UNDERSTANDING MUSSEL AGGREGATION AND DENSITY ON PATCH SCALE

Optimizing seeding density in mussel bottom culture

Final thesis

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ABSTRACT

The seeding of mussel seeds is an important factor within the mussel culture as losses can be as high as 75% within the first 4 weeks after seeding. Therefore it is important to get more knowledge about the factors that can cause mortality within the first weeks after seeding. With seeding high local densities arise. It is important to gain more knowledge about what happens within these higher densities. Therefore this study focusses on mussel density within a patch scale. The study consisted out of seven repetitive field experiments on a subtidal culture plot in the eastern Scheldt between March 2017 and April 2018. The experimental set-up consisted out of 30 gabions with three replicates of ten different mussel densities. with a four week duration of every experiments. within this experiment the chlorophyll-a levels, water temperature, water turbidity, aggregation, patch complexity, initial growth and survival where measured. No relation between starting density and aggregation was found but rather influenced by water current. Furthermore the optimal mussel density on a patch scale seemed to be between 7 and 9 kg/m^2 as this was the density in which the highest performance of the mussel was found in relation to growth and survival.

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1. INTRODUCTION

1.1 BACKGROUND.

The blue mussel (*Mytilus edulis*) is a filter feeding animal filtering phytoplankton and detritus in the water column (Thompson & Bayne, 1972). The mussel is widely cultured for human consumption. Mussel culture in Europe produces about 50% of the annual world-wide harvest of mussels and consist out of two species: blue mussels and Mediterranean mussels (*Mytilus galloprovincialis*) (Smaal, 2002). The Netherlands culture has the second largest production of the blue mussel and consist almost entirely out of bottom culture (Smaal, 2002), that takes place on leased culture plots in the Wadden sea (3560 ha) and the Eastern Scheldt (2040 ha) (Capelle, et al., 2016; Smaal, 2002). Small mussels (seeds) are fished from natural mussel beds and are collected by using suspended spat mussel collectors (SMCs) (Dolmer, et al., 2012; Kamermans, et al., 2002). In the timespan between seeding and harvest the mussel farmers have to cope with high losses that can exceed 90% (Capelle, et al., 2016). The high mussel losses are probably caused by a combination of different factors like currents washing away the mussel seeds during seeding, damage caused by handling of the mussels, predation and intraspecific competition (Capelle, et al., 2016).

Seeding of the mussel seed is carried out by mussel vessels. The seeding is done as fast as possible in circular patterns to prevent mussels being lost by the tidal currents. The seeding in circular patterns leads to a heterogenous distribution over the mussel plot and this results in highly concentrated mussel formations (Capelle, et al., 2014). In the first period after seeding high density dependent losses of mussels occur , this can most likely be reduced by a more even spread of mussels (Capelle, et al., 2014). Research is needed to get more insight in the optimal seeding density in relation to spreading so mussel farmers will obtain higher yield in the cultured mussels. To get insight in what happens within these concentrated mussel formations this research is conducted on a patch scale with 10 different densities.

A research project (INNOPRO) carried out by several research institutes together with the mussel industry aims to increase the efficiency of the mussel culture cycle. Within this project several topics are examined including seeding density.

1.2 OBJECTIVE.

The overall objective of this study is to examine the relation between different initial mussel densities, food levels and mussel performance at patch scale. To investigate optimal seeding densities that can be applied by mussel farmers to increase yield.

2. THEORETICAL FRAMEWORK

2.1 MUSSEL BEDS ON SOFT-SEDIMENT

The blue mussel (*Mytilus edulis*) thrives in both rocky and soft-bottom habitats in the northeast and northwest Atlantic (Commito, et al., 2006) and can form dense beds that range in size from tens of meters to square kilometers in which dense patches alternate with sediment containing hardly any mussels (Van de Koppel, et al., 2005). these patches are formed by interconnecting to each other by the use of byssal threads (Van de Koppel, et al., 2005). Aggregation and the attachment to each other protects the mussels from dislodgement by hydrodynamic forces and predation (Van de Koppel, et al., 2008; Bertness & Grosholz, 1985; Widdows, et al., 2002).

2.2 AGGREGATION, FACILITATION AND COMPETITION

Cooperation is an adaptation to survive in harsh environments and is found in many species, this also counts for mussels (de Jager, et al,. 2017). Mussels benefit from any attachment of byssus threads with neighboring individuals (de Jager, et al., 2017) it helps mussels to exist under conditions that would otherwise be lethal (de Jager, 2015; Van de Koppel, et al., 2008).

Mussels actively search for large enough substrates to attach to (de Jager, et al., 2011), on soft sediments substrate which are large enough to attach to is scarce and mussels aggregate into clumps and patches by binding themselves togheter using byssus threads (Snover & Commito, 1998; van de Koppel, et al., 2012). The byssus threat of a mussel is an extraorganismic polymeric structure, it has no living cells and is used as a holdfast or tethering device (Wait, 1992).

Mussels aggregate to facilitate each other to prevent dislodgement by wave impacts, tidal currents and minimize predation. When aggregation occurs, density increases, which might lead to a decrease of per capita space and/or food availability (Frechette et al., 1992), due to this intraspecific competition growth rate and survival can reduce at higher densities (Bertness & Grosholz, 1985; Frechette et al., 1992).

Several factors affect the relation between density and competition, at higher density the competition for space, position and food increases. Position within a patch has an effect on the growth rate. Mussels located in the center of groups often have a reduced growth relative to mussels located on the edges of groups (Svane & Ompi, 1993; Okamura, 1986). The reduction of growth rate in the center of a mussel patch can be caused by crowding and a decreasing phytoplankton concentration over a mussel patch (food depletion) (Svane & Ompi, 1993). The competition for food within an patch can vary depending food availability and temperature and is therefore season dependent. Furthermore mussels within higher densities compete for space and have to withstand external pressure on its shell from its competitors due to crowding. The pressure on the shell leads to an decreased shell gape (Frechette et al., 1992). The reduced shell gape reduce the capability off food uptake. Isolated clumps show a higher growth rate than dense homogeneous beds, probably because of reduced competition (Van de Koppel, et al., 2008) and food depletion. The results of crowding can cause higher mortality especially with smaller mussels (Svane & Ompi, 1993).

2.3 SPATIAL DISTRIBUTION

It was found that patch sizes relate to mussel density, with a low mussel density mussels have more space available and distribute in more smaller patches while with higher densities mussels lack space to distribute in smaller patches so a large patch is formed (Capelle, et al., 2014).

In young, natural mussel beds it was found that aggregation results in the formation of regular patterns (Van de Koppel, et al., 2005). Van de Koppel et al. (2005) found out that mussel beds with patterns results in a higher

productivity, resilience and accommodate mussels to sustain themselves at lower algal concentrations than in a homogenous bed. The patterns increase turbulence over the mussel beds, that increases mixing and food delivery to the mussels (Van de Koppel, et al., 2005).

Mussel beds may appear in banded patterns (Figure 1) within these banded patterns more patchy net-shaped patterns occur (Figure 2) (Van de Koppel, et al., 2005; Liu, et al., 2014). The banded patterns are often found perpendicular to the flow of the water (Van de Koppel, et al., 2005). The banded patterns are formed due to aggregation and physical forcing by tidal currents (van de Koppel, et al., 2012). The net shape patterns are formed by a self organization process between mussel interactions and aggregation (Liu, et al., 2014).

FIGURE 1, BANDED PATTERN (LIU, ET AL., 2014).

FIGURE 2, NET SHAPED PATTERN (VAN DE KOPPEL, ET AL., 2008)

2.5 IN RELATION TO MUSSEL CULTIVATION

the Mussel culture in the Netherlands dates back to the 1860s when mussel plots where assigned to fishermen and mussel banks where privatized (Van Ginkel, 1990). Mussel seeds are collected in the Wadden sea by fishing and the use of SMCs (Kamermans, et al., 2002). Recently fishing activities on natural mussel beds are decreasing and the use of SMCs is increasing because of an agreement signed between environmental NGOs, and the mussel producers trade organization in 2008 (van Hoof, 2012). The mussel seeds are transplanted to the lease sites and are harvested when the mussels reach a commercial size (Smaal, 2002; Dolmer, et al., 2012). The seeding of mussel seeds is done with the use of vessels which move in circular patterns to spread out the mussel seeds over the lease sides (Capelle, et al., 2014). The seeding of the seeds is often done quickly and results in high concentrations of mussels where competition can lead to a loss in a short period post-seeding (Capelle, et al., 2014).

Capelle, J. J. et al. (2014, 2016) found out that the highest loss of mussels occurs within the first 4 weeks after seeding, they found losses up to 75%. Also in Denmark loss rates decreases with time after fishing (Christensen, et al., 2015). A reduction in mussel losses post-seeding may be accomplished by an even spread of mussels (Capelle, et al., 2014). The suggested average plot scale seeding density by Capelle, J. J. et al. (2014) is between 2.5 and 5 kg/m^2 which corresponds with the recommended seeding density by Dolmer, et al. of 3.5 kg/m^2. This density may be higher in patch scale.

3. RESEARCH QUESTION & HYPOTHESIS

What is the effect of different densities at plot scale on mussel aggregation?
Hypothesis: Aggregation activities will take place more within the lower densities when there is space available, within higher densities less aggregation activities will take place due to lack of space.

What is the effect of density at patch scale on growth and survival of mussels in relation to season and food levels?
Hypothesis: there will be a threshold density in which there is an optimal performance of the mussel with intraspecific competition in which there is a limited loss in mussels. This threshold density can fluctuate over the season as the food levels change.

Sub questions.

- What is the effect of the different starting densities at plot scale on density at patch scale?
  Hypothesis: with the lower densities the mussels will form a higher mean within patch density compared to the starting situation. At increasing densities mean within patch density will approach the starting situation.

- Do seasonal effects influence the aggregation process and patch complexity in relation to the different whole plot starting densities?
  Hypothesis: the mussels will form patterns if space is available, to increase turbulence over the patch for a more even food distribution of phytoplankton. And is not affected by food levels.

- What is the effect of different densities at patch scale on the growth off the mussels and are there seasonal influences on the growth?
  Hypothesis: at increasing densities the growth rate will decrease due to competition, and a higher growth rate and competition will occur in seasons with higher food levels (spring & summer).

- Does mussel position within a patch affect condition index and growth , is there a seasonal trend?
  Hypothesis: at increasing patch size a lower growth rate and condition index occurs in the middle of the patch, but at small patch scale edge effects only occur when food and temperature are low.

- Is there a relation between mussel losses and density at patch scale, if so is this relation affected by season?
  Hypothesis: Losses are affected by competition and facilitation: with higher patch densities mussel losses will increase, due to competition especially if food levels are low and food depletion will occur over the patch. however, if the density is to low the mussels will not be able to facilitate each other and higher mussel loss will occur as well.

4. METHOD

4.1 EXPERIMENTAL DESIGN

In this research seven repetitive field experiments were conducted on a subtidal culture plot at the Zandkreek in the eastern Scheldt (Figure 3) between March 2017 and April 2018. This location is a mussel cultivation plot which is no longer in use. To match condition of a professional cultivation plot a subtidal zone was chosen. However, whit a strong eastern storm the plot fell dry. The starting date, end date, duration of every experiment and the number which corresponds with the experiment is shown in Table 1. The planned duration of the experiments was four weeks, but weather conditions influenced the duration of the experiments. The experiments were conducted at the Zandkreek shown in Figure 3.

TABLE 1, NUMBER OF EXPERIMENT, EXPERIMENT CODE, START DATE, END DATE AND DURATION OF THE EXPERIMENTS THAT ARE PART OF THIS STUDY.

Experiment number	Experiment code	Start experiment	End experiment	Duration in days
1	MA17	10-March-2017	09-April-2017	30
2	AM17	18-April-2017	14-May-2017	26
3	Jn17	01-Jun-2017	23-June-2017	22
4	JA17	27-July-2017	28-August-2017	32
5	SO17	20-September-2017	19-October-2017	29
6	ND17	09-November-2017	11-December-2017	32
7	FM18	21-February-2017	30-March-2017	37

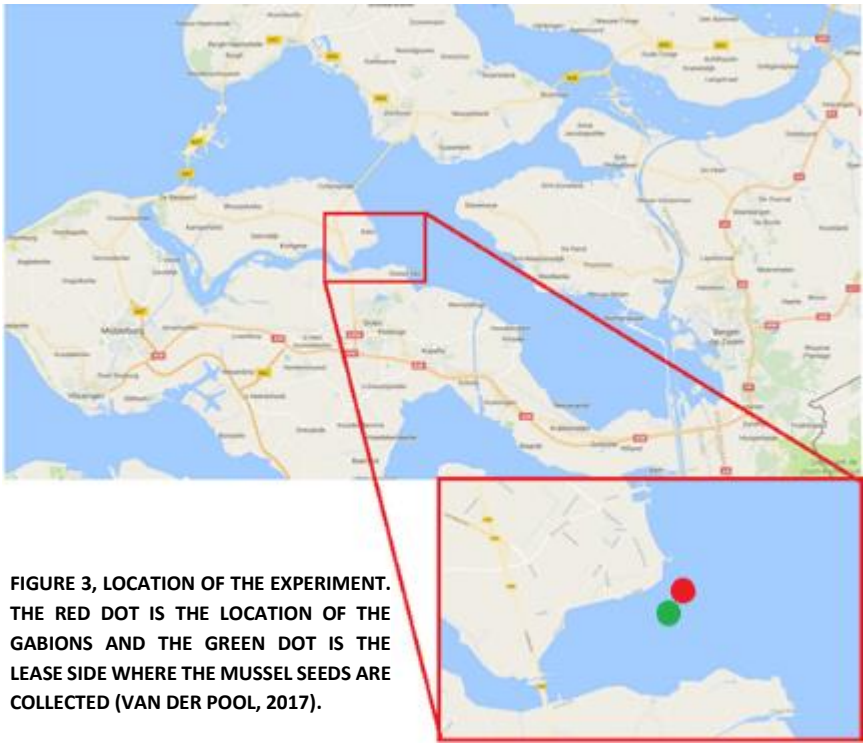

FIGURE 3, LOCATION OF THE EXPERIMENT. THE RED DOT IS THE LOCATION OF THE GABIONS AND THE GREEN DOT IS THE LEASE SIDE WHERE THE MUSSEL SEEDS ARE COLLECTED (VAN DER POOL, 2017).

Mussel seed for the experiments was collected at a culture plot next to the experiment site (Figure 3) except for the mussels that were used in experiment 2 that were collected in the Wadden sea. After collection the mussel seed, the mussels were cleaned off tare and divided in the necessary densities which were calculated by the following

formula: $Area\ quadrant_{(m^2)} * desired\ density_{(n)}$. 3 samples of 0.5 kg were counted to estimate the number of mussels per gabion. Also the mean Condition Index was determined at the beginning of each experiment from a sample of at least 50 mussels.

The experimental set-up consisted of 30 steel gabions (0.55m x 0.60m x 0.25m). The gabions were placed randomly over the plot to account for effects of position, buried 0.1 to 0.2m in the sediment and, anchored with steel hooks of approximately 30cm long, and were coated in polypropylene light wire netting (18 mm mesh size) to prevent predation. On top of every gabion a lid was placed to open and close the gabions. Every lid was closed with cable ties to prevent it from opening. Each gabion was marked with a wooden pole which was hammered into the sediment, furthermore every gabion had a label attached to it which correspondents with the gabion number. An overview of the experiment is shown in Figure 4

one of three replicates of ten different mussel densities was placed in each gabion (1,3,5,7,9,11,13,15,17,19 kg/m^2). The mussel density for each gabion was assigned randomly to each gabion using the RAND() function in Excel. The mussels were evenly spread within a quadrant of 0.09m^2 in the middle of each gabion.

FIGURE 4, RANDOM PLACEMENT OF THE GABIONS WITH WOODEN POLES TO INDICATE THE LOCATION OF EACH GABION

4.2 DATA COLLECTION

Photographs were taken of the mussels with an underwater camera, directly after putting the different densities in the corresponding gabions (this was only done for experiments: ND17 and FM18). The photographs were taken under water at approximately to avoid light reflection. A screw with a length of 153 mm, wrapped in colored tape was placed as reference for later analysis next to the mussels, an example is shown in Figure 5. after the photographs were taken, gabions were closed using cable ties. After approximately four weeks a second set of photographs were taken. The perimeter and the area of every patch could be calculated from the pictures that were taken at the beginning and end of every experiment with the use the image analyzing software MIPAR (www.mipar.com). Two samples of both the center and the edge of every patch were taken by pushing a PVC pipe (D=45mm) through the patch and collecting the mussels, to calculate the condition index of the mussels within the gabions. To determine if a patch had a clear center the following guideline was used: when taking the sample of the center of the patch, mussels of the patch need to be present surrounding the sample pipe. If no center could be defined only 2 samples of the edge were taken. After the samples were taken. The mussels were placed separately

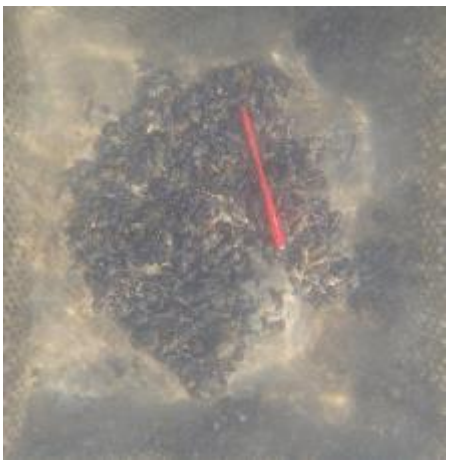

FIGURE 5, EXAMPLE OF A PHOTOGRAPH THAT WAS TAKEN AFTER PLACING THE MUSSELS IN THE CORRESPONDING GABION. THE SCREW WRAPPED IN RED TAPE IS A REFERENCE POINT FOR LATER ANALYSIS.

in a bag to prevent mixing treatments. The mussels were first cleaned and subsequently weighed to determine the mussel biomass and density of each gabion at the end of the experimental run.

Analysis of seasonal influences

During the experiments chlorophyll a content (µg/l), temperature (^{0}C) and turbidity (FTU) of the water were measured with a chlorophyll measuring device (JFE Advantech Ocean Instruments Datalogger) at a 10 minute interval at approximately 1440 meter from the experimental site (Figure 6). The chlorophyll-a content is an indication of the phytoplankton concentration and thus the quantity of food. Turbidity will be a proxy for Current or water velocity as it was found in Belgium that turbidity is related to high winds and waves and that the turbidity increases during and after storms (Fetweiss, et al., 2010), neglecting the influence of algae blooms on the turbidity. Due to storms the water current can be higher than the mean water current caused by the tidal influences. The mean value of these parameters over the experiments are used in the analysis. Seasonal effects on different patch densities could be determined by comparing the mean Chlorophyll a content, mean temperature and mean turbidity with the aggregation ratio, patch complexity, growth and survival of the mussels.

FIGURE 6, MAP WITH THE LOCATION OF THE EXPERIMENT SITE AND THE LOCATION OF THE CHLOROPHYLL MEASUREMENTS.

Aggregation ratio

The aggregation ratio (AR) was calculated for every gabion by dividing the final patch density with the starting density ($\frac{\mathrm{D_{end}}}{\mathrm{D_{start}}}$). If the AR is higher than 1 (>1) aggregation took place. the aggregation ratio could only be calculated for the experiments ND17 and FM18 as this were the only experiments were photographs of the beginning of the experiment were taken and thus only a good comparison between the patch at the start of the experiment and the end of the experiment could be made.

Patch complexity

The patch complexity indicates if there is any effect of density or seasonal influences on mussel pattern formation . The patch complexity is expressed as a perimeter to area ratio (P/A ratio, m^{-1}). The P/A ratio is calculated by dividing the perimeter (m) by the area (m^2) of the end of the experiment ($\frac{P_{end}}{A_{end)}}$). A higher P/A ratio means a higher patch complexity.

Growth and condition index

Somatic growth was estimated by the difference of the mean condition index (CI, mg/cm^3) of the gabion and the mean CI from the start of the experiment ($dCi = CI_{end} - CI_{start}$). The mean CI of the gabions is determined by the samples that were taken in the end of every experiment. The CI is calculated according to Hopkins formula with the shell length (cm) and ash free dry weight (AFDW,mg) $CI = \frac{AFDW}{Shell\ length^3}$. The AFDW was determined according to the protocol in Appendix II – Protocol for determining the ash free dry weight of crustaceans. The growth of experiment MA17 and AM17 could not be determined because the CI at the start of the experiment was not determined.

The difference in growth between edge and the center is estimated by comparing the change in CI at the edge of the patch with the growth in the center of the patch. This comparison could only be made with patches that had a clear center.

Mussel survival

Each sample taken at the end of the experiment was weighted, the shell length measured and the number of mussels in each sample was determined. The mean weight of a mussel from each gabion could be determined with the weight and the number of mussels from every sample. With the weight of the samples and the weight of the mussels that were taken out of the gabions the total amount of mussels within a gabion was estimated. With this information the mussel survival (%) for each gabion was calculated with the following formula: $\frac{n_{t1}}{n_{t2}} \times 100$

Data Analysis

An explorative data analysis was carried out with the use of Microsoft Excel and R software. An overview of the formulas that were used are given in Table 2 and table 3 shows the symbols with the definition and the units.

Different parameters were tested against each other to test the hypothesis. to find the difference between the starting densities and the patch densities the aggregation ratio was tested against the different starting densities. The seasonal influences (turbidity, temperature and chlorophyll a level) where tested against the AR and P/A ration to find a relation between the seasonal influences and the aggregation process and the patch complexity. The difference in CI was tested against the starting densities and the seasonal influences to find out which the important factors influencing the growth are. Also the difference in CI was tested to the position within the patch to find if the position within the patch affects the growth. furthermore the survival was tested against the different starting densities and the seasonal influences to find if the survival is density dependent and if the season has an effect on the survival.

TABLE 2, OVERVIEW OF FORMULAS

CALCULATING	FORMULA
AFDW	$(DW - AW)$
DENSITY T_1	$\frac{N_{t1}}{A_{t1}}$
AR	$\frac{D_{t1}}{D_{t0}}$
CI	$\frac{AFDW}{L^3}$
SURVIVAL	$\frac{\ln(n_{t0}) - \ln(n_{t1})}{t}$
P/A	$\frac{p_{t1}}{A_{t1}}$
STARTING DENSITY	$\frac{n_{t0}}{A_{t0}}$
SURVIVAL (%)	$\frac{n_{t1}}{n_{t2}} \times 100$

TABLE 3, THE SYMBOLS WITH DEFINITIONS AND UNITS.

SYMBOL	DEFINITION	UNIT
A	**Patch area**	**m^2**
AFDW	**Ash free dry weight**	**mg**
AR	**Aggregation ratio**	**-**
AW	**Ash weight**	**mg**
CI	**Condition index**	**mg/cm^3**
D*CI*	**Difference in condition index**	**mg/cm^3**
D	**Density**	**n/m^2**
DW	**Dry weight**	**mg**
L	**Shell length**	**cm**
N	**Number of mussels (individuals)**	**-**
P	**Patch perimeter**	**m**
P/A	**Perimeter to area ratio**	**m^{-1}**
T	**Time in days**	**d^{-1}**
T_0	**Start of experiment**	**-**
T_1	**End of experiment**	**-**

5. RESULTS

5.1 ENVIRONMENTAL CONDITIONS

In Figure 7 the Chlorophyll a levels from January 2017 to April 2018 are shown, the different colors indicate the time of the different experiments. The Chlorophyll levels are an indicator of the phytoplankton concentration in the water and thus a indications of the food availability in the water. Algae blooms in April 2017 and march 2018 are clearly visible.

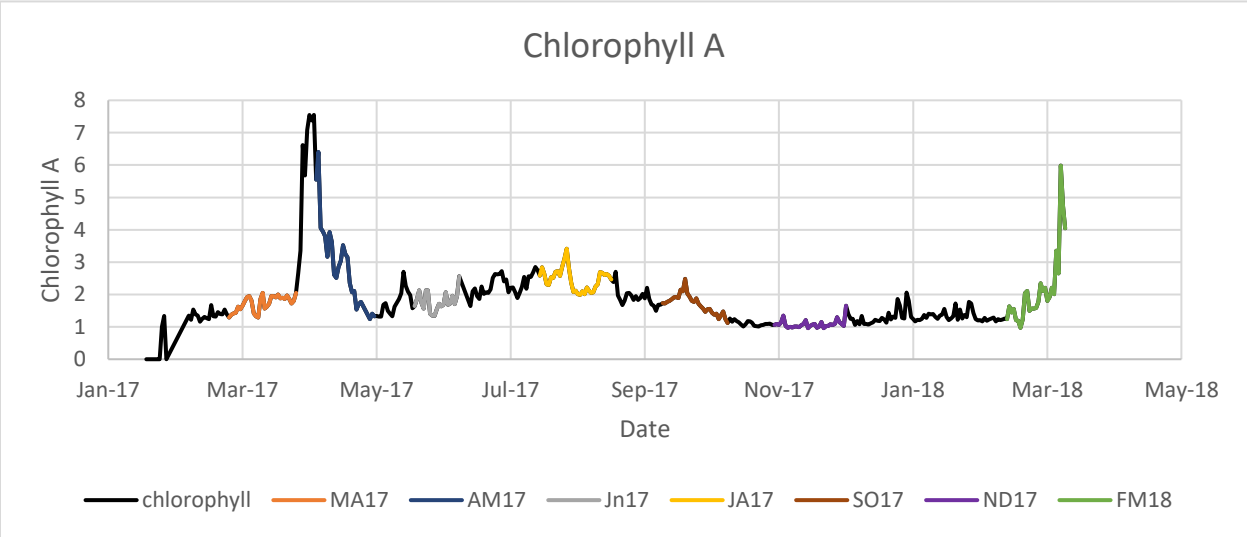

FIGURE 7, CHLOROPHYLL-A LEVELS FROM JANURY 2017 TO APRIL 2018, EVERY EXPERIMENT IS INDICATED WITH A DIFFERENT COLOR.

Water temperature fluctuated with the season, with the water temperature rising during spring, warmest during summer, cooling down with fall and coldest during winter. The lowest water temperatures were during the experiment of February/March 2018 were the mean temperature was 2.3 ^{0}C (Figure 8; Table 4).

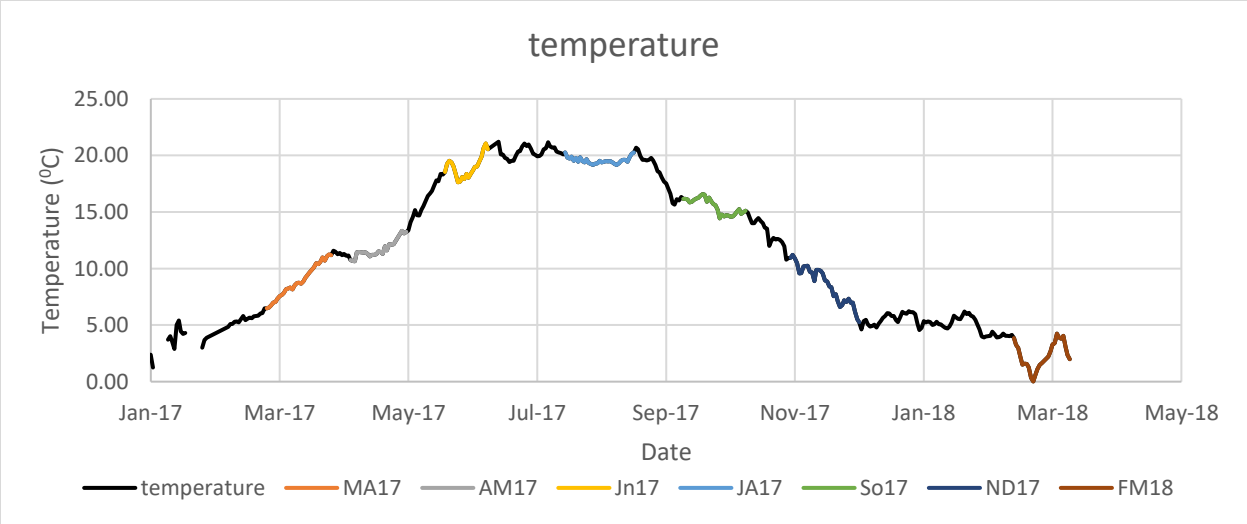

FIGURE 8, TEMPERATURE FROM JANURY 2017 TO APRIL 2018, EVERY EXPERIMENT IS INDICATED WITH A DIFFERENT COLOR.

Turbidity is an indication of currents in the water. A high turbidity is an indication of strong currents. These strong currents are caused by storms. Turbidity varied throughout the experimental period with the highest mean turbidity occurring in February/March 2018 (Figure 9; Table 4).

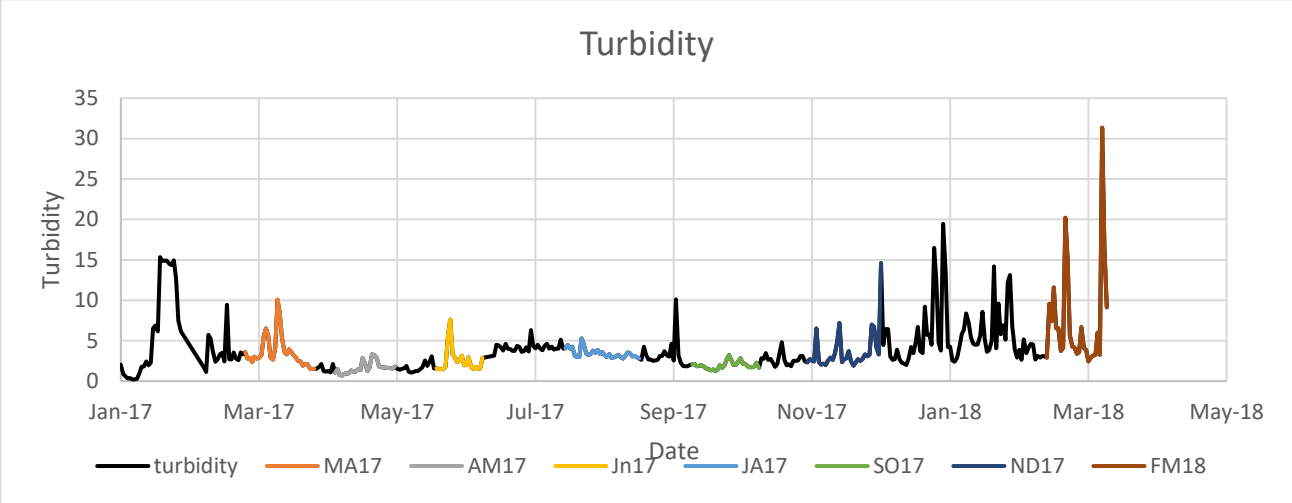

FIGURE 9, TURBIDITY FROM JANURY 2017 TO APRIL 2018, EVERY EXPERIMENT IS INDICATED WITH A DIFFERENT COLOR.

The mean and 95% confidence interval of the chlorophyll-a, temperature and turbidity during the experiments are calculated and shown in table 4.

TABLE 4, THE MEAN AND 95% CONFIDENCE INTERVAL OF THE CHLOROPHYLL A, TEMPERATURE AND TURBIDITY DURING THE EXPERIMENTS

Experiment	Mean chlorophyll a (µg/l)	Conf. int +/-	Mean Temperature (^{0}C)	Conf. int +/-	Mean turbidity	Conf. int +/-
MA17	1.73	0.09	8.89	0.55	3.57	0.72
AM17	2.96	0.55	11.63	0.31	1.63	0.32
Ju17	1.79	0.14	19.08	0.44	2.57	0.70
JA17	2.46	0.12	19.56	0.1	3.43	0.20
SO17	1.70	0.11	15.51	0.26	1.97	0.17
ND17	1.08	0.05	8.59	0.59	3.64	0.87
FM18	2.15	0.46	2.30	0.46	7.64	2.61

5.2 SPATIAL ORGANIZATION

Aggregation Ratio

Figure 10 shows the aggregation ratio compared to the different starting densities of November/December 2017 and February/March 2018. Contrary to what was expected, No trend in aggregation ratio is visible and it fluctuates over the different densities. Moreover the majority of the measurements is less than 1 (<1) which indicates that no aggregation took place and mussels have distributed more evenly over the area rather than aggregate or the mussels have grown which results in wider patches.

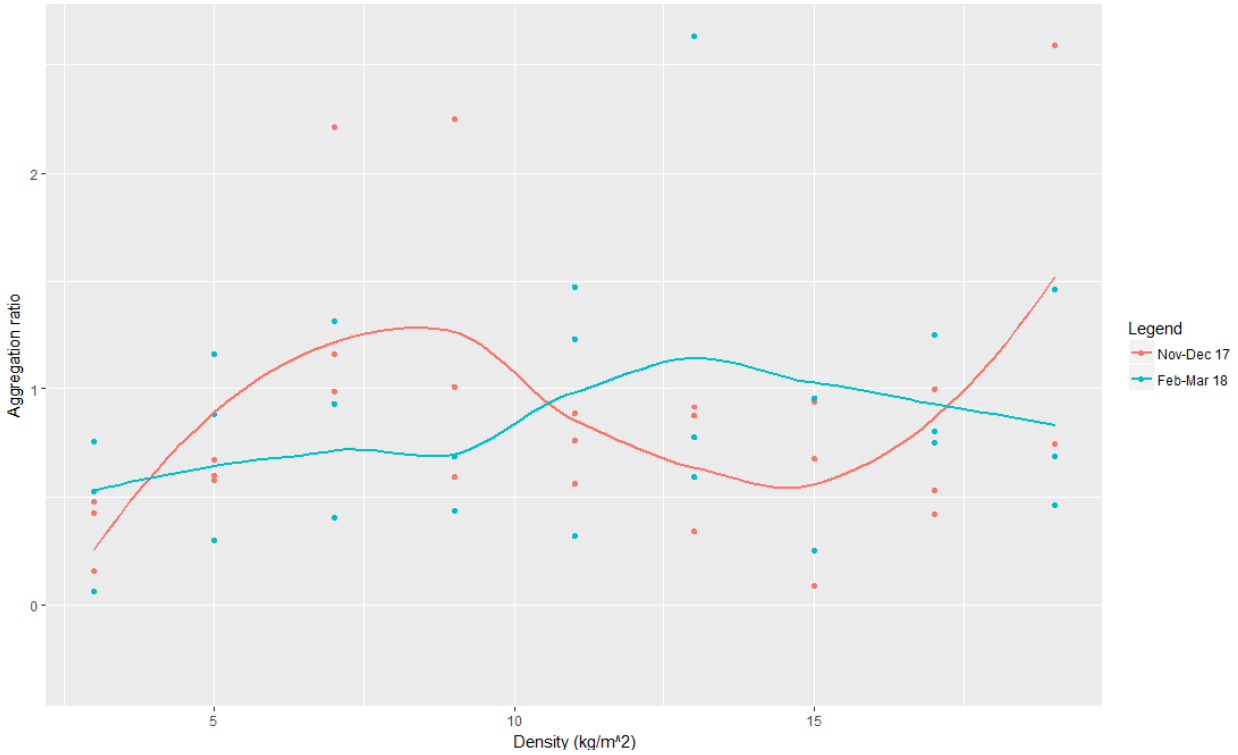

FIGURE 10, AGGREGATION RATIO OVER THE DIFFERENT STARTING DENSITIES. THE DIFFERENT COLORS INDICATE THE DIFFERENT EXPERIMENTS.

Patch complexity

the p/a ratio is visually compared between different starting densities In Figure 12. The different initial starting densities had a limited effect on the perimeter-to-area ratio, only the experiments that took place in July/august 2017 and March/April 2017 show a negative trend, with a decrease in p/a ratio at higher densities.

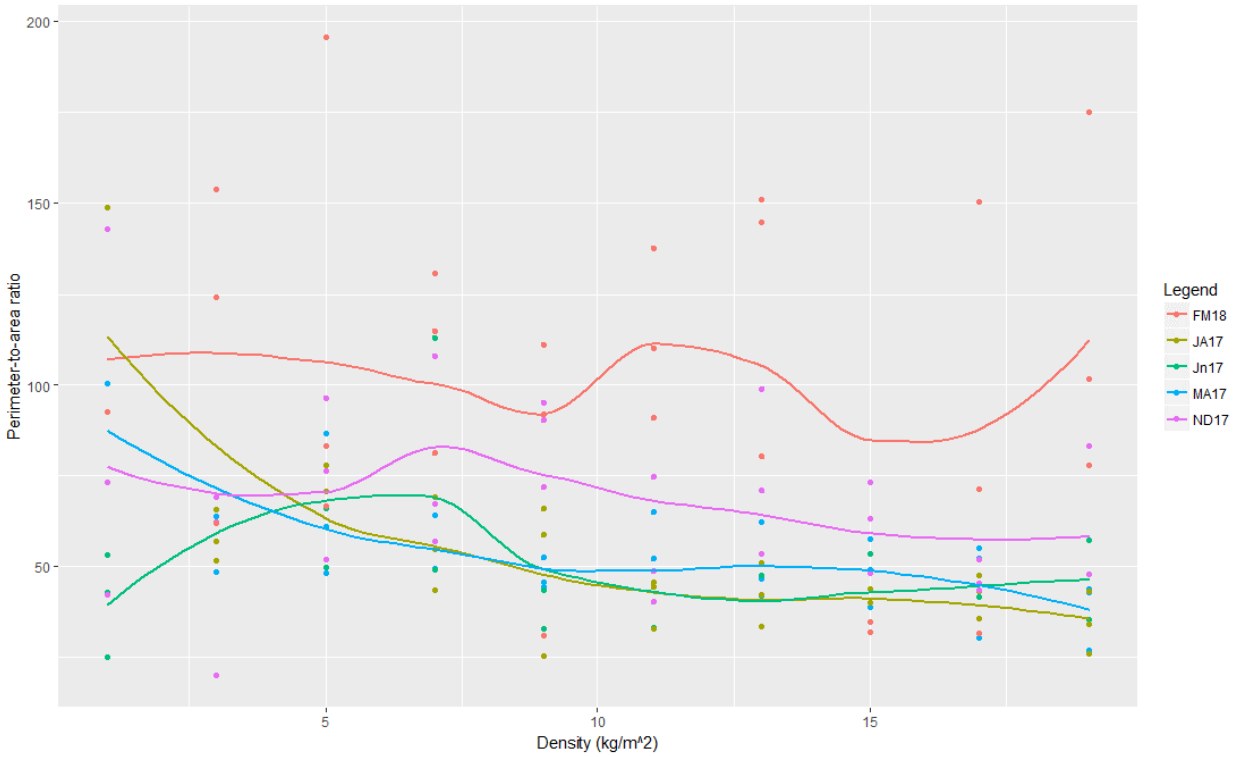

FIGURE 12, PERIMETER-TO-AREA RATIO COMPARED TO THE DIFFERENT STARTING DENSITIES.

If the p/a ratio is compared to temperature a clear negative trend is visible (Figure 11). The mussels in lower temperature formed more complex patterns than the mussels in higher temperature. This can be an indication that mussels are more active in lower temperatures compared to mussels in higher temperatures. No trend between the

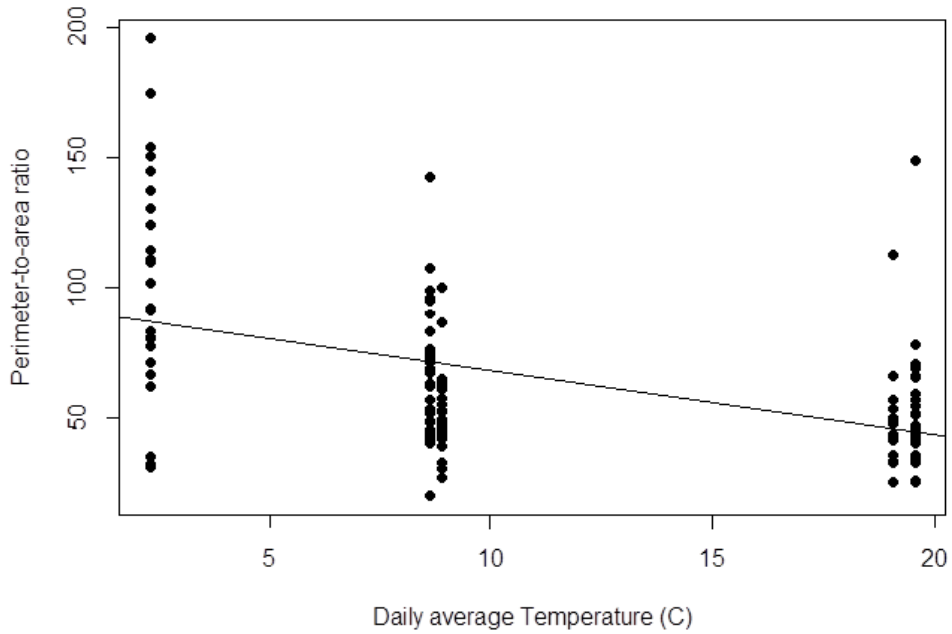

FIGURE 11, PERIMETER-TO-AREA RATIO COMPARED TO THE DAILY AVERAGE TEMPERATURE.

p/a ratio and chlorophyll-a level was found which indicates that the pattern formation is not influenced by food availability.

However if the p/a ratio is compared to the turbidity (Figure 13) it shows a higher p/a ratio with a higher turbidity this can be an indication that the water currents can influence the patch formations as the direction in which the patch will be formed or the water currents can force the formation into different patterns.

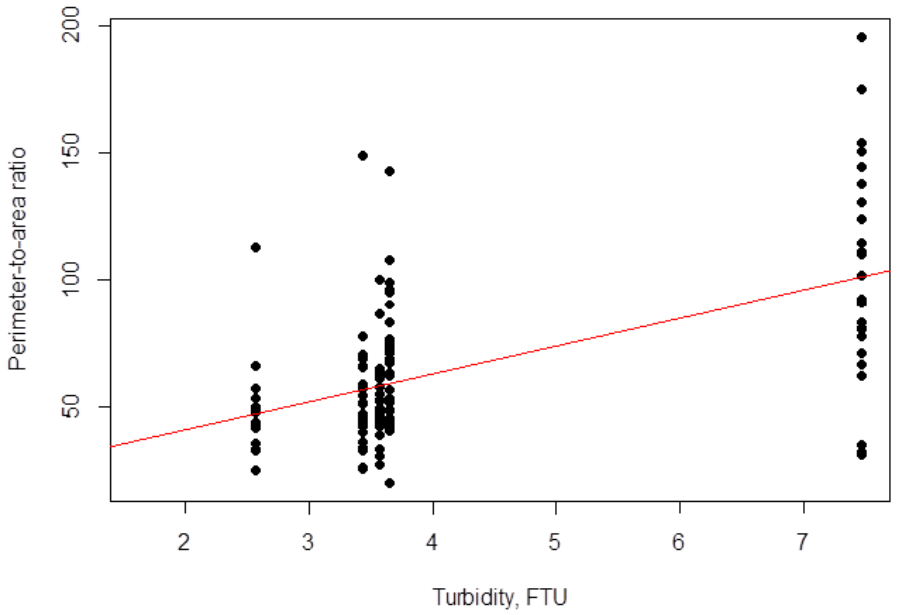

FIGURE 13, PERIMETER-TO-AREA RATIO COMPARED TO THE TURBIDITY.

5.3 Change in condition index (dCI)

Figure 14 shows the dCI over the starting densities. A trend is visible in the data from JA17, SO17 and FM18 where a more dCI takes place in the lower densities and the growth decreases until the densities 9, 11 kg/m^2 where it levels out and the growth of the mussels stays the same for the rest of the densities. This can be an indication of a threshold where the dCI is limited due to intraspecific competition for space. However the data from Ju17 and ND17 deviate from this trend as the growth stays more or less the same over the different densities.

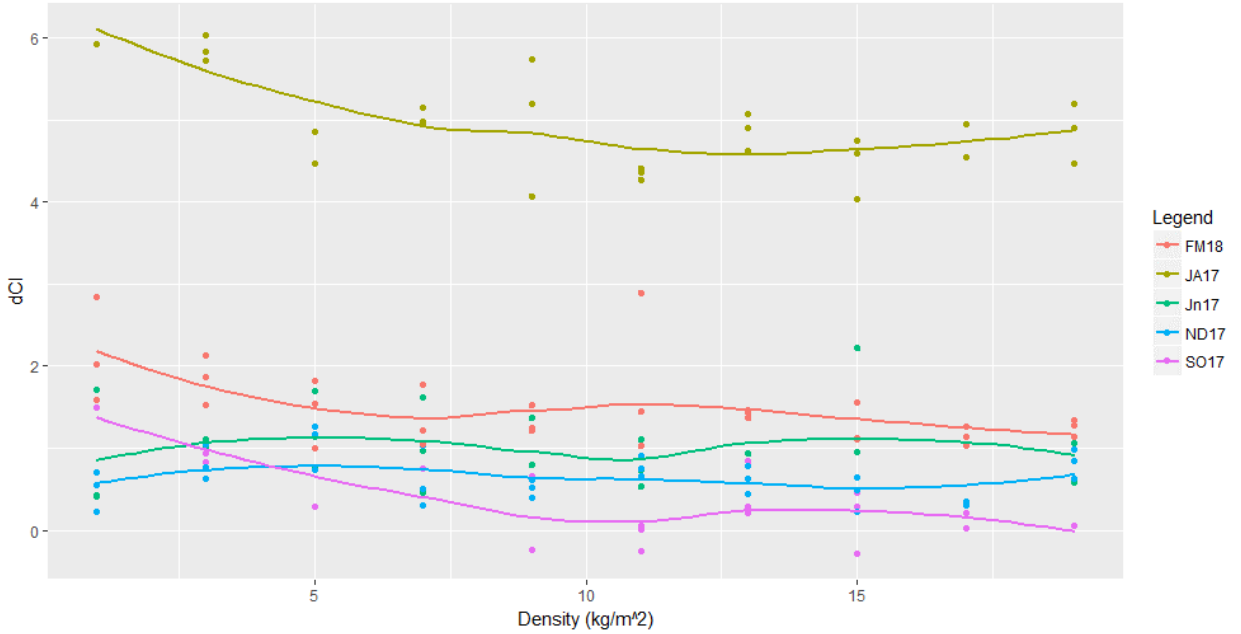

FIGURE 14, GROWTH COMPARED TO THE DIFFERENT INITIAL STARTING DENSITIES. THE COLORS INDICATE THE DIFFERENT EXPERIMENTS.

the dCI over the mean chlorophyll-a levels is shown in Figure 15. It appears that an exponential increase occurs with increasing chlorophyll levels. This indicates that the growth is strongly related to the amount of food that is availible.

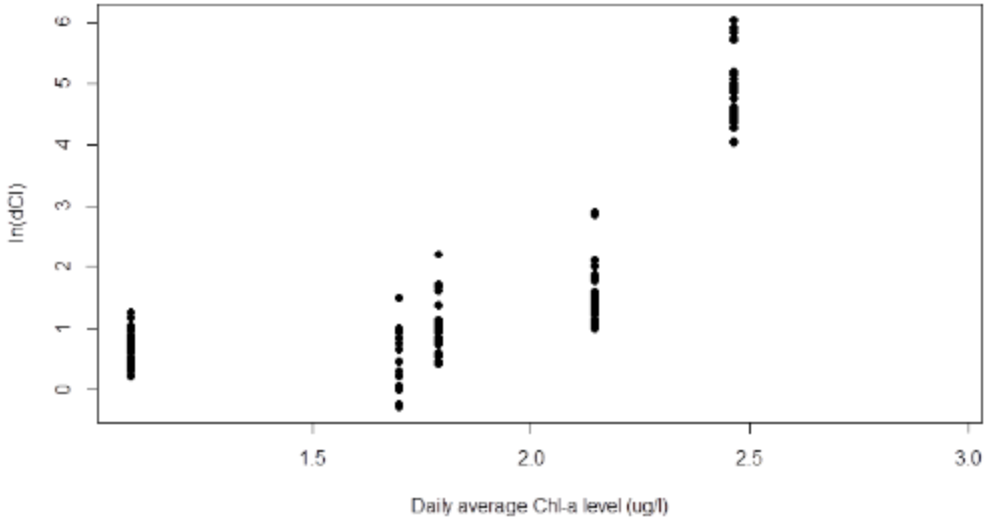

FIGURE 15, GRAPH OF THE GROWTH OVER THE MEAN CHLOROPHYLL LEVELS, THE GROWTH IS SET IN A LOG SCALE.

To explore if there are any differences in dCI over the chlorophyll levels within the different densities all the densities are put in a separate graph with the dCI and chlorophyll levels, an linear trendline is included in the graphs (Figure 16). The same positive trend is visible at every density, with an increasing chlorophyll levels an exponential growth occurs. Until a density of 15kg/m^2 the same growth takes place, only with the starting densities of 17,19 kg/m^2 there seems less difference in growth over the chlorophyll level.

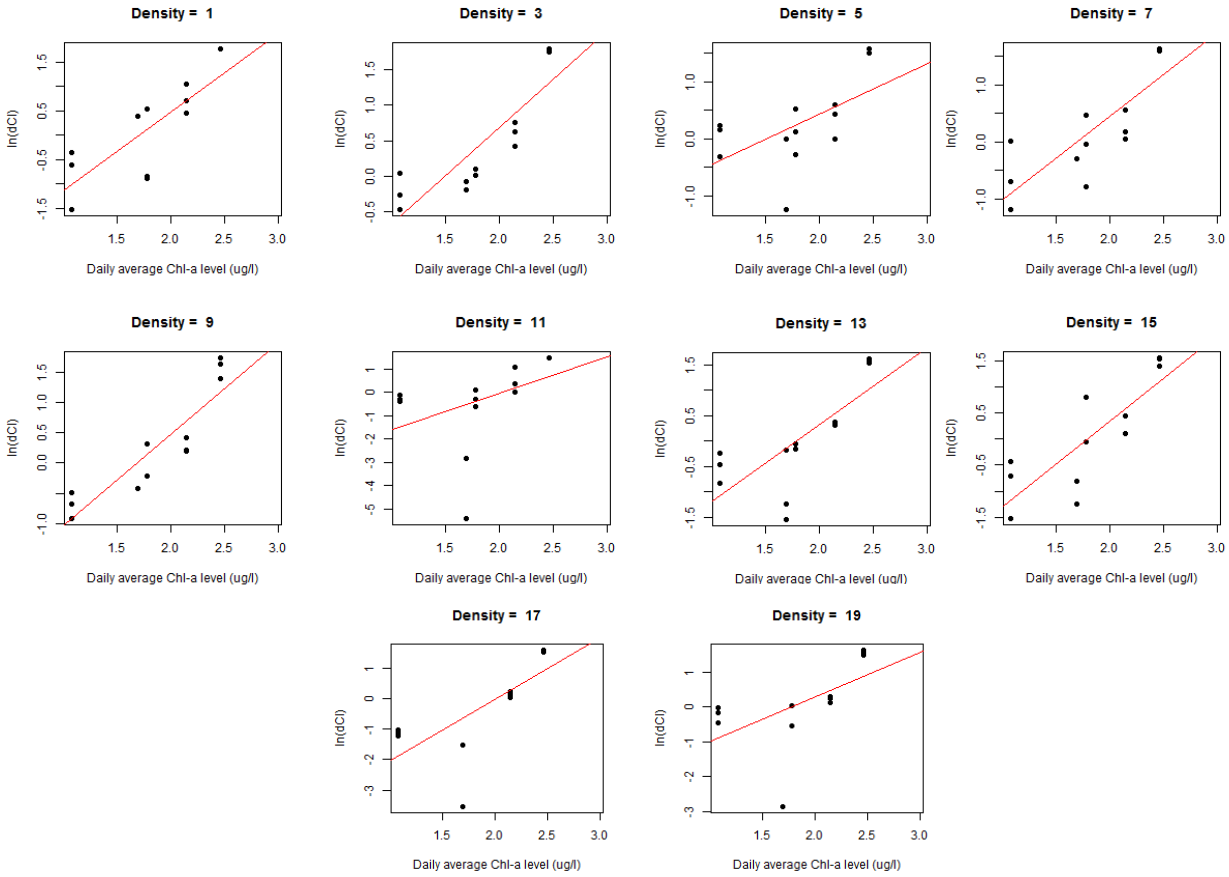

FIGURE 16, ALL THE DIFFERENT STARTING DENSITY WITH THE CHLOROPHYLL-A CONTENT AND DIFFERENCE IN CI (LOG SCALE) NOTE THAT THE Y-AXIS HAVE DIFFFERENT SCALES.

Position

Figure 17 shows a boxplot with difference in growth between the center and edge of a patch. The boxplot suggests that there is no real difference of growth in relation to the location of the mussels within a patch. The mussels in the center of the patch have more variation in dCI than the mussels at the edge of a patch. There is slightly more dCI in the center of the patch.

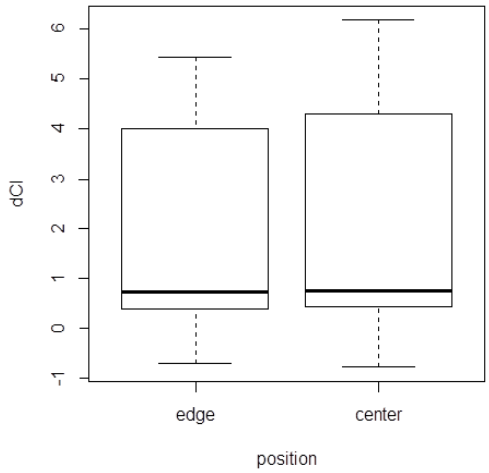

FIGURE 17, A BOXPLOT SHOWING THE DIFFERENCE IN GROWTH BETWEEN THE EDGE AND CENTER OF A MUSSEL PATCH. . WITHIN THE BOXPLOT THE MEDIAN IS SHOWN AS THE LINE WITHIN THE BOX, THE SPACE BETWEEN THE LOWEST POINT OF THE BOX AND THE MEDIAN IS 25% OF THE DATA THAT IS BELOW.

5.4 SURVIVAL

The survival of the mussels is an important factor for the mussel farmer to optimize yield as the farmers have to cope with high mussel losses. In Figure 18 the different starting densities are compared to the survival in percentages. The experiments SO/17 and ND17 show a similar trend. From the lower starting densities a positive trend is visible until 7kg/m^2 which is a threshold point from where the survival starts to decrease until 11kg/m^2, from where the survival stays stable. However the other experiments deviate from this trend especially experiment MA17 as this experiment shows an opposite trend where the survival increases with the higher densities.

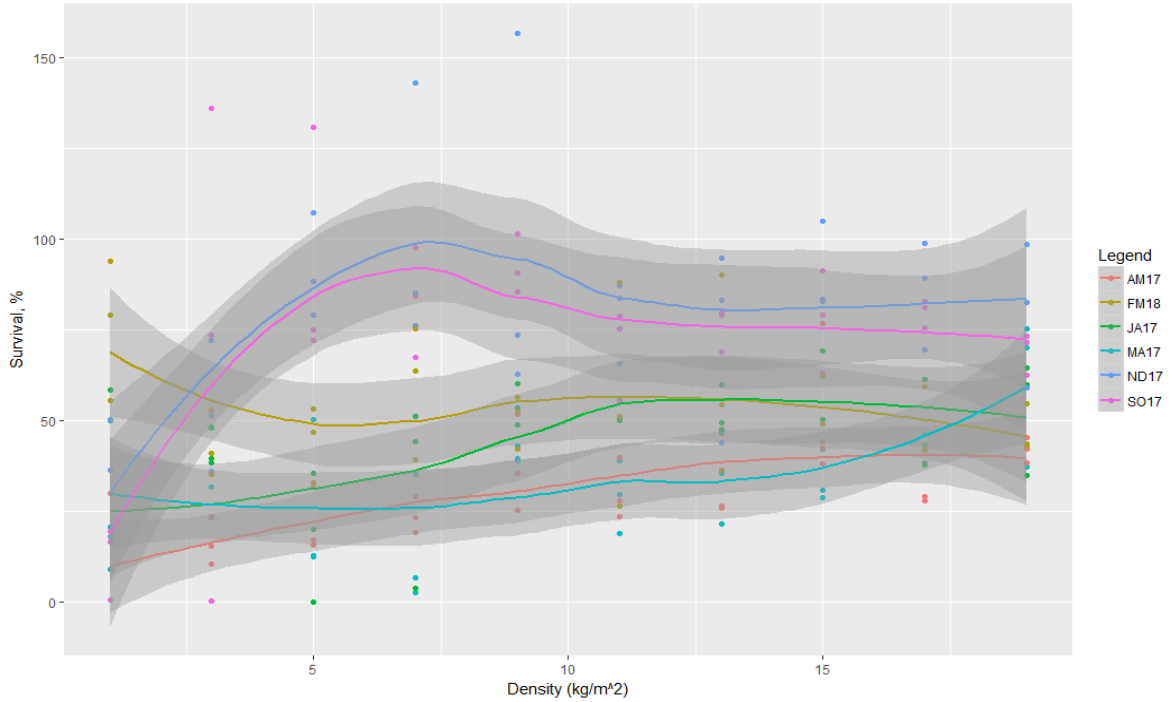

FIGURE 18, SURVIVAL OVER THE DIFFERENT STARTING DENSITIES, THE DIFFERENT COLORS INDICATE THE DIFFERENT EXPERIMENTS.

When this is further explored plotting survival against the chlorophyll levels (Figure 19) a negative trend is found. when chlorophyll-a levels are higher less mussel survive

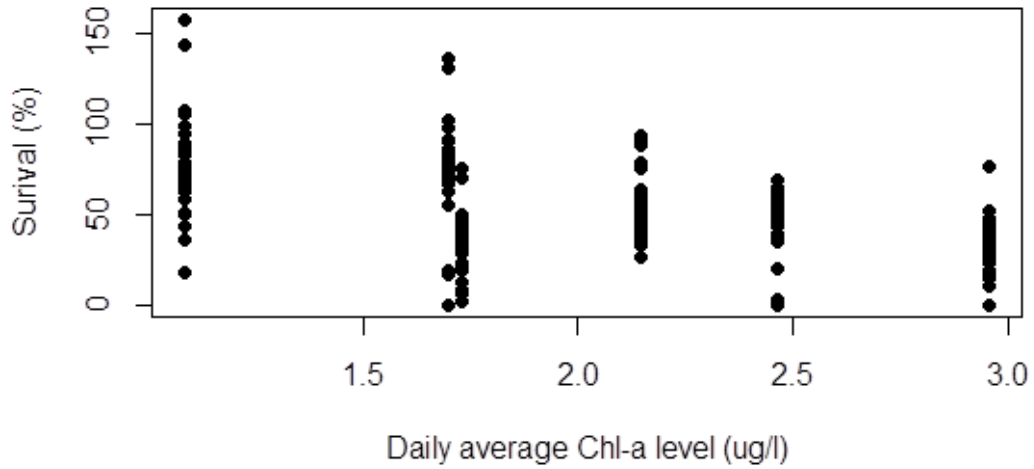

FIGURE 19, SURVIVAL IN PERCENTAGE COMPARED TO THE DAILY AVERAGE CHLOROPHYL-A LEVEL.

In Figure 20 the survival at different densities are plotted against chlorophyll-a level the same negative trend is seen over all the different densities but the negative trend increase when the density increase which suggest that when the chlorophyll and density increases it has negative effect on the survival of the mussels Which indicates a strong relation between survival and the food availability.

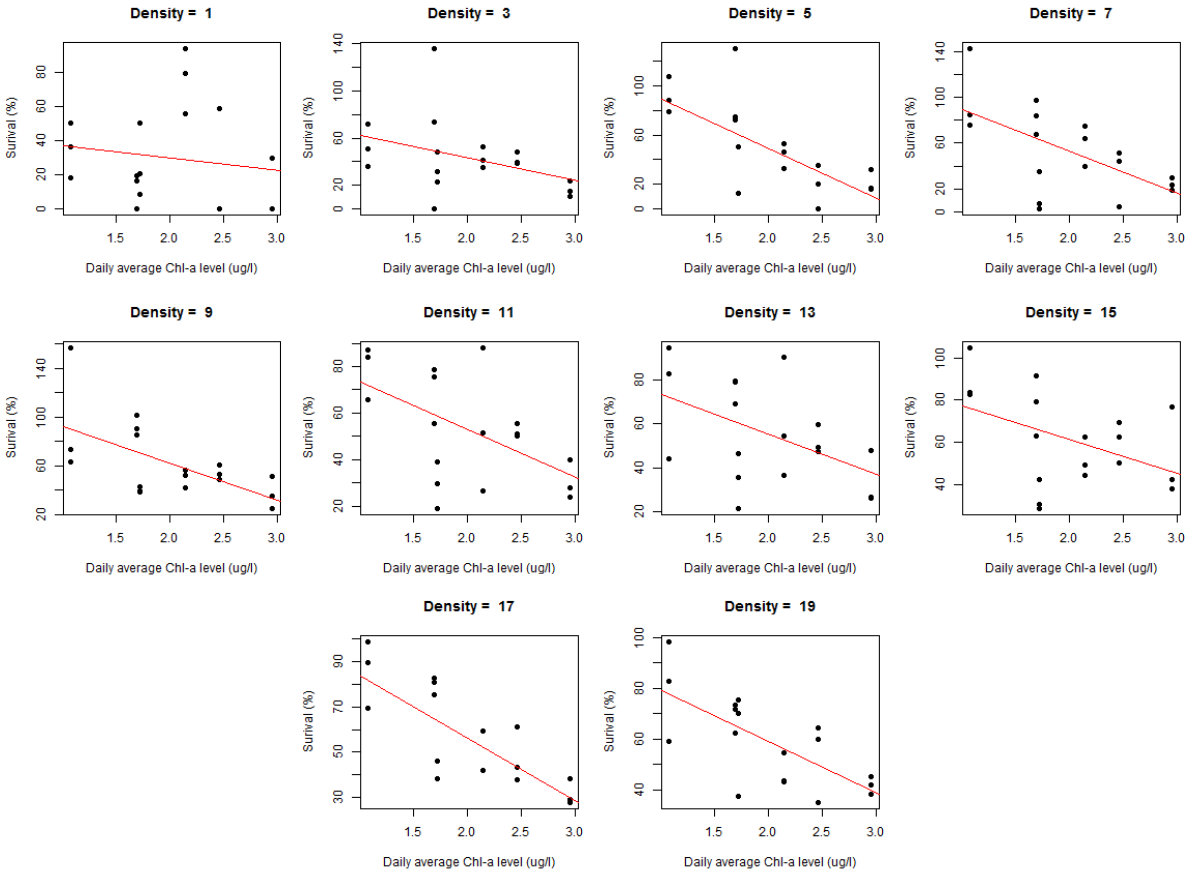

FIGURE 20, SURVIVAL COMPARED TO THE AVERAGE CHLOROPHYLL-A LEVELS FOR THE DIFFERENT STARTING DENSITIES. NOTE THAT THE SCALE OF THE Y-AXIS DEVIATES OVER THE DIFFERENT GRPAHS.

6. DISCUSSION

Spatial organization

On plot scale mussels within lower densities distribute in more patches while in higher densities mussel distribute around a large patch (Capelle, et al., 2014). Which was ground for the hypothesis: "with the lower starting densities the mussels will form a higher mean within patch density compared to the starting situation. At increasing densities, mean within patch density will approach the starting situation". However, By testing the spatial organization on a patch scale no trend between aggregation and starting densities is found but aggregation took place, which suggest that on a patch scale aggregation takes place but is not dependent in which starting density the mussels are in. But, influences from abiotic factors could have played a role in this outcome. Only 2 data sets were useable to test the aggregation ratio, because only picture were taken at the beginning of experiments ND17 and FM18. In which experiment FM18 had extreme conditions with a mean water temperature of 2.3 ^{0}C and a mean turbidity of 7.64 FTU due to storms that passed by. During an eastern storm an extreme low tide of -2.6m was measured and the experimental side was exposed. During this storm temperatures below 0 ^{0}C where measured and ice sheets observed in the eastern Scheldt. It is known that mussel patches can be lifted of its substratum and relocated due to ice forming (Snover & Commito, 1998; Bertness & Grosholz, 1985). these extreme conditions could have overruled the aggregation process and forced the mussels into different shapes. Figure 21 gives an example, these pictures where taken of the same gabion the picture left is at the start of the experiment and the right picture is taken 7 days later. A storm passed by where maximum wind speeds of 40 knots where measured (measured in Vlissingen, weergegevens.nl). The waves that where caused by the storm have probably caused turbulence within the gabions and forced the mussels to the side of the gabion.

FIGURE 21, MUSSELS WITHIN THE SAM GABION, THE PICURE LEFT IS AT THE START OF EXPERIMENT FM18 (21-02-2018) THE RIGHT PICTURE IS TAKEN 7 DAYS LATER (28-02-2018) ALMOST ALL THE MUSSELS ARE FORCED TO THE SIDE OF THE GABION.

In a model study of Van de Koppel et al (2005) mussel beds with pattern formation can withstands lower phytoplankton concentrations than dense homogenous beds. On patch scale the same effect was expected and formulated as a hypothesis: the mussels will form patterns if space is available, to increase turbulence over the patch for a more even food distribution of phytoplankton. And is not affected by food levels. To determine the patch complexity the perimeter to area ratio of the patches was determined at the end of every experiment. A declining trend was found between the p/a ratio and the starting densities from the starting density of 7kg/m^2. This declining trend can be an indication that lack of space within a patch withholds the mussels from forming patterns. The only deviation from this trend was experiment FM18 but can be explained due to the extreme condition this experiment encountered. No trend was found between the p/a ratio and the chlorophyll-a levels which is in contradiction with the hypothesis, and that the patterns within a mussel bed are formed for a more even distribution of food over the

mussels (Van de Koppel, et al., 2005). The pattern formation seems more influenced by abiotic factors as with lower temperatures a higher patch complexity is formed and this also counts for turbidity. There seems to be a relation between temperature and turbidity, in autumn and winter time more storms pass by which increase water currents and influences the patch formation which indicates that the spatial is not influenced by food availability but rather by water flow.

Somatic growth/change in condition

In this paper somatic growth is measured as change in condition index over the experimental period. It is found that mussels in higher densities often have a lower growth rate than mussels within lower densities, when food becomes limited (Bertness & Grosholz, 1985; Okamura, 1986). Therefore, it was expected that "at increasing densities the growth rate will decrease due to competition, and a higher growth rate and competition will occur in seasons with higher food levels (spring & summer)". This trend is evident at most trials. The somatic growth decreases until a starting density of 9/11 kg m^2 from where it levels out at higher densities. this decrease in somatic growth and leveling out of higher somatic growth at higher densities can be an indication of intraspecific competition (Van de Koppel, et al., 2008; Frechette, et al., 1992; Bertness & Grosholz, 1985). Only the experiments Jn17 and ND17 deviate from this trend and show a comparable growth at all the different starting densities. This deviation can be explained due to low chlorophyll levels during the experiment of ND17 and the mussels that came from the Wadden sea for experiment Jn17 and more likely were not physiologically adapted to the condition in the Eastern Scheldt (Bayne, et al., 1987)

It is obvious that with more food availability a higher growth rate occurs (Alunno-Bruscia, et al., 2000) A probable exponential trend is found between the change in condition index of the mussels and the chlorophyll-a levels. Within the different densities the same pattern occurs, with the higher densities (17/19 kg/m^2) the growth seems less influenced by the chlorophyll level as there is less difference in somatic growth between the different experiments. Competition for food and space increases with density which leads to decrease in growth rate (Bertness & Grosholz, 1985; Frechette et al., 1992).

No difference between growth and position within a patch (edge vs center) was found which means that no support was found for the hypothesis that "at increasing patch size less growth occurs in the middle of the patch, but at small patch scale only when food levels and temperature are low". Which is in contradiction with Svane & Ompi (1993) who studied patches within a mussel bed and suggest that the position within a patch has an effect on growth rate and that the mussels in the edge of a patch have a higher growth rate than the mussels in the center of a patch. This is a result of depletion of phytoplankton concentration over a patch (Svane & Ompi, 1993). Because the experiments were on a patch scale without a mussel bed surrounding it, the effect of food depletion over a patch would be minimal. Also the light wire netting that is attached to the gabions could have caused a turbulence in the water flow over the patches and resulted in an increased food supply.

survival

At decreasing food levels and an increasing mussel density a higher mortality will occur due to food depletion (Alunno-Bruscia, et al., 2000) also within higher density because of intraspecific competition (Frechette, Aitken, & Page, 1992). This is also stated in the hypothesis: Losses are affected by competition and facilitation: with higher patch densities mussel losses will increase, due to competition especially if food levels are low and food depletion will occur over the patch. however, if the density is to low the mussels will not be able to facilitate each other and higher mussel loss will occur as well. The experiments SO17 and ND17 show a trend that corresponds with the hypothesis, the survival is low with lower densities but increases with higher densities until a starting density of 7kg/m^2 which seems a threshold density because when the density increases survival decreases until a starting density of 11kg/m^2 from where the survival stays more or less the same over the remaining densities. However the

other experiments do not show this trend. The trend which occurs in SO17 an ND17 may be seasonal influenced as the temperature and chlorophyll-a level gradually drops while with the other experiments the temperature and/or chlorophyll-a level rises or stayed the same this can be an indication for the different trends.

Chlorophyll-a level show a negative trend with survival, which deviates from the hypothesis and the literature (Alunno-Bruscia, et al., 2000). With lower starting densities (1/3kg/m^2) a small negative trend in survival and chlorophyll levels is found this suggests that within the lower densities the chlorophyll-a levels did not affect the survival but that the mussel could not facilitate eachother within such a low density (de Jager, 2015; Van de Koppel, et al., 2008). Within the higher density (5 to 19 kg/m^2) a more obvioud negative trend is found. this can be an indication that within this density the mussels can facilitate eachother. However with higher food levels more growth occurs what eventually can lead to more intraspecific competition for space (crowding). This intraspecific competitition can lead to a higher mortality (Svane & Ompi, 1993).

7. CONCLUSION

No relation between the starting densities and the aggregation process was found so no evidence was found for the hypothesis that aggregation activities will take place more within the lower densities when there is space available, within higher densities less aggregation activities will take place due to lack of space. However, only the data of two experiments could be used to test the hypothesis. More research is necessary to find out if there is a relation between aggregation activities and the different starting densities on patch.

For the hypothesis: there will be a threshold density in which there is an optimal performance of the mussel with intraspecific competition in which there is a limited loss in mussels. This threshold density can fluctuate over the season as the food levels change. the optimal patch density seems to be formed between 7/9 kg/m^2 within this starting density the highest survival was found from this density the change in CI stayed the same over the different experiments. This density does not seem to fluctuate over the seasons but the season has effect on the growth and survival within a patch.

8. RECOMMENDATIONS

8.1 RECOMMENDATIONS FOR MUSSEL CULTURE

As a recommendation for the mussel culture it is important to keep track of the weather for the coming week when seeding the mussel seeds. Strong winds can be a source for strong currents in the water which may flush away the mussel seeds, as it this study suggest that the spatial organization is influenced by water currents. Furthermore it seems to be important to keep the seeding density between 7/9kg/m^2 as this seemed that this was the density with the least mortality, however this density can be different on a plot scale. With growth the mussels will have intraspecific competition for space which will lead to a lower yield. Also the season seemed to be an important factor on the survival of the mussels so it is recommended to seed in fall as this was the period whit the least mortality.

8.2 recommendations for further research.

It is recommended to further conduct this research to find if the same trends occur within the same season. When repeating this experiment it will be recommended to use bigger gabion to prevent the mussels to use the side of the gabions as a hard substrate to attach to. It would also be important to improve the way how the gabions close as many crabs where found while emptying the gabions. It is also recommended to keep attention to the weather forecast when putting the mussels in the gabions as with strong currents the mussels will be pushed to one side of the gabions. Furthermore it is important to clean the gabions before putting the mussels in the gabions and to find a way to keep the gabions clean of algae and seaweed without interfering the experiment as it is important to take clear photographs of the mussels at the beginning and the end of every experiment.

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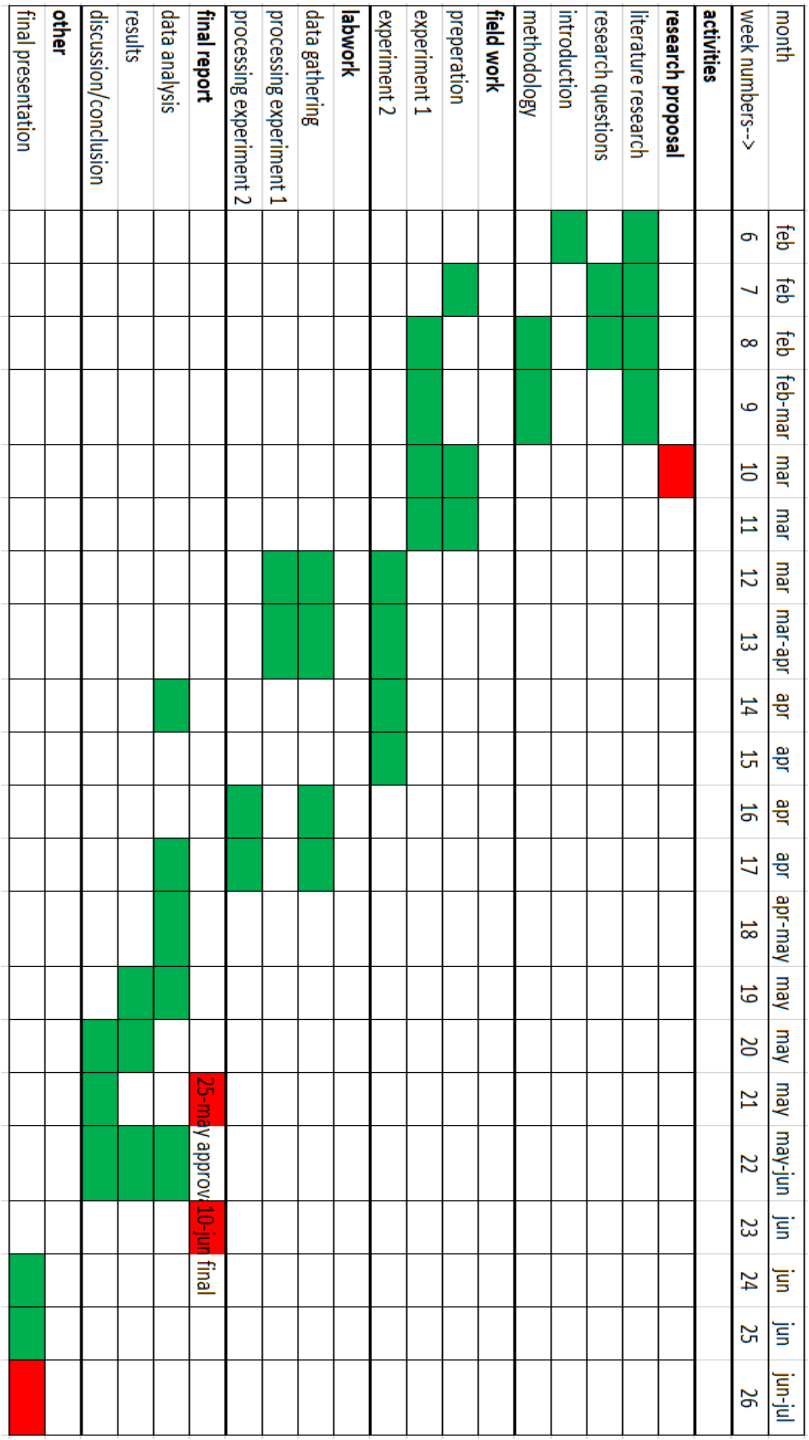
APPENDIX I – PLANNING



APPENDIX II – PROTOCOL FOR DETERMINING THE ASH FREE DRY WEIGHT OF CRUSTACEANS.

Werkvoorschrift: bepalen asvrijdrooggewicht van schelpdieren met moffeloven

Document nummer: 2.16.2.07
Procesbeschrijving: -

1. ONDERWERP EN TOEPASSINGSGEBIED Section Break (Continuous)

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

2. TERMEN EN DEFINITIES

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

3. BEGINSEL

Schelpdieren worden met of zonder schelp gedroogd en verast met behulp van droogstoven en een moffeloven. ~~Uitvoering is conform ISO 5984 (1978) en NEN 3323 (1969).~~

4. Apparatuur er hulpmiddelen

4.1 Moffeloven bereik tot minimaal 600 °C
4.2 Droogstoof minimaal 150 °C
4.3 Exicator met actieve silicagel
4.4 Analytische balans
4.5 Hitte bestendige bakjes of kroezen, genummerd.
4.6 Roestvrijstalen plateaus voor deze bakjes voor plaatsing in droogstoof
4.7 Speciale metalen plateau voor in moffeloven
4.8 Hittebestendige handschoenen
4.9 Metalen tang om bakjes en plateau mee vast te pakken

5. Werkwijze

5.1 Voorbereiding

1 Kalibreer de balansen met de gewichten die bij de balans staan.
2 Noteer de waarden op de aftekenlijsten.
3 Zet de computer aan. (gebruikersnaam en wachtwoord staan op een sticker op de computer)
4 Start Excel en Mettler balance op door deze dubbel aan te klikken.
5 Maak een lijst in Excel met daarin de: sample, kroes nr., as1, netto as1. Bijv.:

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

6 Let erop dat het drooggewicht van de samples al bepaald is en genoteerd staat. (zie voorschrift drogestof bepaling bij schelpdieren versie 2).

5.2 Verwerking samples

1 Zet de moffeloven op 520°C (was 560°C). Dit doe je door aan de tweede blauwe knop (van links) te draaien. Om te weten hoe warm het binnen in de oven is moet de tweede blauwe knop iets uitgetrokken worden. Als de oven al aan staat deze eerst af laten koelen tot beneden de 200°C.
2 Zet de te bepalen samples op een speciale metalen ovenschaal voor in de moffeloven.
3 Schuif vervolgens het plateau met samples in de moffeloven.
4 De moffeloven gaat aan wanneer je de eerste blauwe knop (links onderaan) naar rechts draait. (let op de oven gaat dan langzaam zichzelf opwarmen tot het aangegeven aantal graden)
5 De oven warmt zich op tot de juist ingestelde aantal graden en de samples zullen er, afhankelijk van de hoeveelheid, wordt nog uitgezocht) 2 tot 10 uur in (er stond 8 uur)

¶

moeten·blijven·staan.·Kontroleer·het·as,·dit·moet·grijs·verbrand·zijn.·Als·het·nog·teerachtig·zwart·is·de·verastijd·verlengen.·¶

6 → De·volgende·dag·is·de·oven·afgekoeld·en·kunnen·de·kroezen·eruit·gehaald·worden·en·in·de·droogstoof·van·70°C·geplaatst·worden·voor·60·minuten.·Als·je·meerdere·series·doet·de·kroezen·eerst·van·de·hete·speciale·ovenschaal·overzetten·op·de·daarvoor·bestemde·metalen·plateaus.·De·hete·ovenschaal·niet·in·de·stoof·zetten·want·daardoor·kan·de·temperatuur·in·de·stoof·plaatselijk·te·hoog·worden.¶

7 → Hierna·kunnen·de·kroezen·60·minuten·afkoelen·in·de·exicator.·.·Zet·de·samples·in·een·exicator·met·het·ventiel·open,·draai·het·ventiel·na·30·seconden·dicht·en·de·exicator·trekt·vacuüm.¶

¶

5.3·Weging·samples→¶

1 → Weeg·de·bakjes/kroezen·met·het·as.¶

2 → Zet·de·gevonden·waarden·in·het·Excel·bestand·en·bereken·het·netto·verschil·uit·in·procenten.¶

¶

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

¶

3→ Is·er·een·afwijking·gevonden·die·groter·is·dan·1%·dan·moet·de·kroes·nog·3·uur·in·de·oven·verhit·worden·op·560°C.·Let·op·de·oven·zal·warm·zijn·na·die·3·uur.·Gebruik·dan·hitte·bestendige·handschoenen,·tang·en·de·plank·met·gaas·dat·op·de·stoof·staat.·¶

4→ De·kroes·gaat·daarna·60·minuten·in·de·stoof·en·vervolgens·60·minuten·in·de·exicator.¶

5→ Hierna·kan·het·ruw·as·gewicht·berekend·worden.·¶

6→ De·stappen·vanaf··5.2·worden·herhaalt·tot·de·afwijking·minder·dan·1%·is.¶

Nog· herzien,· gebaseerd· op· 560°C· maar· door· kalk· verbranding· misschien· lang· instabiele· gewichten.¶

6. → Berekeningen¶

6.1·Netto·asvrijdrooggewicht:¶

Bruto·drooggewicht·–·gewicht·kroes·¶

¶

6.2·Percentage·afwijking:¶

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

¶

6.3·Asgewicht¶

Asvrijdrooggewicht·-·Drooggewicht¶

¶

6.4·Voorbeeld·spreadsheet:¶

Sample¤	kroes·(=nr)¤	Gewicht·kroes·(g)¤	Gewicht·kroes·+·ovenvocht,·Sample·1·(g)¤	Netto·ovenvocht,·Sample·1·(g)¤	Gewicht·kroes·+·ovenvocht,·Sample·2·(g)¤	Netto·ovenvocht,·Sample2·(g)¤	Verschil·(%)¤	Asvrije·drooggewicht¤	Asgewicht¤	¤
bv:·mossel¤	5¤	20.01¤	31.22¤	=(31.22-20.01)¤	30.11¤	=(30.11-20.01)¤	=(((11.21-10.10)*100)¶/11.21)¤	10.1¤	=(20.01-10.10)¤	¤

¶

7. → BIJLAGEN·EN·VERWIJZINGEN¶

Literatuur¤	Titel¤
Drooggewicht¤	WERKVOORSCHRIFT·VOOR·DROGESTOF·BEPALING·BIJ·SCHELPDIEREN¶ ¤