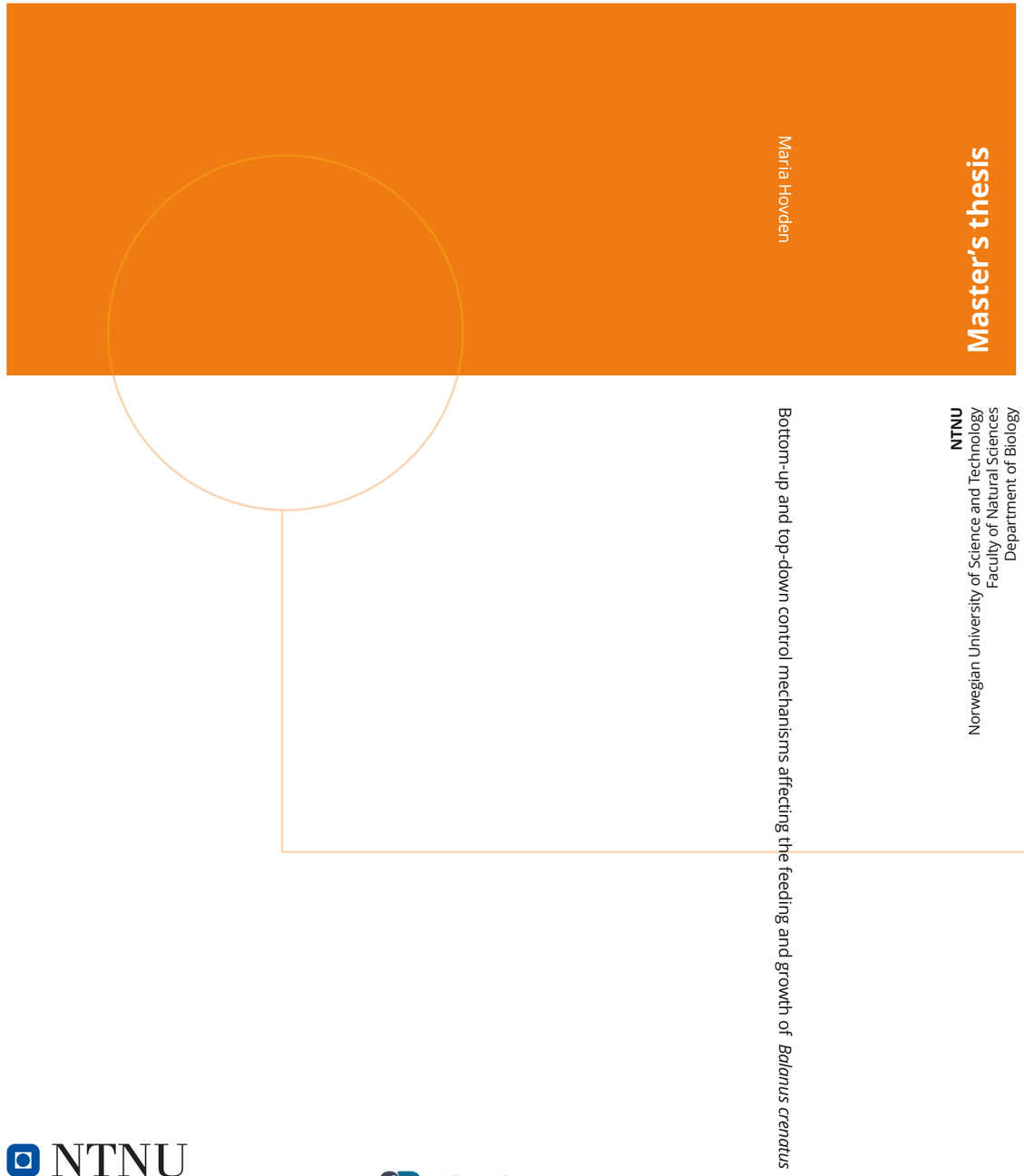

Maria Hovden

Bottom-up and top-down control mechanisms affecting the feeding and growth of the cultivated barnacle *Balanus crenatus*

Master's thesis in Ocean resources, Ecosystems – Biology, ecology and biogeochemistry
Supervisor: Nicole Aberle-Malzahn
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Abstract

Cultivating the barnacle *Balanus crenatus* to produce nauplii as start feed for marine juveniles has proven to be a challenge. The problem is not being able to cultivate them but rather to fully understand their biotic and abiotic conditions to ensure survival during the winter months before harvest.

The aim of this thesis was to study bottom-up and top-down control mechanisms that affect the survival and growth potential of the cultivated barnacle *Balanus crenatus.* Barnacles cultivated at Hemnesberget, Norway, were monitored through the seasons 2020-2021 and 2021-2022. Two Top-down experiments were conducted using the predators *Asterias rubens* and *Nucella lapillus,* on juvenile and adult barnacles. One Bottom-up experiment was conducted to study the growth potential of barnacles, using a dry formulated salmon-fry feed, frozen *Semibalanus balanoides* nauplii and a control group that were not fed. The non-fed group gave insight in the barnacles ability to withstand starvation. In addition to the experiments, six cultivation bands were counted and categorized for live and dead barnacles to get an understanding of where in the water column mortality occurred, and to see how salinity and temperature could have affected their performance.

The counting of live vs dead barnacles showed that the survival seemed to be higher in the upper water layers with lower salinity (-1, -3 meters). *A. rubens* salinity tolerance could explain the pattern of mortality along the cultivation bands, but in situ studies are needed to confirm this as a main cause. The Top-down experiments proved that there was a significant difference in ingestion rate between the predators in both experiment 1 and 2, and that *A. rubens* exerted a high predation pressure, consuming on average six juvenile barnacles, or two adult barnacles per day. In comparison *N. lapillus* consumed on average three juvenile barnacles or one adult barnacle per day. The mean ingestion ratio between the predators was 38 % (SD 15%) on juvenile barnacles, for adult barnacles a mean ingestion ratio was 34 % (SD 11%).

The results from barnacle growth rates in the bottom-up experiment, showed that the Zooplankton treatment had the overall best results both on shell growth and showed the highest increase in gonad mass in addition to an impressing increase in gonadosomatic index (GSI) of 16% compared to the control group. The carbon-to-nitrogen (C:N) ratio of the feeds did not translate to the C:N ratio of body tissue and gonad biomass, indicating the particle size of the Salmon-fry feed was possibly being too small for the barnacles to catch, resulting in lower C:N values for the Salmon-fry fed barnacles.

The barnacles fed Zooplankton and Salmon-fry feed grew significantly in shell size compared to the Control group. Zooplankton fed barnacles also grew in gonad mass and body tissue in relation to their shell size, while Salmon-fry feed barnacles maintained similar gonad mass and body tissue mass from start to end of the experiment. The gonad and body tissue of the Control group decreased. The gonadosomatic index of the Control group increased 1% (to 43%) compared to the Start-population which had a GSI of 42%, whereas Zooplankton and Salmon-fry fed barnacles had a GSI of 59% and 47%, respectively. In this experiment, the natural zooplankton diet gave the best results in shell growth and production of gonad mass through late fall/early winter. Salmon-fry feed also shows an overall increase in gonad mass when taking increased shell size and GSI into consideration.

The results in this thesis gave valuable insight to some of the factors controlling growth and mortality, but no concluding results was made as to why the barnacles at the Hemnesberget suffer from high mortality in water layers with higher salinity.

Sammendrag

Kultivering av skipsrur (*Balanus crenatus*) som blir benyttet til produksjon av levende-fôr til marin fiskeyngel, har vist seg å være utfordrende. Problemet er ikke å få dem til å vokse, men å forstå de biotiske faktorene som påvirker overlevelse gjennom vintermånedene før høsting.

Denne oppgaven har hatt som mål å se på ovenfra og ned- og nedenfra og opp styringsmekanismer for overlevelse og vekst potensial hos skipsruren ved å studere kultiverte skipsrur fra Hemnesberget, Norge gjennom sesongene 2020-2021 og 2021-2022. Dette ble gjort ved å utføre to ovenfra og ned predasjonsstudier på skipsruren. Her fikk predatorene *Asterias rubens* og *Nucella lapillus* fri tilgang på små nyslåtte- og på voksne rur. Et nedenfra og opp forsøk ble også utført for å se på rurens vekst potensiale og sult toleranse, ved å bruke tørrfôr beregnet på laksesmolt og fryste nauplier fra *Semibalanus balanoides*. I tillegg til forsøkene ble seks lengder med kultiveringsbånd talt og kategorisert ut fra overlevelse for å øke forståelsen av hvor i vannsøylen dødeligheten var høy. Salinitet og temperaturmålinger ble sett på sammen med dødeligheten langs båndene for å se om det kunne indikere en sammenheng.

Tellingen av ruren på kultiveringsbåndene viste at overlevelse ser ut til å følge de ferskere overflatevannmassene (-1, -3 meter). *A. Rubens* toleransegrense for lav salinitet kan forklare mønsteret for dødelighet langs kultiveringsbåndene, men det trengs *in situ* forsøk for å kunne bekrefte *A. Rubens* som hovedårsak. Predasjons eksperiment 1 og 2 viste at det var en signifikant forskjell mellom predatorene i begge eksperimentene. *A. rubens* viste et høyt predasjonstrykk og konsumerte gjennomsnittlig seks stykk små nyslåtte rur eller to voksne rur per dag. *N. lapillus* konsumerte omtrent halvparten, med tre stykk nyslåtte rur eller en voksen rur per dag. Den konsumerte gjennomsnitts ratioen mellom predatorene på små rur var 38% (ST 15%) og for voksen rur 34% (ST 11%).

Vekst resultatene fra fôringsforsøket viste at Zooplankton ga gjennomgående best vekstresultater, både for skallvekst og økning i mengde gonade, som ga en imponerende økning i Gonadosomatic index (GSI) på 16% sammenlignet med kontrollgruppen. Karbon-til-nitrogen (C:N) ratio resultatene fra fôret var ikke reflektert i C:N resultatene fra rurens

kroppsmasse og gonade i de ulike forbehandlingene. Det mistenkes at partikkelstørrelsen til Smoltfôret var for liten til at ruren fikk tak i fôret og resulterte i lavere C:N verdier for ruren fôret med smoltfôr.

Ruren som fikk Zooplankton og Smoltfôr hadde en signifikant økning i skallvekst sammenlignet med kontrollgruppen. Ruren foret på Zooplankton økte også i gonade og kroppsmasse i relasjon til skallstørrelse, mens ruren foret på Smoltfôr beholdt den samme størrelsen. Kontrollgruppens kroppsstørrelse og gonademengde krympet. Kontrollgruppens GSI økte med 1% etter syv ukers forsøk, sammenlignet med start-populasjonen fra uke 0 som hadde en GSI på 42%. Her hadde Zooplankton 59%, og den Smoltfôrede ruren 47%. Disse resultatene viste at et naturlig fôr som zooplankton ga de beste resultatene for skallvekst, og økning i gonademasse gjennom fôring i høst/vintermånedene. Smoltfôret viste også en økning i gonademengde når den økte skallveksten og GSI tas i betraktning.

Resultatene fra de utførte eksperimentene ga en innsikt i faktorer for vekst og død, men ingen endelig konklusjon kan trekkes som hovedårsak for dødeligheten i de mer saltholdige vannmassene hvor ruren kultiveres.

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I would like to thank Planktonic AS for the opportunity to write my master thesis in collaboration with them. In addition to supplying me with all the barnacles I needed for my study, they founded most of the economical expenses connected to it.

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Trondheim, May 2022

Maria Hovden

List of abbreviations

NTNU	Norges teknisk-naturvitenskapelige universitet
TBS	Trondhjem Biologiske Stasjon
PVC	Poly vinyl cloride
C:N	Carbon and Nitrogen analysis
GSI	Gonadosomatic index
B:S	Body tissue biomass (B) to shell size (S)
G:S	Gonad biomass (G) to shell size (S)

Explanation of shortened vocabulary

Barnacle	Referring to: *Balanus crenatus* in this thesis, unless otherwise stated
Zooplankton	Referring to frozen nauplii from *Semibalanus balanoides* (not cryopreserved)
(B)	Body tissue biomass
(G)	Gonad biomass
(S)	Shell size

1 Table of contents

All photographs, lists and figures are by Maria Hovden unless otherwise stated.

2 Introduction

Aquaculture and a better utilization of the marine environment is thought of as one of the solutions to feed the increasing population on earth, and especially aiding in the production of a healthy protein source. The Norwegian coastline is blessed with good conditions for fishing and production of seafood of high quality, and salmon farming has been a success story both for the national and local economy. (Nærings- og fiskeridepartementet, 2021)

However, according to Hagemann *et al.*, (2021) cultivating marine species can be challenging, and providing the larvae a good start has shown to be really important for health and good growth later in life. Even though the knowledge of cultivation of fish and marine larvae has grown alongside the increased amounts of fish produced in the world today, the mortality of marine fish larva is still high and feeding regimes are still under development. The mortality could be reduced by ensuring the fish larva gets food that is appropriate for them at the right time. Today rotifers and *Artemia salinas* are widely used as first live feed, although they are not considered to be an ideal solution. A relatively new feed, made from cryopreserved barnacle nauplii has shown good results e.g., for the liver and gut health in feeding trials with ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*). These early developmental stages of liver and gut are really important for later development, growth and generally good fish health (ibid).

Planktonic AS is the first company to commercially cultivate barnacles for production of start feed for marine juveniles and has developed a way to cryopreserve the nauplii of *Balanus crenatus* and *Balanus balanoides* to replace rotifers and *Artemia sp*. (Planktonic AS, no date a). Their cultivation site at Hemnesberget (Norway) is on the one hand side, good growth of the barnacles can be achieved on this site. However, high losses caused by mortality during the winter months in the deeper saltier water layers also occur. The cultivation of barnacles as live feed is still in its infancy, with the first cultivation season in 2018/2019. But with good feedback from fish farmers and good results in feeding trials it has a high potential to be one of the preferred first-feeding diets for aquaculture

industries (Nils Tokle, personal communication 15.010.2021; Hagemann *et al.*, 2021). But to make this feed a success, they depend on the survival of their barnacles.

2.1 Objectives

The objective of this thesis have been to study bottom-up and top-down control mechanisms that affect the survival and growth potential of the cultivated barnacle *Balanus crenatus.* The first aim was to study the predation pressure by the predators *Asterias rubens* and *Nucella lapillus* on *Balanus crenatus* to find the predator's effect on population size. The second aim was to study this barnacle's growth potential and starvation tolerance during times of natural food scarcity, through a feeding trial using formulated salmon-fry feed and zooplankton (nauplii of *Semibalanus balanoides*).

3 Background

3.1 Related work

Barnacles have received their share of academic attention, starting with Charles Darwin in *The Origin of Species,* Professor Dennis Chrisp has also contributed greatly to the study of barnacles in general and his studies are represented in the book Barnacle Biology (Southward, 1987). The information on the species *Balanus crenatus* in this book has however not been prioritized as much as the species *Semibalanus balanoides.* But there are some general information about the biology of *Balanus crenatus*.

Much of the recent research on different barnacle species generally focuses on aspects concerning biofouling in the shipping industry, such as cypris settlement and barnacle cement. The importance of these studies are easy to understand when taking a look at the statement made by (Alsaab, Aldred and Clare, 2017) that it can reduce propulsion efficiency up to 86%. It also costs lots of time and money to remove them.

But when it comes to research done on cultivation only two studies have been found. Both of them are done on different species, and due to the location and method used it is hard to actually make a comparison to the cultivation done at Hemnesberget, Norway. The first study on cultivation, was done in Chile, conducted by (Lopez *et al.*, 2012) they studied cultivation of the giant barnacle *Austromegabalanus Psittacus* for human consumption. The second study was performed by Swedish scientists, (Jonsson *et al.*, 2018) who have developed a method to cultivate the barnacle *Balanus improvisus* for repeated studies on their nauplii.

The reasons Planktonic AS experience difficulties when cultivating *Balanus crenatus* is mainly because of a knowledge gap. We don't understand all the biotic and abiotic factors affecting their survival, which this thesis is aiming to help piece together by studying some of the factors concerning top- down and bottom-up mechanisms.

3.2 The barnacle *Balanus crenatus*

The barnacle species *Balanus crenatus* is found all along the Norwegian coast (Moen, 2020). The global distribution is described by White, (2004) to reach from the Northeast Atlantic in the Arctic down to Bordeaux, Northwest in France, in the east *B. crenatus* is found on the west coast of North America and in Japan. It is a sublittoral species that seems to prefer protected waters, but they are also found on exposed shores and live in cold to temperate waters with a salinity ranging from full (30-40) down to 14 (White, 2004).

They belong to the Phylum of Arthropoda, Crustacea in the class of Cirripedia, order of Thoracica, Sessilia and the family Balandiae. *Balanus crenatus* is an obligate-cross-fertilizing hermaphrodite and their lifecycle starts as nauplii larvae. They go through six nauplii stages before reaching a cypris stage (figure 1). (White, 2004)

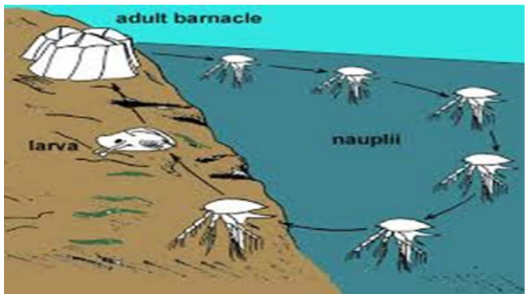

Figure 1 Life stages of Balanus crenatus (Tokle, 2019). With 6 naupliar stages and one cypris stage before settling down on the substrate of choice and availability.

B. crenatus are described as an early colonizer of sublittoral rock surfaces by Kitching, 1937 in White, (2004). The growth pattern is described by Barnes and Powell, (1953a) to mainly have a rapid growth in spring and fall, with a stagnation of growth in winter. It is also mentioned that the barnacles most likely reach their full size the first year, the exception to this is the barnacles that settled in fall instead of spring. They show more growth the following spring and summer until their full size has been reached in fall. The fully grown size is described to reach a mean of 21,7 mm, and the maximum size measured was only a few mm more. According to Henry, 1940,. in Rudy *et al.*, (2013) the largest recorded barnacle is 28 mm. The life span is described by Barnes and Powell, (1953b) to be 18 months, but (White, 2004) mentions that they can live up to two years. Their shell consists of six, usually smooth and white plates, with a calcareous base (White, 2004) (figure 2).

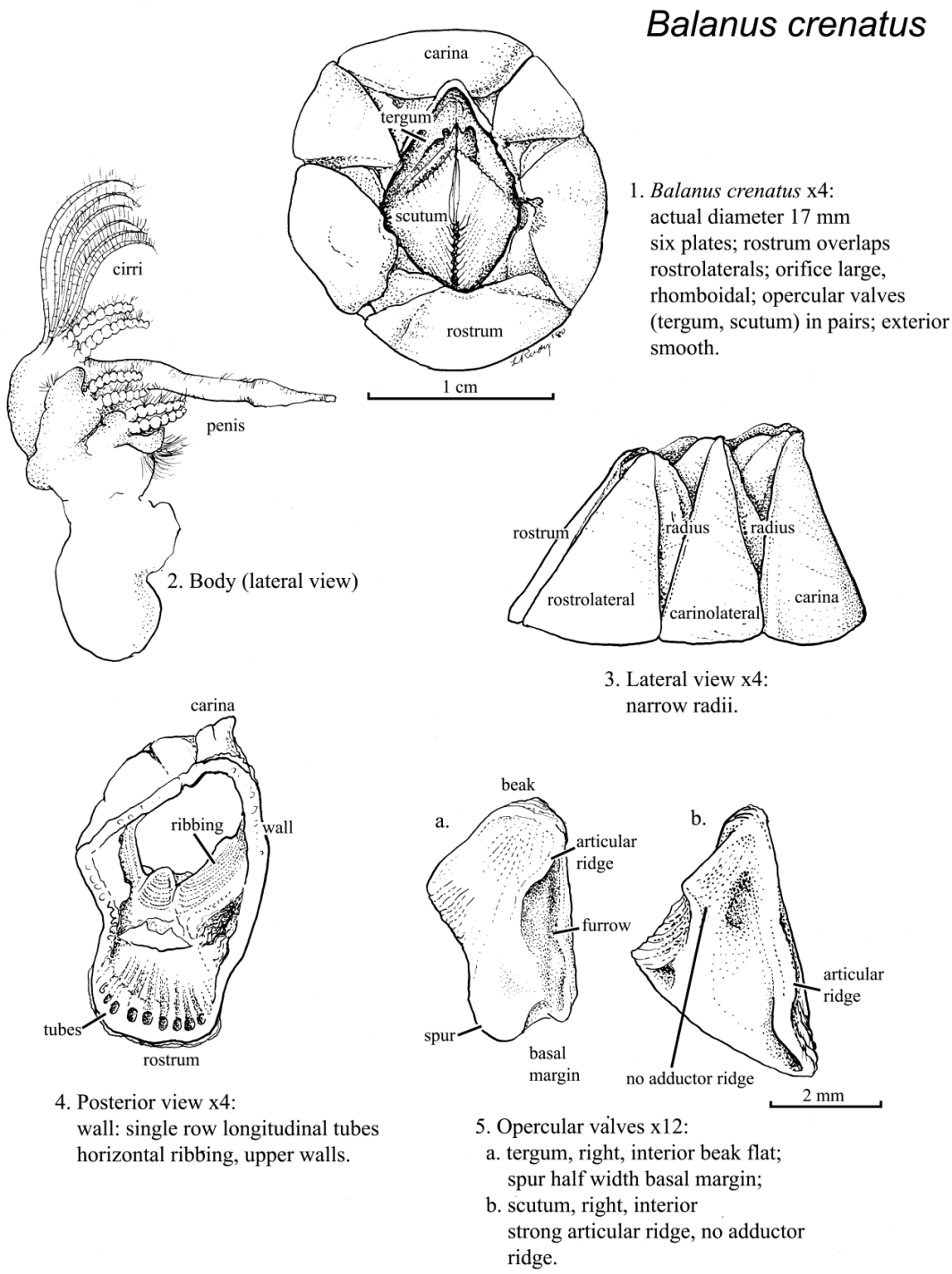

Figure 2 Illustration of the body and shell plates of Balanus crenatus (Rudy et al.*, 2013)*

To feed the barnacles either use their cirri in a waving motion to catch zooplankton, or if there is a strong current they extend their cirri, using it as a net and let the current bring the zooplankton to them (White, 2004). The nauplii of *Balanus crenatus* (figure 3) mainly eat phytoflagellates and diatoms, they are primarily herbivores and the release of nauplii seems to be connected to phytoplankton blooms (Turner *et al.*, 2001).

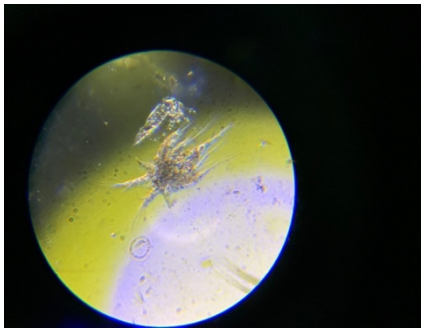

Figure 3 Nauplii larvae of Balanus crenatus

3.3 Live feed for marine juveniles

Cultivating marine fish larvae is still challenging because of the different fish larvae's environmental and nutritional needs during their first live stages to ensure survival, normal development, and good growth (Conceição *et al.*, 2010).

Due to availability and standardized protocols for production, the most used species for live feed are rotifers (*Brachionus sp*) and brine shrimp (*Artemia sp*) and in some cases microalgae, or "green water" is added, as it has shown a positive effect on the larva's digestive development and feeding behaviour (Conceição *et al.*, 2010). Rotifers are often used as the *first* feed due to their small size of 70 – 350 µm depending on strain and age, followed by the larger *Artemia* before formulated diets are introduced (Yùfera, Rodriguez & Lubiàn., 1984: Polo, Yùfera &Pascual., 1992; Olsen *et al.*, 2000 in Conceição *et al.*, 2010) The downside to these organisms is that they have nutritional deficiencies, and it is mainly the lack of the essential n-3 fatty acids that have shown to be critical for larval development. But with enrichment of the feeds diet, good results can be obtained for the larvae (Conceição *et al.*, 2010). One of the challenges of using rotifers and *Artemia* as feed, is the high bacterial load that follow these species, as a high bacterial load can be fatal to the larvae (Benavente & Gatesoupe, 1988; Reitan et al., 1998 in Vadstein, Mo and Bergh, 2014. p, 52).

Copepods and other zooplankton functions as a natural prey for marine larva in the wild, and better results on survival are obtained when compared to Rotifers and *Artemia*

(Conceição *et al.*, 2010). The reason for this that marine zooplankton naturally contain n-3 fatty acids that are bound to the phospholipids, which provides bioavailability of these precious fatty acids for the larvae (Coutteau & Mourente, 1997; Izquierdo , Socorro, Arantzamendi & Hernandes-Cruz, 2000; Gisbert *et al.* 2005 in Conceição *et al.*, 2010). According to (Conceição *et al.*, 2010) the bottleneck for providing live marine feed in time for the hatching of the marine larvae. In 2008 the company Planktonic AS in Trondheim solved this by cryopreserving the nauplii from *Balanus crenatus* (CryoPlanktonSmall), and *Semibalanus balanoides* (CryoPlanktonLarge), to use as a replacement of rotifers and *Artemia (Planktonic AS, no date c)*. A fresh study on feeding regimes of larvae of lumpfish (*C. lumpus*), and ballan wrasse (*Labrus bergylta)* by (Hagemann *et al.*, 2021) showed that added (CryoPlanktonLarge) to the diet seemed to improve the capacity of the digestion of ballan wrasse. This experiment also showed that a mix of CrypoPlanktonLarge and CrypoPlanktonSmall in a 50/50 ratio, had the highest mortality rate, but the assumed reason for the high mortality was believed to be because of a possibly lower availability to feed, as CrypoPlanktonLarge is described to be too large for newly hatched ballan wrasse larvae, leaving them with only half of the amount of feed. For the lumpfish, the results showed that larvae fed cirripeds (CryoPlanktonLarge) showed very good growth later on when given formulated feed, and during weaning, while copepods/formulated feed gave the lowest result in growth. (Nils Tokle from Planktonic AS, personal communication, 15.10.2021) informs that the feedback on their feed from hatcheries are very positive and that all the hatcheries in Norway and in the United Kingdom that produce (*L. bergylta)* now use CryoPlankton either alone or in combination with other types of feed. Nils Tokle also mentioned that producers of yellowtail Kingfish (*Seriola lalandi*) in Europe have gone from using rotifers to CryoPlankton, and by doing so they have increased the survival rate from 2-3% to an average of 25%. Planktonic AS state on their webpage that their feed suits species such as cod, Amberjack, Brass, Bream and Wrasse (Planktonic AS, no date b, no date c). Barnacle nauplii naturally contain taurine (Planktonic AS, no date a). And according to a study by (Gaon *et al.*, 2021) the effect of added taurine to rotifer and *Artemia* diets shows that taurine seems to be important for developing of the eyes of sea bream larvae (*Sparus aurata*), aiding in better capabilities to prey, and probably increased growth. (Hagemann *et al.*, 2021) also found that lumpfish larvae fed CryoPlanktonLarge had a higher rate of successful prey attack on day 28 compared to larvae fed Artemia and cirripeds. It was not tested for eye development in

the lumpfish, but it could possibly have a connection to development of the eyes due to taurine content in the CryoPlanktonLarge, such as the taurine enriched *Artemia* and sea bream study.

3.4 Cultivating *Balanus crenatus*

Planktonic AS in Norway, are the first firm to cultivate the species *Balanus crenatus* to produce live feed for marine juveniles. They have patented a way of cryopreserving the nauplii and have been selling CryoPlankton since 2016 ;Tokle and Aakerøy, 2019). They cultivate their barnacles in Ranfjorden in Norland, Norway. The cultivation process is inspired by the blue mussel industry, and they are using PVC-pipes, concrete pipes or *Swedish bands* (used for cultivation of blue mussels (*Mytilus edulis)*) to cultivate their barnacles (Minnhagen, 2017). These bands are 5 cm wide and 6 meter long. They are fastened to an anchoring line connected to buoys and weighed down by iron bars (figure 4). The exact depth of the bands varies through season, depending on the weight of the barnacles with approximately -30 cm below the surface at the start of season, to – 50 cm at the end.

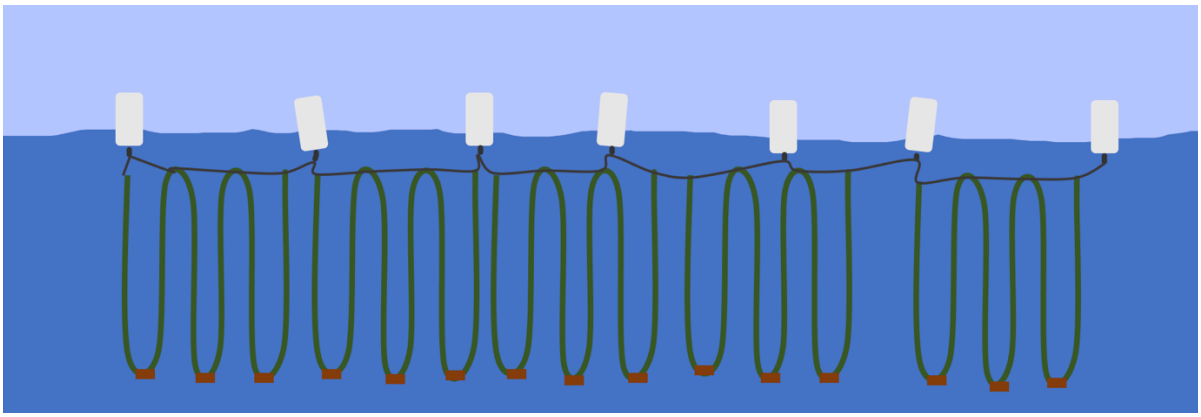

Figure 4 illustrates the setup of the Swedish bands used for cultivating Balanus crenatus at Hemnesberget.

The bands are put into the sea to mature prior to the release of nauplii and settlement of cypris. The development and growth of cultivated barnacles are illustrated in figure 5, where the main nauplii release on location usually occurs in mid-March, with a following cypris settlement in mid-April to start of May. Most of the growth happens in spring and

fall, and the gonads develop and mature during the darker colder winter months. Harvest happens in beginning of March before the barnacles release their nauplii.

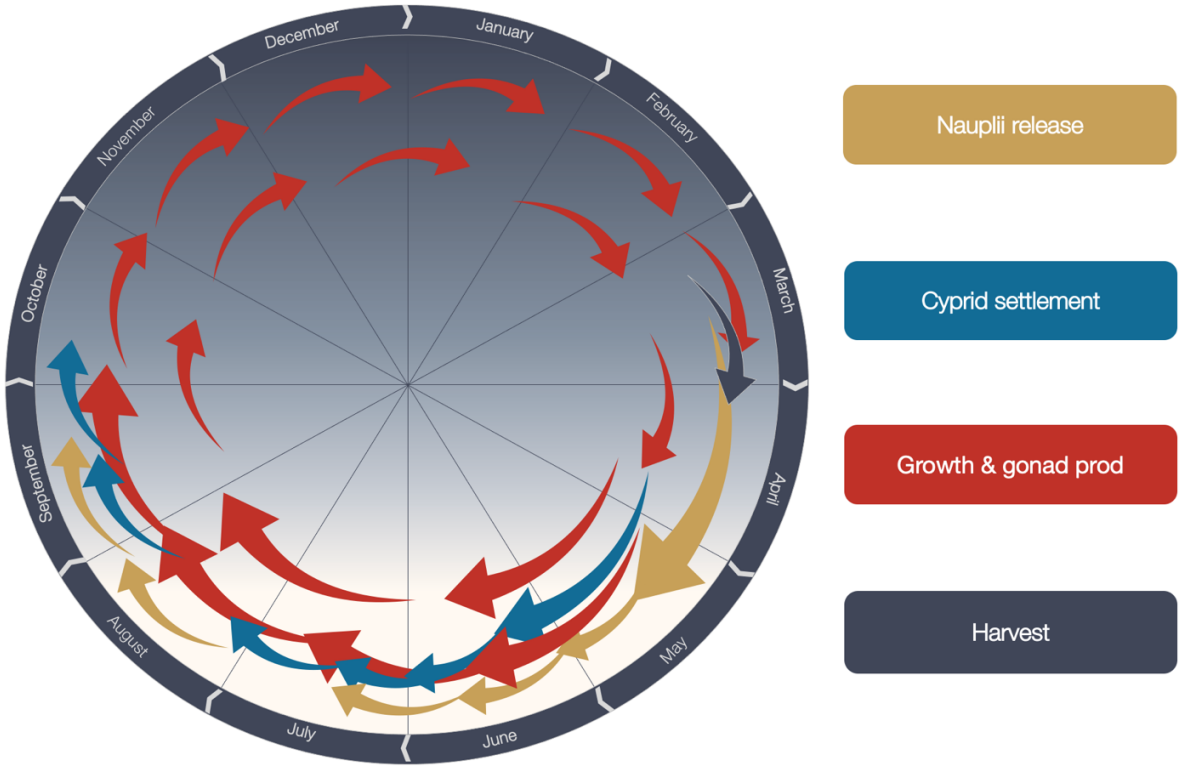

Figure 5 illustrates the lifecycle of one- and two-year-old cultivated Balanus crenatus.

In 2020 Planktonic missed the short window of harvesting the barnacles that had settled in May 2019 over a period of two to four weeks (figure 6), and the barnacles released their nauplii before Planktonic had the chance to harvest them. These barnacles were then left in the sea for one more year (illustrated by the inner read circle in fig 5). During winter eider ducks ate all the *M. edulis* that had settled on the PVC pipes together with the barnacles, resulting in PVC-pipes with only barnacles left at harvest time (26.03-2021), now 22 months old. The size of the barnacles was larger than previously recorded, (Barnes and Powell, 1953b) that found their maximum size to be between 20-25 mm in their study, and (Henry, 1940 in Rudy *et al.*, 2013) found the largest recorded specimen to be 28 mm. Measurements taken at Planktonic AS, was taken from a randomly picked PVC-pipe during harvest (26.03.2021), Barnacles in Figure 7 had larger barnacles with a maximum length

Figure 6 shows newly settled B. crenatus (Photo by Håvard Aakerøy, Planktonic AS, 26.05.2019)

of 31 mm (table 1 for sizes of three random barnacles). This maximum size in Rostro-carinal length may not actually be the maximum size obtained at Hemnesberget, as it was not actively searched for the largest ones to measure. It was mostly done for fun, hence only three barnacles measured. (Håvard Aakerøy from Planktonic AS, personal communication 26.03.2021) also described their growth to have increased a lot in size the second season, which contradicts the findings of (Barnes and Powell, 1953b) stating that barnacles settled in spring grow almost exclusively in the first season.

Table 1 shows the size of three 22-month-old barnacles, cultivated at Hemnesberget. Harvested 26.03.2021

	Length in mm	Width in mm	Height in mm
Barnacle 1	21	20	25
Barnacle 2	31	28,5	19
Barnacle 3	29	23	22

Figure 7 PVC pipe showing large 22-month-old barnacles on top of the pipe. Pen for size reference. The ''normal'' sized (10-13 mm in length) barnacles in lower right corner are one year younger than the large ones.

3.4.1 Locations

Ranfjorden, located in Nordland is where Planktonic AS are cultivating *Balanus crenatus.* This fjord is according to (Thorsnæs, 2022b) 68 km long, reaching from Mo i Rana to

Huglneset. The maximum depth of the fjord is -525 m close to the Hemnes peninsula, where the fjord is narrow, and the surrounding terrain is steep. The area around Hemnes is according to (Thorsnæs, 2022a) surrounded by 12 hydro power-plants that in total produces 578 MW annually. Meaning there is a lot of fresh water entering the fjord. The surrounding area is reported to get a mean 1439 mm of rain/snow annually, the amount of precipitation in the area is illustrated in appendix VII p. 3 . Data from salinity and temperature measurements done by Åkerblå and Planktonic AS (appendix V and VI) shows that the locations Planktonic AS uses to cultivate *B. crenatus* (Lassevika, Mastervika and Storsteinvika just outside Hemnesberget) have a distinct freshwater layer especially during the period of snow melt in spring and early summer, with a salinity as low as 4,5 in the first meter. Further down at around -5 m depth, the salinity is around 33, but the salinity changes with season. According to (Miljødirektoratet, 2021) the condition of the sediments in the inner parts of the fjord at Mo is contaminated by heavy metals, especially PHA. Even though the levels have dropped lately it is still a problem. In addition to this, studies done by NIVA (Øxenvad, Borgersen and Brkljacic, 2017) point to the high values of toxic Lilaflot D 817M from the industry in their sediment samples of the inner part of the fjord, but the measuring stations in the sediments outside Hemnesberget showed good conditions. The water quality on the location where *B. crenatus* is cultivated (figure 8) is categorized as very good by (Fiskeridirektoratet *et al.*, 2021).

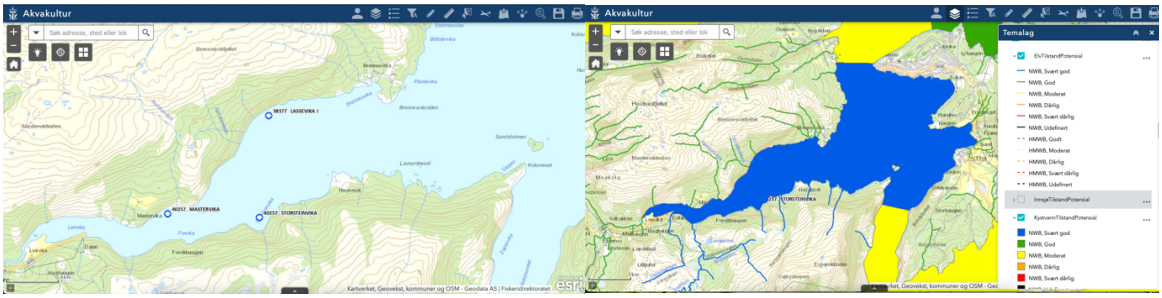

Figure 8 To the left the photo shows the placement of the growing sights marked by rings. The right photo illustrates the state of the water quality (Fiskeridirektoratet et al.*, 2021)*

Trondheimsfjorden is located in the middle of Norway and is where the experiments of this thesis were conducted. It is Norway's third largest fjord, and it is 126 km long, have a volume of 235 km^3, a surface of 1420 km^2 and an average depth of 165 m. The topography

around Høvringen (orange ring to the left in figure 9) has a steep slope and, the bottom sediment consists of clay, mud and silt. (Bakken, 2000, p. 12, 15). When looking at figure 9 we can see that (Fiskeridirektoratet *et al.*, 2021) have categorized the water quality at Høvringen as moderate, but the area is very close to the area of good water quality. Large parts of the water in this fjord comes from rivers in the area with an annual average of 21,9km^3 (Sakshaug and Killingtveit, 2000, p. 65). According to (Bakken, Holthe and Sneli, 2000 p. 42, 47, 49 and 51) the sea water entering the fjord comes from the Atlantic ocean, and the temperature at depth is 7 – 7,5 year round, the salinity is 34 or higher. There are good oxygen levels in the whole fjord with levels of 90-100% below the upper thermocline, except in some closed off basins. The average tidal difference in Trondheimsfjorden is 1,6 m.

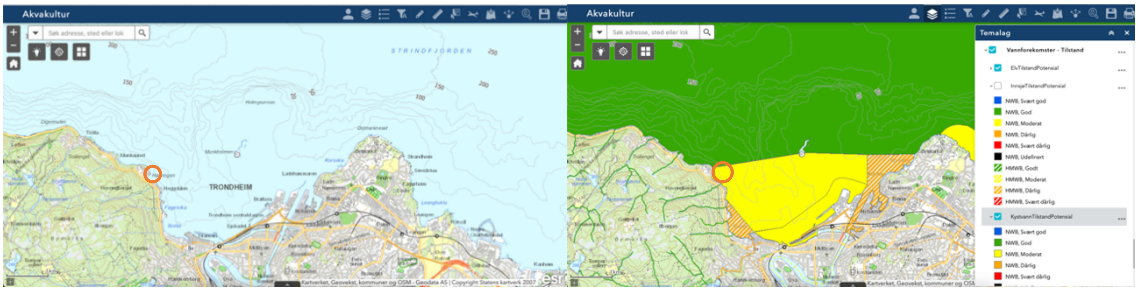

Figure 9 To the left the photo shows Where Planktonic lies in Trondheim. The right photo illustrates the state of the water quality (Fiskeridirektoratet et al.*, 2021)*

3.5 Predators

3.5.1 Asterias rubens

Asterias rubens (figure 10) is according to (Budd, 2008) "the most common starfish in the north-east Atlantic region". They are often found on sandy bottoms, coarse gravel, and rock from the intertidal zone down to 650 m, from in Norway in the north to Senegal in the south. They can reach a size up to 52 cm and get up to 7-8 years old. Since they shrink in size when there is little food,

Figure 10 Asterias rubens in a typical feeding position with the body elevated over its prey

their size is not the best way of determining their age. They usually become sexually mature after two years and when having reached a size of 5 cm in diameter. *A. rubens* have a well-developed olfactory sense that helps them find food and avoid predators. Their diets usually consist of bivalves, small crustaceans, and polychaetas, but according to Agüera *et al.*, (2012) the size of *A. rubens* limits the size of their prey. Their salinity preference lies between 40 to 18. In a study done by Dickey *et al.*, (2021) they found that a salinity of 12 is where *A. rubens* stop feeding. This is illustrated in figure 11, that is borrowed from the same study to illustrate the effect of salinity on predation rate on *Mytilus edulis*. Since they are stenohaline and have a poor ability to osmoregulate, they may have problems if the salinity changes to rapidly (Smith, 1940, in Lawrence, 1995, in Budd, 2008).

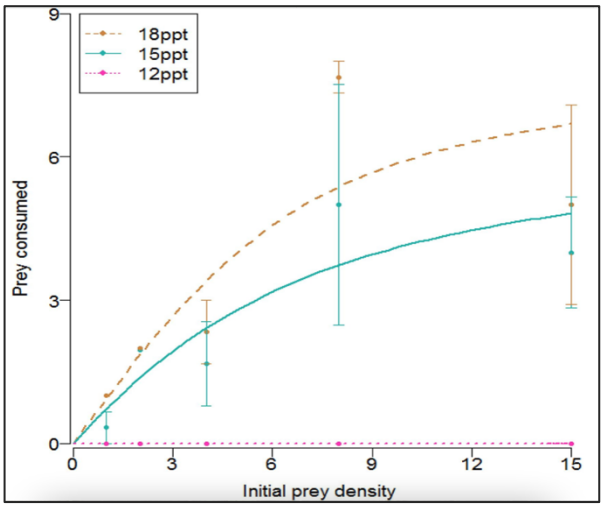

Figure 11 Showing how Asterias rubens feeding rate is connected to salinity, with no feeding activity at a salinity of 12 ((figure is borrowed from the study of Dikey et al.,2021)

3.5.2 Nucella lapillus

This predatory snail (figure 12) is according to Tyler-Walters, (2007b), found in the North Atlantic. It is distributed form Long Island in the north to Greenland in the west, and from the Arctic to the Algarve in east. In Norway, *N. lapillus* are found from Oslo to Finmark (Sømme, 2017). According to Tyler-Walters, (2007b), this species is found in intertidal areas, and can show high abundances on wave exposed shores, and areas with strong tidal stress. *N. lapillus* can get up to five to ten years of reach

Figure 12 Nucella lapillus

maturity after 2,5 years. Copulation occurs both in early spring and through summer, and the vase-shaped egg capsules are fastened on the underside of rocks and overhanging surfaces. The number of capsules laid depends on the age, temperature and energy reserves of the female. During mating and spawning they stop feeding and this starvation period can last up to four to five months. They feed mainly on barnacles and bivalves, and are reported to grow faster in sheltered areas (Osborne, 1977; Crothers, 1985; Etter, 1989 in Tyler-Walters, 2007a) and as Etter, (1996) in Tyler-Walters, (2007a) found that an important factor for growth is the amount of wave exposure, since waves reduce the feeding time. As for feeding strategies *N. lapillus* relies on a drilling technique where they create a circular hole in the shell of their prey (figure 13) and eat through the hole using their proboscis, or by pressing their proboscis between the plates of the barnacles or bivalves and using the rasping radula to remove the flesh. The last method is the most time effective, but it has been shown by (Rovero, Hughes and Chelazzi, 1999) that the method used may depends on the previous diets of bivalves or barnacles. As it seemed *N. lapillus* was unable to change from drilling to use the gape penetration method instead, when specimens collected on barnacle dominated sights were introduced to bivalves.

Figure 13 The circular mark on the closing plate of these Semibalanus balanoides are made by feeding Nucella lapillus during the acclimatization period of experiment 2

4 Material and Method

4.1 Flowchart

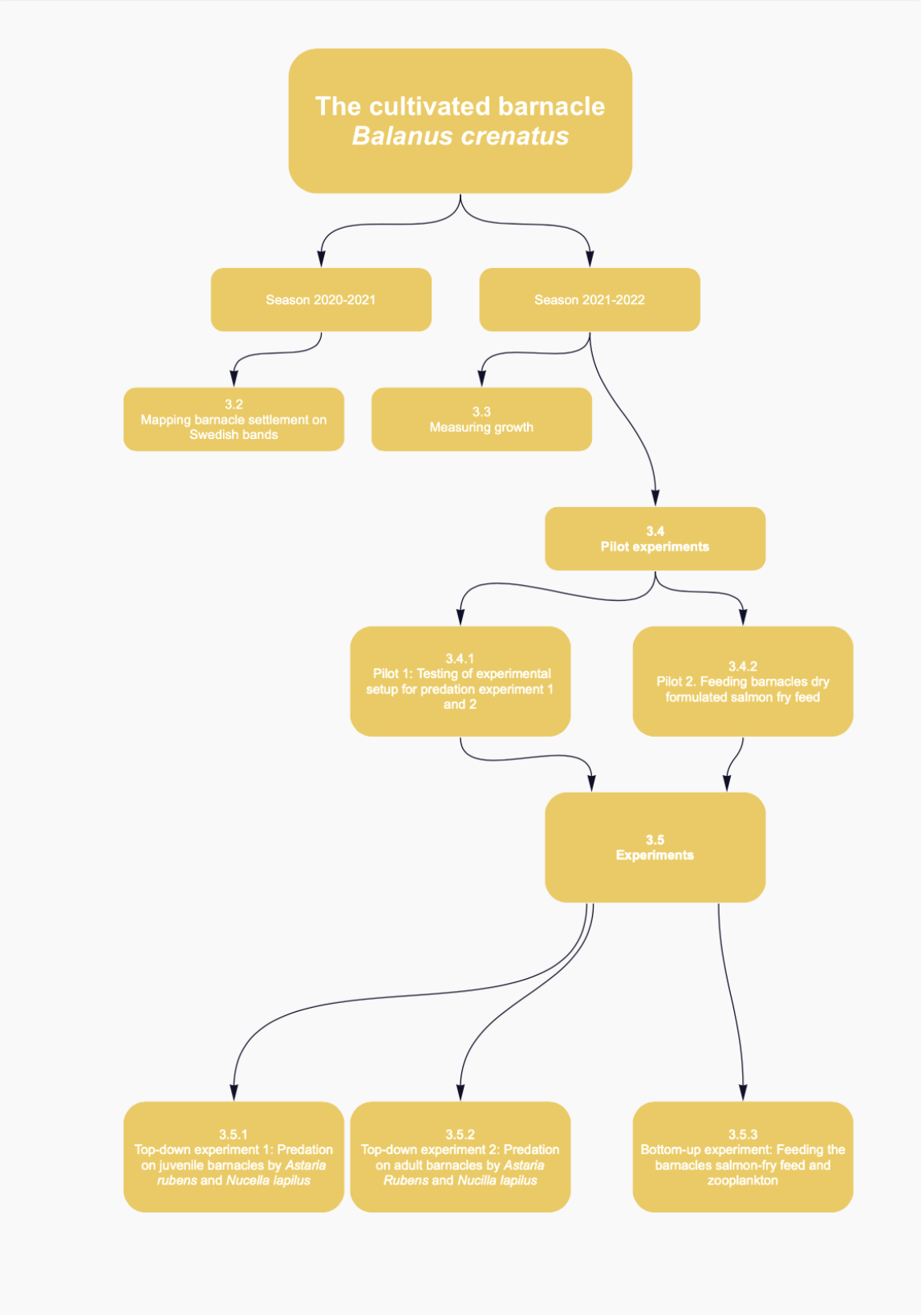

Figure 14 Flowchart of the work done in this thesis

4.2 Mapping barnacle settlement on Swedish bands (season 2020-2021)

To get to know the material better, six settling bands from Lassevika at Hemnesberget in Nordland, Norway (figure 15) from the season spring 2020 to spring 2021 were studied. The bands were analysed to get a better understanding of barnacle settling distribution, growth pattern and death zones along the cultivation bands throughout the year.

Figure 15 Location Lassevika, were Planktonic AS cultivate Balanus crenatus

The settling bands harvested on 15.03.2021 were counted between 22.03.2021 - 25.03.2021. The six lengths (figure 4) of one band that were counted were collected from one of Planktonic AS harvesting bags that had been brought to Trondheim for harvest (figure 4). The bands were damaged during harvest, resulting in many dead barnacles that were damaged. The shapes of the barnacles got divided into three categories: (1) pyramid, (2) cube, and (3) tower. The shape categories used in this study were defined by the author to their shape and geometric measures. The barnacles were then sorted into live and dead individuals. When barnacles were missing, their bottom plates were counted instead and, categorized as dead. Some areas had to many damaged and dead barnacles to be categorized into a certain shape. Here the remaining barnacles were scraped off and the back plates counted instead.

4.2.1 Counting procedure

To count the barnacles, the six lengths of bands was placed on a table together with a measuring band and counted from the end that had stayed closest to the surface towards the bottom. Only one side was counted. Segments of 10 cm were counted at a time. In high density areas a thin white cotton string was used to divide the 10 cm even further to keep track of the counted individuals. A photo was taken every 1 meter with panorama function

on a iPhone XR, iOS 15.3.1 for documentation (apple.com, 2021). Other epifauna were also noted as they showed up on the band during the counting. The sizes of *Asterias* rubens were measured using their radius, as done in (Agüera *et al.*, 2012). As the number of dead barnacles increased difficulties in categorizing the different shapes occurred, the categorizing part was left out, but the remaining barnacles and back plates were counted. Further down the band, the broken shells were removed, and the number of back plates counted instead.

The method for measuring the barnacles was done as described in (Barnes and Powell, 1953a) but with some alterations, such as using the tergum and scutum closing line as a guideline for both length and width measurements. In addition, a calliper was used instead of a microscope and measured to nearest 0.5 mm. The size measurements were taken as shown in figure 16. Length was measured from carina to rostrum, in a 90° angle

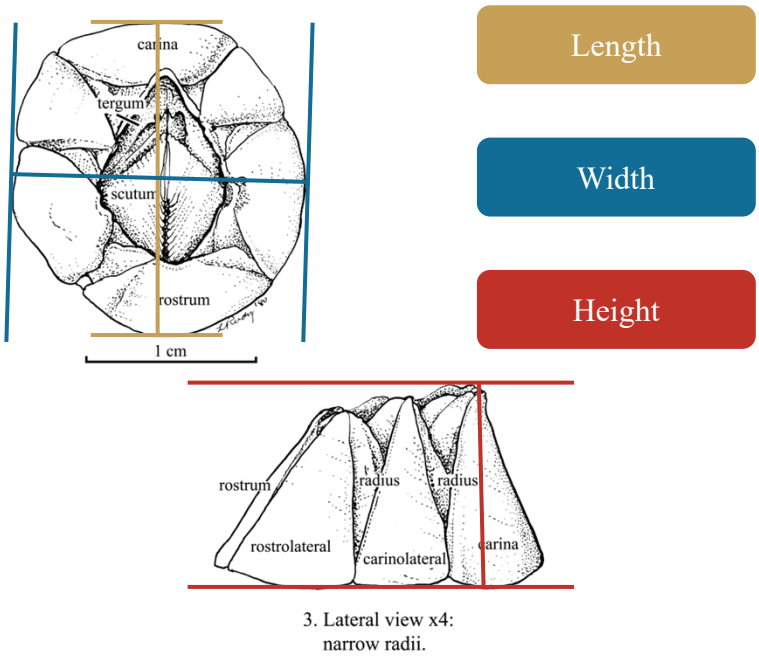

Figure 16 Procedure of barnacle measurements. Illustration of the barnacle is taken from (Rudy et al., 2013)

to the tergum and scutum closing line. The width was measured in parallel line with the tergum and scutum closing line at the widest part of either the carinolateral or the rostrolateral plates. The height was measured from base to the highest point of either of

the following plates: carina, rostrum, carinolateral or rostrolateral. Not using the tergum and scutum plates for this measure.

Initial shape categories were tested during the pilot experiment but proved to be accurate throughout the process and therefore adopted adapted for the entire work. The categories are pyramid, meaning a shape where the largest diameter of bottom plate being larger than total height of the barnacle. Square, where largest diameter is the same as total height and where diameter on top is the same or smaller than bottom diameter. Tower are taller than they are wide at the bottom diameter, and those that have a larger diameter at top than at bottom. Se figure 17 for an illustration of the three categories.

Figure 17 The different growth shapes of barnacles from left to wright: pyramid, cube and tower

On the first three lengths, the barnacles were measured on all sides, where it was not obvious what category they belonged to. This was done for training purpose, and to understand when the shapes changed and how the distribution between the shapes was spread along the band following the depth gradient. The 3 bands that followed were inspected visually and not all barnacles were measured. This was done to avoid the barnacles to break thus making the counting even more difficult, especially where they had grown in hummock formation.

The program Numbers, version 11,2 was used to make an illustration (figure 28) of the six lengths of the counted band, to show mortality and settlement density. Salinity and temperature measurements from the locations at Hemnesberget, provided by Planktonic AS and Åkerblå (Appendix V and VI) was used together with figure 28 to see if it could have caused the observed mortality of the barnacles and the death zone.

4.3 Measuring growth (season 2021-2022)

After the start of season 2021, around 01.06.21, two randomly chosen lengths of Swedish bands with newly settled barnacles (figure 18) were transferred on 18.06.2021 from the location Lassevika at Hemnesberget to the pier of Planktonic AS at Høvringen (Trondheim, Norway) located by the Trondheimsfjord. One length was used for measuring growth throughout the summer period, the second was used in experiment 1.

Figure 18 09.06.2021 Was the start of the season at Hemnesberget. Newly settled Balanus crenatus. Time of settlement: between 01.06.2021 and 09.06.2021.

To be sure that the same barnacles were photographed every time, a frame for the band and the iPhone camera was built using Lego bricks, (figure 19). Two small pieces of batten were stapled to the band to get the same area photographed every time. The frame was placed in a white plastic box to achieve an even light distribution, and a calliper was placed next to the barnacles to later have a reference for size, to use in Image J software, version 1.53. Following the procedure described by (Barnes and Powell, 1953a), the barnacles were cleaned from blue mussels with a soft toothbrush prior to each photo session to get precise measurements. The predator *Asterias rubens* was removed from the band when photographing, once per week. The recurring high amount of *A. rubens* was assumed to be a result of the band hanging too low in the water column, and part of it lying on the seafloor. And thus, giving easy access for *A. rubens.* Raising the band helped. The band were photographed weekly from 09.07.21 to 22.09.21. After the growth period 15 barnacles remained that were measured using Image J and a calliper. On 22.09.21 the measuring of this band was terminated when the rope holding the band

detached from the pier (somewhere between 23.09. and 29.09.21). The mean growth was calculated using length start and end data.

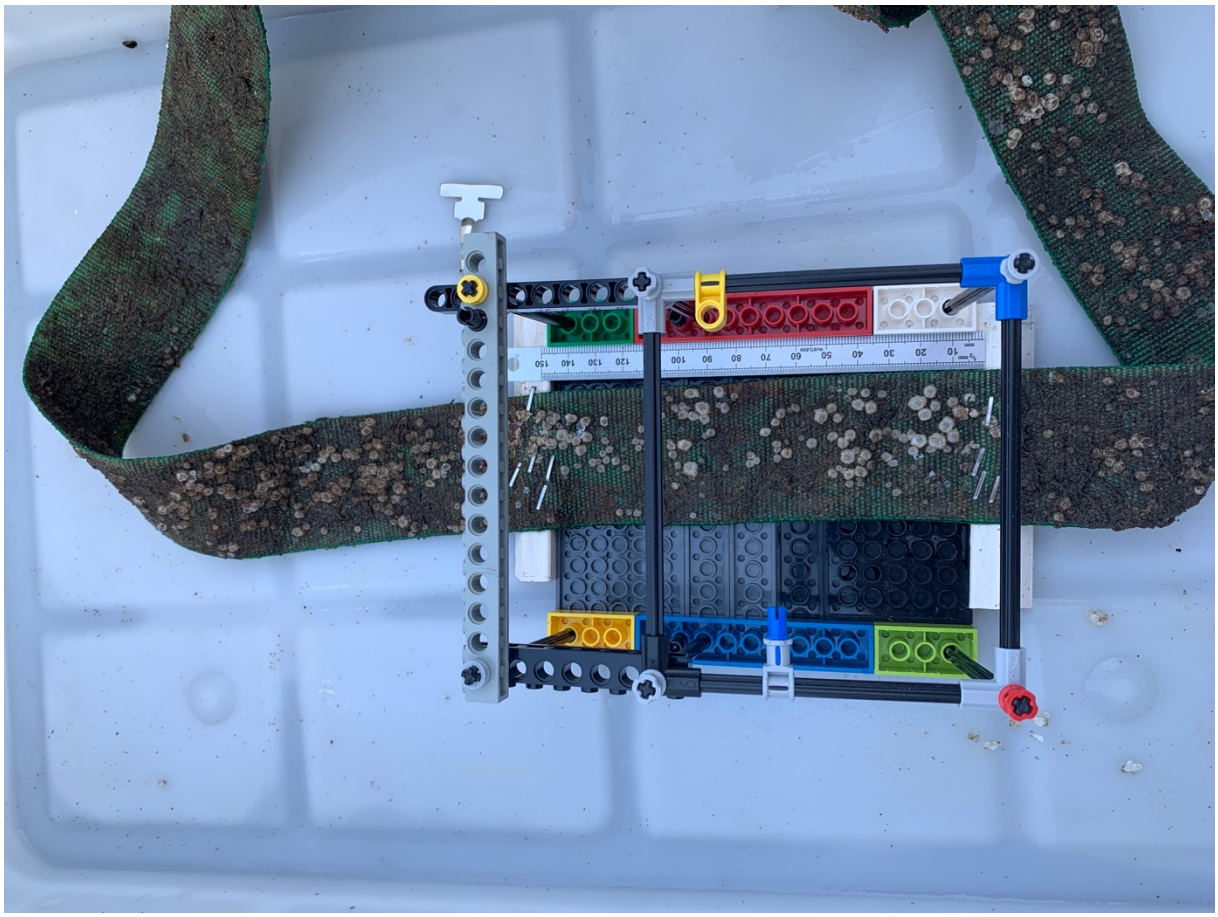

Figure 19 The setup for photographing Balanus crenatus for studying growth in field. The Lego frame was used as a camera tripod.

4.4 Pilot experiments

4.4.1 Pilot 1: Testing of experimental setup for predation experiment 1 and 2

To test the experimental setup for experiment 1 and 2, a pilot experiment was conducted to see if starving the barnacles would affect their cirri activity negatively. The aim was to investigate if the barnacles cirri activity would decrease when starved for eight days, as indicated in Budd, (2008) that states that *A. rubens* have a well-developed olfactory sense. It was suspected that the predators, and especially *A. rubens* would find active barnacles

more attractive due to microturbulences created in the water and the barnacles smell if they showed normal cirri activity.

Pilot 1 was conducted in the wet lab facilities at Trondhjem Biologiske Stasjon (TBS) and lasted for eight days (23.06.2021 - 01.07.2021). The setup consisted of six ten litre aquariums, where three replicate aquariums were fed, and three replicates were starved. The feed used, was frozen nauplii from *Semibalanus balanoides,* provided by Planktonic AS, and the barnacles were fed 1 g wet weight nauplii per day. Every day prior to feeding, the aquariums were cleaned, and two thirds of the water was exchanged. After cleaning the aquariums the barnacles were filmed for one minute on both sides using an I-phone camera (iPhone XR, iOS 15.3.1) (apple.com, 2021). The filming was done shortly after feed had been distributed and the barnacles showed a normal feeding behaviour after being disturbed.

The band with barnacles used for pilot 1. was harvested at Hemnesberget (26.01.2020) but had not been used for production. Instead, it had been hung out in the Trondheimsfjord at the pier of Planktonic AS until the start of pilot 1 (22.06.2021). Before Planktonic AS put this band into the fjord it had been stored in a fridge for seven days (27.10.2020 - 04.11.2020) at 7°C, then at 2°C (04.11.2020 - 12.11.2020) for another seven days before being hanged out in the fjord. This band had both adult barnacles, and newly settled ones, thus allowing to study both ages/sizes during pilot 1. The band was divided

Figure 20 Before and after cleaning the barnacles used for the Pilot (left). Bands of barnacles used for the Pilot 1. Barnacles covered by newly settled blue mussels (right

into smaller pieces, containing at least 25 young and 25 adult individuals for each aquarium (approx. 12 cm long bands). Two aquariums got two pieces of band to ensure there were sufficient numbers of each age/size. Prior to being added into the aquariums, the barnacles were cleaned to remove newly settled blue mussels (figure 20). This was done using a soft toothbrush and rinsed with seawater. The bands were then fastened to a piece of batten that were placed across the aquarium using a staple hammer, so that the band with barnacles could hang freely in the water column. Air pumps with aeration stones were added to each aquarium.

The seawater used in the aquariums was sand-filtered seawater pumped from 80 m depth from the Trondheimsfjord in the vicinity of TBS. Temperature and salinity were measured with a conductivity meter (WTW Cond 3310 SET1). These parameters were initially measured daily and found to have a stable salinity of approximately 34 and an initial temperature of 8°C, the measurements of intake water was then just checked sporadically to control for any changes. The seawater was tempered in the lab for two days in 50 litre plastic buckets to reach a temperature of around 14 °C before being added to the aquariums, here the temperature was measured daily. The ambient temperature and salinity at the seawater surface in the Trondheimsfjord was 13°C at high tide and had a salinity of 26,2 when starting pilot 1. To clean the aquariums, a 4 mm rubber hose and a 60 ml syringe was used to make a siphon to "vacuum" the bottom of the aquarium before adding new seawater and feed. Two thirds of the water was manually exchanged every day since it wasn't possible to have flow-through with the desired temperature at this lab. To make the rubber hose used for cleaning easier to handle, a barbeque skewer was taped onto it making it more rigid.

The wet lab used was a temperature-controlled room with a stable temperature of 15-16°C. It had dimmed light that ranged from PAR 0,5 µ to1,6 µmol m^{-2} s^{-1}, measured by a Walz ULM – 500 universal light meter. To film the barnacles, a torch was used to get more light locally in the room without disturbing simultaneously running experiments in the same lab. The strength of this light was measured to be 1733,1 µmol m^{-2} s^{-1}. The filming was done for 70-90 seconds on each side, and the filming was executed more systematically as the pilot progressed in time. Filming of the barnacles before feeding and cleaning of the aquariums was only done the last couple of days, the same with filming the

same side of all the band's first (side X, side Z). Some of the barnacles used in this pilot released nauplii during the duration of pilot 1. To avoid them becoming food the nauplii were removed whenever it was noticed. This was done by turning off the air for 2-5 minutes to stop the water movement and a torch was used to lead them into a corner. They were then removed using a syringe. This was done to try to prevent the aquariums that was supposed to starve from eating.

Pilot 1 was terminated on 01.07.2021, the bands of barnacles were connected back together using strips and put back into the fjord outside the pier of Planktonic AS on the next day.

4.4.2 Pilot 2: Feeding barnacles dry formulated salmon fry feed

Pilot 2 was done to study the filter feeding performance and activity of the barnacles when given dry formulated salmon fry-feed.

Pilot 2 lasted for 4 days (05.07.2021 until 09.07.2021) and was done using one piece of band with barnacles, no replicates. This piece of band belonged to the same band used in pilot 1. The pilot 2 band stayed in the lab during pilot 1 and had gotten the same treatment and feed as the bands used in pilot 1. It was also put back into the fjord when terminating pilot 1 but brought back into the lab for pilot 2 the following day (02.07.2021). The piece of band used in pilot 2 had about 100 individuals of first-year barnacles and 45 newly settled barnacles. It was fed the same diet as before until the start of pilot 2 (05.07.2021). The salmon-fry feed used was EWOS – Micro Start size 015 and came from Cargill (Appendix III p. 2 for nutritional value). The initial plan was to give them 0,5 g of salmon-fry feed two times per day (1 g total per day), but this amount had to be halved after day one since it seemed to be exsessive. 0,25 g two times per day seemed to be *ad libitum,* so that amount of feed was used for the rest of this pilot. The feed was soaked and mashed to get smaller particles since the smallest salmon-fry feed size was too big for the largest barnacles. Their consumption and beating activity were documented by filming them for 70-90 seconds on each side. This was done before the daily feeding and seawater exchange procedure, and after the first feeding event per day. Before filming, the

barnacles were allowed time to get into to feeding mode again after being disturbed. The aquarium was emptied and cleaned every day as long as the barnacles got salmon-fry feed to reduce bacterial growth from excess feed particles that lined the aquarium walls.

4.5 Top-down and bottom-up control experiments

Experiment 1 and 2 were two top-down comparative experiments done on juvenile barnacles in early summer 2021 and on adult barnacles in fall 2021. The aim was to analyse the effect of barnacle size for a variety of predators (figure 21). The two experiments had the same setup in lab and similar predator sizes were used. Since experiment 2 had a focus on the growth of the predators, they were measured more thorough in experiment 2 than in 1. The lab, seawater source, water/room temperature, light regime and cleaning routines was the same as in pilot 1 and 2 (for details see chapter 3.4.1) Experiment 3, the bottom-up experiment was conducted to analyse the feeding performance and starvation potential of barnacles using a formulated diet.

Figure 21 The predators Asterias rubens and Nucella lapillus used in experiment 1 and 2.

4.5.1 Experiment 1: Predation on juvenile barnacles by *Asterias rubens* and *Nucella lapillus*

The experiment was conducted at TBS and lasted for 5 days (05.07.2021-10.07.2021). The acclimatisation period of the predators *Asterias rubens* and *Nucella lapillus* started 16 days prior to the experiment, including 2 days of starvation. *Balanus crenatus* used in the experiment got no acclimatisation period. *A. rubens* and *N. lapillus* were collected on 21.06.2021 on low tide on the beach outside TBS. In total, 35 individuals of each species. The size of *A. rubens* was chosen based on observations from bands at Hemnesberget and

their radius, measured from body centre to tip of arm, ranged between 9-25 mm (n= 35). *N. lapillus* did not differ much in size with a length between 27-36 mm (n=30), see figure 22 for illustration of size range of the predators. The predators were put in two separate 10 L aquariums with aeration. The high amount of *A .rubens* in a 10 L aquarium made it necessary to change the seawater every day. During the acclimatisation period the predators were given rocks containing *Semibalanus balanoides* to feed on, with the aim to let them feed *ad libitum* both during the acclimatisation period and throughout the experiment, except the two days of starvation in between. The rocks were replaced whenever the predators had almost completely removed the *S. balanoides*.

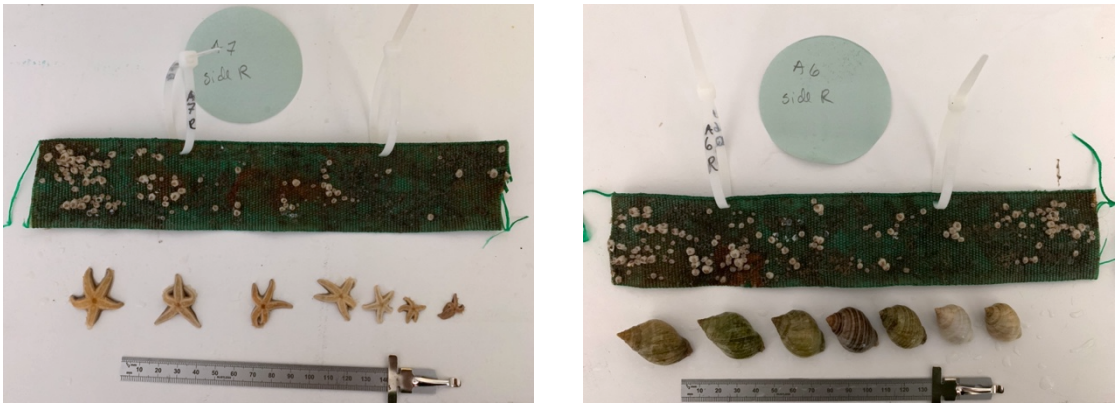

Figure 22 Size ranges of Asterias rubens and Nucella lapillus s used in experiment 1.

The barnacles were sampled at Lassevika at Hemnesberget on 18.06.2021, 17 days prior to the experiment and were kept on the bands hanging in Trondheimsfjorden until they were retrieved on 05.07.2021 at the start of the experiment and added to their aquariums. The ambient sea surface salinity of the Trondheim's fjord was 24,2 and the temperature 18°C. In the aquariums the salinity was 34,7 and the temperature 14,4 °C. One Swedish band was used and the first 1,7 metres of the band had a barnacle density that was considered as suitable to keep track of the barnacles that were eaten. The band was cleaned with a soft toothbrush to remove blue mussels. At the start of experiment 1, the bands were photographed on both sides before being added into the aquariums. The placement of treatments followed a randomised design, and the replicate number was added from left to right. The predators were sorted by size in falling order, and seven individuals were added into each aquarium with predators. See table 2 and figure 23 for the experimental setup.

Table 2 Setup of the aquariums in experiment 1: A= aquarium number R= replicate number. Placement followed a randomized design.

Aquarium number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Predator/ control	*Nucella lapillus*	*Asterias rubens*	Control, *Balanus crenatus*	Control, *Balanus crenatus*	*Asterias rubens*	*Nucella lapillus*	*Asterias rubens*	*Nucella lapillus*	Control, *Balanus crenatus*
Replicate number	R1	R1	R1	R2	R2	R2	R3	R3	R3
Aquarium code	A1-R1	A2-R1	A3-R1	A4-R2	A5-R2	A6-R2	A7-R3	A8-R3	A9-R3

Figure 23 The aquariums before adding barnacles and predators for experiment 1.

To keep the bands easily reachable for the predators, they were attached with strips and strings so that they could be hung over the aquarium, but still touch the bottom. The predators were placed on either side of the band, with the same distance to the band and aquarium wall (figure 24).

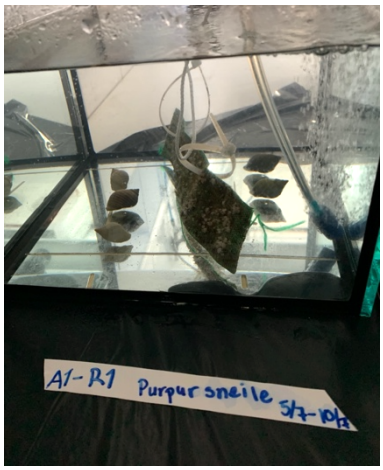

Figure 24 the placement of predators, and barnacles in the aquariums

Daily routines included, cleaning the aquariums, exchange of seawater and visual inspection to see if the barnacles were still active. The bands were photographed on both sides every day, trying to disturb the predators as little as possible.

A Welch's t-test for two groups with unequal variance (ratio of 4>) was chosen to analyse the data of Experiment 1, to check for differences between the mean number of barnacles eaten in each treatment. Microsoft Excel version16.60 (22041000) was used to perform this test.

H_0 = There is no difference between the groups ($p < \propto 0,05$)
H_1 = There is a difference between the groups ($p < \propto 0,05$)

4.5.2 Experiment 2: Predation on adult barnacles by *Asterias rubens* and *Nucella lapillus*

The experimental set-up used in 3.5.1 was also used in this experiment. The main difference was the size of the barnacles. The predator *N. lapillus* had a size between 29 and 37 mm in total shell length and the *A. rubens* had a radius between 7 – 30 mm. The predators were sorted by size from small to large in three replicates, and divided into the aquariums

Experiment 2 started with the acclimatisation period of the predators that were collected on 16.08.2021 on the beach at TBS. The ambient sea surface temperature was 16,6°C with a salinity of 28. The seawater temperature during the experiment was 14,7 °C with a salinity of 34,6. The barnacles were sampled at Lassevika on 02.09.2021 and arrived in

Trondheim on 03.09.2021. They were then cleaned and kept in the lab in one large container with aeration (40 hours) until the experiment started. Dead barnacles were removed prior to the start of the experiment on 06.09.2021. The placement of predators followed the same design as in experiment 1.

To analyse predator growth and biomass increase during the experiment, wet weight measurements were taken before and after the experiment as well as dry weight after the experiment . *A. rubens* were measured with a calliper using their radius. Before weighing them on a Mettler Toledo ME analytical scale, they were transferred to a paper towel and gently blotted to remove excess water. *N. lapillus* were marked with numbers written on their shell for identification. For estimating their body and shell weight the method developed by (Palmer, 1982) was used. This was done by taking the snail and tease the snail further inside their shell to release air bubbles, transferring them to a beaker with seawater placed on the balance and weighing them in water. Thereafter, the snail was removed from the beaker and teased back into the shell again thus pushing out excess seawater from the shell. The shell was then dried from outside with a paper towel and left in an upright position to drain water for 30 minutes before weighing them again. Then the shell weight could be calculated. The snail was also measured in size using a calliper. The total length was measured, and the distance of the opening was measured. After measuring and weighing the predators, counting the barnacles and photographing them (for picture identification after the experiment), they were added to their respective aquariums without further disturbance. The daily cleaning routine and photos were taken in the same manner as in experiment 1 but the barnacles had to be taken out on day two of the experiment for one more removal of dead barnacles. After the termination of the experiment, the predators were collected and measured in the same manner as in the beginning of experiment 2. The predators were euthanized by cooling their body temperature through freezing, before drying. To obtain dry weights, they were subsequently placed in a dry oven at for two days at 60°C and weighed again. Due to difficulties in removing the snail tissue entirely from the shell using a verity of methods, it was decided to dry the snails in their shell.

A Welch's t-test for two groups with unequal variance (ratio of 4>) was also chosen for analysing the data of Experiment 2, to check for differences between the mean number of

barnacles eaten in each treatment. Microsoft Excel version16.60 (22041000) vas used to perform this test.

H_0 = There is no difference between the groups ($p < \propto 0,05$)
H_1 = There is a difference between the groups ($p < \propto 0,05$)

Since Experiment 2, also measured the predators, they were first tested with a Shapiro-Wilks normality test in Past 4.04, using the dry weight of the predators. Before a Two-way ANOVA was run to check for differences in growth between the different size groups (1-7) for the two predators. Microsoft-Excel version16.60 (22041000) was used to perform this test.

H_1 = That the means of observations grouped by the factor predators, are the same
H_2 = That the means of observations grouped by the factor size group (1-7) are the same
H_3 = That there is no interaction between the two factors
($p < \propto 0,05$) for all hypotheses

This test was followed by a Tukey's post-hoc test to find out if there were differences between the factor's predator and size group. This test was performed in the program Past4 ($p < \propto 0,05$).

4.5.3 Experiment 3: Feeding barnacles salmon-fry feed and zooplankton

The experiment was conducted at the facility of Planktonic AS in Trondheim, in an unheated work tent. Prior to the experiment, 95 barnacles were measured for comparative reasons. The total duration of the experiment lasted for 14 weeks (11.10.2021 - 14.01.2022). This period was divided into a feeding period and a starvation period. The feeding period lasted for seven weeks (11.10.2021 - 26.11.2021) and the starvation period from 26.11.2021- 14.01.2022. The experimental setup consisted of 3 x 3 replicates where each replicate contained a piece of Swedish band with 50 individuals on it. Of these 50 barnacles, 30 from each replicate was used for dry weight measurements, and 10 of these 30 went further to a Carbon-to-Nitrogen (C:N) analysis to

see how the different diets and starvation affected the barnacles body mass and gonad mass composition as carbon content reflects lipid content in marine animals (Post *et al.*, 2007). The remaining barnacles were starved for the remaining time of the experiment to check for starvation tolerance.

For this experiment Swedish bands from Mastervika at Hemnesberget was collected 05.10.2021 and kept at Planktonic pier until the start of the acclimatisation period, starting on 11.10.2021. The bands were chosen at a random place on sight, but with the criteria of having a growth pattern with individual barnacles growing with no, or few connected barnacles to each other in the lower 3 meters of the band. This was done to make sure the barnacles could be measured for individual growth without being affected by their neighbour's growth during the experiment. It was the 2-4 metres from the bottom of the band that was used, and where the density was possible to work with. The barnacles were thinned with an aim of having only single standing barnacles left, so that the barnacles had room to grow in their natural shape. The description of how the dry weight, wet weight and C:N measurements of the gonads and animal were done are described in chapter 3.5.3.1.

At the same time as the barnacles for the experiment was prepared, 95 barnacles were randomly/haphazardly selected from one band for comparative reasons. The measurements taken was size (length width, height) wet and dry weight, of gonad and animal in addition to shell weight. 21 of these 95 specimens were randomly selected for C:N analysis.

The bands for the experiment were divided into nine 23 litre plastic boxes by Nordiska Plast (Nordic, 410 x 330 x 225 mm). Aeration was provided by one large pump, SuperFish Kol-Flow 60, with several outputs. Due to the pump being slightly over-dimensioned, the air hoses needed restrictors to reduce and control the air flow. In addition to this, one of the outputs on the aerator stayed open to reduce the pressure coming out into the boxes. The seawater used for the experiment was collected from a large holding tank on the pier containing unfiltered seawater pumped up from 80 meters. It had a temperature of 9,0-10,4°C for the duration of the experiment. The water was distributed to the boxes by a custom-made flow through system. Consisting of a water hose siphon connected to a PVC-

pipe with smaller pipes distributing the water. The flowrate was adjusted to 1,33 L per minute.

The barnacles were brushed clean with a soft toothbrush before they were randomly distributed among the 9 boxes. Each box had one to two Swedish bands containing 50 individuals. The barnacles were distributed on both sides of the band. The bands were held suspended in the middle of the water column by a piece of batten and strips see figure 25 for a visual of the setup. Acclimatisation period lasted for one week, and the barnacles were given their respective diets throughout the duration of this week as well as during the experiment. The treatments had a control group for starvation (control starvation) which was given no food. The control group for food (zooplankton) was given frozen nauplii from *Semibalanus balanoides* provided by Planktonic AS (and is not to be confused

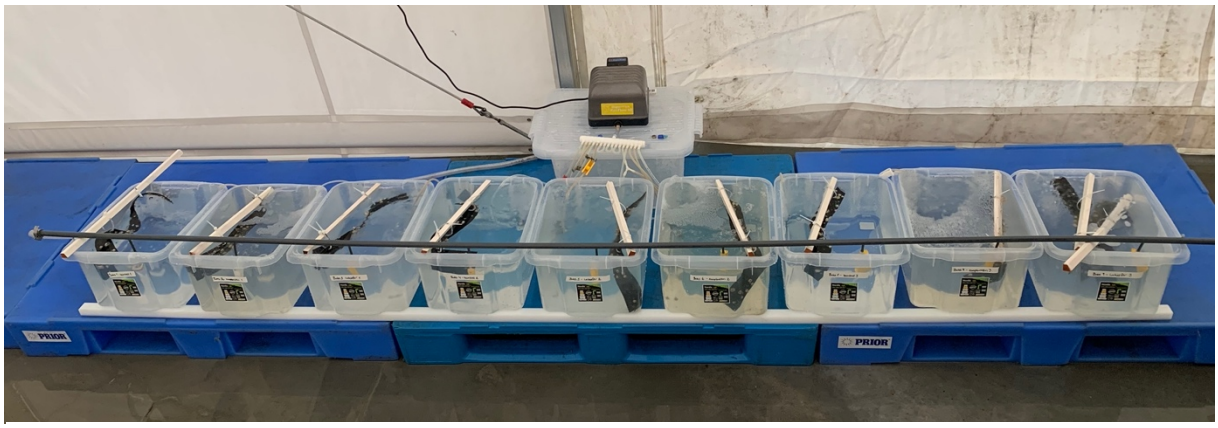

Figure 25 Setup of the experiment with the 9 replicates, the barnacles, aeration, and flow-through system

with the cryopreserved product that Planktonic AS produces, even though the nutritional value would be the same). The formulated feed group (salmon-fry feed) was given pellets from Cargill, EWOS – Micro Start size 015 (for nutritional values of the feeds and calculations for the amount of feed given, see appendix III).

The feed amount was based on experience from pilot 2, and the approximate dry weight of the zooplankton to provide equal amount of food in the different treatments. The amount of feed given was between 9,5-10 g of zooplankton and 1-1,2 g of salmon-fry feed. The dry feed was soaked in 2 ml freshwater and mashed with a small silicone spatula to a thick paste to break up the structure of the pellets. The thick paste needed to be furthered watered out to make sure that it was evenly distributed in the seawater of the replicate

boxes, so that the feed particles could circulate freely in the water. The zooplankton was added as ice cubes, but after thawing the water was stirred to make sure the feed was evenly distributed. Whenever working in the tent during feeding time, all the boxes were stirred gently with a wooden stick. This was to distribute the feed that had fallen to the bottom of the boxes into the water masses again. The aeration was set high to make sure most of the feed particles were suspended during feeding hours. But not so high that the water was "boiling" since this resulted in the barnacles staying closed.

Since the barnacles only were fed once per day, the flow through system had to be temporarily stopped by lifting off the PVC-pipe manually to avoid the feed being washed out by the water during daytime. It was turned back on again before leaving for the day to ensure the temperature didn't drop too low. This gave the barnacles a period of seven hours when feed was available *ad libitum* (09.00-16.00), with a dilution of the food concentration by the flow-through system during the night (16.00-09.00). This pause in flow-through resulted in a daily temperature drop down to between 5,3 – 7,0 °C, depending on the temperature outside (the experiment was done in an unheated tent from October 2021 to January 2022). Contiguous aeration was used to keep most of the food suspended in addition to oxygenating the water. The light regime was 7 hours light/13 hours dark, with light from 09.00- 16.00. The source of light was the lamps in the tent, but the strength of this light was not measured for this experiment.

The daily routines consisted of checking the flow-through, aeration, water and air temperature making sure it was stable. The activity of the barnacles was visually inspected together with the amount of faeces and number of moults. The replicate boxes were cleaned before feeding. To do this a spare box was used to keep the barnacles in seawater to keep them at the same temperature during the cleaning process. A dishwashing brush was used for cleaning the sides and bottom of the boxes before they were emptied. Then new seawater was added, and the barnacles was returned to their boxes and fed.

Once a week the barnacles were cleaned gently from feed particles with a dishwasher brush prior to the growth measurements. This measuring was done inside in the lab using a calliper. To keep track of the individuals' photos each band, both sides were used, and

each individual got a number that followed them throughout the experiment. The weekly measurements consisted of length, width and height and were done in the same manner as when counting the bands earlier (see chapter 3.2). At the end of the experiment, the barnacles were measured one final time. In addition, gonad tissue biomass (G), body tissue biomass (B) and shells (S) were weighed, both wet and dry, and C:N analysis were conducted.

4.5.3.1 Preparation for C:N analysis on Balanus crenatus

To be able to analyse the growth of the body tissue biomass (B) and gonad biomass (G), the barnacle biomass needed to be extracted from the shells and separated into (B) and (G). To do so they needed to be heat-treated first. With the aim of avoiding excess proteins dissolving into the water it was assumed that poaching would be a good method for heat treating to make the barnacles pliable to work with. According to (Innli, 2000 p. 161) and personal experience, poaching is a gentle way of heat treating a product that retains moisture in the product. Since this method retains moisture, it was assumed that the amount of proteins leaking out into the water would be reduced compared to boiling and steaming, that are some commonly used heat shocking methods to remove meat from the shell (Gökoğlu, 2021 p.144). The wish to keep the proteins in (B) and (G) was to keep the weight and C:N values as correct as possible.

The barnacles were carefully removed from the band trying to keep the backplate intact to avoid excess proteins from leaking out into the water. To keep track of the individuals, the barnacles were put in a large silicone ice tray where the number of each individual was written on the mold. A mesh was attached to the mold with bamboo sticks on either side of the mold and were fastened with rubber bands to keep the barnacles in place during the poaching (figure 26).

Figure 26 The left photo shows the marked ice tray mold containing the individual barnacles. The nettingon on top was to keep track of the individual barnacles during poaching. The middle and right photo shows the poaching prosess.

The barnacles were added to boiled water and poached for 10 minutes. In this case 6 L of water was used per 30 barnacles. New water was used for each tray. The barnacles were then laid upside down on a paper towel to wick away excess water. After poaching and pre-drying with paper towel, the individuals were frozen in an -80°C freezer before weighing and separation for dry weight, to prevent them to dry out and or go off.

Figure 27 Here the (B) and (G) has been removed from the shell. The (G) in the right corner is pale yellow and the (B) is lying in the top part of the photo together with two pair of adductor muscles.

The procedure for removing (B) and (G) out of the shell was done similarly as in the method for dissection of barnacles on the barnacle *Balanus improvisus* described by (Jonsson *et al.*, 2018). The difference was that their method did not use heat treated barnacles. Heat treating the barnacles made it easy to open the shell and to divide (B) from (G). To do so, a tweezer was used to tilt the scutum plates back towards the rostrum plate. This resulted in raising of the scutum and tergum plate making it easy to get a hold on the animal to lift it out. The animal was removed from the closing plates and added to a pre weighed tin together with the two pairs of adductor muscles, that sometimes stayed behind in the shell when pulling out the (B). The (G) when

connected to the animal was scraped off and added to its own tin capsule. See figure 27 for a visual over the barnacle, adductor muscles and gonad when separated.

There was a difference in the way the initial specimens and the ones from the experiment trials were treated before dry weight measurements. The initial specimens were not frozen before the final measurements and sampling for dry weight, but the experiment individuals were. This change was done because of the time it took to measure, poach, and separate the gonad and animal for dry weight measurements, the increased numbers of barnacles to measure for the final measurements and to keep the material fresh. These adjustments may have affected the data but were considered as necessary to obtain reliable results.

Each specimen was weighed on a Mettler Toledo ME analytical scale (g) before separating the animal and gonads by using tweezers for the C:N samples and transferring them into pre weighed tin capsules from Säntis analytical (Tin capsules for solids 5 x 9 mm). Thereafter, the samples were sorted into cell culture boxes (Thermo scientific, Nunclon ™ Delta Surface. Microsoft-Excel version16.60 (22041000) was used to keep track of the individuals along the way. The tin capsules, with (B) and (G) were weighed (wet weight) using a Mettler Toledo UMT2 fine scale weight (mg). The samples were dried for two days at 60°C before being weighed in for dry weight measurements. The samples were stored in a desiccator until running the C:N-analyses. Microsoft-Excel was also used to calculate average and to make growth curves of the different treatments.

A standard C:N method was conducted at TBS in a Elementar Vario EL Cube, where the total nitrogen (N) and Carbon (C) was measured. New weight-time categories were established especially for this experiment since due to the large verity in sample sizes and weight, resulting in an increased incineration with increasing sample size. The weight-time categories are described in appendix IV p. 7-8 . Ten individuals were randomly chosen from each treatment and 20 from the start population samples. They were divided into (B) and (G), resulting in 220 samples in total. In addition, samples from their feed, and one barnacle that had mature nauplii were run.

The statistical tests used for experiment 3 was: A One-way ANOVA, for testing for difference of mean shell growth in the three diet treatments. Here the length, width and height were combined to include all dimensions of growth over the period of seven weeks. (n =150) ($p < \propto 0,05$).

Hypotheses for the One-way ANOVA

H_0 = There is no difference between the growth of the three feeding groups.
H_1 = There is a difference between the growth of the three feeding groups.

Since Experiment 3, measured the barnacles every week they were tested with a Two-way ANOVA to check for differences in growth between the weakly measurements (week 41-47) for the three treatments, control, salmon-fry feed, and zooplankton. Microsoft-Excel version16.60 (22041000) was used together with Past4 to perform this test, and the following Tukey's post-hoc test.

H_1 = That the means of observations grouped by the factor predators, are the same
H_2 = That the means of observations grouped by the factor size group (1-7) are the same
H_3 = That there is no interaction between the two factors
($p < \propto 0,05$) for all hypotheses

This test was followed by a Tukey's post-hoc test to find out if there were differences between the factor's predator and size group. This test was performed in the program Past4 ($p < \propto 0,05$).

To test for difference in dry (B) and (G) (n =60) of the three different feed treatments, the ratio of dry weight to shell size was used (S). This was done to be able to compare the treatments to the alternative population that was measured at the start of the experiment. A Sahpiro-Whilk test was used to check for normality in the dataset, including the alternative population ($p < \propto 0,05$). Since the data of the alternative population was not normally distributed a Kruskal-Wallis test for non-parametric data ($p < \propto 0,05$) was run, followed by Dunn's post-hoc test on both datasets.

Before analysis on the C:N data was conducted, some individuals were removed from each treatment. This removal was done to exclude possible mistakes and to balance the dataset. Leaving n=8 per replicate in each treatment.

Individuals from the Start-population was also reduced to n=17. Later when comparing the Start-population to the Control group, six more individuals were randomly removed from the Control group to balance the dataset to the Start-population before continuing with a T-test. But this was done after the analysis on the treatment groups.

For the analysis on C:N ratio on body tissue and gonad, a Shapiro-Wilk test was run to test for normality, before proceeding with a One-way ANOVA on the three different treatments Salmon-fry feed, Zooplankton and Control. Then a Tukey's pairwise test was run to find the difference.

The gonadosomatic index (GSI) was used to assess development of gonads in fish using the measurement of % of gonad to total body mass (West, 1990). The method was tweaked to be used on poached barnacles (chapter 3.5.3.1), using the dry weight of gonad and total body mass, excluding the shell weight. This was done for Start-population (n=95) from week 0, and the three treatments Salmon-fry feed, Zooplankton and Control from week 7 (n=30, all treatments).

5 Results

5.1 Mapping barnacle settlement on Swedish bands (season 2020-2021)

When looking at figure 28 keep in mind that the figure shows the length of the bands and not the depth where the barnacles grew. To get depth of the settlement and the live/death zones add 50 cm to the start of the bands. These 50 cm is the approximate length of the ropes and buoys holding the bands.

Barnacles

		Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Average Band 2-6	Average Of records
0	- 10 cm	3	0	0	4	3	4	2	2
10	- 20 cm	2	3	1	1	10	6	4	4
20	- 30 cm	10	14	4	6	4	5	7	7
30	- 40 cm	7	12	3	5	11	6	7	7
40	- 50 cm	3	10	4	13	18	5	10	9
50	- 60 cm	14	15	1	16	8	22	12	13
60	- 70 cm	23	18	11	15	20	13	15	17
70	- 80 cm	18	23	7	15	8	31	17	17
80	- 90 cm	12	17	16	33	16	22	21	19
90	- 100 cm	20	32	10	46	35	20	29	27
100	- 110 cm	15	23	47	51	41	14	35	32
110	- 120 cm	22	37	27	37	56	24	36	34
120	- 130 cm	23	51	48	32	46	28	41	38
130	- 140 cm	16	31	29	32	53	35	36	33
140	- 150 cm	45	26	21	33	15	79	35	37
150	- 160 cm	41	34	49	41	51	39	43	43
160	- 170 cm	35	49	50	62	47	82	58	54
170	- 180 cm	44	50	59	55	47	66	55	54
180	- 190 cm	45	98	78	53	98	46	75	70
190	- 200 cm	46	84	51	75	55	51	63	60
200	- 210 cm	148	63	90	102	60	67	76	88
210	- 220 cm	141	75	63	128	60	83	82	92
220	- 230 cm	114	78	92	94	97	111	94	98
230	- 240 cm	129	84	62	123	94	192	111	114
240	- 250 cm		102	79	119	120	99	104	104
250	- 260 cm		97	69	128	109	114	103	103
260	- 270 cm		99	126	136	80	110	110	110
270	- 280 cm		153	141	175	89	113	134	134
280	- 290 cm		116	109	127	106	98	111	111
290	- 300 cm		94	152	122	87	100	111	111
300	- 310 cm		107	176	138	97	107	125	125
310	- 320 cm		107	146	135	107	130	125	125
320	- 330 cm		124	130	134	143	148	136	136
330	- 340 cm		159	110	173	138	131	142	142
340	- 350 cm		55	101	206	140	101	121	121
350	- 360 cm		174	143	159	101	102	136	136
360	- 370 cm		148	105	127	129	95	121	121
370	- 380 cm		112	119	109	132	36	102	102
380	- 390 cm		138	119	137	154	92	128	128
390	- 400 cm		77	124	137	139	95	114	114
400	- 410 cm		100	86	100	103	79	94	94
410	- 420 cm		113	88	112	111	80	101	101
420	- 430 cm		85	98	120	98	80	96	96
430	- 440 cm		69	109	103	87	102	94	94
440	- 450 cm		115	43	70	62	79	74	74
450	- 460 cm		91	36	78	67	95	73	73
460	- 470 cm		114	38	45	87	96	76	76
470	- 480 cm		70	24	39	48	47	46	46
480	- 490 cm		61		33	64	69		57
490	- 500 cm		59		37	63	47		52
500	- 510 cm		71		36	36	52		49
510	- 520 cm		27		30	33	50		35
520	- 530 cm				28	27	37		31
530	- 540 cm						13		13
540	- 550 cm								
550	- 560 cm								
560	- 570 cm								
570	- 580 cm								
580	- 590 cm								
590	- 600 cm								
	Sum, 1 side	976	3764	3294	4165	3710	3648	3542	3779
	Std							278	
	Sum band 2-6, green to mid yellow (0-230 cm)							854	Aprox 24 %
	Sum band 2-6, brown to mid yellow 240-470 cm)							2687	Aprox 76 %
	Sum band 2-6 (0-470 cm)							3542	

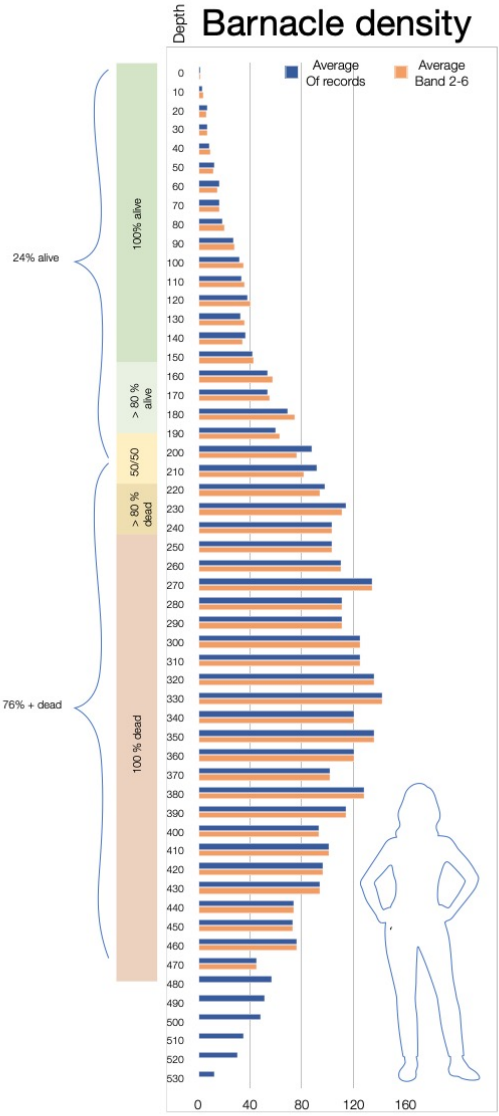

Figure 28 To the left, an overview over barnacle settlement per 10 cm, along the length of the six counted Swedish bands (season 2020-2021). The average of records for settlement along all the bands and average of band 2-6 is shown on the right. The colors indicate number of live and dead barnacles. Average number of barnacles on band 2-6 (n=3542, SD 278) Only one side was counted.

Figure 28 is an overview over the six lengths of counted Swedish bands (season 2020-2021) with colour coded areas of the % of alive and dead barnacles. The green area has 100% alive barnacles. The light green area has more than 80% alive. Yellow means 50 % alive 50 % dead. The mustard yellow has more than 80% dead, and brown means all the barnacles were dead. The most radical change on these bands were noted between 200

cm -250 cm, meaning that -2,5 to -3 meters depth is the area where the barnacles went from alive to dead. Calculated from band 2-6 (total n=2542) the estimated percentage of live barnacles was 24%, and dead barnacles 76%. Here the area between 0-230 cm was used to calculate live barnacles and 240-470 cm to calculate dead. On the right side of the figure, the average density of barnacle settlement on the bands is shown together with the colour coding for mortality.

The different shapes of the barnacles changed with density of settlement and size of barnacles. Pyramids only was observed on areas of the band with 1- 45 barnacles per 10 cm. These areas were found at the top and bottom of the bands with generally larger barnacles at the lower part of the band.

Cube shaped barnacles were found where the density was between 45 – 120 barnacles per 10 cm. These areas had a settlement of mixed shape in a transition zone between pyramid and tower shaped barnacles in hummock formation. This hummock formation with tower shaped barnacles were found at densities over 120 barnacles.

In tables 3 and 4 The datapoints of favourable conditions for *Asterias rubens* have been highlighted in the tables.

Table 3 Salinity measurements Lassevika 2021. The salinity tolerance of Asterias rubens is highlighted in yellow. With 12 as the lower limit, where Asterias rubens stops feeding (Dikey et al., 2021).

Date / Depth	**- 1 m**	**- 2 m**	**- 3 m**	**- 4 m**	**- 5 m**	**- 5,5 m**
18.6.	4,5	6	8,3	11,9	22	25,5
7.7.	6,8	20,3	26,8	28,5	29,5	30
13.8.	16	18,5	23,2	26,2	27,3	28,5
24.8.	14,24	17,5	22	24	26	27
30.8.	11,82	24,8	27,5	28,3	29,1	
9.9.	17,7	17,68	17,9	18,67	18,95	
17.9.	9,74	24	27,3	28,8	30,2	30,7
24.9.	22,6	23,5	24,4	25,9	29,2	29,2
11.11.	22	24	26,1	26,5	27,2	27,3
24.11.	19,7	24,1	27,3	28,8	30,2	30,7
2.12.	7,1	23,5	29,2	31	32,8	33,1

Table 4 Sea temperature at Lassevika 2021. Optimal feeding temperatures according to for Asterias rubens are highlighted in yellow.

date	- 1	- 2 m	- 3 m	- 4 m	- 5 m	- 5,5 m
18.6.	11,5	11,3	11	10,8	10,6	10,5
7.7.	19	13,2	10,5	9,5	8,8	8,3
13.8.	13	13	12	12	11,5	11
24.8.	11,8	12,1	12,4	11,5	11,4	11
30.8.	12,3	10,6	9,9	9,5	9,2	
9.9.	11,4	10,9	10,7	10,6	10,5	
17.9.	8,8	9,6	9,4	9,3	9	8,8
24.9.	8,6	8,8	8,9	9	9,3	9,4
11.11.	5,5	6	6,7	7	7,2	7,3
24.11.	2,4	3,2	4,7	6,3	7	7,3
2.12.	0,2	3,5	5	6,1	7	7

5.2 Growth in Trondheimsfjorden through summer

The difference in growth from start to end (02.07.2021-22.09.2021, 9 weeks) was measured on 15 individuals. The mean growth in length was 3,16 mm (SD 1,36 mm), for width 2,99 mm (SD1,33 mm). Figure 29 shows the start and end size of the barnacles used, in addition to a comparison of growth to Hemnesberget over the same time period.

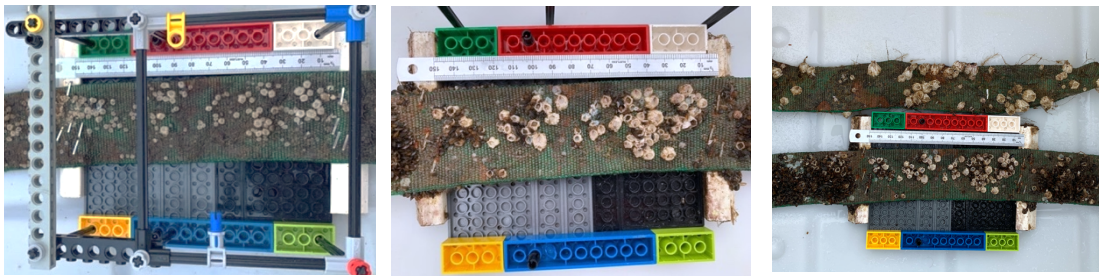

Figure 29 to the left, size of barnacles at start of measuring 09.07.2021. In the middle, size of barnacles at end of measuring (22.09.2021),and to the right barnacles from Trondheimsfjorden at the bottom and from Hemnesberget at the top, clearly showing larger barnacles from Hemnesberget (same age)

5.3 Pilot studies

5.3.1 Pilot 1: Testing of experimental setup for predation experiment 1 and 2

The observation and filming of the barnacles during pilot 1, showed that the barnacles were active in both treatments. This result was true for both adult and juvenile barnacles. Based on clear observations of cirri beating in both treatments at the end of the pilot, it was decided not to go any further in analysing the activity. The setup of the aquariums worked as intended and no adjustments needed to be done to the parameters.

Other observations made during the pilot (time period, July 2021) was mating activity, assumed sperm casting and nauplii release. The mating activity showed a clear tendency to one barnacle being the subject of interest for the surrounding individuals trying to fertilize this individual. The individual of interest did not always seem interested, and often closed for prolonged periods of time and only occasionally coming out for short periods of time. This behaviour went on for several days. When this barnacle was being fertilized, it closed holding the penis for a prolonged period of time. Resulting in the barnacles being exposed when trying to mate. The period was observed to last for over 30 seconds before the penis was released (figure 30).

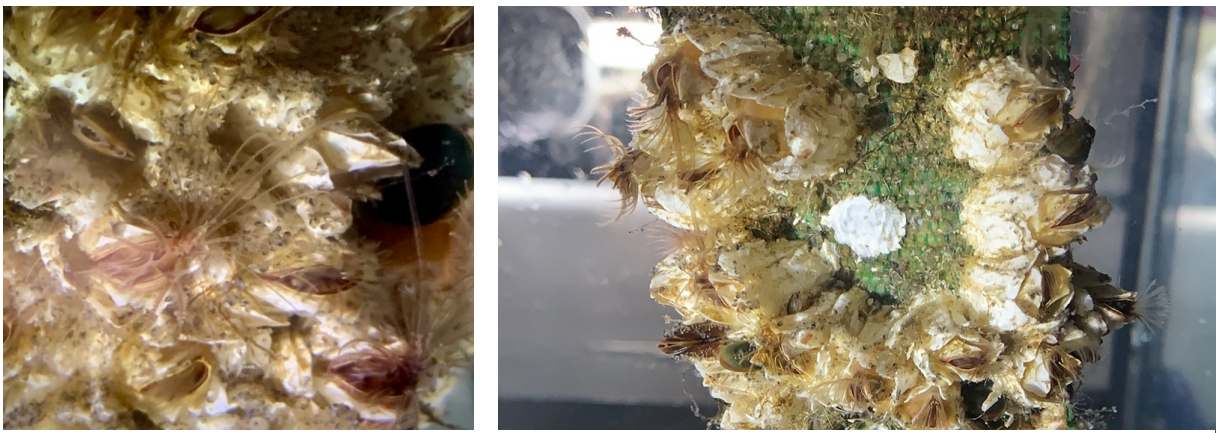

Figure 30 Left. Two barnacles reaching over backwards to mate, exposing their bodies. The barnacle being visited closed up, holding the penises from retracting up to 30 seconds or more. To the right. Here 5 barnacles have determined that the barnacle in the middle is the one worth mating with. This barnacle was particularly interesting for several days, but it seemed to close every time the neighbour's tried to mate. It was not observed that they try to mate with the other neighbouring barnacles, only this one.

Another interesting event was the assumed observation of sperm casting done by one individual. It was seen as a small white cloud being pumped out, observed against the back light of the setup in the lab. The release of nauplii was observed on several occasions. Sometimes by observing the release of nauplii by them being pumped out, other times by observing nauplii in the water masses of the no feed aquariums (fig 31).

Figure 31 The released nauplii has swarmed towards the light of the torch.

5.3.2 Pilot 2. Feeding barnacles dry formulated salmon fry feed

During the short period of time this experiment lasted it was obvious that the barnacles ate the formulated feed. They became really active after feed had been distributed, and there were faeces in the aquarium every morning. The newly settled barnacles had a higher beating rate than the older and bigger barnacles.

5.4 Experiments

5.4.1 Experiment 1: Predation on juvenile barnacles by *Nucella lapillus* and *Asterias rubens*

The results of experiment 1 showed that *Asterias rubens* consumed significantly more juvenile barnacles compared to *Nucella lapillus* (p - 0,0049), with a mean ratio between the ingestion rate of the predators, *N. lapillus : A. rubens* was 38% (figure 32).

N. lapillus consumed in mean 12 barnacles (SD 4,6), and *A. rubens* 31 barnacles (SD 0,9). Using a Welch's t-test for two groups with unequal variance, the two-sided p-value was

0,0049, resulting in rejection of H_0 ($p < \propto 0{,}05$), finding a significant difference in predation rates between the two predators in Experiment 1. Additional details of the statistical data are included in appendix I.

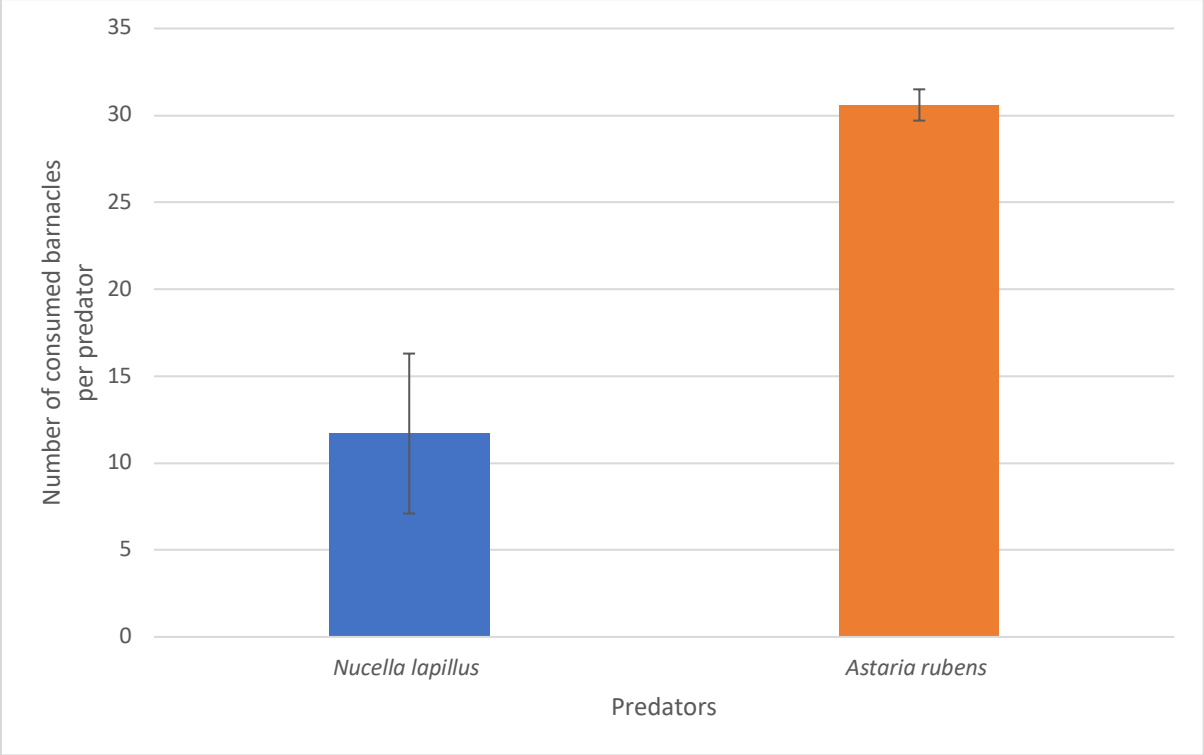

Figure 32 Experiment 1. Shows the mean predation rate (n = 21, duration 5 days) across all three replications with standard deviation (SD) .

5.4.2 Experiment 2: Top down, Predation on grown barnacles by *Nucella lapillus* and *Asterias rubens*

Asterias rubens consumed significantly more barnacles compared to *Nucella lapillus* (p - 0,012). This time on adult barnacles (fig 33). *N. lapillus* mean ingestion rate was 4 barnacles (SD 2), and for *A. rubens* , 11 (SD 4,2). The mean ratio between the ingestion rate of the predators, *N. lapillus : A. rubens* was 34%.

per day was 4 barnacles (SD 2), for *Asterias rubens*, 11 barnacles (SD 4,2). The mean ratio between the predators was 34%.

The p-value (0,012) of Experiment 2, resulted in rejection of H_0 ($p < \propto 0{,}05$), finding a significant difference between the predation rates of the two predators (figure 33), additional details of the statistical data is included in appendix II.

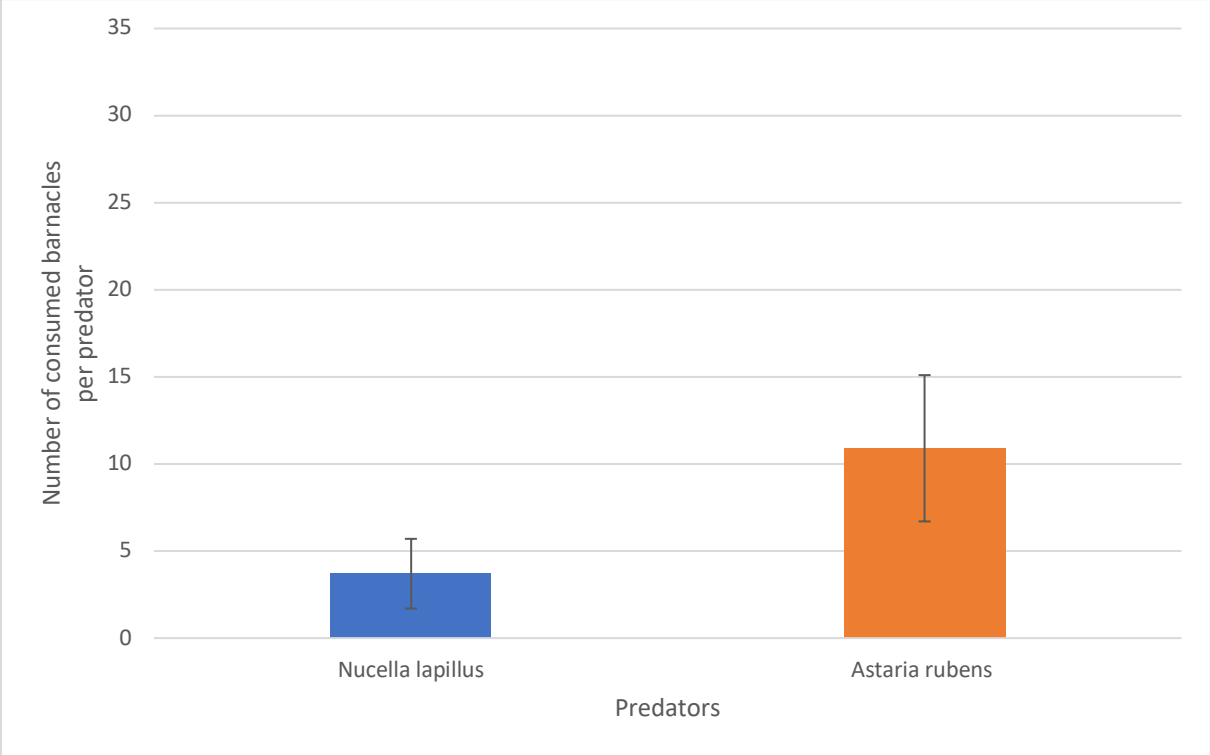

Figure 33 Experiment 2. Shows the mean predation rate (n = 21, duration 5 days) across all three replications with SD.

The results of predator growth in the seven different size groups showed that the growth of the size groups is dependent on the type of predator, and the effect of growth is dependent on the size of the predator. *A. rubens* grew more with increasing start size while *N. lapillus* did not (figure 34). A significant difference between the predators was detected for size groups six and seven.

The Shapiro-Wilks test, on normality showed that all replicates of both predators were normally distributed with p-values of 0,88 for (R1), 0,95 (R2), 0,26 (R3) for *A. rubens.* For *N. lapillus* the p- values were 0,71 for (R1), 0,80 (R2), 0,26 (R3). The results from the Two-way ANOVA on the increased weight of the seven different size groups of *N. lapillus* and *A. rubens* used in experiment 2, showed that there was a significant difference between the two predators with a p-value of 0,0000006 between the size groups with a p-value of

0,004. For interaction a p-value of 0,0002 ($p < \propto 0,05$) rejecting H_1, H_2, H_3. This showed that the effect of growth in the size groups are dependent on the type of predator, and the effect of growth is dependent on the size of the predators. Further the Tukey's post-hoc test showed that there was no difference between the size categories of *N. lapillus*, but there was a significant difference between *N. lapillus* Size 6 and *A. rubens* Size 6 with a p-value of 0,008 ($p < \propto 0,05$), also between *N. lapillus* Size 7 and *A. rubens* Size 7 with a p-value of 1,80 E07. For *A. rubens* there is also difference within the size groups. *A. rubens* size 1 and *A. rubens size* 6 had a p-value of 0,016, *A. rubens* size 1 and *A. rubens size* 7 had a p-value of 1,8 -E07, *A. rubens* size 3 and *A. rubens* size 7 had a p-value of 0,0002, *A. rubens* size 4 and *A. rubens* size 7 had a p-value of 0,0007, *A. rubens* size 5 and *A. rubens* size 7 had a p-value of 0,001 and Asterias size 6 and *A. rubens* size 7 had a p-value of 0,004.

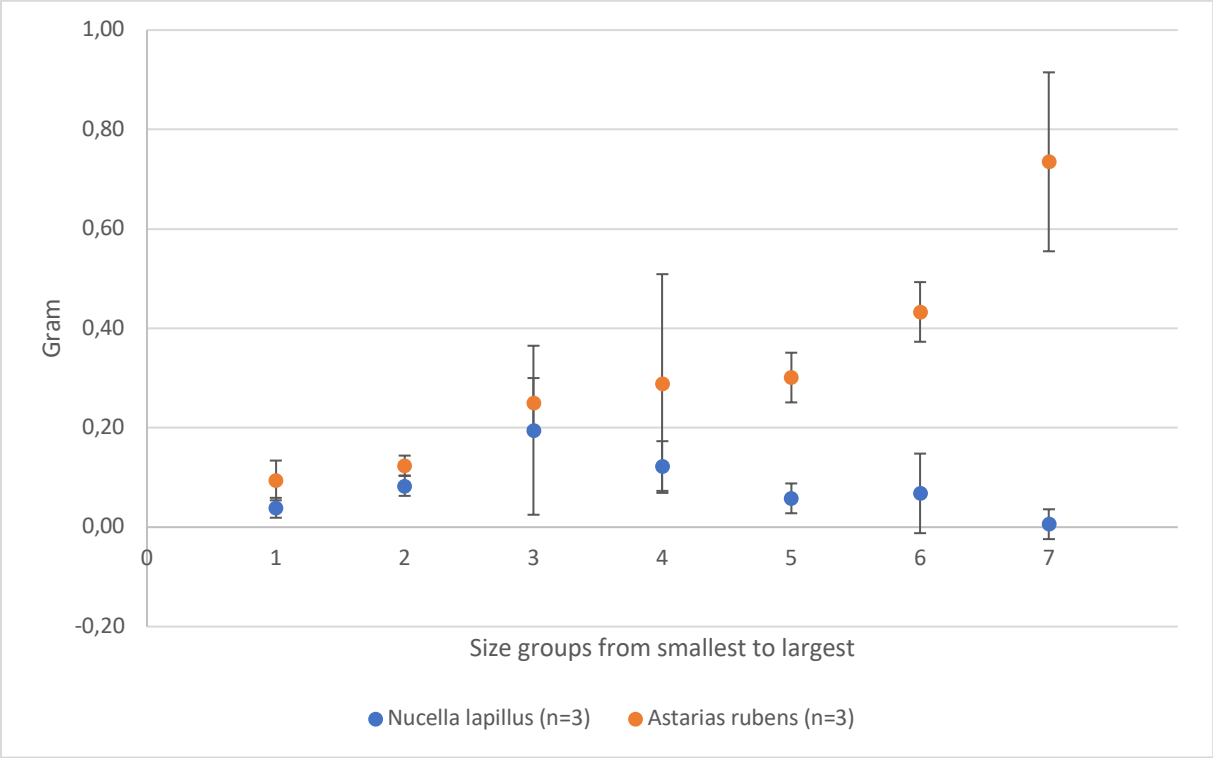

Figure 34 Mean weight increase of the seven size groups (n = 3) of Nucella lapillus and Asterias rubens, with SD..

5.4.3 Experiment 3: Bottom-up experiment: Feeding the barnacles salmon-fry feed and zooplankton

5.4.3.1 Shell growth

The results of the barnacles' shell growth were based on the mean increase of the combined measurements: length, width, and height after seven weeks of the experiment (n=50), times three replicates.

The Zooplankton-fed barnacles had a significantly higher growth compared to salmon-fry feed barnacles, and from week six the Salmon-fry fed barnacles showed a significantly higher growth compared to the Control group. During the seven-week measuring period it was noted that length was the variable that increased the most, followed by width, then height. Salmon-fry fed barnacles showed a mean growth of 1,24 mm (SD 0,99) and a 4,7 % increase in growth, Zooplankton grew 2,96 mm (SD 1,75) and had a 10,7 % increase in growth, the Control group grew 0,44 mm (SD 0,47) and increased 1,6 % in growth (figure 35).

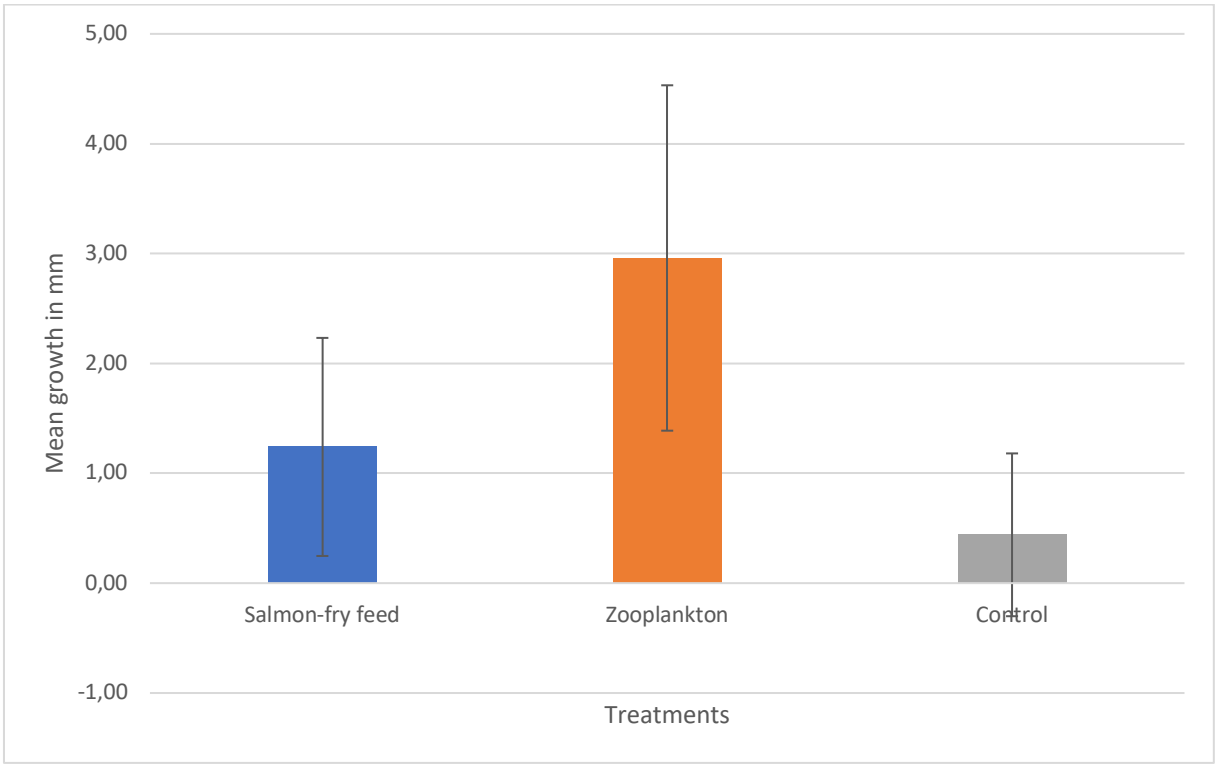

Figure 35 Mean shell growth increase in length, width, and height (n =150), after 7 weeks of feeding, with SD.

A one-way ANOVA was run to check for differences between the means of the three groups. A p-value of 7,648E59 resulted in rejection of H_0 ($p < \propto 0{,}05$), finding a significant difference in shell growth between the treatments.

The results from the Two-way ANOVA showed that the effect of growth in the week categories are dependent on the type of feed, and the effect of growth is dependent on the time (week number). The p-value for the feed was 1,09E-20, 4,11E- 20 for the different weeks and 8,73-E13 for the interaction. Resulting in rejecting all three hypotheses (p-value $< \propto 0{,}05$). (See appendix IV for additional details).

These results were followed up by a Tukey's post-hoc test done on the mean value on growth of the different treatments (length, width, and height n=150), using weekly measurements over a time period of seven weeks The highest increase in size was detected in the Zooplankton treatment (figure 36). The SD values for the figure is found in table 5. (Additional details are provided in appendix IV). The results from this test showed that Zooplankton had a significant difference within the groups between week 41 and 43 - 47, week 42 and 34 – 47, week 43 and 45 – 47, week 44 and 46- 47, week 45 and 47. Salmon-fry feed had a significant difference within the groups between week 41 and 45 – 47, week 42 and 45 -47, week 44 and 46 -47, week 45 and 47, week 46 and 47. The control group did not have any significant difference within the group. Between groups there was a significant difference between Salmon-fry feed and Zooplankton for week 44 – 47, between Salmon-fry feed and Control week 46 and week 47, and for Zooplankton and control there was a significant difference between week 43 – 46.

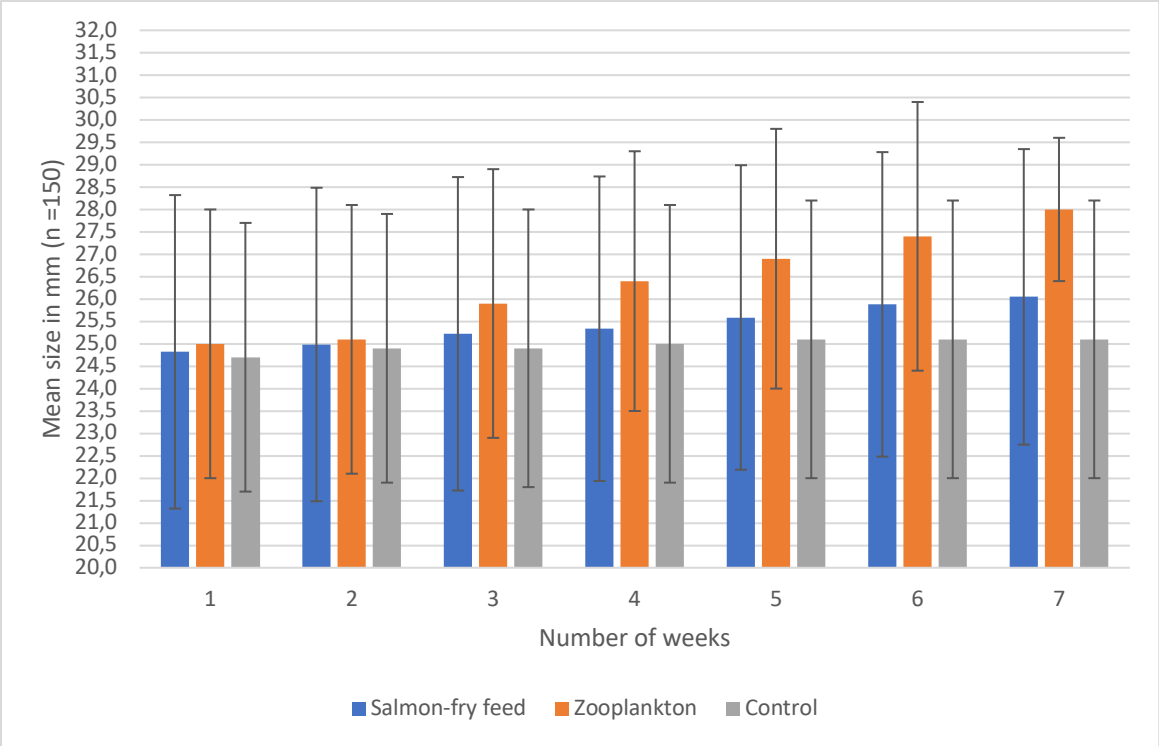

Figure 36 Mean size of the barnacles and their weekly increase over seven weeks (n = 150). The measurements length, width and height were combined to get the total growth, on the three diets: salmon-fry feed, zooplankton, and a starved control group. Standard deviation (SD) is shown in table 5

Table 5 Showing the SD for figure 34

SD for combined length, width, and height (n=150)							
Week number	**41**	**42**	**43**	**44**	**45**	**46**	**47**
Salmon-fry feed	3,5	3,5	3,5	3,4	3,4	3,4	3,3
Zooplankton	3	3	3	2,9	2,9	3	1,6
Control	3	3	3,1	3,1	3,1	3,1	3,1

5.4.3.2 Dry weight of body tissue biomass and gonad biomass

The ratios of body tissue biomass (B) to shell size (S) and gonad biomass (G) to shell size (S) showed significant differences between the groups for B:S and G:S (n=20). For B:S, a significant difference was found between all treatments except between Start population and Zooplankton. For G:S, significant differences between Start-population and Zooplankton, Start-population and Control, Salmon-fry feed and Zooplankton, Salmon-fry feed and Control, and between Zooplankton and Control were found. As seen in figure 37, the Control group showed a decrease in B:S and G:S after 7 weeks of starvation. The Salmon-fry fed group did not show any significant difference to the Start-population

meaning they had the same ratio to shell size after being fed salmon-fry feed for seven weeks. Since the Salmon-fry fed group's shell size grew significantly compared to the Control group when tested for shell growth means they grew some but kept the same ratio in regard to the measurements of the Start-population. The Zooplankton fed barnacles showed a significant increase in body mass and grew significantly more than both Control and the Salmon-fry fed group when tested for shell growth (figure 35). But most interestingly the gonad mass showed a higher weight than body mass weight for the Zooplankton fed barnacles, this was not observed in the other treatments (figure 37).

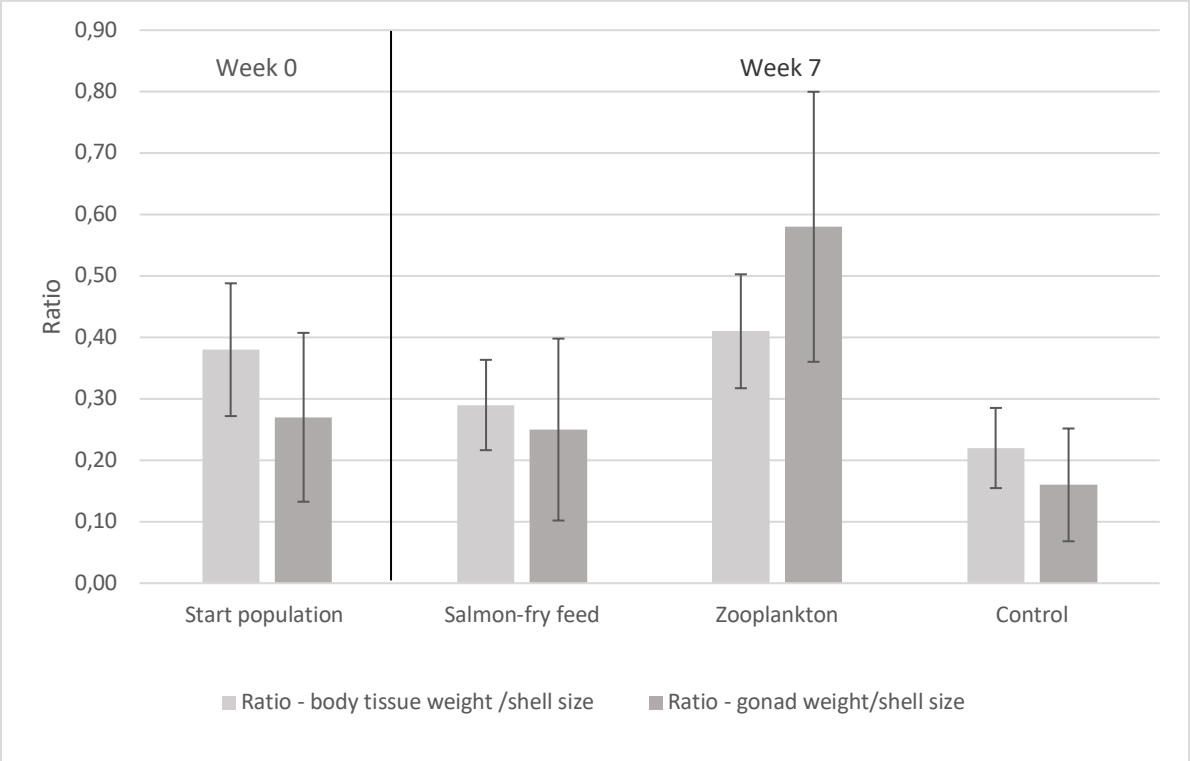

Figure 37 Weight-size-ratio (mg/mm) providing the relationship between dry weight (mg) and total shell size (S) (mm) of body tissue biomass (B)and gonad tissue biomass (G), respectively. For comparison measurements from a Start-population from week 0 is displayed together with ratios for the different treatments from week 7. (n=20) three replicates, with SD..

The results on dry body weight to shell size ratio using the Kruskal-Wallis test, showed a significant difference between sample medians of the different treatments (p-value 2,694E-24). Further results from Dunn's post-hoc test showed that there was a significant difference between the following combinations: The Start-population and salmon-fry feed (p-value 6,42E-06), Start-population and Control (p-value 5,791E-16), Salmon-fry feed and Zooplankton (p-value 3,42E-09) and between Salmon-fry feed and Control (p-value 0,00034). Lastly between Zooplankton and Control (p-value 2,274E-21).

The results on G:S using the Kruskal-Wallis test, also showed a significant difference between sample medians of the different treatments (p-value of 7,366E-26). The Dunn's post-hoc test showed a significant difference between the following combinations: Start-population to Zooplankton (p-value 9,438E-11), Start-population to Control (p-value 3,019E-05), Salmon-fry feed and Zooplankton (p-value 5,288E-14), Salmon-fry feed and Control (p-value 0,00179) and between Zooplankton and Control (p-value of 1,787E-26).

The mean gonadosomatic index (GSI) for the Start-population (n=95) at week 0, was 42 %. The rest of the treatments from week seven was 47 % for Salmon-fry feed (n=30), 59 % for Zooplankton (n=30) and 43% for Control group (n=30).

5.4.3.3 C:N values of the feed

The *feed* used in the experiment was also tested for C:N ratio (table 6). Where the C:N values of the feed showed that the Salmon-fry feed had higher values of both µg N/mg and µg C/mg than the Zooplankton feed. Comparing these to the values to the body tissue biomass and gonad tissue biomass values from Experiment 3 (table 7) it looks like the effect of the feed only reflects in the µg N/mg values of the gonad tissue biomass, while the rest of the µg C - and N/mg is higher in the Zooplankton fed group compared to the Salmon fry fed group.

Table 6 Overview over the C:N values of the two feeds: Salmon-fry feed and Zooplankton (nauplii from Semibalanus balanoides).

Feed values	µg N/mg	µg C/mg	C:N Ratio
Salmon-fry feed	95	454	7,19
Zooplankton (nauplii from *Semibalanus balanoides)*	86	362	6,3

Table 7 Overview over the mean C and N values/mg weighed in body tissue and gonad in the different treatments (n=24), The start population (n=17) from week 0 is added for comparison, together with the ratio of C:N.

Mean values	µg N/mg Body tissue	µg C/mg Body tissue	C:N Ratio	µg N/mg Gonad	µg C/mg Gonad	C:N Ratio
Start-population (n=17)	96	423	4,4	91	478	5,3
Salmon-fry feed (n=24)	104	425	4,1	100	470	4,7
Zooplankton (n=24)	109	463	4,2	96	495	5,2
Control (n=24)	109	437	4,0	97	457	4,7

5.4.3.4 C:N ratio of feed treatments

The results from the Shapiro-Wilks normality test showed that the C:N ratio for body biomass was normally distributed with p-values of 0,45 for Salmon-fry feed, 0,78 for Zooplankton and 0,65 for the control group. The results on data from C:N ratio on gonad biomass was also normal with p-values of 0,053 for Salmon-fry feed, 0,80 for Zooplankton and 0,35 for the control group. Resulting in using a One-Way ANOVA followed by Tukey's pairwise test (Appendix IV for additional details on the statistical data)

The results showed that there was a significant difference between the C:N of body tissue biomass (B) and the C:N of gonad tissue biomass (G) within the treatment groups. So, when the Zooplankton treatment showed significantly higher C:N than both the Salmon-fry feed and Control groups, both body tissue and gonads. No significant difference between the CN ratio of Salmon-fry feed and Control was observed. The results on CN ratio for body tissue was 4,08 (SD 0,13) for Salmon-fry feed, Zooplankton had 4,24 (SD 0,18) and the Control group had 4 (SD 0,1). For gonads, the C:N ratio was 4,72 (SD 0,3), Zooplankton 5,18 (SD 0,31), and Control group 4,73 (SD 0,34), (figure 38).

The p-values for the One-way ANOVA on C:N ratio of body tissue biomass were 3,99925E-07, showing a significant difference between the treatments. The p-value from the Tukey's pairwise the C:N ratio of body tissue biomass between groups was: (0,00079) for Salmon-fry feed and Zooplankton, (2,60E-07) for Zooplankton and Control, and (0,087) Salmon-fry feed and Zooplankton.

The p-values for the One-way ANOVA on C:N ratio of gonad tissue biomass were 1,1246E-06, showing a significant difference. The p-value from the Tukey's pairwise the C:N ratio of gonad tissue biomass between groups was: (1,08E-05) for Salmon-fry feed and Zooplankton, (1,20E-05) for Zooplankton and Control, and (0,999) Salmon-fry feed and Zooplankton

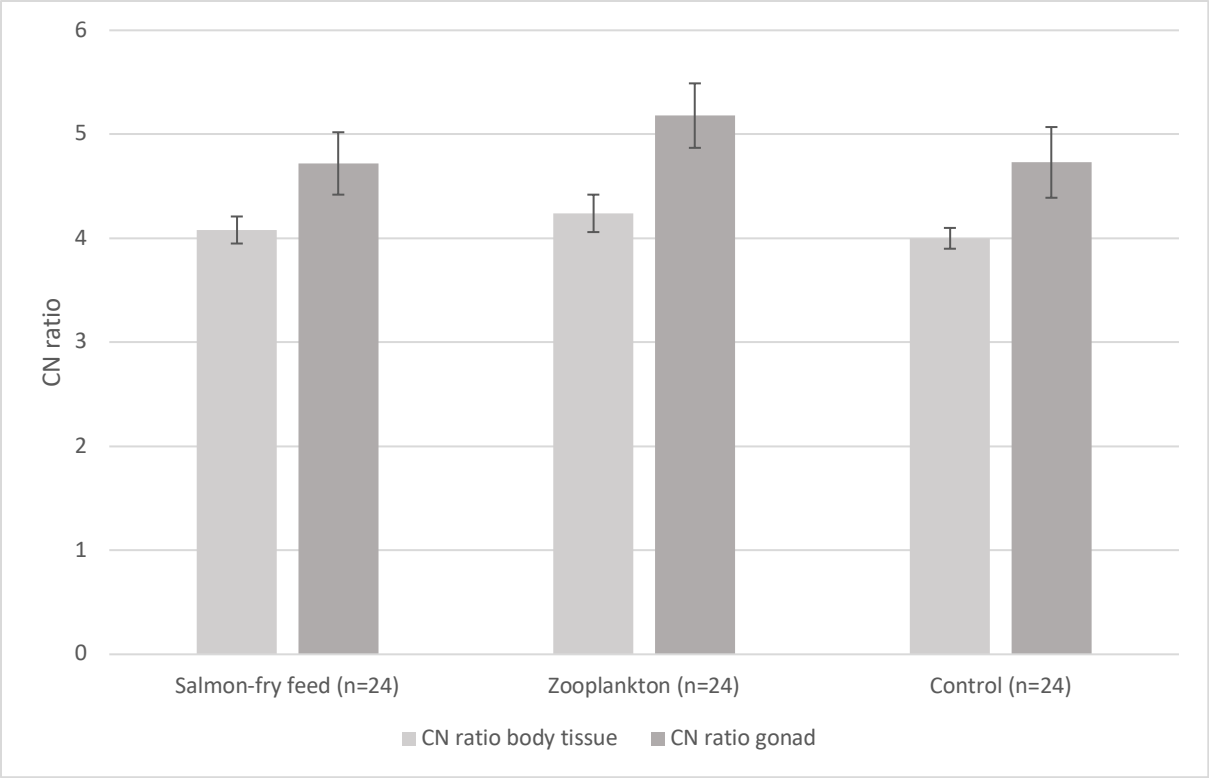

Figure 38 C:N ratio of the three different treatments on body tissue biomass (B) and gonad tissue biomass (G) at week 7 of the experiment (n=24), with SD.

5.4.3.5 C:N ratio analysis on treatments compared to start population

The results from the Shapiro-Wilks normality test showed that the data on C:N ratio for body tissue biomass (B) was not normally distributed for the Start-population with a p-value of 0,020. Resulting in using Kruskal-Wallis test and Dunn's test for pairwise comparison. The results from gonad tissue biomass (G) data showed that the data was normally distributed, but a Kruskal-Wallis test was used for both (B) and (G), followed by Dunn's post hoc test.

The results showed that there was a statistical difference between the same treatment groups for C:N ratio on (B) and (G). The groups with significant combinations were the same as when only the treatments were tested against each other using more replicates than when comparing the end treatments to the start population. However, comparing the Start-population to the end of seven weeks of feeding showed that there was a statistical difference between Start-population and Salmon-fry feed, also for Start-

population and Control, but there was no statistical difference between Start-population and Control. At the start of the experiment the barnacles had a C:N ratio of 4,45 (SD 0,32) in body tissue biomass, and 5,27 (SD 0,45) in the gonad tissue biomass. At week seven of the experiment Salmon-fry feed had a C:N ratio on body tissue biomass of 4,08 (SD 0,13) and for gonad tissue biomass 4,74 (SD 0,32). Zooplankton had a C:N ratio of 4,25 (SD 0,20) for body tissue biomass and 5,25 (SD 0,32) for gonad tissue biomass . Control had a C:N ratio of 3,99 (SD 0,11) for body tissue biomass and 4,70 (SD 0,27) for gonad tissue biomass (figure 39). Table 7 shows that the start population had the highest C:N ratio for body tissue biomass and gonad tissue biomass but the lowest µg C/mg weighed, and µg N/mg weighed body tissue biomass values. The values for gonad tissue biomass showed that the µg N/mg weighed gonad tissue biomass also was lowest for the Start-population, but not for the µg C/mg weighed gonad tissue biomass, where the control group had the lowest values. The N values increased after 7 weeks for body tissue biomass and gonad tissue biomass. The µg N/mg weighed gonad tissue biomass showed increased values for Zooplankton but decreasing values for control and Salmon-fry feed.

The results on the Kruskal-Wallis test showed that there was a significant difference between the body tissue biomass of the different treatments, including the data from the Start-population (p-value 8,779E-07). Going further to the Dunn's post hoc test. The results showed that there was a significant difference in the median values for C:N ratio on body tissue biomass between: Start-population and Salmon-fry feed (p-value 0,00012), Start-population and Control (p-value 4,143E-07), Salmon-fry feed and Zooplankton (p-value 0,0218) and between Zooplankton and Control (p-value 0,00043). There was however not a difference between the Start-population and Zooplankton (p-value 0,121) and between Salmon-fry feed and Control (p-value 0,221).

The results on the Kruskal-Wallis test showed that there was a significant difference between the gonad tissue biomass of the different treatments, including the data from the Start-population (p-value 3,015E-06). Going further to the Dunn's post hoc test. The results showed statistical differences in the median values for the same pairs as for the C:N ratio body mass: Start-population and Salmon-fry feed (p-value 0,00038), Start-population and Control (p-value 7,741E-05), Salmon-fry feed and Zooplankton (p-value 0,00037) and between Zooplankton and Control (p-value 7,33E-05). There was however

no difference between the Start-population and Zooplankton (p-value 0,986) and between Salmon-fry feed and Control (p-value 0,685).

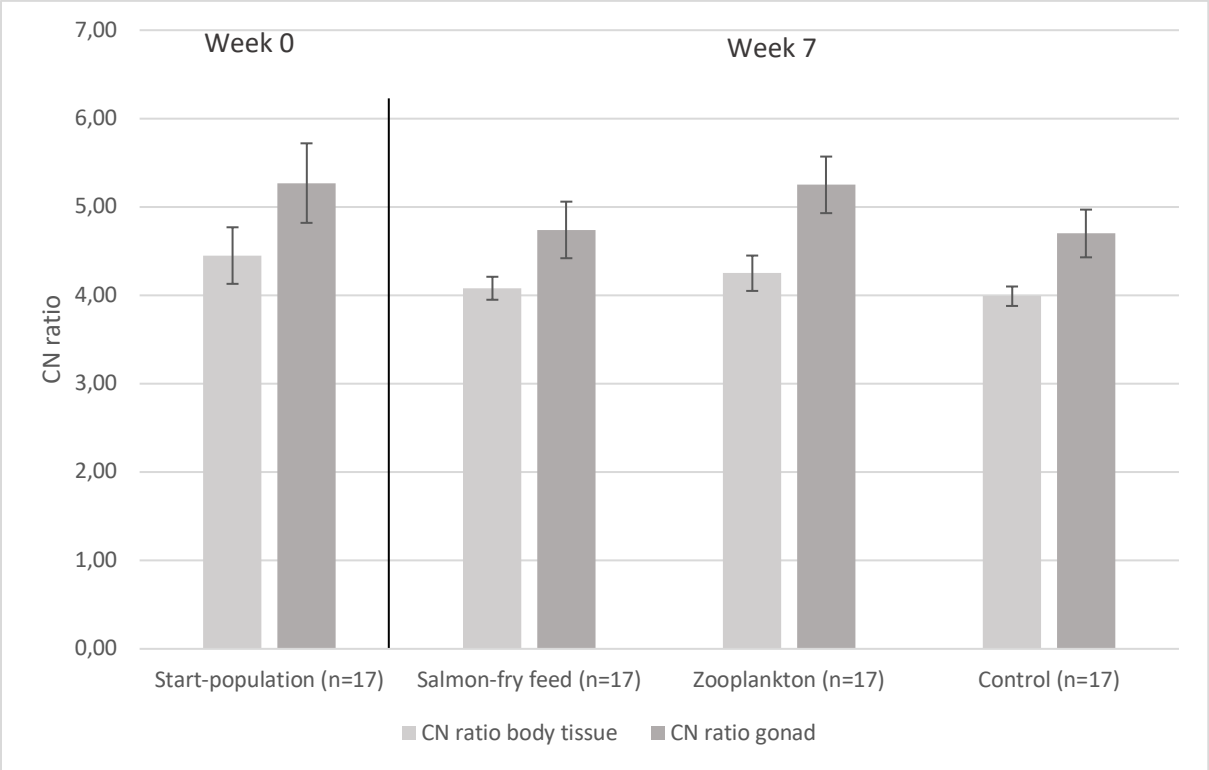

Figure 39 C:N ratio of the three treatments from week seven together with C:N values from the start population at week 0 (n=17), with SD. Showing the same significant differences between the treatments as in figure 36, chapter 4.4.3.3 but also showing significant differences between the Start-population to Salmon-fry feed and Control for both body tissue biomass and gonad tissue biomass.

5.4.3.6 Comparing mean gonad weight (mg) to total mean individual content of C and N (µg) in gonads

When comparing the mean gonad weight to the total mean individual content of C and N (without adjusting for shell size as is done in chapter 4.4.3.2), the graphs in figure 40, 41 and 42 shows that the C content in Zooplankton is very low compared to the other treatments. The N columns follow the same distribution as the weight columns. The mean weight of the Start-population was 7,23 mg (SD 6,18), for Salmon-fry feed the weight was 7,55 mg (SD 8,04), for Zooplankton 18,19 mg (SD11,42), and for Control the gonad weight was 2,83 mg (SD 1,81). The mean C content across all specimens showed that the Start-population had 3370 µg C (SD 3003), for Salmon-fry feed had 3557 µg C (SD 3816), Zooplankton 1993 µg C (SD5704), and for Control 1312 µg C (SD 781). The mean N content across all specimens showed that the Start-population had 633 µg N (SD 564), for

Salmon-fry feed had 754,23 µg N (SD 822), Zooplankton 1746 µg N (SD1102), and for Control 274,26 µg N (SD 177).

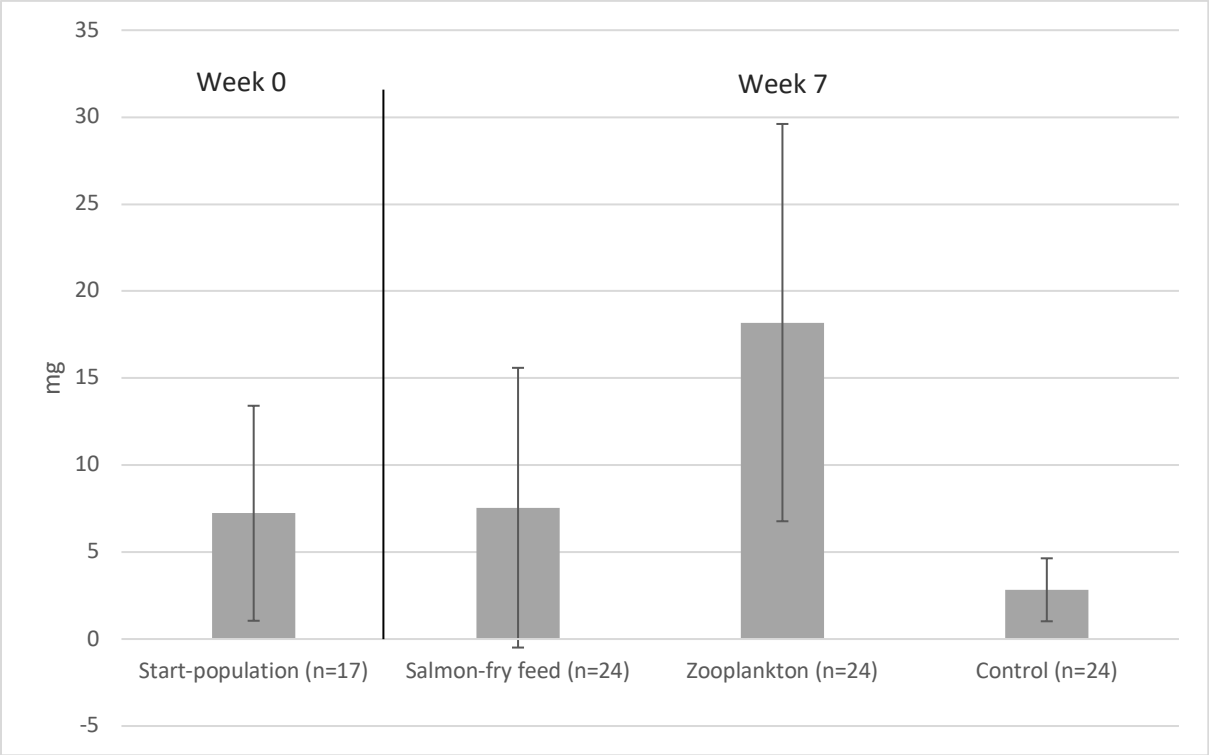

Figure 40 Mean gonad weight (mg) across all replicates at week 0 for the Start-population and week 7 for the three treatments, with SD.

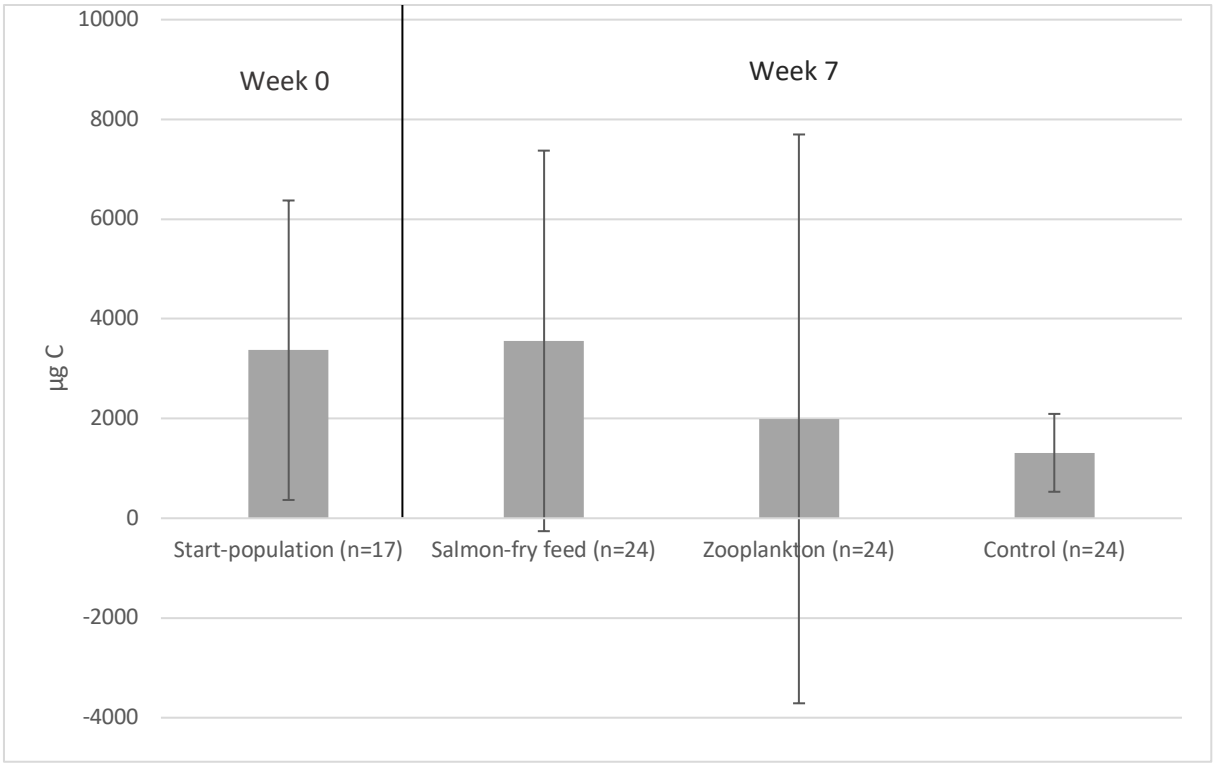

Figure 41 Mean content of C in the gonad tissue biomass across all replicates at week 0 for the Start-population and week 7 for the three treatments.

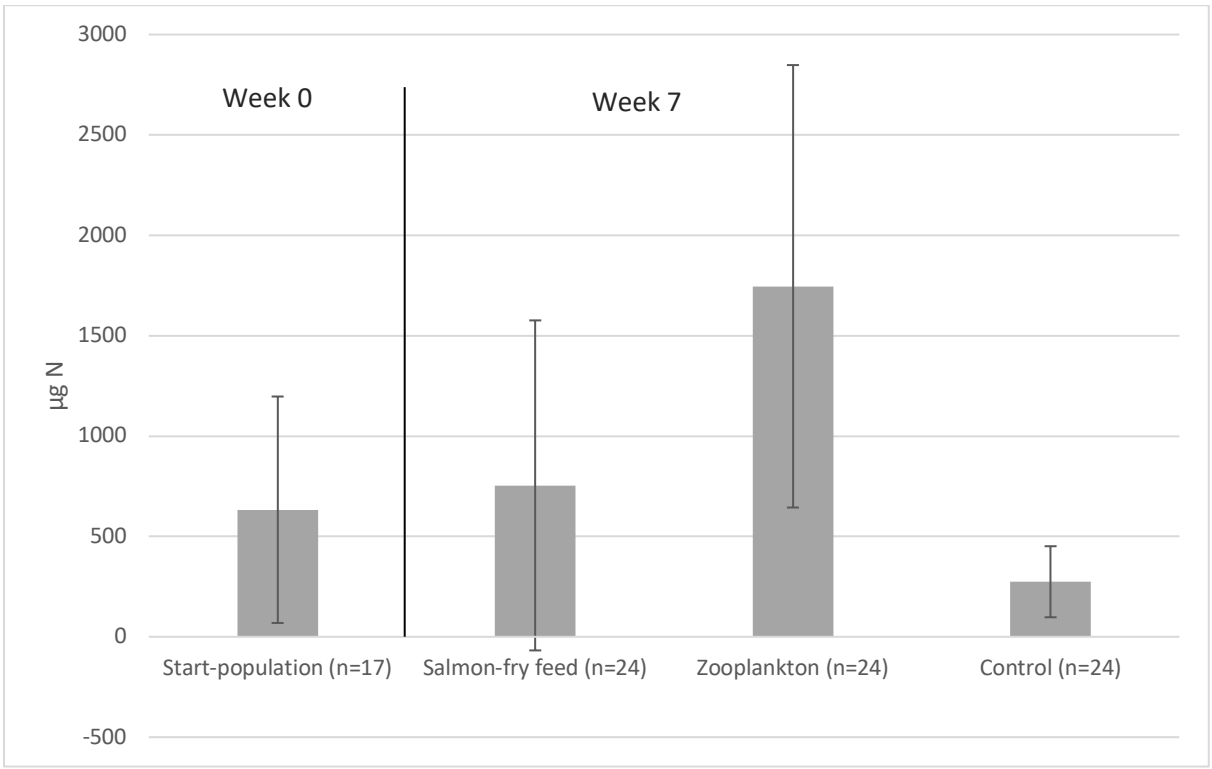

Figure 42 Mean content of N in the gonad tissue biomass across all replicates at week 0 for the Start-population and week 7 for the three treatments.

6 Discussion

6.1 Mapping barnacle settlement on Swedish bands season 2020-2021

The categorizing of shapes was initially done to get some background knowledge as a side part of the study. The information is kept in this thesis to give the reader a better understanding the of the shapes and how the barnacles are affected by the density that follows the preferred settling places. This seems to be between 2-5 meters, with a sweet spot between 3-4 meters. The Baltic Sea Rapport by Minnhagen, (2017) found that in most studies done on the preferred settlement of *Mytilus edulis,* was a depth between 2-5 meters. That is the same as the cultivated *Balanus crenatus* and could indicate that they prefer the same conditions and feed. A previous study done by Santhakumaran, (1984) showed that *B. crenatus* in Trondheimsfjorden showed a preference of the three meter depth, which is also the depth where they showed the best growth.

Considering *Asterias rubens* could be one of the reasons for mortality along the Swedish bands it interesting to look at figure 28. table 3 and 4 in chapter 4.4 together. It becomes obvious that *A. rubens* had a favourable salinity and optimal feeding temperature for large periods of the season. Giving them the possibility to feed on both *Mytilus edulis* and *B. crenatus* along most of the Swedish bands. Since the barnacles and *M. edulis* grew together on the bands and they shared the zones of live and dead individuals, it is fair to assume they have the same cause of death. If A*. rubens* is the main reason for mortality, they would probably eat *M. edulis* first as this is described to be one of their preferred food sources, before moving on to the barnacle (Agüera, 2015).

This year Planktonic removed most of the *M. edulis* of the bands during summer so that the barnacles had less competition for space and food. This year the barnacles died several months earlier in October, not in December. The removal of *M. edulis* could also have resulted in less available food for *A. rubens* so that they ate more *B. crenatus* while the temperature was still favourable at 10-13°C (Agüera *et al.*, 2012), resulting in barnacles dying earlier.

6.2 Growth in Trondheimsfjorden through summer

The barnacles that were moved from Hemnesberget to Trondheimsfjorden for measuring growth through summer showed little growth, and the survival rate was also low with only 15 individuals left at the end of period. The poor growth in Trondheimsfjorden may be caused by an assumed lower than normal spring bloom, that co supervisor Nils Tokle noticed when doing plankton samples through spring and summer (2021). According to (Sakshaug and Tangen, 2000, p 88) there was also an absence of spring bloom in 1966 because of extremely cold conditions resulting in the water masses mixing all the way down to 100 m. Plankton samples was not collected for this thesis, so the absence of the spring bloom in Trondheimsfjorden during spring 2021 is just aspeculation. Comparing the growth from Trondheimsfjorden and Hemnesberget was not intentionally planned, nor done in a scientifically way, but it was hard to not comment on the obvious difference in growth of these two locations when viewed together both from the summer growth at Hemnesberget the same season.

During late summer (2021), more cultivation bands were brought to Trondheim and hung out on the pier. These were still alive when checked on in March 2022, while most of the barnacles at Hemnesberget had died in October (2021). This further strengthen the theory about the mortality being caused by an unknown factor that follows the saltier water layers at Hemnesberget.

6.3 Pilot 1: Testing of experimental setup for Experiment 1 and 2

Based on clear observations of actively cirri beating in both treatments at the end of pilot 1, it was decided not to analyse the activity by running any further statistics on the filmed material to test for difference. And to go ahead with the top-down experiment 1 and 2 without feeding the barnacles. This decision was made even though the starved barnacles may have eaten some of their own nauplii whenever they were

Figure 43 Nauplii swarming towards the light (Light used for removal from aquarium).

released. But these nauplii were removed to best of ability as soon as they were discovered (figure 43). It was believed that starving would not affect the activity of the barnacles for the short amount of time it took to run the experiments (5 days).

Since the experimental setup worked as planned in pilot 1, no adjustments in the parameters light, salinity, temperature, water exchange and cleaning routines were made for top-down experiment 1 and 2.

The execution of pilot 1 seemed to be unintentionally perfectly timed for observing their behaviour, and both footage and filming of mating and nauplii release was obtained. The seemingly observation of sperm casting is of special interest, since results from a study done by (Barazandeh *et al.*, 2013) on gooseneck barnacle, *Pollicipes polymerus* showed that this species uses sperm casting as a mean of reproducing. They were previously thought to be a self-fertilizing species, resulting in questions about reproductivity biology in barnacles might have to be revisited. *B. crenatus* is not considered to be self-fertilizing, nevertheless if they also use sperm casting as a way of fertilizing this would be new knowledge regarding *B. crenatus.* It would be interesting to try to test further to possibly confirm this observation as a means of reproduction. Since this pedicular barnacle was not observed before the observation of "sperm casting" it might just be an observation of excess sperm being pushed out of the shell cavity from after copulation, as described in (Crisp and Southward, 1961). Another study done on *B. balanoides L* by (Crisp and Patel, 1960) describes observed sperm pools, that they concluded was an indication on copulation probably had occurred. There might be a video of the observed incident of possible sperm casting, but the time it would take to go through all that footage was not possible to do before the delivery of this thesis.

6.4 Pilot 2. Feeding barnacles dry formulated salmon fry feed

Pilot 2. was based on the idea that if the barnacles were fed during times of low food availability in winter, mortality might go down, and/or the gonad production might increase. By feeding them dry formulated salmon-fry feed in a pilot one could see if they actually ate it, and if it was any point in conducting an experiment later on using this feed

as one of the variables for testing their filter feeding performance. If the barnacles would eat formulated feed and grow on it, it could also be possible to include barnacle cultivation as a part of multitrophic aquaculture as the barnacles could eat particles from feed spill.

In lab the circulation in the aquariums were controlled to keep the food particles suspended in the water column. With no current from the aerators the feed quickly sank to the bottom. If feeding the barnacles are to be taken in situ, feed spill will surely be a challenge, and the amount of feed distributed would have to be increased to ensure enough feed is available for the barnacles.

The band chosen for this experiment was an extra band prepared for pilot 1 but that was not used. It was kept in a spare aquarium during pilot 1 in case it was needed later. When pilot 1 was terminated the bands were connected back together using strips and thrown back into the fjord. The next day it was decided to run pilot two and the extra band was taken back into the lab for pilot 2. The larger barnacles seemed to be a bit affected by the night in the ocean. Probably due to the different salinity in the fjord and lab (lab salinity was 34,6 and temperature 14-15 °C. The fjord had a salinity of 26,7 and a temperature of 15,1 °C), but the barnacles behaved normal again after a couple of days. The newly settled barnacles seemed less affected and showed normal activity right away. The smaller barnacles seemed more eager to feed in general and had a more rapid beat than their one-year-old older neighbours.

The smallest size feed from Ewos (appendix III) was too big for both the newly settled and the one-year-old barnacles, and it was observed that when a large barnacle finally managed to catch one pellet (size 015) it was released back into the water shortly after. The solution was to soak and mash the pellets to reduce the particle size to better suit the barnacles. This seemed to work well but the water ended up looking like a mud puddle. To make sure the water quality was good throughout the pilot, and to prevent bacterial growth that might harm the barnacles, the aquarium it had to be cleaned every day. The amount of food was also reduced by half and distributed twice per day to ensure the conditions were water with feed, and not a muddy soup. The amount still seemed to be ad libitum as the barnacles did not manage to be able to clear the water of particles before next feeding.

Even though this only was a small pilot it showed that the barnacles ate the feed when soaked and mashed. Leading to going ahead with the bottom-up experiment, Experiment 3, feeding the barnacles dry formulated salmon-fry feed and zooplankton together with testing the barnacles' starvation potential.

6.5 Experiment 1 and 2: Predation on Balanus crenatus by Nucella lapillus and Asterias rubens

In Experiment 1, a statistical difference between the two predator's feeding rate on juvenile barnacles were observed with *A. rubens* consuming almost twice the number of barnacles compared to *N. lapillus.* The large difference between the predators and how much barnacles they consumed in experiment 1, lead to taking more measurements of the predators in experiment 2, to be able to study the predators as well to find out which size classes they preferred. The results showed that the effect of growth in the different size categories are dependent on the type of predator, and the effect of growth is dependent on the size of the predator. *A. rubens* showed an increased consumption with increased size for *A. rubens.* In contrast, such a pattern could not be observed for *N. lapillus,* which could be explained by their shell size, and that they probably had reached maturity. It is mentioned by Fretter & Graham, (1994) in Tyler-Walters, (2007a) that there is a stop in point shell growth after reaching maturity and a size of 29,5 mm. An observed increase in weight for different size groups of *A. rubens* was found in a study by Smith, (1940) who's results showed that *A. rubens* fed mussels increased as much as 34 % for the size class of 42 mm in diameter (duration, Sept- Oct) and a 27% increase for 56 mm large *A. rubens*. The tendency showed a decline in increasing size with increasing size. And the 90 mm ones only increased 11%. This information combined with the information from Experiment 2, that the largest *A. rubens* seems to increase in weight more rapidly compared to the smaller *A. rubens* could indicate a sweet spot for explosive growth around a size between 40 and 50 mm. The idea of this sweet spot must be regarded with some caution, as the sample size of the size classes in Experiment 2 only had three replicates each. In addition, this experiment only lasted for five days which makes it hard to draw any conclusion on their growth over time. Also a study by Agüera, (2015) points to the

feeding rate potentially being a function of size for *A. rubens* in the size categories 80-160 mm in diameter, leaving contradictory conclusions on the theory for a sweet spot on growth.

Even though this was a comparative study of the two predators *A. rubens* and *N. lapillus,* it is important to note that the cultivated barnacle *B. crenatus* is only affected by predation by *A. rubens* and not *N. lapillus*. The reason for choosing *N. lapillus* together with *A. rubens* for the predation experiments was to have something to compare against since these two predators' prey on similar species such as *Balanus balanoides (Tyler-Walters, 2007a).* And are reported for being able to empty large areas along the shore line together with *N. lapillus* (Budd, 2008).

Experiment 1 and 2 could be viewed together as they are both similar and the results are connected through the predator's effect on barnacle settlement, but they could not be statistically compared due to two major confounding effects. Meaning comparisons between the predators from experiment 1 and 2, *A. rubens* to *A. rubens* and *N. lapillus* to *N. lapillus* to look for change in feeding rate on juvenile and adult barnacles was not done. Because of these two confounding effects the results of experiment 2 are not sound and can only indicate a decreased number of barnacles consumed with increased size of the barnacles (Figure 32 and 33). The reduction of consumed barnacles could be a result of increased barnacle size, or it could be the result a confounding effect caused by the extra measuring and weighing of the predators before the start of experiment 2, causing extra stress for on the predators that may have reduced their appetite. This confounding effect was caused by inconsistency in method by the author when weighing and measuring the predators, as this extra step was not done in the same way for experiment 1 where only length was measured. The other major confounding effect was the poor quality of the barnacles in experiment 2 that lead to having to disturb the predators even further by taking them out of the aquariums on day 1 of the experiment, to remove dead barnacles and clean the aquariums before continuing with the experiment. It was hard to separate the cause of death for all the dead barnacles, some were visibly dead without sign of the predators, other barnacles were harder to assess. This likly added to the uncertainty of the results in Experiment 2

Nevertheless, the results of these two experiments can give an approximate feeding rate of the predators of the chosen size range. Aiding in a rough estimation on maximum number of tolerated *A. rubens* per Swedish band. When *A. rubens* can consume six juvenile barnacles per day as done in experiment 1, and the average amount of barnacles with 3700 individuals per band (figure 28) it would take ten *A. rubens* two months to empty one band with juvenile barnacles. Or four months on adult barnacles in fall as the number of consumed barnacles were halved for Experiment 2. Se appendix I and II for data on the estimated individual consumption rate of the predators.

But the results of the estimated time it takes ten *A. rubens* to empty one band must be considered alongside other abiotic factors that affect *A. rubens* at Hemnesberget such as salinity and temperature. The temperature in experiment 1, was 14 °C. According to Agüera *et al.*, (2012) their optimal feeding temperature lies between 10-13 °C, and decreases with lower temperatures. They stop eating at 2 °C. For salinity, the water in experiment 1 had a stable value of 34, at Hemnesberget it fluctuated between 4,5 and 33 through the season of 2021. According to Budd, (2008) their preferred salinity range lies between 18-40. And as Dickey *et al.*, (2021) found out, they stop eating at a salinity of 12. The cultivation bands at Hemnesberget also contain another food source, tiny *M. edulis,* that grow alongside the barnacles. When adding this factor on top of the list of factors affecting the *A. rubens* predation rate on the barnacles, it is fair to assume it will take *A. rubens* longer to empty one band than calculated in this thesis. That said, dry summers with a salinity over 12 high up in the water column could indeed be a threat to the cultivated barnacles considering the high predation rate of *A. rubens.*

Other factors that were considered when choosing predators for these two experiments were the feeding habits of *A. rubens*. During spring they will stop their feeding rate for the duration of the spawning period (Sloan 1980 in Agüera, 2015) but the specimens used in these experiments were probably too small to be sexually mature, with a maximum size of 44 mm in diameter. According to Budd, (2008) sexual maturation is

described to be after individuals being two years of age and reaching 50 mm in diameter. But even then, it would be difficult to determine their age since they show plasticity in growth according to food availability. This led to assume the size chosen

Figure 44 On day 5 of the acclimatization period in experiment 1, Nucella lapillus to lay egg capsules on the rock in their aquarium.

would not be affected by going through spawning period and following decreased appetite as they were smaller than 50 in diameter at the start of the experiments, or close to 50 mm. On the other hand, *N. lapillus* were obviously sexually mature, as on day 5 of the acclimatization period for experiment 1 they started to lay egg capsules on the rocks in their aquarium (figure 44) This may have affected the appetite as *N. lapillus* are reported to stop eating during spawning (Crothers, 1985 in Tyler-Walters, 2007). But the knowledge about this fact was not learned until after Experiment 1 and 2 was finished. One more thing that could have been a confounding factor regarding *N. lapillus,* is the assumed different feeding rate according to wave action. In sheltered areas the snails have more time to feed resulting in increased growth, compared to areas with more wave action Osborne (1977); Crothers, (1985); Etter, (1989) in (Tyler-Walters, 2007b).*N. lapillus* in Experiment 1 and 2 had to feed when submerged and this may have

affected the feeding rate, as it may not have been optimal, but they did feed when submerged so if it is a difference in feeding rate between being submerged in still water where they don't need to attach themselves from not being washed away by the waves, or when being out of water is not known by the author as no source was found on the topic.

The laying of egg capsules was only observed during Experiment 1, not in Experiment 2. And is one more confounding effect between Experiment 1 and 2. Largen, (1967) mentions observing the connection of laying egg capsules, to the mean sea temperature exceeding 9 °C. And that the rise in mean temperature from 9-10 °C is stimulating the onset of oviposition. In Experiment 1 and 2 the temperature was held at a stable 14 °C. This stable temperature could be the reason for the laying of eggs in the aquarium, or it could simply be that they would have done it anyway as it was a similar temperature outside in the sea as well. The assumed reason for not laying egg capsules in experiment 2, may be because they were done with laying egg capsules for the season. The statement of *N. lapillus* stopping to eat when laying egg capsules seems to go against the findings in Experiment 1 and 2 where *N. lapillus* ate more barnacles, but smaller ones while laying egg capsules, than when thy didn't lay egg capsules when eating larger barnacles later in Experiment 2. This difference could of course be a result of the barnacles in Experiment 2 containing more food, leading to the need to feed less frequently, or that the barnacles took longer to open, as they were bigger, or the fact that they were went through more stressful measuring prior to the experiment that may have affected their appetite. The snails used in Experiment 2 seemed to use the method of drilling a hole to feed on *S. balanoides,* which is described by Rovero, Hughes and Chelazzi, (1999) to be the slowest method of feeding. The feeding method for Experiment 1, was not noted, but as the snails were collected at the same beach it is assumed they would choose the same method to feed. The inability to change feeding technique for the snails that were used to drill holes to feed, was also mentioned in Rovero, Hughes and Chelazzi, (1999).

Largen, (1967) also found different feeding rates for *N. lapillus* at different temperatures. Compared to the results of the present study, the author found that the mean number of *S. balanoides* consumed was 1,45 barnacles per day, at 15 °C. The numbers in Experiment 1 had 2,34 barnacles (juvenile *B. crenatus*) per day, at 14 °C, in

Experiment 2 on larger barnacles, *N. lapillus* ate 0,74 barnacles per day at 14 °C (appendix I and II). Largen did not state if the prey was submerged when feeding the snails, but it is assumed when looking at their method.

N. lapillus seemed to prefer the larger individuals of juvenile *B. balanoids* they got as feed during the acclimatisation period before Experiment 2 (27.06.2021).

A. rubens may have had too little food at the end of acclimatization period prior Experiment 2, as one of them may have turned to cannibalism during the two days of starvation before the Experiment start. One of the smaller specimens had lost one arm and got excluded from the experiment.

6.6 Experiment 3

This experiment was conducted for several reasons. Firstly, we knew from the short pilot number 2 that the barnacles ate salmon-fry feed. But we wanted to see if they could live and grow on it. In addition, the experimental setup with a starvation group, a salmon-fry fed group and a group fed zooplankton (nauplii from *S. balanoides*) would give us an indication on whether starvation could be the cause of death during winter, and if the barnacles grew and developed on their respective diets. If starvation turned out to be a main course of death, then the possibility of being able to feed them with salmon-fry feed would be of utmost interest. This would be interesting for Planktonic AS as a cultivator of barnacles, but it could also be interesting to utilize feed spill from smolt farms for growth on lower trophic levels.

Zooplankton as feed, clearly gave the best shell growth and showed a significant difference within groups from week 43, two weeks into the experiment. Salmon-fry feed showed a significant difference in growth at week 45, four weeks into the experiment. While the control group did not show any significant growth during the seven weeks the experiment lasted, even though they showed some growth. The most interesting part was to see if salmon-fry feed could be used to increase growth in times of food scarcity, and five weeks into the experiment, at week 46 and 47 there was a significant difference in

growth between the control group and salmon-fry feed. There was also a significant difference between the control group and zooplankton at week 43, two weeks into the experiment. Meaning if Planktonic AS wants to feed their barnacles formulated salmon-fry feed it could be possible to increase the shell growth after 5 weeks if they can find a way to keep the feed suspended in the water column, as the feed was observed to sink to the bottom of the boxes if the aeration did not provide enough circulation. Distributing the feed at the cultivation site instead of in small plastic boxes could prove to be challenging and time consuming, so the cost/benefit should be carefully considered. Planktonic AS have mentioned the possibility of putting out a bubble plant at the cultivation sight and this might help with keeping the feed suspended a while longer. There is already a bubble facility at the harbor at Hemnesberget (appendix VIII) to add circulation in the water masses during winter to keep the harbor from freezing.

As for the growth of the animals and gonad, the salmon-fry fed group kept the same body and gonad mass to shell size ratio after seven weeks, this may indicate that they got approximately the same amount of feed as the barnacles had access to in the sea before being taken up for experiment 3. However, they did grow and showed significant difference to the control group both in body mass and in gonad mass which was what this thesis wanted to test for. If there is a way of feeding the barnacles salmon-fry feed in the sea, they will possibly grow resulting in more gonad compared to the starved individual in the Control-group. But the most interesting results was the zooplankton fed barnacles. They increased the most of all treatments and in addition showed a larger gonad mass than body weight mass.

The comparison between the gonadosomatic index (GSI) of the start population with (42%) and the starved Control group after 7 weeks with a GSI of 43%, only shows an increase of 1%. This could mean that it still was too early in the season for the gonad to grow and mature as an assumed natural diet would have with little to no food at that time of year. But when the barnacles were fed, the GSI increased with 5 % for the Salmon-fry feed and 17% for the Zooplankton fed barnacles compared to the Start-population (week 0). Showing that the barnacles will not only use extra energy for shell growth as shown in chapter 4.4.1.3 but also utilize the extra energy for developing their gonad. When the barnacle tissue and gonads were separated, some of the gonads of each treatment were

compared under a microscope, but no obvious difference was noted. Because the gonad development, apart from the sheer mass of it was not a part of the focus of this thesis, the development of the gonads was not studied any further.

When looking at the C:N values of the feed in table 7 and 8 in chapter 4.4.3.5, the amount of µg N/mg and µg C/mg of the values in the feed, seemed only to be reflected for the N value of the gonad, but was reversed for the other measurements. Since the Zooplankton fed group increased in value for both of µg N/mg and µg C/mg for body tissue compared to Salmon-fry fed barnacles, despite higher of µg N/mg and µg C/mg values in the Salmon-fry feed it looks like the barnacles may have had problems catching the Salmon-fry feed during the experiment, as the particles of the salmon-fry feed were smaller than the Zooplankton feed. Problems with catching the Salmon-fry feed was already suspected during the experiment as the boxes had less feces compared to the Zooplankton fed boxes. And the barnacles were not as active as the Zooplankton fed barnacles, especially at the last weeks of the experiment. When looking at the Control group values against the Salmon-fry fed group the values are higher for µg N/mg and µg C/mg values except for µg C/mg body tissue where the starved Control-group had higher value than the Salmon-fry fed group. This could indicate that the barnacles distribute their recourses differently regarding growth of shell, body tissue and gonad depending on availability to feed and nutrition. Another possible reason for lower C and N values in the Salmon-fry fed barnacles, could be inability to utilize the nutrition provided, and that the nutrient in the Zooplankton is easier to utilize for growth.

6.6.1 Other observations made during the experimental period

As Experiment 3 progressed and through daily observation of the barnacles it became more obvious that the boxes fed zooplankton had a higher activity level than the control and salmon-fry feed boxes. In the morning, before light has been turned on and the barnacles got their box cleaned and been given food, most of them were usually all inside their shell. After being disturbed is seemed they woke up and came out fanning their cirri in search for food. The individuals that just had gone through a moulting seemed more careful the first day or two. They also showed restricted cirri movement compared to the

barnacles that hadn't gone through this process in a while. See figure 45 for a visual of this difference. In a study done by Crisp and Patel, (1960) they tested *B. balanoides* for starvation for the duration of one year to see if it affected the moulting cycle. But it was not made clear if this starved group was starved for one year, or if it was done in batches for a shorter period. It does not say for how long this starving was done but the measurements lasted from July-to-July next year. They mention a decreased activity in the starved individuals, with only beating of the cirri after changing of water. This reduction in activity of the starved individuals was also experienced in this bottom-up experiment.

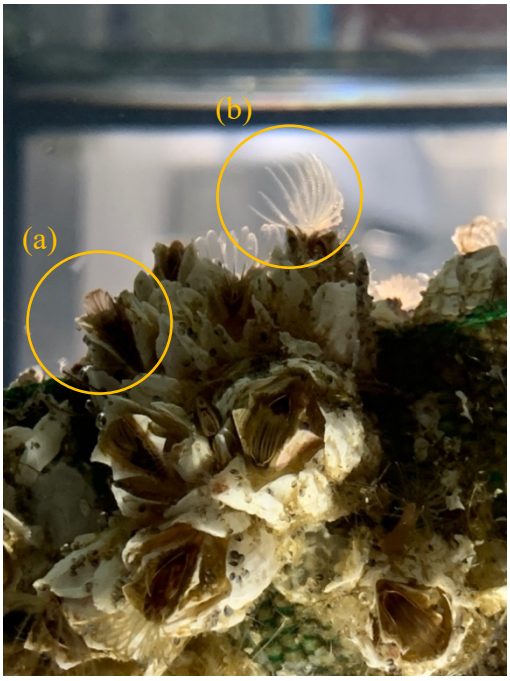

Figure 45 Appearance of the cirri when (a) the barnacle recently has gone through a moult, with restricted movement (b) a while after with full extension and movement of the cirri.

This restricted pumping movement was also observed in the control boxes as a more general way of cirri movement. Which is logic after being starved for a while. Almost all the barnacles in the zooplankton boxes on the other hand were actively fanning before feed were given. The boxes with salmon-fry feed behaved somewhere in the middle. This might be a result of less available food than in the zooplankton boxes. The aim of giving the fed barnacles the same amount of feed may have failed because of the particle size of the feed. The amount in dry weight was calculated to be close to the same with 9,5-10 g of zooplankton to 1-1,2 g of salmon-fry feed. But the zooplankton size is 320 µm and the salmon-fry feed seemed to be only around 10-20 µm when checked under a microscope, after it was soaked, mashed and dissolved in water (Planktonic AS, no date c).

A study done on *B. balanoides* by Crisp and Patel, (1960) showed that the frequency of moulting decreased with lower temperature and no access to food. But the moulting did not stop entirely. The exception was recently fertilized individuals that stopped moulting altogether for a period of six to eight weeks before they resumed moulting again. They believed this was to prevent the fertilized eggs from being shed with the skin. *B.*

balanoides also had a distinct fall in moulting rate by the end of November, which was the same time Experiment 3 was terminated. They suggested the pause in moulting activity could be caused by a basic physiological rhythm, same as the loss of penis by *B. balanoides* after the fertilisation period. They had no data on loss of penis in *B. crenatus* after copulation, and it was not observed during the time of this master thesis, even though there were both copulations and releasing of nauplii during the time of the pilot 1 period.

According to Håvard Aakerøy, Cofounder of Planktonic AS (personal communication 2022) the barnacles started to die around 15/11-2021. This was a lot earlier than during the fall/winter of 2020 when they died during mid-December. This year it was estimated that the barnacles died during October. With most dead barnacles found furthest down on the band. Compared to last year when the surviving barnacles were practically empty this time of year, they now had plenty of gonad. An explanation of the early deaths could be the seemingly larger population of *A. rubens* this year, as it was larger than last year, and the year before when there was practically none.

The remaining barnacles that were left to test for starvation potential at the end of Experiment 3 showed no mortality in any of the treatments. The experimental setup had both higher salinity (34) and no food (for 14 weeks, 11.10.2021-14.01.2022) compared to Hemnesberget which might have had some but little food availability at the time of the experiment. The barnacles at Hemnesberget however died in the lower part of the band where salinity was higher than at the surface sometime during October. This was during the same time period as Experiment 3. Håvard Aakerøy (personal communication, 2021) informed that a small test on elevating a few bands to the fresher water layers showed good survival and filling grade of gonad mass. These experiences combined with Experiment 3 exclude high salinity or lack of food as cause of death in the lower saltier water layers at Hemnesberget. And leads toward a possible reason for mortality being caused by organisms that is limited by the salinity gradient and-or temperature. Like predators, or perhaps parasites, viruses, or fungi.

7 Conclusion

This master thesis has studied the bottom-up and top-down control mechanisms that affect the survival and growth potential of the cultivated barnacle *Balanus crenatus*.

The introductory results of barnacle counting and salinity measurements together with the top-down experiment results, indicate that *Asterias rubens* could be one of the main causes for the high mortalities at Hemnesberget. But further studies need to be done, preferably in situ to confirm this as the main cause of barnacle mortality in the saltier water layers at sight. Of the two predators *A. rubens* consumed in general twice the number of barnacles compared to *Nucella lapillus*.

During the bottom-up experiment, when the barnacles tolerance to starvation was tested, the results showed that they lost both gonad and body mass, but starvation did not kill them. The bottom-up results also showed that feeding the barnacles during a period of food scarcity will increase growth and gonad production. The best results were seen in the Zooplankton treatment with an increase of gonadosomatic index (GSI) of 17%, but Salmon-fry feed also showed significant results on growth and gonad production and had a (GSI) of 5% compared to the starved control group. The C:N content of the barnacles body tissue and gonad biomass did not mirror their feed source content of C:N, indicating the barnacles could have had problems with either catching the Salmo-fry feed, or that the barnacles could not utilize the nutrients in the Salmon-fry feed to build body and gonad mass.

If these results are to be applied in in cultivation of barnacles, it is recommended to address the mortality aspect before feeding the barnacles. The cost of increasing gonad output, together with the assumed difficulties of distributing the feed in situ due to sinking rate and particle size of the feed particles. This would be outweighed by the loss of barnacles due to mortality, even though the results of feeding the barnacles were good.

8 Future work

During the time periode of this master thesis the largest and oldest *Balanus crenatus* that has ever been recorded was harvested by Planctonic AS. They were both larger and older than described in previous litterature, at least to the authors knowledge. These large and old barnacles should be studied further on aspects regarding feed, growth, and gonad production. They were killed for harvest, so we actually don't know if they could get even older than 22 months. Why do they get so old at Hemnesberget?

In situ observations of the predator *Asterias rubens* on the cultivation bands shows potential as a follow up study to the predation experiments 1 and 2 to see if their precense is the cause of the general mortality in the saltier layers of the cultivation site.

Looking at the death zone along the cultivation bands, a rotating of depth on the cultivation bands according to best settlement and growth rate in summer and fall should be tested, with a change to fresher water for winter maturation of gonads before harvest.

Since experiments showed that the barnacles ate formulated feed and grew compared to the starved control-group. This promotes a future exploration on how to feed them in field. Challenges would be finding the right particle size so that they stay suspended long enough for the barnacles to catch the feed. The feed used in the feeding experiment also produced slime on the bands. This could possibly be a problem for the barnacle health and the environment close by. But if barnacles could be fed leftovers from the salmon industry it could actually be beneficial on several ecological aspects.

Since barnacles are crustaceans, they could possibly be vulnerable to delousing chemicals. And since they are an important source of food in the food web it would be beneficial to establish their tolerance levels. Both for adults, nauplii and cypris larvae.

Since taurine have shown good effect on eye development in Sea bass and possibly increases successful prey attack for the larvae. It should be possible to examine if the taurine levels in barnacle nauplii show the same effect, and if taurine is important for eye development in all cultivated marine larvae.

Assuming Planktonic AS will expand to several facilities, developing a GSI rating system for barnacles, as a quantitative method to use for communication about the barnacle's gonad mass to body ratio, and the developmental stage of the gonad, could be a useful tool for estimating when to harvest and how much product one could expect during harvest. Especially if used as a training tool for those who lacks the tacit knowledge about the maturation process of cultivated barnacles.

9 References

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Appendix

Appendix I - Data used for analysing the results in Experiment 1

Table 8 The results of a Welch's t-test for two groups with unequal variance ($p < \propto 0,05$), used to test for difference between the two predators used in Experiment 1

A Welch's t-test for two groups with unequal variance (ratio of 4>)

	***Nucella lapillus*, mean consumed**	***Asterias rubens* mean consumed**
Mean	82	217
Varians	1027	32
Observations	3	2
Assumed difference between means	132	
df	2	
t-Stat	-14,10482352	
P(T<=t) one-sided	0,002494452	
T-crit, one-sided	2,91998558	
P(T<=t) two-sided	0,004988905	
T-crit, two-sided	4,30265273	

Table 9 Mean values of consumed barnacles during Experiment 1 (5 days, n=7), and (SD) for all treatments. The mean ratio between the predators in % is also shown.

Replicate number	***Nucella lapillus* mean consumed**	***Asterias rubens* mean consumed**	**Controll/ dead, mean**	**Mean Ratio %**
R1	84	221	0	38
R2	113	213	0	53
R3	49	208	0	24
Mean	82	214	0	38
SD	32,0	6,6	0	
Mean per individual	12	31	0	
Mean per individual per day	2	6	0	

Table 10 Mean values of consumed barnacles (n=1) across all three replications during Experiment 1, and (SD) for all replications. The mean ratio between the predators in % is also shown.

Replicate number	***Nucella lapillus***	***Asterias rubens***	**Mean Ratio %**
R1	12,0	31,6	38
R2	16,1	30,4	53
R3	7,0	29,7	24
Mean	11,7	30,6	38
SD	4,6	0,9	
Mean per ind per day	2	6	

Table 11 Overview over data collected in Experiment 1

	Experiment 1 (05.07.21-10.07.21)								
Treatment	***Nucella lapillus***	***Asterias rubens***	**Controll**	**Controll**	***Asterias rubens***	***Nucella lapillus***	***Asterias rubens***	***Nucella lapillus***	**Controll**
Aquarium code - Replicate number	**A1-R1**	**A2-R1**	**A3-R1**	**A4-R2**	**A5-R2**	**A6-R2**	**A7-R3**	**A8-R3**	**A9-R3**
Barnacle number start	333	598	105	147	458	505	400	357	134
Barnacle number end	249	377	104	147	245	392	192	308	134
Difference/eaten	84	221	1	0	213	113	208	49	0
/7 individuals	12	32	0	0	30	16	30	7	0

Appendix II - Data used for analysing the results in Experiment 2

Table 12 The results of a Welch's t-test for two groups with unequal variance ($p < \propto 0,05$), used to test for difference between the two predators used in Experiment 2

A Welch's t-test for two groups with unequal variance (ratio of 4>)

	***Nucella lapillus,* average eaten**	***Asterias rubens,* average eaten**
Mean	26	76
Varians	196	853
Observations	3	3
Assumed difference between means	50	
df	3	
t-Stat	-5,347772003	
P(T<=t) one-sided	0,006394198	
T-crit, one-sided	2,353363435	
P(T<=t) two-sided	0,012788395	
T-crit, two-sided	3,182446305	

Table 13 Total mean values of consumed barnacles during experiment 2, (5 days, n=7), and (SD) for all treatments. The mean ratio in % is also shown.

Replicate number	***Nucella lapillus,* mean consumed**	***Asterias rubens,* mean consumed**	**Controll/dead, mean**	**Mean Ratio %**
R1	40	80	6	50
R2	12	103	0	12
R3	26	45	3	58
Mean	26	76	3	34
SD	14,0	29,2	3,0	
Mean per individual	4	11	0	
Mean per individual per day	1	2	0	

Table 14 Mean values of consumed barnacles (n=1) across all three replications during Experiment 2, and (SD) for all replications. The mean ratio between the predators in % is also shown.

Replicate number	***Nucella lapillus***	***Asterias rubens***	**Controll, average dead**	**Mean Ratio %**
R1	5,7	11,4	0,9	50
R2	1,7	14,7	0,0	12
R3	3,7	6,4	0,4	58
Mean	3,7	10,9	0,4	34
SD	2,0	4,2	0,4	
Mean per ind per day	1	2		

Table 15 Overview over data collected in Experiment 2

	Experiment 2 (06.09.21-11.09.21)								
Predator	***Nucella lapillus***	***Asterias rubens***	**Controll**	**Controll**	***Asterias rubens***	***Nucella lapillus***	***Asterias rubens***	***Nucella lapillus***	**Controll**
Aquarium code-Replicate number	**A1-R1**	**A2-R1**	**A3-R1**	**A4-R2**	**A5-R2**	**A6-R2**	**A7-R3**	**A8-R3**	**A9-R3**
Barnacle number start	283	235	280	331	237	211	156	222	209
Barnacle number end	243	155	274	331	134	199	111	196	206
Difference/eaten	40	80	6	0	103	12	45	26	3
/7 individuals	6	11			15	2	6	4	

Table 16 Weight increase for Nucella lapillus in all size classes and replicates, with mean values and (SD)

Weight increase, *Nucella lapillus*

Size class	A1-R1	A6-R2	A8-R3	mean	SD
1	0,0590	0,0387	0,0191	0,039	0,02
2	0,0731	0,1109	0,0640	0,083	0,02
3	0,4249	0,0435	0,1155	0,195	0,17
4	0,0881	0,1910	0,0896	0,123	0,05
5	0,0930	0,0601	0,0208	0,058	0,03
6	0,1834	0,0293	-0,0082	0,068	0,08
7	0,0417	-0,0207	-0,0034	0,006	0,03

Table 17 Weight increase for Asterias rubens in all size classes and replicates, with mean values and (SD)

Weight increase, ***Asterias rubens***

Size class	A2-R1	A5-R2	A7-R3	mean	SD
1	0,1010	0,1391	0,0411	0,094	0,04
2	0,1169	0,1048	0,1496	0,124	0,02
3	0,1872	0,2734	0,2907	0,250	0,05
4	0,3750	-0,0178	0,5104	0,289	0,22
5	0,2447	0,2964	0,3605	0,301	0,05
6	0,3579	0,4273	0,5149	0,433	0,06
7	0,5010	0,9533	0,7500	0,735	0,18

Table 18 Mean weight gain for the predators Nucella lapillus and Asterias rubens for all 7 size classes (1-7), with (SD)

Mean weight gain in gram (n = 3)

Size class	*Nucella lapillus*	*Asterias rubens*	SD *Nucella lapillus*	SD *Asterias rubens*
1	0,04	0,09	0,02	0,04
2	0,08	0,12	0,02	0,02
3	0,20	0,25	0,17	0,05
4	0,12	0,29	0,05	0,22
5	0,06	0,30	0,03	0,05
6	0,07	0,43	0,08	0,06
7	0,01	0,74	0,03	0,18

Table 19 An overview over the predator's weight increases of all size classes (1-7) with three replicates

Size class	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Nucella lapillus	0,0590	0,0731	0,4249	0,0881	0,0930	0,1834	0,0417
	0,0387	0,1109	0,0435	0,1910	0,0601	0,0293	-0,0207
	0,0191	0,0640	0,1155	0,0896	0,0208	-0,0082	-0,0034
Asterias rubens	0,1010	0,1169	0,1872	0,3750	0,2447	0,3579	0,5010
	0,1391	0,1048	0,2734	-0,0178	0,2964	0,4273	0,9533
	0,0411	0,1496	0,2907	0,5104	0,3605	0,5149	0,7500

Table 20 ANOVA Two-factor with replication. Testing difference between predators and their feeding rate in seven different size classes.

SUMMARY	1	2	3	4	5	6	7	Totalt
Nucella lapillus								
Count	3	3	3	3	3	3	3	21
Sum	0,1168	0,2480	0,5839	0,3687	0,1739	0,2045	0,0176	1,7134
Mean	0,0389	0,0827	0,1946	0,1229	0,0580	0,0682	0,0059	0,0816
Varians	0,0004	0,0006	0,0411	0,0035	0,0013	0,0103	0,0010	0,0092
Asterias rubens								
Count	3	3	3	3	3	3	3	21
Sum	0,2812	0,3713	0,7513	0,8676	0,9016	1,3001	2,2043	6,6774
Mean	0,0937	0,1238	0,2504	0,2892	0,3005	0,4334	0,7348	0,3180
Varians	0,0024	0,0005	0,0031	0,0753	0,0034	0,0062	0,0513	0,0563
Total								
Count	6	6	6	6	6	6	6	
Sum	0,398	0,619	1,335	1,236	1,076	1,505	2,222	
Mean	0,066	0,103	0,223	0,206	0,179	0,251	0,370	
Varians	0,002	0,001	0,019	0,040	0,020	0,047	0,180	

ANOVA: Two-Factor with replication

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-crit*
Selection	0,5867	1	0,5867	40,9862	0,0000006	4,1960
columns	0,3588	6	0,0598	4,1771	0,0040279	2,4453
Interaction	0,5518	6	0,0920	6,4240	0,0002418	2,4453
within	0,4008	28	0,0143			
Total	1,8980	41				

Table 21 Tukey's post-hoc on the results of the Two-way ANOVA from experiment 2, Factor B, p- values – size class of the predators Nucella lapillus and Asterias rubens (1-7)

Size classes	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1		0,9964	0,6688	0,2958	0,5403	0,07483	0,0006066
2	0,9964		0,9434	0,6426	0,8756	0,2395	0,00293
3	0,6688	0,9434		0,9947	1	0,8153	0,03723
4	0,2958	0,6426	0,9947		0,9994	0,9898	0,1459
5	0,5403	0,8756	1	0,9994		0,9034	0,05923
6	0,07483	0,2395	0,8153	0,9898	0,9034		0,4742
7	0,0006066	0,00293	0,03723	0,1459	0,05923	0,4742	

Table 22 Tukey's post-hoc, Factor A, p- values - Predators

	Nucella lapillus	***Asterias rubens***
Nucella lapillus		2,469E-08
Asterias rubens	2,469E-08	

Table 23 Interactions between predators Nucella lapillus and Asterias rubens and their size groups (1-7) Significant interactions are marked in yellow

Predator – size group	***Predator – size group***	**Q**	**p**
Nucella lapillus - 1	*Nucella lapillus* - 2	0,7086	1
Nucella lapillus - 1	*Nucella lapillus* - 3	0,7086	1
Nucella lapillus - 1	*Nucella lapillus* – 4	1,361	0,9923
Nucella lapillus - 1	*Nucella lapillus* – 5	0,3084	1
Nucella lapillus - 1	*Nucella lapillus* - 6	0,4737	1
Nucella lapillus - 1	*Nucella lapillus* - 7	0,5358	1
Nucella lapillus - 1	*Asterias rubens - 1*	0,888	0,9997
Nucella lapillus - 2	*Nucella lapillus - 3*	0	1
Nucella lapillus – 2	*Nucella lapillus - 4*	0,6519	1
Nucella lapillus – 2	*Nucella lapillus - 5*	0,4002	1
Nucella lapillus – 2	*Nucella lapillus - 6*	0,235	1
Nucella lapillus - 2	*Nucella lapillus - 7*	1,244	0,9959
Nucella lapillus – 3	*Asterias rubens - 2*	0,666	1

Nucella lapillus – 3	***Nucella lapillus - 4***	**0,6519**	**1**
Nucella lapillus – 3	*Nucella lapillus - 5*	0,4002	1
Nucella lapillus – 3	*Nucella lapillus - 6*	0,235	1
Nucella lapillus – 3	*Nucella lapillus - 7*	1,244	0,9959
Nucella lapillus – 4	*Asterias rubens - 3*	2,718	0,6552
Nucella lapillus – 4	*Nucella lapillus - 5*	1,052	0,9989
Nucella lapillus – 4	*Nucella lapillus- 6*	0,8869	0,9997
Nucella lapillus – 4	*Nucella lapillus - 7*	1,896	0,348
Nucella lapillus – 5	*Asterias rubens - 4*	2,695	0,6657
Nucella lapillus – 5	*Nucella lapillus - 6*	0,1653	1
Nucella lapillus – 5	*Nucella lapillus - 7*	0,8442	0,9998
Nucella lapillus – 5	*Asterias rubens - 5*	3,93	0,1919
Nucella lapillus – 6	*Nucella lapillus - 7*	1,009	0,9992
Nucella lapillus – 6	*Asterias rubens – 6*	5,918	0,008148
Nucella lapillus – 6	*Asterias rubens - 7*	11,81	1,806E-07
Asterias rubens - 1	*Asterias rubens - 2*	0,4867	1
Asterias rubens - 1	*Asterias rubens – 3*	2,539	0,7325
Asterias rubens – 1	*Asterias rubens – 4*	3,167	0,4553
Asterias rubens – 1	*Asterias rubens – 5*	3,351	0,3797
Asterias rubens - 1	*Asterias rubens – 6*	5,503	0,01685
Asterias rubens - 1	*Asterias rubens - 7*	10,39	2,166E-06
Asterias rubens - 2	*Asterias rubens – 3*	2,052	0,8996
Asterias rubens - 2	*Asterias rubens – 4*	2,681	0,6719
Asterias rubens - 2	*Asterias rubens – 5*	2,864	0,5897
Asterias rubens - 2	*Asterias rubens – 6*	5,017	0,0382
Asterias rubens - 2	*Asterias rubens – 7*	9,9	5,198E-06
Asterias rubens - 3	*Asterias rubens – 4*	0,6282	1
Asterias rubens - 3	*Asterias rubens – 5*	0,8188	0,999
Asterias rubens - 3	*Asterias rubens – 6*	2,964	0,5447
Asterias rubens – 3	*Asterias rubens – 7*	7,848	0,00023
Asterias rubens - 4	*Asterias rubens – 5*	0,1836	1
Asterias rubens – 4	*Asterias rubens – 6*	2,336	0,8117
Asterias rubens - 4	*Asterias rubens – 7*	7,22	0,00074
Asterias rubens - 5	*Asterias rubens - 6*	2,152	0,872
Asterias rubens - 5	*Asterias rubens – 7*	7,036	0,00105
Asterias rubens - 6	*Asterias rubens - 7*	4,884	0,04737

Appendix III - Estimation of feed given during experiment 3, and datasheets of feeds

The weight for salmon-fry feed that was distributed in experiment 3 was based on pilot 2 where 0,5 g of feed per 10 liters of water were given per day. In experiment 3 the boxes of water held 23 liters, so the amount of feed was doubled, plus a little extra (1-1,2 g per day) to ensure they could feed ad libitum during feeding time. The zooplankton distributed, (9,5-10 g wet weight) was calculated by using their dry weight, approximately 10% of their wet weight according to Nils Tokle, (personal communication 2021). To match the dry weight of the salmon-fry feed, the average % of water in the salmon-fry feed was calculated to be 7%, Resulting in the dry weight of 1 gram of salmon-fry feed to be 0,93 g. Hence the 9,5-10 g of zooplankton.

To ensure the barnacles had a sufficient amount of feed, the weight of zooplankton was compared to data on copepods (*Calanus*) where the estimated incipient limited concentration (ILC) started to have a limiting accessibility to feed from 200 mg c/litre. In dry weight this was 400 mg c/liter (personal communication, Nils Tokle. 2021)

Using a feed ratio of 1:10 in experiment 3 (approximately 1 gram salmon-fry feed to 10 grams of zooplankton in wet weight) meant that 10 g of zooplankton / 23 l of water = 0,5 g per liter, or a feed concentration of 500 mg zooplankton/l/7 hours. Compared to the experiment on copepods this experiment had 150% more feed per liter.

Ewos Micro Start, salmon-fry feed size 015
Estimated % of water content in grams, based on average values from:

Average values of content	921 g
Average value of water	65 g
Water content in %	7,06

Figure 45 Information sheet on nutritional value of salmon-fry feed produced by EWOS AS, Cargill.

EWOS MICRO START

Ekstrudert mikropellet, startfôr til laksefisk i ferskvann

Sammensetning g/kg fôr:

	015	040	1
Protein	530-570	520-560	510-550
Fett	145-185	165-205	175-215
Vann	50-80	50-80	50-80
Aske	100-140	95-135	90-130
Fiber	0-10	0-10	0-10
Fosfor	16-18	15-17	14-16
Brutto energi MJ/kg	20.5-21.5	21.0-22.0	21.5-22.5

Råvarer:

Fiskemel, fiskeolje, hvete*, tapioka**, hvetegluten, soyaproteinkonsentrat*, mineraler, vitaminer og nukleotider

*I EWOS MICRO START 1
**I EWOS MICRO START 015 og 040

Brukerveiledning:

Produktnavn	Fiskestørrelse
015	0.15g – 0.3g
040	0.25g – 2.0g
1	1.0g – 5g

Med forbehold om små endringer

Dato: 09.10.2020

EWOS AS
Adresse: Thormøhlens gate 51 B, 5006 Bergen, Norway

Figure 46 Information sheet on nutritional value of CryoPlanktonLarge produced by Planktonic AS.

Figure 47 Information sheet on nutritional value of CryoPlanktonLarge produced by Planktonic AS.

planktonic

How to use CryoPlankton

1. ORDER
Using CryoPlankton is extremely easy. You simply order the desired amount of nauplii from Planktonic. We ship the containers to your hatchery in due time for the first feeding of your larvae.

2. PREPARE
Preparing the live feed is done in a few simple steps, you just collect the frozen nauplii from the container and revitalize them in seawater. The nauplii will then resume their normal swimming activity, ready to be fed to the fish larvae.

3. FEED
The whole process takes about one hour each day. There is no need for cultivation, no need of live algae, algae paste or other food items for the nauplii before being fed to the fish larvae.

NUTRITIONAL CONTENT	
Average content in g/100 g dry weight	
Protein	67
Lipid	11
Ash	12,5
Typical fatty acid content (% of total fatty acids)	
DHA	22
EPA	25
Total saturated fatty acids	18
Total monounsaturated fatty acids	19
Total polyunsaturated fatty acids	62
Total omega-3 fatty acids	50

PACKAGING
CryoPlankton is frozen in 12 g pellets, stored in an easy-to-ship cryogenetic dewar. Each dewar contains 80 kg CryoPlankton, approx. 4 bill. nauplii.

SHELF LIFE / STORAGE
CryoPlankton is stored in liquid nitrogen at -196°C and have unlimited storage time. Liquid nitrogen will evaporate and the container/dewar has to be refilled on a regular basis.

LOT NUMBER
Each package or container is marked with a lot number, securing traceability of harvesting location, date of harvest and detailed information about the preservation procedure.

PLANKTONIC AS
Planktonic AS is a Norwegian company dedicated to the development and production of live feed for the aquaculture industry. Founded in 2008, based on extensive research into marine zooplankton combined with a thorough understanding of the nutritional needs of marine species, the company aims to be a recognized leader within its market. Planktonic is supervised by the Norwegian Food Safety Authority, registration number NO13051054.

Co-funded by the European Union

PLANKTONIC AS Bynesveien 48, 7018 Trondheim +47 93 43 89 28 info@planktonic.no planktonic.no

Appendix IV - Data used for analysing the results in Experiment 3

Table 24 Mean shell growth and (SD) of the combined measurements, length, width, and height of the three feed treatments in experiment 3 (n = 150)

	Salmon-fry feed	**Zoo plankton**	**Control**
Mean growth of shell	1,24	2,96	0,44
SD	0,99	1,57	0,74

Table 25 of One-way ANOVA used to test for difference in shell growth in the tree different feed treatments in experiment 3 ($p < \propto 0{,}05$).

One-way ANOVA

Analysis of variance: single factor

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
Salmon-fry feed	149	185	1,24161074	0,985148739
Zooplankton	147	435,2	2,96054422	2,471309291
Control	149	66	0,44295302	0,549088518

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-crit*
Between groups	489,225909	2	244,612955	183,9137987	7,64827E-59	3,016128428
Within groups	587,878271	442	1,33004134			
Total	1077,10418	444				

Table 26 Mean value of all individuals (all 3 replicates) for growth during the seven-week period of experiment 3.

Mean value, all individuals (all 3 replicates)									
Week number		41	42	43	44	45	46	47	Growth %
Length -->	Salmon-fry feed	9,1	9,2	9,2	9,3	9,3	9,4	9,4	3,1
	Zooplankton	9,4	9,4	9,7	9,9	10,0	10,2	10,4	9,6
	Controll	9,3	9,3	9,3	9,3	9,3	9,3	9,3	0,0
Width -->	Salmon-fry feed	8,2	8,3	8,4	8,4	8,5	8,6	8,6	5,4
	Zooplankton	8,2	8,3	8,6	8,8	9,0	9,2	9,4	12,8
	Controll	8,1	8,2	8,2	8,3	8,3	8,3	8,3	2,4
Height -->	Salmon-fry feed	7,5	7,5	7,6	7,6	7,8	7,9	8,0	5,8
	Zooplankton	7,4	7,4	7,6	7,7	7,9	8,0	8,2	9,8
	Controll	7,3	7,4	7,4	7,4	7,5	7,5	7,5	2,7
Total growth/	Salmon-fry feed	24,8	25,0	25,2	25,3	25,6	25,9	26,0	4,7
measurements	Zooplankton	25,0	25,1	25,9	26,4	26,9	27,4	28,0	10,7
combined	Controll	24,7	24,9	24,9	25,0	25,1	25,1	25,1	1,6

Table 27 Two-way ANOVA. Significant values are marked in yellow

	sum of sqrs	**df**	**Mean square**	**F**
P(same)				
A: feed	14,6856	2	7,34281	166,4
1,09E-20				
B: weeks	18,1781	6	3,02969	68,66
4,11E-20				
Interaction:	9,4727	12	0,789392	17,89
8,73E-13				
Within:	1,85333	42	0,044127	
Total:	44,1898	62		

Table 28 Showing the p-values for the different types of feed used in experiment 3. Significant values are marked in yellow

Factor A, feed p- values			
	Salmon	**Zooplankton**	**Control**
Salmon		1,06E-12	9,84E-05
Zooplankton	1,06E-12		1,06E-12
Control	9,84E-05	1,06E-12	

Table 29 Showing the p-values for the week number in experiment 3. Significant values are marked in yellow

Factor B, week number p-values							
	41	**42**	**43**	**44**	**45**	**46**	**47**
41		0,5729	0,0001	3,048E-08	5,175E-12	1,064E-12	1,059E-12
42	0,5729		0,0188	0,0000	8,591E-10	1,269E-12	1,061E-12
43	0,0001	0,01879		0,2056	0,00006792	6,023E-09	7,133E-12
44	3,048E-08	0,00001013	0,2056		0,0803	0,0000	1,122E-12
45	5,175E-12	8,591E-10	0,0001	0,0803		0,0867	0,0001
46	1,064E-12	1,269E-12	6,023E-09	0,0000	0,0867		0,2894
47	1,059E-12	1,061E-12	7,133E-12	1,122E-12	0,0001	0,2894	

Table 30 Tukey's Post-hoc interactions table showing where the difference between the treatments lies. Significant values are marked in yellow

Tukeys post hoc interactions table. Shows the size, not growth		Q	P
Salmon - 41	**Salmon - 42**	1,3190	0,9991
Salmon - 41	**Salmon - 43**	3,3260	0,4951
Salmon - 41	**Salmon - 44**	4,2880	0,1512
Salmon - 41	**Salmon - 45**	6,3490	0,0033
Salmon - 41	**Salmon - 46**	8,7130	0,0000
Salmon - 41	**Salmon - 47**	10,1100	0,0000
Salmon - 41	**Z.plankton - 41**	0,0000	1,0000
Salmon - 41	**Control - 41**	0,0000	1,0000
Salmon - 42	**Salmon - 43**	2,0060	0,9652
Salmon - 42	**Salmon - 44**	2,9680	0,6638
Salmon - 42	**Salmon - 45**	5,0300	0,0444
Salmon - 42	**Salmon - 46**	7,3930	0,0003
Salmon - 42	**Salmon - 47**	8,7950	0,0000
Salmon - 42	**Z.plankton - 42**	0,2474	1,0000
Salmon - 42	**Control - 42**	0,1374	1,0000

Salmon - 43	**Salmon - 44**	**0,9620**	**1,0000**
Salmon - 43	**Salmon - 45**	3,0230	0,6382
Salmon - 44	**Salmon - 46**	5,3870	0,0229
Salmon - 45	**Salmon - 47**	6,7890	0,0013
Salmon - 46	**Z.plankton - 43**	4,0950	0,1997
Salmon - 47	**Control - 43**	1,2090	0,9996
Salmon - 44	**Salmon - 45**	2,0610	0,9576
Salmon - 44	**Salmon - 46**	4,4250	0,1226
Salmon - 44	**Salmon - 47**	5,8270	0,0099
Salmon - 44	**Z.plankton - 44**	7,3110	0,0004
Salmon - 44	**Control - 44**	1,3190	0,9991
Salmon - 45	**Salmon - 46**	2,3640	0,8949
Salmon - 45	**Salmon - 47**	3,7650	0,3076
Salmon - 45	**Z.plankton - 45**	10,1400	0,0000
Salmon - 45	**Control - 45**	3,2430	0,5339
Salmon - 46	**Salmon - 47**	1,4020	0,9983
Salmon - 46	**Z.plankton - 46**	2,0900	0,0000
Salmon - 46	**Control - 46**	5,2770	0,0281
Salmon - 47	**Z.plankton - 47**	14,5900	0,0000
Salmon - 47	**Control - 47**	6,4590	0,0026
Z.plankton - 41	**Z.plankton - 42**	1,5670	0,9954
Z.plankton - 41	**Z.plancton - 43**	7,4210	0,0003
Z.plankton - 41	**Z.plankton - 44**	11,6000	0,0000
Z.plankton - 41	**Z.plancton - 45**	16,4900	0,0000
Z.plankton - 41	**Z.plankton - 46**	20,8100	0,0000
Z.plankton - 41	**Z.plancton - 47**	24,7100	0,0000
Z.plankton - 41	**Control - 41**	0,0000	1,0000
Z.plankton - 42	**Z.plankton - 43**	5,8540	0,0092
Z.plankton - 42	**Z.plankton - 44**	10,0300	0,0000
Z.plankton - 42	**Z.plankton - 45**	14,9200	0,0000
Z.plankton - 42	**Z.plankton - 46**	19,2400	0,0000
Z.plankton - 42	**Z.plankton - 47**	23,1400	0,0000
Z.plankton - 42	**Control - 42**	0,1099	1,0000
Z.plankton - 43	**Z.plankton - 44**	4,1780	0,1777
Z.plankton - 43	**Z.plankton - 45**	9,0700	0,0000
Z.plankton - 43	**Z.plankton - 46**	13,3800	0,0000
Z.plankton - 43	**Z.plankton - 47**	17,2900	0,0000
Z.plankton - 43	**Control - 43**	5,3050	0,0268

Z.plankton - 44	**Z.plankton - 45**	**4,8290**	**0,0566**
Z.plankton - 44	**Z.plankton - 46**	9,2070	0,0000
Z.plankton - 44	**Z.plankton - 47**	13,1100	0,0000
Z.plankton - 44	**Control - 44**	8,6300	0,0000
Z.plankton - 45	**Z.plankton - 46**	4,3150	0,1451
Z.plankton - 45	**Z.plankton - 47**	8,2180	0,0001
Z.plankton - 45	**Control - 45**	13,3800	0,0000
Z.plankton - 46	**Z.plankton - 47**	3,9030	0,2588
Z.plankton - 46	**Control - 46**	17,3700	0,0000
Z.plankton - 47	**Control - 47**	21,0500	0,0000
Control - 41	**Control - 42**	1,4570	0,9976
Control - 41	**Control - 43**	2,1160	0,9490
Control - 41	**Control - 44**	2,9680	0,6638
Control - 41	**Control - 45**	3,1060	0,5992
Control - 41	**Control - 46**	3,4360	0,4447
Control - 41	**Control - 47**	3,6550	0,3504
Control - 42	**Control - 43**	0,6596	1,0000
Control - 42	**Control - 44**	1,5120	0,9967
Control - 42	**Control - 45**	1,6490	0,9928
Control - 42	**Control - 46**	1,9790	0,9686
Control - 42	**Control - 47**	2,1990	0,9338
Control - 43	**Control - 44**	0,8520	1,0000
Control - 43	**Control - 45**	0,9894	1,0000
Control - 43	**Control - 46**	1,3190	0,9991
Control - 43	**Control - 47**	1,5390	0,9961
Control - 44	**Control - 45**	0,1374	1,0000
Control - 44	**Control - 46**	0,4672	1,0000
Control - 44	**Control - 47**	0,6871	1,0000
Control - 45	**Control - 46**	0,3298	1,0000
Control - 45	**Control - 47**	0,5950	1,0000
Control - 46	**Control - 47**	0,2199	1,0000

Table 31 Showing the ratio for the dry body tissue biomass (B) and dry gonad tissue biomass (G) to total shell size (S), for the different treatments.

Mean all replicates (n=20)	Ratio – B:S	Ratio – G:S	Ratio – G:B
Alternative population	0,38	0,27	0,70
Salmon-fry feed	0,29	0,25	0,87
Zooplankton	0,41	0,58	1,42
Control	0,22	0,16	0,74

Kruskal-Wallis test on body tissue biomass /Shell size ratio (B:S) (p-value < ∝ 0,05), showed a p-value of 2,694E-24 stating there is a significant difference between sample medians, leading to Dunns post-hoc test in table 32.

Table 32 Dunns post-hoc test showing significant values for dry body tissue to total shell ratio for the different treatments.

Dunns post-hoc test	
Body tissue /shell size ratio B:S	
Alternative population- Salmon-fry feed	6,42E-06
Alternative population -control	5,79E-16
Salmon-fry feed - Zooplankton	3,42E-09
Salmon-fry feed - Control	0,00034
Zooplankton – Control	2,27E-21

Kruskal-Wallis test on gonad tissue biomass/shell size ratio (G:S) (p-value < ∝ 0,05), showed a p-value of 7,366E-26 also stating there is a difference between the sample medians, leading to Dunns post-hoc test in table 33.

Table 33 Showing significant values for dry gonad tissue biomass (G) to total shell ratio (R) for the different treatments.

Dunns post-hoc test	
Gonad/ shell size ratio G:S	
Alternative population- Zooplankton	9,44E-11
Alternative population -control	3,02E-05
Salmon-fry feed - Zooplankton	5,29E-14
Salmon-fry feed - Control	0,00179
Zooplankton – Control	1,79E-26

Table 34 Values for reference samples made for C:N analysis of barnacle body tissue biomass and gonad tissue biomass. Developed by TBS Department Engineer, Siv Anina Etter. Weight- time categories used for the running of the C:N samples of Experiment 3.

Sample size, up to	**Incineration time in seconds**
2 mg	80
5 mg	90
10 mg	120
20 mg	150
30 mg	180

Table 35 Shows the data of the reference samples that were made to be able to run N:C analysis of the barnacles used in Experiment 3.

2mg80sek	**Nitrogen**	**Carbon**
slope	0,03023825	0,04394342
intercept	-0,706039	-6,9767197
corr	0,99991768	0,99998286

5mg 90sek	Nitrogen	Carbon
slope	0,02976147	0,04396405
intercept	1,20172642	-21,397708
corr	0,99994411	0,99993401

5 mg 90sek	Nitrogen	Carbon
Slope	0,02957738	0,04375839
Intercept	4,23741719	-5,8373603
Corr	0,99992882	0,99992168

Table 36 One-way ANOVA for C:N ratio on body tissue biomass (n=24)

C:N ratio Body mass n=24						
Analysis of variance: single factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Salmon-fry feed	24	98,0	4,1	0,0		
Zooplankton	24	101,7	4,2	0,0		
Control	24	95,9	4,0	0,0		
ANOVA						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-crit*
Between groups	0,7	2	0,36	18,38	3,99925E-07	3,13
Within groups	1,4	69	0,02			
Total	2,1	71				

Table 37 C:N ratio on body tissue biomass using Tukey's pairwise, with Tukey's Q below the diagonal, P(same) above the diagonal. Significant comparisons are yellow

	Salmon-fry feed	Zooplankton	Control
Salmon-fry feed		0,00079	0,0875
Zooplankton	5,424		2,599E-07
Control	3,039	8,462	

Table 38 One-way ANOVA for C:N ratio on gonad tissue biomass (n=24)

C:N ratio gonad	**n=24**					
Analysis of variance: single factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Salmon-fry feed	24	113,3	4,7	0,1		
Zooplankton	24	124,4	5,2	0,1		
Control	24	113,4	4,7	0,1		
ANOVA						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-crit*
Between groups	3,40	2	1,70	16,82	1,1246E-06	3,13
Within groups	6,97	69	0,10			
Total	10,37	71				

Table 39 C:N ratio on gonad tissue biomass, using Tukey's pairwise, with Tukey's Q below the diagonal, P(same) above the diagonal. Significant comparisons are yellow

	Salmon-fry feed	Zooplankton	Control
Salmon-fry feed		1,079E-05	0,9996
Zooplankton	7,122		1,196E-05
Control	0,038	7,083	

Table 40 C:N ratio on body tissue biomass of the feed treatments from week 7, compared to Start population from week 0 (n=17).

	Start-population	Salmon-fry feed	Zooplankton	Control
Start-population		0,00012	0,1218	4,143E-07
Salmon-fry feed			0,02189	0,2214
Zooplankton				0,00043
Control				

Table 41 C:N ratio on gonad tissue biomass of the feed treatments from week 7, compared to Start population from week 0 (n=17).

	Start-population	Salmon-fry feed	Zooplankton	Control
Start-population		0,000389	0,9896	7,7491E-05
Salmon-fry feed			0,0003701	0,6856
Zooplankton				7,33E-05
Control				

Appendix V – Salinity and temperature measurements at Hemnesberget, performed by Åkerblå

Salinity and temperature measurements taken by Åkerblå. Tables and figures are reproduced with permission by Åkerblå.

Table 42 Salinity measurements from Åkerblå (2017-2019), see figure 49

Salinity measurements from Åkerblå							
Sted	Dato / Dybde	- 1 m	- 2 m	- 3 m	- 4 m	- 5 m	- 5,5 m
Lassevika	**19.06.2017**	6,64	6,95	7,62	8,31	10	14
Mastervika	**11.05.2018**	12,19	16,84	22	22,8	26,28	26,84
Storsteinvika	**11.05.2018**	10,73	10,87	11,74	13,84	17,78	21,74
Aunevågen	**06.11.2018**	31	32	32	32	32	32
Blåvika	**06.11.2018**	31	31,55	31,72	31,89	32	32,05
Ransskjæret	**17.01.2019**	28	28	28	29	29	29
Mastervika	**01.03.2019**	16,81	21,48	25,42	26,83	28,33	29,1
Storsteinvika	**01.03.2019**	17,07	20,68	25,69	27,73	28,11	28,77

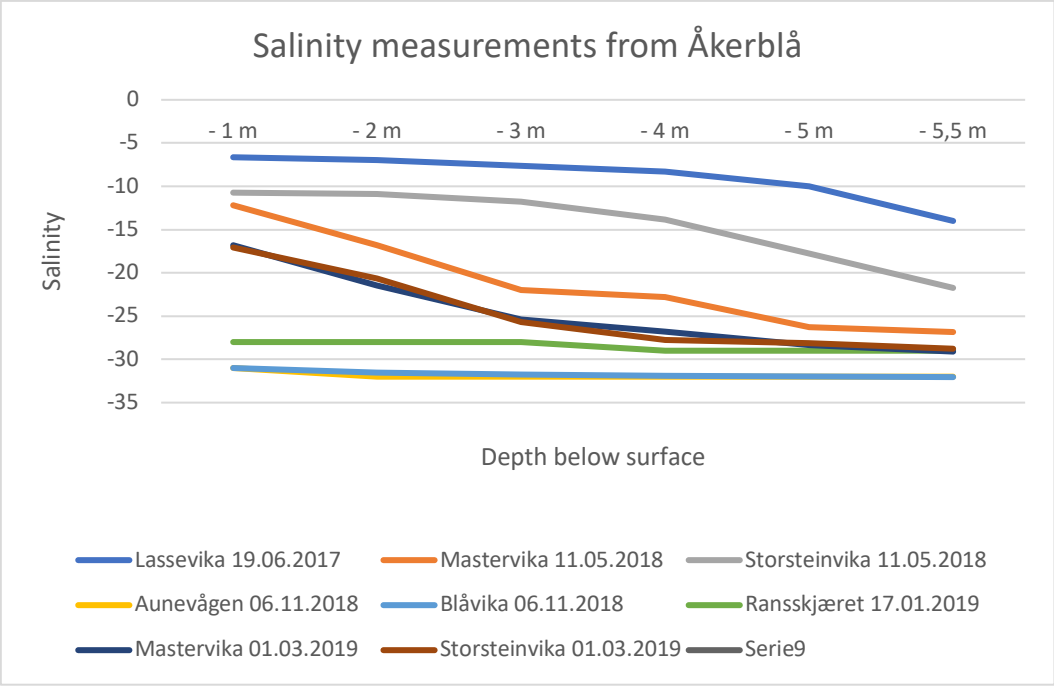

Figure 48 Salinity measurements from Åkerblå, showing the salinity gradient on sights of possible cultivation of Balanus crenatus. (Negative salinity values were made to get the direction of the salinity gradient in the sea with fresh water at the surface)

Table 43 Temperature measurements from Åkerblå, Taken at the same time as salinity measurements in table 42

Temperature measurements from Åkerblå							
Sted	Dato / Dybde	**- 1 m**	**- 2 m**	**- 3 m**	**- 4 m**	**- 5 m**	**- 5,5 m**
Lassevika	**19.06.2017**	10,75	10,69	10,56	10,47	10,39	10,09
Mastervika	**11.05.2018**	7,6	7,19	6,8	6,8	6,7	6,7
Storsteinvika	**11.05.2018**	8,4	8,3	8,2	8,07	7,6	7,2
Aunevågen	**06.11.2018**	10,2	10,4	10,4	10,4	10,4	10,4
Blåvika	**06.11.2018**	10,4	10,4	10,4	10,5	10,5	10,5
Ransskjæret	**17.01.2019**	4,1	4,3	4,4	4,5	5,1	5,2
Mastervika	**01.03.2019**	1,5	2,6	3,9	4,4	4,7	4,9
Storsteinvika	**01.03.2019**	1,7	2,5	3,9	4,4	4,7	4,8

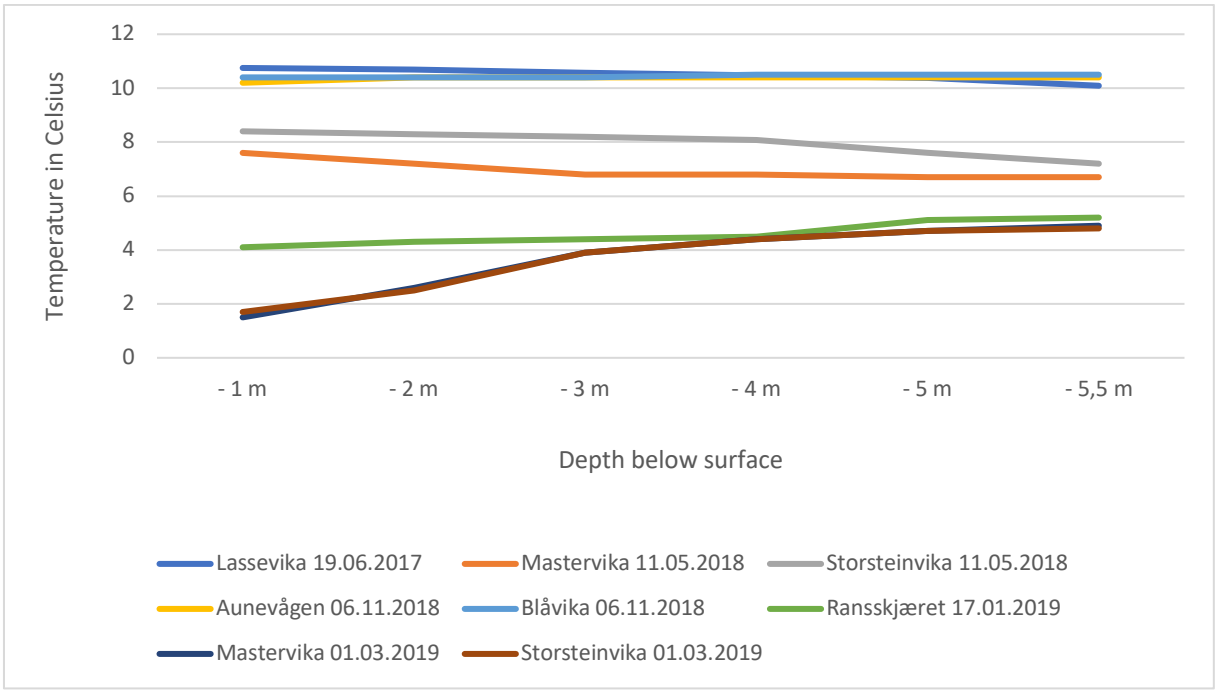

Figure 49 Temperature measurements from Åkerblå, showing the temperature gradient on sights of possible cultivation of Balanus crenatus.

Appendix VI – Salinity and temperature measurements by Plancktonic AS

Salinity and temperature measurements done by Planktonic AS. Tables and figures are reproduced with permission by Planctonic AS.

Table 44 Salinity at Lassevika through season 2021

Salinitet 2021 Lassevika						
dato	- 1 m	- 2 m	- 3 m	- 4 m	- 5 m	- 5,5 m
18.jun	-4,5	-6	-8,3	-11,9	-22	-23,5
07.jul	-6,8	-20,3	-26,8	-28,5	-29,5	-30
13.aug	-16	-18,5	-23,2	-26,2	-27,3	-28,5
24.aug	-14,24	-17,5	-22	-24	-26	-27
30.aug	-11,82	-24,8	-27,5	-28,3	-29,1	
09.sep	-14,7	-17,68	-17,9	-18,67	-18,95	
17.sep	-9,74	-24	-27,3	-28,8	-30,2	-30,7
24.sep	-22,6	-23,5	-24,4	-25,9	-29,2	-29,2
11.nov	-22	-24	-26,1	-26,5	-27,2	-27,3
24.nov	-19,7	-24,1	-27,3	-28,8	-30,2	-30,7
02.des	-7,1	-23,5	-29,2	-31	-32,8	-33,1

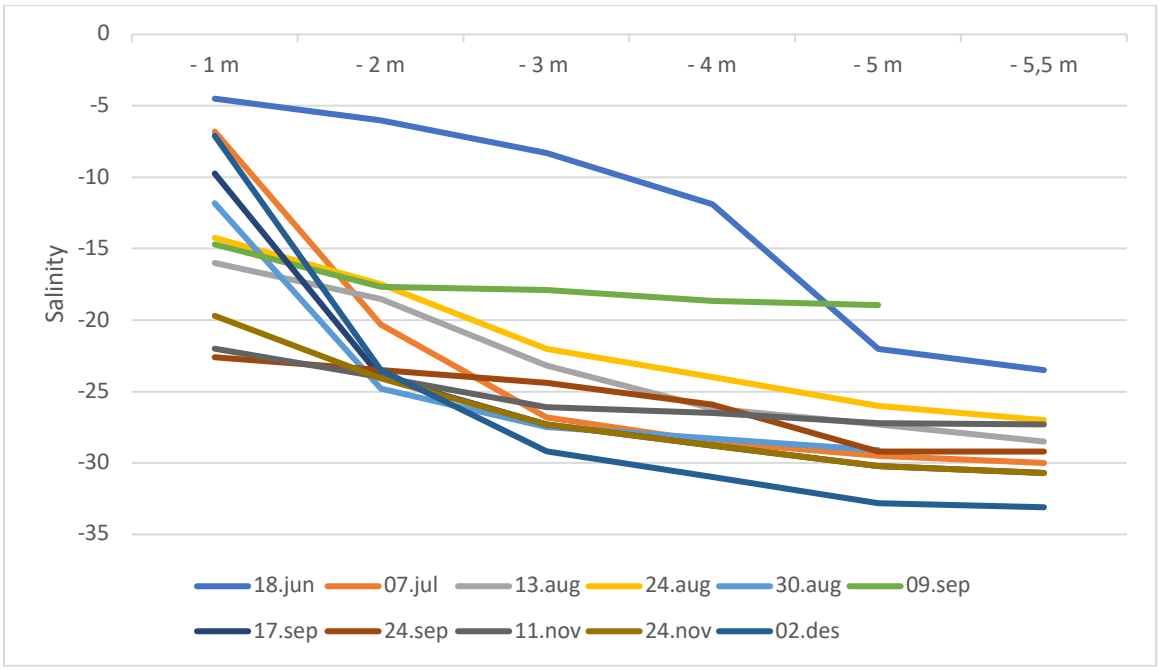

Figure 50 Salinity through the cultivating season, Lassevika 2021.

Table 45 Temperature at Lassevika through season 2021

Temperature						
2021						
Lassevika						
dato	- 1 m	- 2 m	- 3 m	- 4 m	- 5 m	- 5,5 m
18.jun	11,5	11,3	11	10,8	10,6	10,5
07.jul	19	13,2	10,5	9,5	8,8	8,3
13.aug	13	13	12	12	11,5	11
24.aug	11,8	12,1	12,4	11,5	11,4	11
30.aug	12,3	10,6	9,9	9,5	9,2	
09.sep	11,4	10,9	10,7	10,6	10,5	
17.sep	8,8	9,6	9,4	9,3	9	8,8
24.sep	8,6	8,8	8,9	9	9,3	9,4
11.nov	5,5	6	6,7	7	7,2	7,3
24.nov	2,4	3,2	4,7	6,3	7	7,3
02.des	0,2	3,5	5	6,1	7	7

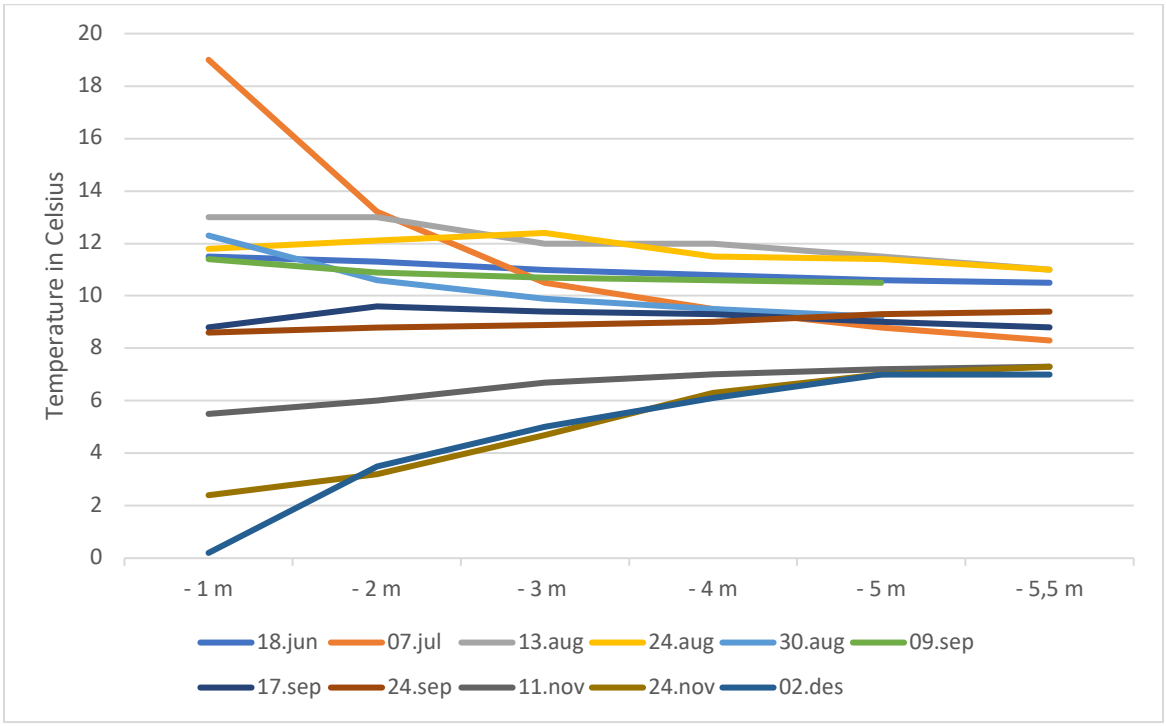

Figure 51 Temperature through the cultivating season, Lassevika 2021.

Appendix VII - Weather data for Hemnesberget for the season 2019-2022. Snow depth, precipitation, and temperature

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Data from the weather station Seljelia, closest observation site to Hemnesberget for historical data, was collected through yr.no (Norwegian Meterological institute *et al.*, 2022).

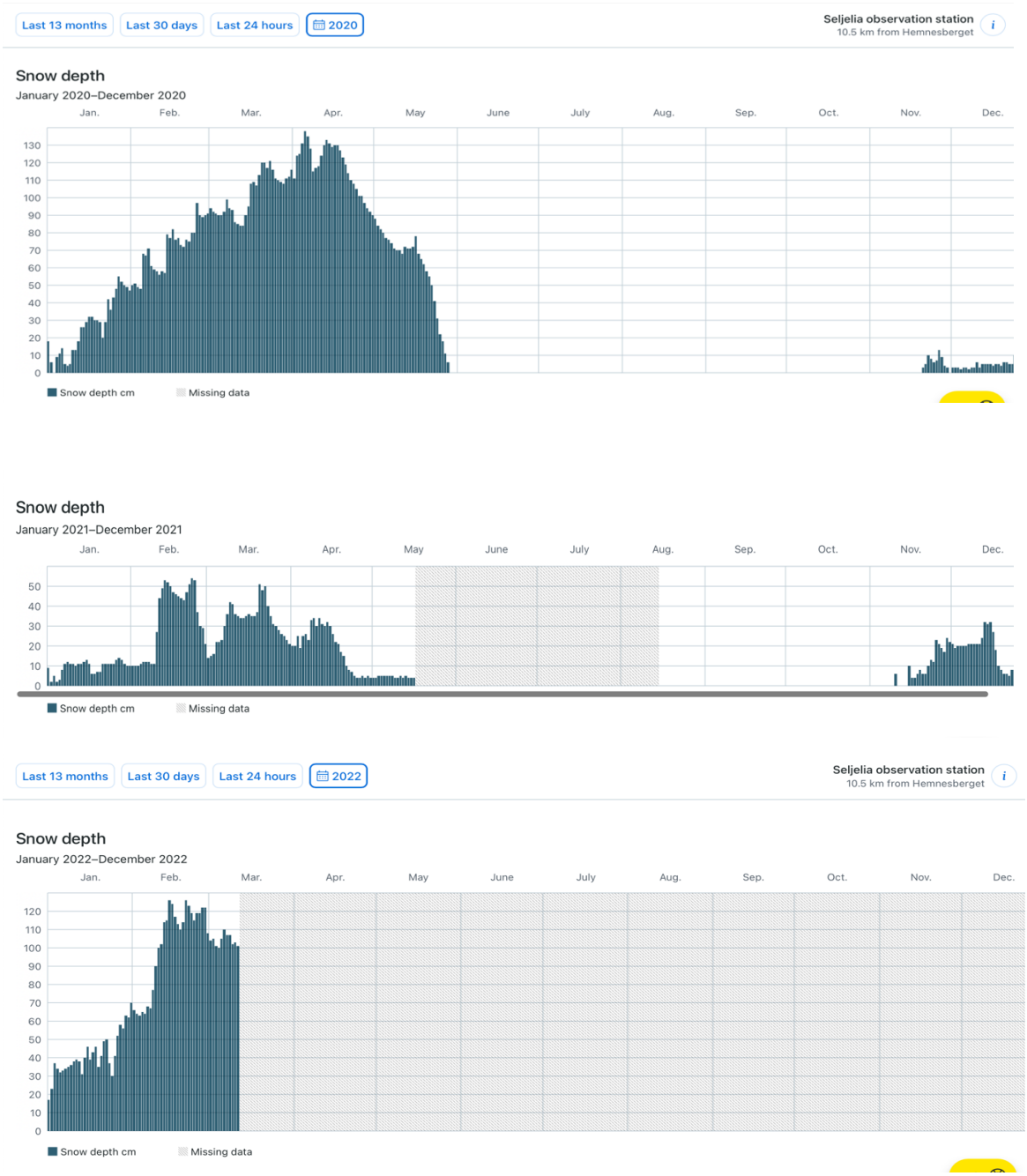

Figure 52 Snow depth data for the seasons 2019-202.

Figure 53 Precipitation data for the seasons 2019-2022

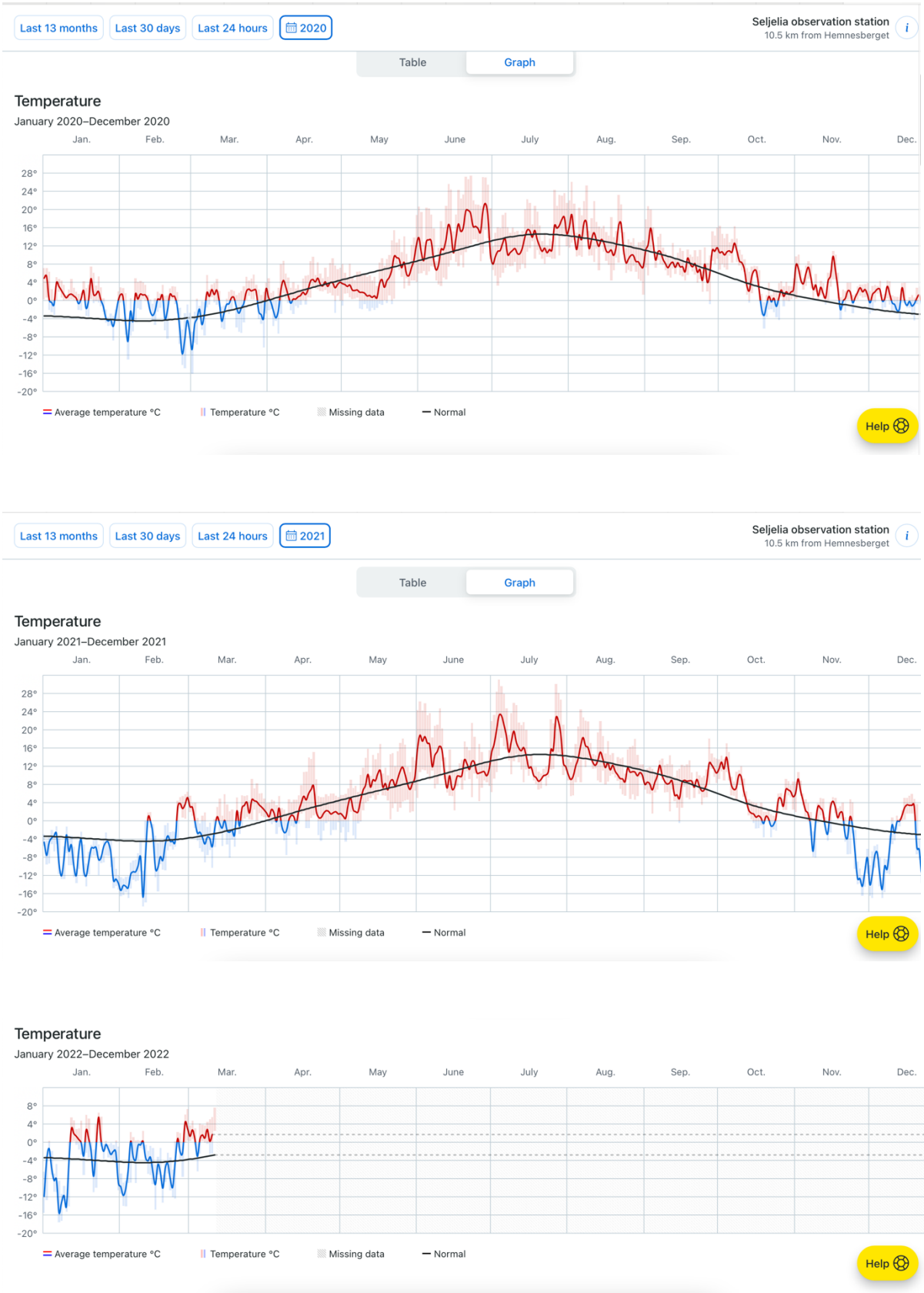

Figure 54 Temperature data for the seasons 2019-2022

Appendix VIII – Sketch of the bubble facility at Hemnesberget harbor

The following information is a personal message from Roger at Hemnesberget Marina, regarding the bubble facility at Hemnesberget harbor.

The bubble facility outside the molo at Hemnesberget has 200 nozzles with an opening of 2mm. There is no exact overview over how much it has been run in 2019, but somewhere around 5-6 weeks. In 2020-2021 about 4 weeks. In November and December 2021, the bubbles were only used inside the marina (7 weeks in total). In 2021 the bubbles have been on for 2 weeks inside the marina.

A new bubble facility is now put in place in the marina (180 m, with 450 nozzles with an opening of 1,5 mm). Hoping to be ready before the next cold period. Be aware that there has been released a significant amount more freshwater from Røssåga the last year. Fresh water gets lighter the colder it gets while saltwater gets heavier. We have seen a freshwater layer of up to 4- 5 meters this winter in the basin outside the molo.

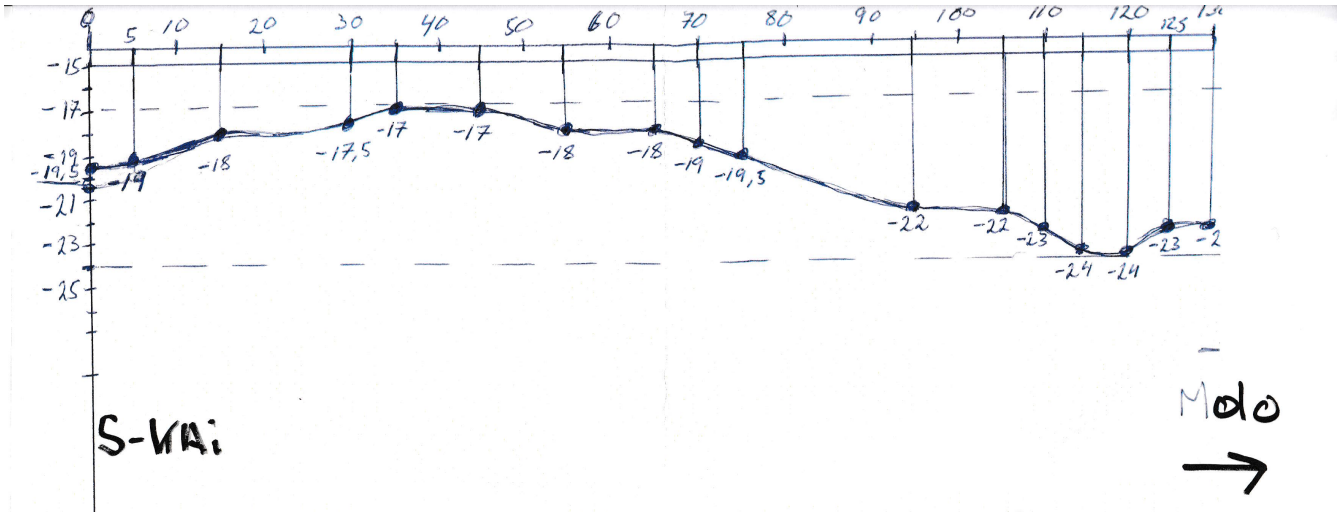

Figure 55 A sketch over the bubble facility outside the molo at Hemnesberget.