on total nu	mber mea	sured)	
System	T0	T34	T74	T116	T161
on-bottom		13,3	8,9	22,2	41,6
	System	System TO	System TO T34	System TO T34 T74	

Table 39 Mortality rate measured on T days after baseline measurement of consumption size Ostrea edulis shown in percentages (%).

Age class System T0 T34 T74 T116 T16		Condition inde	Condition index ( % fish weight on total fresh weight)					
	ge class	ass System	то	T34	T74	T116	T161	
Consumption on-bottom 21,31 16,31 17,77 17,04 17	onsumption	umption on-bottom	21,31	16,31	17,77	17,04	17,51	

Table 40 Condition index measured on T days after baseline measurement of consumption size *Ostrea edulis* shown in percentages (%).

	Total weight (g)					
Age class	System	T0	T34	T74	T116	T161
Consumption	on-bottom	52,51	52,79	52,27	51,79	52,85

Table 41 Total weight measured on T days after baseline measurement of consumption size *Ostrea edulis* shown in grams (g).

	Fish weight (g)					
Age class	System	T0	T34	T74	T116	T161
Consumption	on-bottom	11,06	8,59	9,28	8,81	9,22

Table 42 Fish weight measured on T days after baseline measurement of consumption size *Ostrea edulis* shown in grams (g).

	Shell weight (g)					
Age class	System	T0	T34	T74	T116	T161
Consumption	on-bottom	39,95	38,80	38,68	38,38	37,60

Table 43 Shell weight measured on T days after baseline measurement of consumption size *Ostrea edulis* shown in grams (g).

## Appendix III Biofouling example.

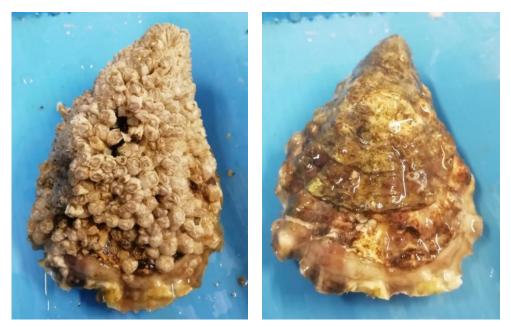


Figure 36 Consumption size oysters (*Crassostrea gigas*) before (left) and after (right) the removal of barnacles 27-05-2021 (L. Sanders, 2021).