The effect of *Laminaria hyperborea* detritus on the growth of key filter feeding species in the littoral zone

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Abstract

Laminaria hyperborea is an important species of kelp comprising large kelp forests along the Norwegian coast. Large quantities of detritus from these forests are exported to other ecosystems. These exports could support high secondary production in neighboring habitats, such as the intertidal zone. Kelp tissue fragments off the ends of the lamina, is degraded by bacteria and can then potentially be consumed by organisms like benthic filter-feeders. *Mytilus edulis* and *Semibalanus balanoides* are two key suspension feeders found in the littoral zone and feed primarily on phytoplankton and organic detritus. Some of this detritus could be derived from *L. hyperborea*. In order to investigate this, a growth experiment was devised to see how the two filter-feeders responded to different diets. One half of the organisms from each species received *L. hyperborea* detritus and the other half received a blend of three phytoplankton species: *Protoceratium reticulatum*, *Prorocentrum minimum*, and *Skeletonema pseudocostatum*. The test organisms were housed in aquaria with an artificial seawater system, while control organisms received natural running seawater from Oslofjord. Organisms were fed regularly with measurements of mussel shell length and barnacle shell diameter taken once a month. *M. edulis* individuals were grouped by small or large size and these measurements were analyzed separately. The large group of mussels on the phytoplankton diet grew significantly more than the detritus diet group. This means that the phytoplankton diet provided a better nutrition source than the detritus from *L. hyperborea*. There was no significant difference in the growth of small *M. edulis* between the two diet types. The small mussel group fed *L. hyperborea* detritus grew just as well as the phytoplankton diet group. There was no difference in size between the diet groups for the barnacles when considering only the effect of diet. Both barnacle diet groups had a significantly smaller size than the control group. Overall, *S. balanoides* grew very little over the duration of the experiment. The result from *M. edulis* shows that this species can survive on *L. hyperborea* detritus and in the case of the small group they can grow equally as well as on a phytoplankton diet. The outcome from *S. balanoides* indicates that more factors may need to be taken into consideration with this species and its feeding activity. This thesis gives a fundamental aim of investigating the relationship between *L. hyperborea* and the filter feeding species living in the littoral zone.

Acknowledgements

Before beginning at the University of Oslo, I had a difficult decision to make. I knew I wanted to study a master's in biology but was unsure of the concentration I wanted to take. I had always been interested in marine biology but after the first lecture in Benthic Ecology, I knew I had made the right decision. This study has taught me a lot but not just what was important for the exams. Cooperation with classmates on the field course, time management, the importance of setting small goals, being creative with solutions, and most importantly asking for help when it is needed.

Throughout the writing of this thesis, there were numerous people whose support and encouragement helped me to complete this work. I am especially grateful for their help as I wouldn't have been able to finish without them.

First, my advisor Stein Fredriksen whose advice and suggestions were essential during the experiment, as well as the editing of numerous drafts and guidance with the statistics. My co-advisor Wenche Eikrem, for her knowledge of microalgae and help with the cultures. Rita Amundsen for help with making medium and loan of the filter. Berit Kaasa for analyzing my samples in the laboratory. My friend, Louise Valestrand, for giving detailed and helpful feedback on my drafts and always giving an answer to my random questions. My partner Åsmund Ervik, without whose encouragement I may not have finished before the deadline. A big thank you to my parents, without their support I would not be at this position in my life. And lastly the students in room 4405, I have had a fantastic two years at UiO and you guys have been the highlight of the experience.

Table of Contents

1 Introduction

The littoral zone describes the area where the terrestrial and marine systems meet, from the high tide to the low tide. Above the littoral zone is the supralittoral, which stretches up to the highest point of the black *Verrucaria* belt. The sublittoral is below the littoral zone down to the area where the deepest algal vegetation can be found. Marine species found in the intertidal area of rocky littoral systems display a specific vertical sequence called zonation (Chappuis et al., 2014). This can be defined as the distribution of species and communities along environmental gradients (Chappuis et al., 2014) for example wave exposure, seawater temperature, salinity, shore slope, nutrient availability, or biotic interactions among organisms (Cefali et al., 2016). Extreme conditions are experienced by organisms living in the littoral zone and species must be highly tolerant to withstand the threats of desiccation, strong currents, temperature and salinity fluctuations, in addition to predation pressure (Marfenin et al., 2013). Instability is the key feature in this environment.

In addition to the stressful conditions, certain processes like contamination, climate change effects and increased ultraviolet radiation are magnified in the littoral system compared to deeper zones (Marfenin et al., 2013). This contributes further to the harshness of the living conditions in this environment.

Despite the number of severe factors species must deal with in the intertidal, there are some benefits to life in this habitat. High levels of nutrients and plenty of light mean that productivity levels are high in the littoral system. Benthic organisms recycle nutrients, tides bring in more nutrients and wind helps with mixing (Demers et al., 1989). An abundance of nutrients and light availability supports high phytoplankton productivity but not necessarily high biomass. Benthic filter-feeders which are abundant in this habitat can influence the amount of particular organic matter found in the water column (Demers et al., 1989). Consequently very little phytoplankton biomass is built up in the littoral due to the high grazing by these organisms (Demers et al., 1989). In contrast to the littoral, grazing activity is reduced offshore which allows phytoplankton biomass accumulation to occur in the mixed layer (Demers et al., 1989).

Filter-feeding organisms such as *Mytilus edulis* (Linnaeus, 1758) and *Semibalanus balanoides* (Linnaeus, 1767) commonly inhabit the rocky littoral zones of coastal Norway. The littoral

zone is generally wave-exposed and therefore makes it a great location for sessile filter-feeders to obtain various food sources. Detritus from local kelp forests can also be exported to these areas. Old kelp tissue fragments off the distal part of the lamina and after being decomposed by bacteria is released as detritus into the water column (Abdullah et al., 2017). In South Africa, a study conducted by Bustamante et al. (1996) found that filter-feeders used particulate kelp detritus as their major source of organic carbon and nitrogen. They also found that average particulate organic matter (POM) levels were significantly higher on exposed shores than sheltered shores. In the Aleutian Islands (Alaska), kelps belonging to the genera *Laminaria* and *Alaria* were identified as supporting high productivity in adjacent ecosystems through the export of their detritus (Duggins, 1989). Since these links between filter-feeders and kelp forests are found in similar climates, it is sensible to investigate further the relationship between filter-feeders and kelp detritus in Norway.

1.1 Study organisms

1.1.1 *Mytilus edulis* Linnaeus

Mytilus edulis are filter-feeding sessile bivalves whose diet consists of phytoplankton, zooplankton, and detritus which are filtered from the water column (Lesser et al., 2010). The blue mussel is a cold-temperate generalist with a nearly cosmopolitan distribution stretching from both sides of the northern Atlantic (Lesser et al., 2010; Sorte et al., 2017) as well as down the western coasts of North and South America (Berge et al., 2006; Suchanek, 1978). In Norway, various species of *Mytilus* including *M. edulis* are found along the entire coast (Figure 1) with *M. edulis* solely found inhabiting intertidal rocky shores in Oslofjord (Brooks & Farmen, 2013). The blue mussels used in this experiment were taken from Oslofjord, therefore it is very unlikely that they belonged to another *Mytilus* species. Blue

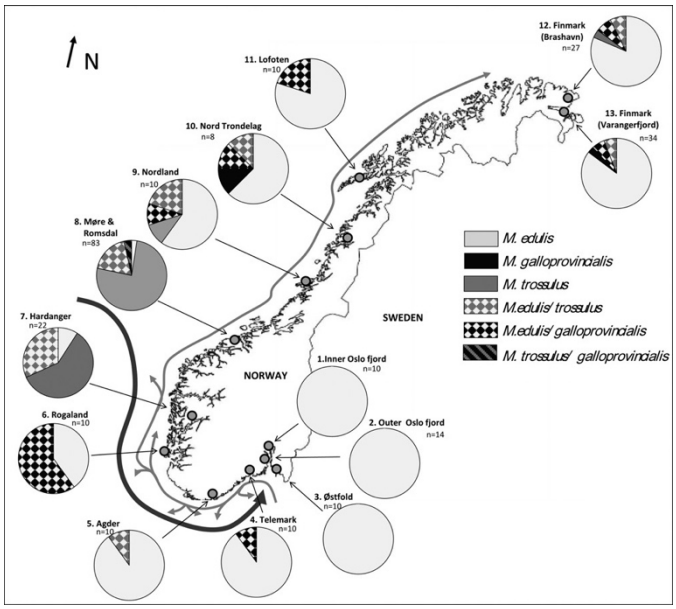

Figure 1: Distribution of various Mytilus *species along the Norwegian coast (From Brooks & Farmen, 2013). The mussels used in the experiment were taken from Oslofjord which only has the species* Mytilus edulis.

mussels are frequently used in growth experiments due to their development being affected by external factors in their habitat. Berge (2006) describes mussel growth as, "*A sensitive parameter of the suitability of the environment…*" and that "*Growth is an unspecified response, but provides a quantitative measure of stress experienced by the mussel*". In addition to the sensitivity of *M. edulis* growth to environmental conditions, there are a few other reasons for conducting experiments with this species. First, *M. edulis* is considered an important foundation species within intertidal food webs and also creates beds that provide essential habitats for diverse assemblages of invertebrates, supporting high biodiversity (Seed, 1969a; Suchanek, 1978, 1992). These beds are made up of living mussels, shells, debris and sediment; they can house as many as 300 species at a specific location (Suchanek, 1978). Mussel beds provide protection to associated species from harsh environmental conditions, shelter from predation, and a vast amount of surface area for settlement for sessile species (Suchanek, 1978). Second, they are capable of tolerating a wide range of environmental conditions because of their external shell and firm attachment to the substrate (Seed, 1969a).

Spawning generally occurs from spring to summer with blue mussel larvae first attaching to filamentous substrates where they are temporarily settled until migrating permanently to adult mussel beds (Seed, 1969b).

As previously stated, *Mytilus edulis* is a key species inhabiting the littoral zone. Its upward expansion is restricted to the littoral zone by abiotic factors, like temperature and desiccation, while a combination of competition for space and predation controls its lower boundary on shore (Suchanek, 1978).

In contrast to what was earlier believed about particle size capture by mussels (Coe & Fox, 1944), mussels do not select food particles based on size (Bayne, 1976). Selection is based on the chemical composition of the particles (Ward & Targett, 1989). Mussels have been observed filtering natural seawater with a particle size ranging between 2-100μm (Bayne, 1976). Food is captured by a mucus secreted over the animal's gills which particles stick to and then the mucus sheet is drawn into the mouth (Coe & Fox, 1944). *M. edulis* does not filter in very dilute suspensions but instead begins filtering when particle concentration reaches an unknown critical threshold (Bayne, 1976). While there have been numerous studies focusing on the diet of *M. edulis* with an emphasis on phytoplankton as the primary food source, more research is needed on the potential link between bivalves and detritus from kelp as nutrition.

Mytilus californianus, a closely related species to *M. edulis*, was found to use organic detritus from various sources as its primary food supply, as living phytoplankton could provide only a small amount of the nutrients required (Coe & Fox, 1944). In a study conducted by Lesser et al. (2010), it was found that *M. edulis* populations in Maine consumed a mixed diet of phytoplankton and detritus originating from macrophytes in the intertidal. The larvae of *M. edulis* were fed four diverse species of algae grown under various conditions to evaluate if there would be a change in growth or mortality (Leonardos & Lucas, 2000). All the larvae survived and there was a significant difference in larval growth with the most nutritious alga containing a low proportion of carbon to nitrogen ratio equal to 16, saturated fatty acids, as well as increased proteins and carbohydrates.

1.1.2 *Semibalanus balanoides*

Semibalanus balanoides is an intertidal, boreal-arctic species of barnacles (Marfenin et al., 2013) which feeds by filtering microscopic organic particles from the water. This animal is found inhabiting the littoral zone on rocky shores and competes with *Mytilus edulis* in their shared habitat. *S. balanoides* has a widespread habitat distribution with organisms found on both coasts of the North Atlantic, as far south as northwestern Spain, as well as individuals found in the Pacific from Alaska to Japan (Bourget et al., 1990). This species has a breeding cycle which is attenuated to fluctuations in temperature and nutrition with fertilization generally taking place in autumn (Crisp, 1964). Habitat and latitude influence breeding times and growth rates of *S. balanoides* meaning that fertilization will occur earlier in northerly latitudes than more southern habitats (Crisp, 1959). Decreases in temperature and nutrients, increase in animal's age and possibly decreased irradiance are some of the factors which prompt maturity (Crisp, 1959). *S. balanoides* is an obligate, cross-fertilizing hermaphrodite (Pineda et al., 2002). The nauplii of *S. balanoides* are released in the spring/summer season in concert with the spring phytoplankton bloom (Davenport et al., 2005). These are nauplius I larvae which will molt six times, producing nauplii II-VI, before molting into a final cyprid stage (Pineda et al., 2002). The planktonic larvae of *S. balanoides* spend weeks in the water column before settling on a permanent location (Bertness et al., 1991).

Sessile filter-feeders are ideal candidates to study secondary production as they cannot move to find nutrition and therefore food resources can be thought of as a flux of food particles available in the water to individuals (Bertness et al., 1991). Labarbera (1984) describes

S. balanoides as a facultative active suspension feeder. This describes the organism's process of actively driving water through feeding cirri or by passively extending its cirri into currents to collect food (Labarbera, 1984).

Bertness et al. (1991) describes acorn barnacles (*Semibalanus balanoides*) specifically as being preferred study organisms for production influence experiments. It was found that barnacle secondary production can be enhanced by high primary production and patterns in barnacle growth and reproduction may reflect food supply rates (Bertness et al., 1991).

The growth of *Semibalanus balanoides* has been studied with regards to geographic variation (Bertness et al., 1991) and oceanographic effects (Burrows et al., 2010) but the effect of feeding this organism different species of algae has not been thoroughly explored.

1.1.3 Phytoplankton

The spring bloom is a common characteristic found in northern temperate coastal ecosystems (Cebrian & Valiela, 1999) with this short event usually taking place in late February/early March in the Oslofjord and in mid-April to end of May in northern Norway (Hegseth et al., 1995). Increasing light, low temperature and high nutrient levels mark the environmental conditions at the time of this event (Hegseth et al., 1995). Sverdrup (1953) first discussed the importance of the critical depth before the onset of the bloom stating that the mixed surface layer must be less than the critical depth if the phytoplankton in the mixed layer will increase. This is because production only occurs above the compensation depth where the rate of photosynthesis production is higher than respiration rate. Nutrients generally become depleted as the phytoplankton rapidly consume and multiply with the exhaustion of nitrate and silicic acid triggering the collapse of the Oslofjord spring bloom (Kristiansen, 1987). Diatoms tend to dominate the phytoplankton community in the Oslofjord (Kristiansen et al., 2001). Silicic acid is vital to diatoms due to its use in the development of the frustule. When silicic acid becomes depleted, the phytoplankton community shifts from being diatom-dominated to flagellate-dominated (Malone et al., 1996).

1.2 Kelp forests

1.2.1 *Laminaria hyperborea* (Gunnerus) Foslie 1884

Laminaria hyperborea belongs to the family Laminariaceae, order Laminariales, (Kain, 1971) class Phaeophyceae. The structure consists of three parts: the lamina, stipe, and holdfast which attaches the kelp to hard-bottom surfaces (Eilertsen et al., 2011). Primary and secondary growth are seasonal. The transition zone between stipe and frond is where primary growth occurs (Kain, 1971). This tissue is comprised of a medulla, inner and outer cortex (Kain, 1963). Secondary cortex is produced below the mature part of the stipe consisting of a meristematic layer and increases the stipe's diameter (Kain, 1963, 1967). Primary growth is fastest from January to June, and then slows down extremely for the remainder of the year (Kain, 1963). Secondary growth speeds up much later than primary growth and results in lines in a vertical section or rings in transverse (Kain, 1963). Cells produced in slow growth are dark and small, therefore the two types of cells can be distinguished from each other and approximate age can be inferred from the number of zones of these cells (Kain, 1963). The cells in the transition zone initiate growth of the frond and a young blade develops distally from this zone. This tissue develops into a rounded lamina with slits eventually materializing with the end result being finger-like processes (Kain, 1963). These developments occur during the fast stipe growth followed by a slow growth period, resulting in a small amount of frond growth at the transition zone which forms a band

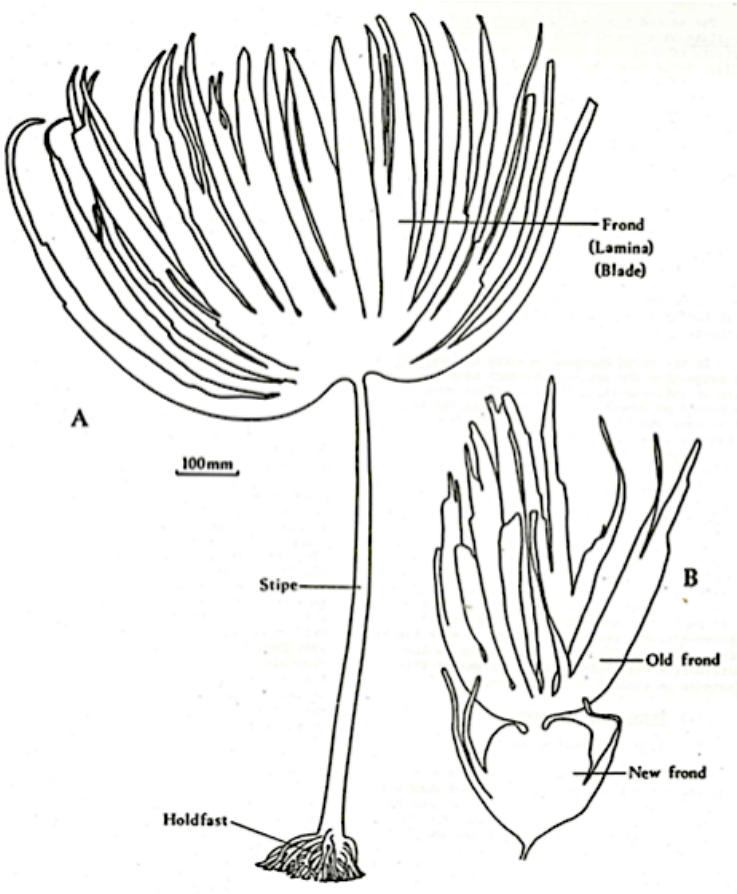

Figure 2: A: Drawing showing the structure of Laminaria hyperborea *sporophyte during the slow growing season. B: Shows a typical plant in February with the old and new fronds (From Kain, 1971).*

of tissue at the base of the old frond. When a new frond emerges below this in a fast growth period the narrow band of tissue joins the new and old fronds (Figure 2) until April or May when the old frond is torn off (Kain, 1963). The fast-growing period begins in the winter when there is the least light available, therefore growth of the frond takes place by use of storage material in the lamina (Kain, 1963). The laminas are covered with reproductive tissue from fall to spring and gametophytes appear during winter and can give rise to sporophytes soon after, usually during winter and spring (Kain, 1963).

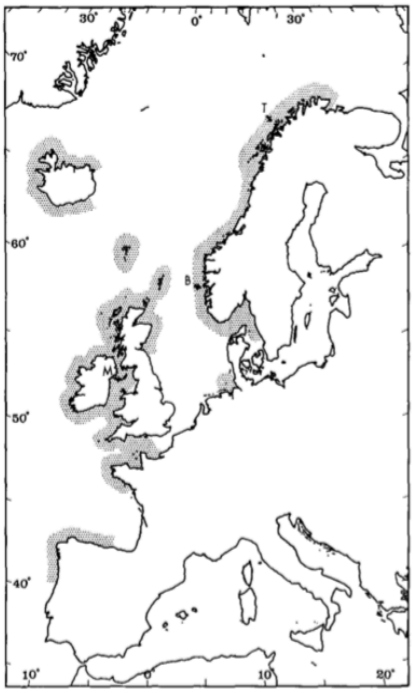

Figure 3: Map showing distribution of Laminaria hyperborea *in Western Europe (From Kain, 1967).*

Laminaria hyperborea is a significant kelp species which comprises large kelp forests along the Norwegian coast and is responsible for high secondary production in adjacent habitats (Norderhaug & Christie, 2011). This species forms dense forests on the substratum where it dominates over other algae and results in a markedly changed environment (Kain, 1962). These forests occur from low water springs down to a variable depth. *L. hyperborea* thrives in semi-exposed areas with unstable gravelly bottoms to fully exposed stable rocky bottoms (Kain, 1962). This species is found as far south as northern Portugal, as far north as northern Norway (71°N) with distribution stretching east to the Murman coast of Russia (Figure 3) (Araújo et al., 2009; Kain, 1967; Schoschina, 2012). According to Kain (1963), most of the advantage in more northerly attitudes (i.e. Norway) is connected with longer life rather than faster growth. The factor which limits the depth maximum of *L. hyperborea* forests acts on their initial establishment and not on their growth (Kain, 1963). Growth conditions do deteriorate with depth and therefore light availability controls the depth limit (Kain, 1963). Despite this, the establishment stage is critical to the success of a new forest even if colonizing on a suitable substratum.

Detritus can be exported from kelp forests to surrounding habitats such as rocky intertidal shores and then enhance secondary production there (Krumhansl & Scheibling, 2012). Along the Norwegian coast, *Laminaria hyperborea* is estimated to cover an area of > 5000 km^2 (Fosså, 1995). Norderhaug and Christie (2011) found that only 8% of primary production was utilized in high to medium exposed kelp forests, suggesting that large quantities of *L. hyperborea* are exported to other systems. Abdullah et al. (2017) determined that kelp was the major source of organic matter in sediment cores taken in the area of a well-established kelp community off the west coast of Norway. The high levels of phenols in kelp make it disagreeable as a food source for most benthic organisms (Norderhaug et al., 2006). Phenols are antiherbivore and antibacterivore which is the reason why kelp may not always be consumed by organisms or bacteria. This would suggest that a substantial amount of POC derived from kelp remains in the aquatic system and may be sedimented or exported to other areas (Abdullah et al., 2017).

If *Laminaria hyperborea* detritus contributes significantly to the growth of either *Mytilus edulis* or *Semibalanus balanoides*, this could help direct future studies to understanding this trophic interaction thoroughly. This kelp species has undergone significant regime shifts in Norway in the last decades and is currently recovering from the barren ground to kelp forest state (Norderhaug & Christie, 2009) and it is uncertain to what extent climate change can exacerbate the recovery of kelp forests. Recent studies have shown a worrying trend of declining kelp forests and reduced stability due to warmer ocean temperatures and eutrophication along coastlines (Filbee-Dexter & Wernberg, 2018). If kelp forests continue to be negatively affected by climate change, especially by increased warming, this could have serious consequences for the multitude of species which depend on kelp forests and their detritus exports.

1.3 Study aims

It is important to see if the growth of these key filter-feeders might be affected by kelp detritus in their diet. Therefore, *Mytilus edulis* and *Semibalanus balanoides* were collected and fed detritus from *Laminaria hyperborea*.

The objective of this study was to determine if detritus from *Laminaria hyperborea* had a significant effect on the growth of *M. edulis* and *S. balanoides* compared to a diet consisting of three different species of phytoplankton.

The following hypotheses were tested:

H_0 = There is no difference in growth of <*the organism*> due to different algal species in their diet.

H_1 = There is a difference in growth of <*the organism*> due to different algal species in their diet.

Where <*the organism*> is *M. edulis* or *S. balanoides*.

2 Materials and Methods

2.1 Collection of organisms

2.1.1 *Mytilus edulis*

Mytilus edulis organisms were collected from the guest harbor at Drøbak on the Oslofjord in February 2017 (Figure 4). A rake was used to scrape the blue mussels from the side of the dock and the organisms were placed in seawater filled buckets. The animals were collected from the exposed side of the dock facing out towards the fjord. Two size groups of mussels were collected: a smaller group of <20mm and a larger group of >20mm. The mussels were taken to the Department of Biosciences at the University of Oslo and placed in the aquarium room in the cellar. A total of 60 mussels were harvested for the experiment with approximately an extra 30 individuals taken as backups.

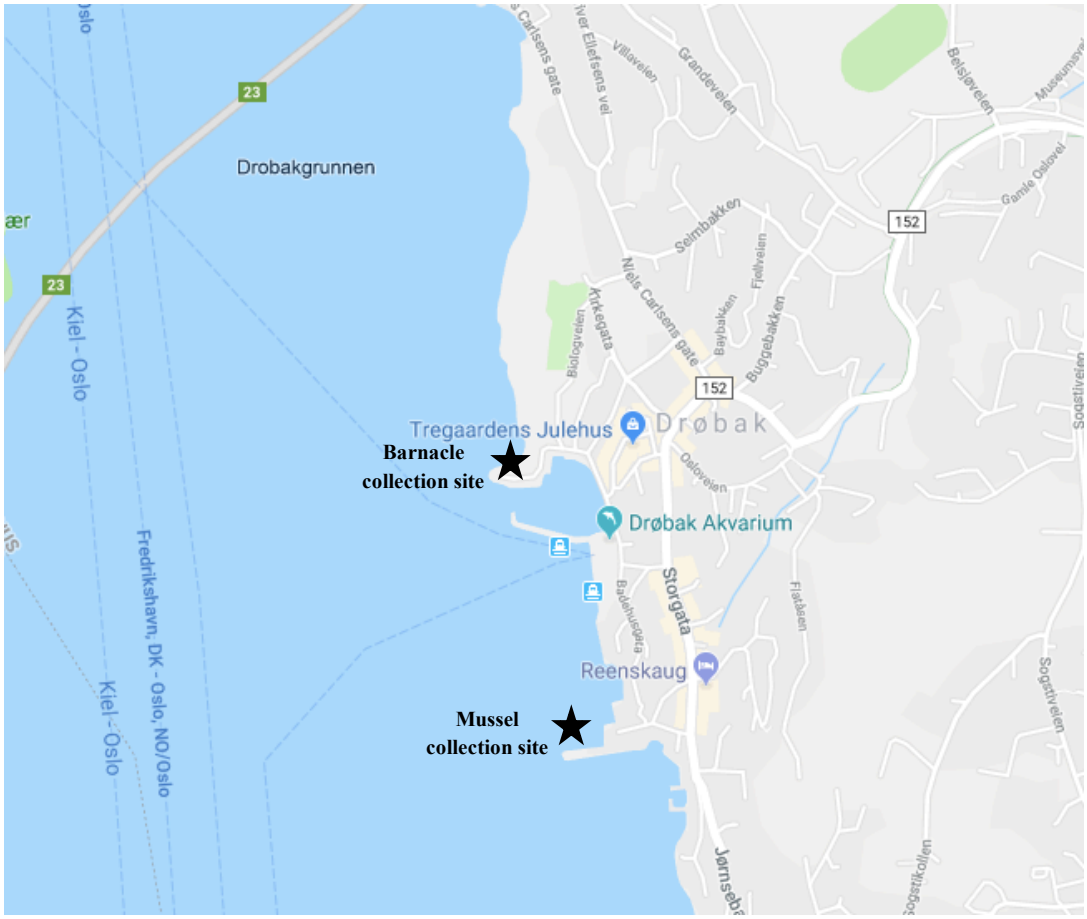

Figure 4: Map showing the sites where Mytilus edulis *and* Semibalanus balanoides *were collected in Drøbak (From Google Maps).*

2.1.2 *Semibalanus balanoides*

Plates were smeared with older *Semibalanus balanoides* organisms and hung over the fjord in Drøbak in March 2017. Barnacle larvae are attracted to surfaces where adult barnacles have settled and will settle on these habitats. An ice scrape was used to collect old barnacles attached to the rocks along the shoreline next to the university's biological station in Drøbak. The old barnacles were crushed between two stone plates and smeared on all the plates attached to the chain. First a chain was laid out and the nine square plates evenly spaced in a row along this. The plates were attached to the chain using plastic ties looped through the hole drilled in the middle of each. Three ropes were attached to the chain with one at each end and one in the middle. The chain was then hung in the middle of an old barnacle settlement` at the edge of the dock next to the biological station by tying the ropes to planks on the dock. In April 2017 the first larvae had settled on the plates and began to grow their shells. By May 2017 the plates were covered with thousands of small *S. balanoides* juveniles. They were then moved from the chain with six of the nine plates placed in the aquarium room in the cellar at the Department of Biosciences in Oslo. The remaining three were set up in aquaria at the biological station in Drøbak to be used as controls. Before placement in the aquaria the plates were thinned using a metal pick to reduce the risk of crowding between the barnacles.

2.2 Aquaria set-up

Six identical aquaria (handmade at UiO) were placed in a room in the cellar of the Department of Biosciences at the University of Oslo (Figure 5). This room had access to the artificial seawater system. Two hoses were connected to the water spouts in the ceiling with each end placed in a separate plastic rectangular container raised on a platform on opposite sides of the room. These containers were raised up higher on one end so that water could drain out a pipe on the opposite end. Three glass aquaria were placed in each of these two containers and filled with the artificial seawater. In each aquarium a water pump (Fluval Nano Aquarium Filter (up to 55L) 6.8W) was placed to continuously circulate the water.

The artificial seawater had a temperature of 9.4°C and salinity of 20 PSU in February 2017. The water in the system had just been replaced and the salinity would adjust after full circulation. When taking down the aquaria in November 2017 the temperature was 10°C and the PSU was 32.

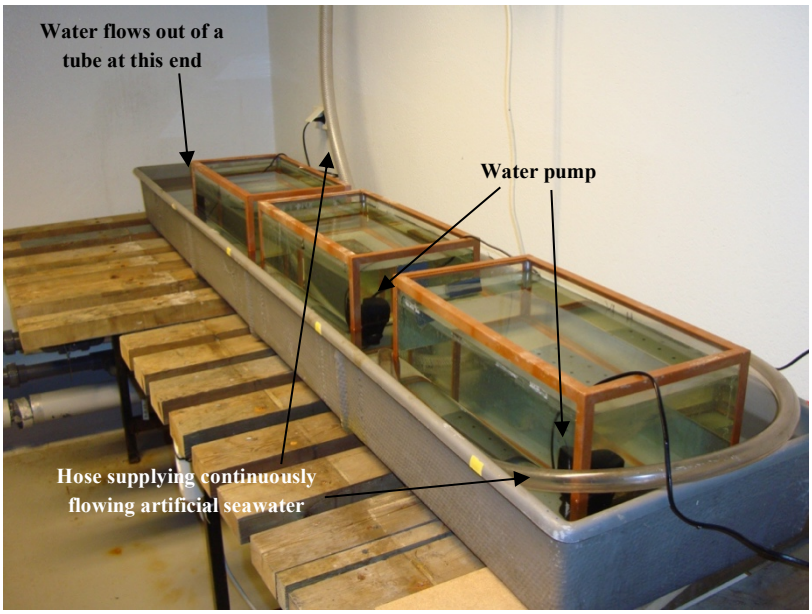

Figure 5: Aquarium set-up in the cellar of The Department of Biosciences at Blindern, Oslo. There was an identical aquarium set-up on the opposite side of the room (not shown in photo) contributing to a total of 6 aquariums that could be used simultaneously for the experiment.

2.2.1 *Mytilus edulis* design

The mussels were dried off with paper towels before being glued to rectangular PVC plates using super glue (Clas Ohlson Universal Super Glue Water-resistant). The organisms were glued in a row with approximately 2cm of space left for growth between them (Figure 6). The first row consisted of the larger mussel group, with the second group composing the smaller group of mussels. Each group consisted of 5 individuals meaning there were a total of ten mussels per plate/aquarium. Each plate was placed in an aquarium with a small plastic cup raising the plate up about 5cm off the aquarium floor. Therefore, each aquarium had ten mussels of two size groups contributing to a total of 60 mussels with half belonging to each respective size group. Water in the aquaria was replaced every 2 weeks with each aquarium emptied and cleaned out every 2-3 weeks.

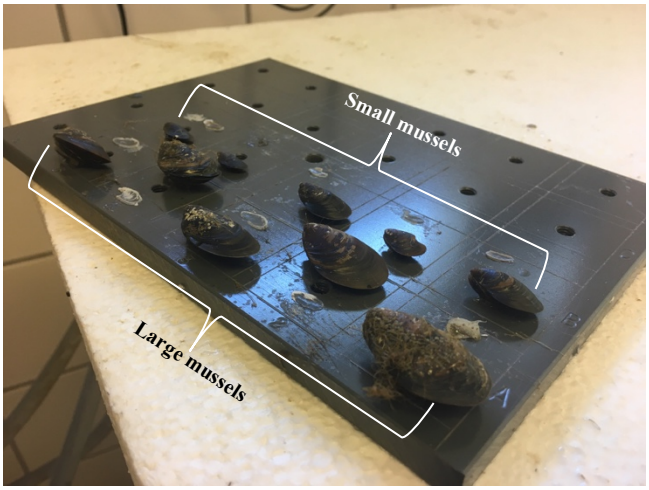

Figure 6: Mytilus edulis *glued to a PVC plate before placement in the aquarium. The first row consists of the larger organisms and the second row is the small group of mussels.*

On 17 March 2017, thirty-one days after the start of the experiment some test mussels at the university had managed to move from their glued positions. The plates were taken out of the aquaria and dried. The loose mussels were measured to determine which group and spot they belonged to. The animals were then dried with paper towels before placing glue on the plates in the correct position. After the glue had dried, the plates were placed back in to their respective aquaria. The final measurements were taken on 2nd of May.

2.2.2 *Semibalanus balanoides* design

The aquaria were organized identically to the mussel experiment, six aquaria with running artificial seawater. The stone plates were raised off the aquarium floor using the same plastic cups as in the mussel experiment (Figure 7). Aquaria were cleaned every 2-3 weeks and received new water every 2 weeks. The barnacles were measured once a month over a four-month period.

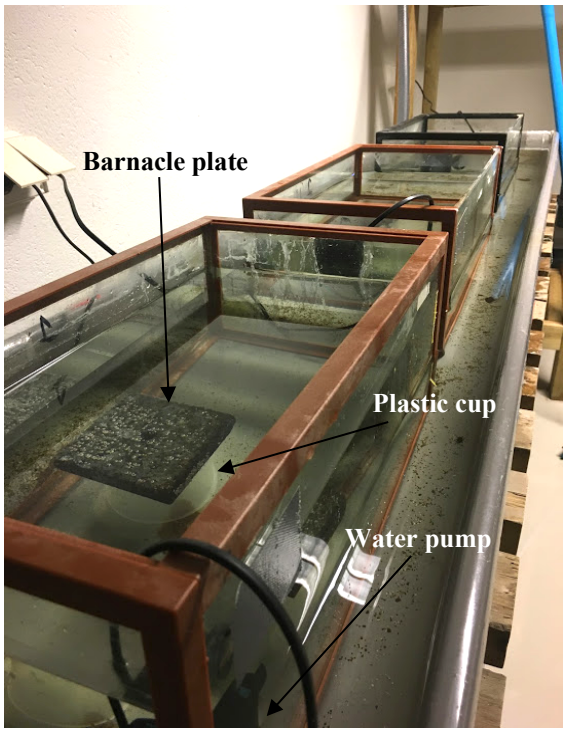

Figure 7: Semibalanus balanoides *covered plates set up in aquaria in the cellar at the Department of Biosciences, Blindern, Oslo. The plates were raised approximately 5cm off the aquarium floor by small plastic cups.*

2.2.3 Control design

Mytilus edulis

A control experiment was set up in parallel to the test experiment at the university's biological station in Drøbak in February 2017. Thirty blue mussels were taken from those collected in the guest harbor and placed in three aquaria at the biological station with some extra individuals placed in a fourth aquarium. The aquaria received natural running seawater from the fjord so that the organisms would receive a normal diet (Figure 8). The animals were glued down to the same plastic plates as described earlier with enough space for growth between individuals. Two size groups had been chosen identical to the test experiment.

Figure 8: Control set-up at Biologen in Drøbak. The aquaria received natural seawater from Oslofjord through plastic tubes. Each Mytilus edulis *control and* Semibalanus balanoides *control shared an aquarium.*

Semibalanus balanoides

A control was set up at the biological station with three aquaria receiving natural running seawater from Oslofjord. This was set up 1st of June and the control barnacles shared aquaria with the second group of control mussels (Figure 9). The control aquaria were set up identically for the barnacles as described for the control mussels. The control barnacles had to be taken back to the Department of Biosciences at Blindern, Oslo for measurement on the microscope in the Algae Lab.

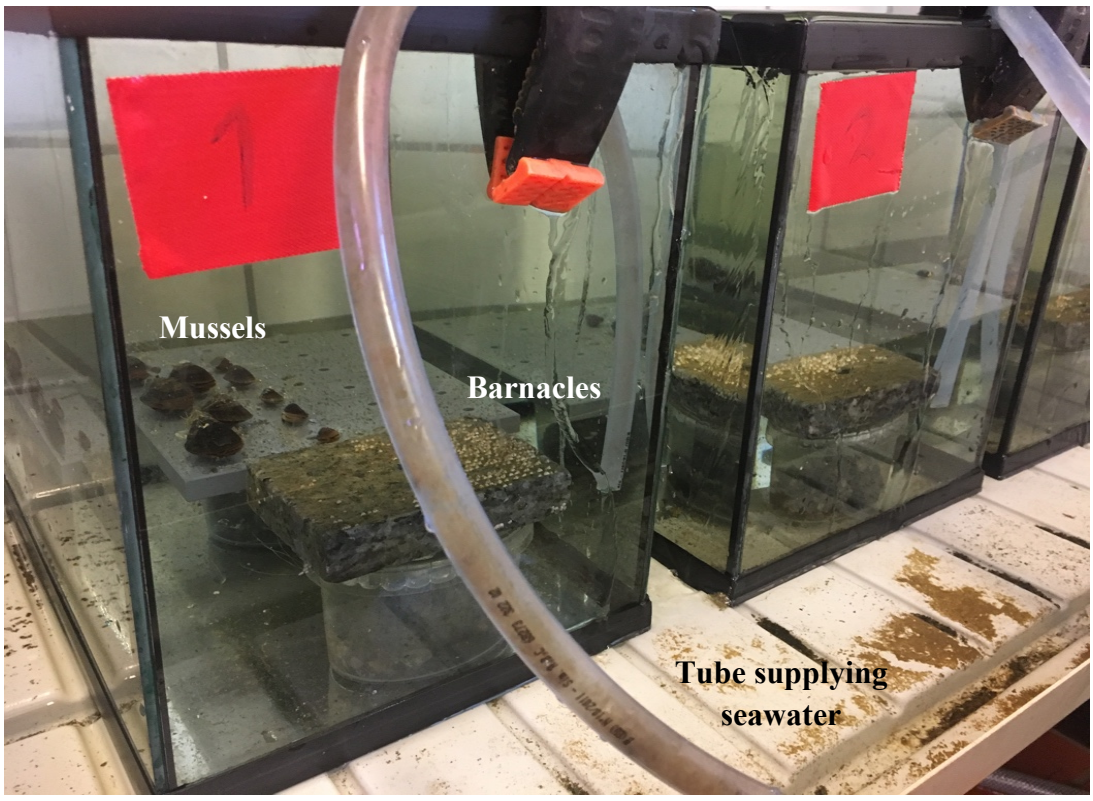

Figure 9: One control for Mytilus edulis *and one control for* Semibalanus balanoides *were placed in each aquarium together. The organisms were nourished with natural seawater continuously flowing from Oslofjord.*

2.2.4 Diet

Both *Mytilus edulis* and *Semibalanus balanoides* were fed the same type of diet over the experimental period. Half of the test organisms from each species received a diet of kelp detritus from *Laminaria hyperborea* while the remaining half received a mixture of three different phytoplankton species. The phytoplankton species used in this study include two dinoflagellates, *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli 1885, *Prorocentrum minimum* (Pavillard) J. Schiller 1933, and a diatom, *Skeletonema pseudocostatum* Medlin 1991. Both species control groups were housed in aquaria with

natural seawater and therefore were nourished by the phytoplankton in the surface water found naturally in Oslofjord.

Preparation of phytoplankton mixture

As stated earlier, the algae cultures used to feed the test animals were: *Protoceratium reticulatum*, *Prorocentrum minimum*, and *Skeletonema pseudocostatum*. Each of these cultures had been grown by Professor Bente Edvardsen (UiO) and maintained throughout the experiment in the Culture room at the Department of Biosciences, University of Oslo. The cocktail created to nourish the test organisms was made by blending 200mL from each culture in an Erlenmeyer flask. Therefore, each of the three phytoplankton aquaria received a total of 600mL of phytoplankton every 3 days. An IMR ½ medium (Eppley et al., 1967) was made to maintain the microalgae cultures.

Preparation of kelp

Laminaria hyperborea used in this experiment were harvested by Professor Stein Fredriksen (UiO) outside Stavanger in March 2016 and were kept frozen (-18°) in plastic containers in the Algae lab at the Department of Biosciences, University of Oslo. The frozen kelp blades were placed in a mortar and liquid nitrogen was added. The blades were crushed into smaller particles with a pestle. These particles were then transferred to plastic trays and placed in a drying oven (Termaks 'Mains': FOH60) at 60°C for a minimum of five days. After being fully dried, the contents of the trays were emptied back into the mortar to be crushed into finer particles. These particles were filtered using a 500-micron filter (handmade by Rita Amundsen, UiO) (Figure 10) which ensured that no particles larger than 500µm were used in the experiment. The majority of the *L. hyperborea* particles were most likely smaller than this size after being grinded multiple times. After being filtered for size, the particles were placed in sealed plastic tubes for storage. When it came time for feeding, the particles were placed in Eppendorf tubes and weighed

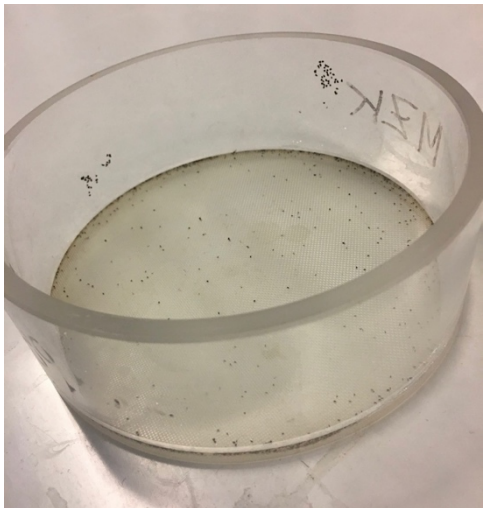

Figure 10: The 500-micron filter used to filter Laminaria hyperborea *detritus. The detritus was blended with liquid nitrogen, dried and then crushed with a mortar and pestle before filtering.*

at 0.9g. Each of the three aquaria receiving kelp were fed with the contents of one tube every 3-4 days.

Carbon nitrogen analysis of diet

An analysis was performed on both diet samples to determine the C:N composition. Five Eppendorf tubes were filled with the detritus from *Laminaria hyperborea* used to feed the test organisms. The three phytoplankton cultures were blended together and prepared as described earlier. Vacuum filtration was used to filter five replicates of this mixture onto GF/C filter paper. These ten samples were submitted to the Toxicology Lab at the Department of Biosciences, University of Oslo. Berit Kaasa (UiO) performed the analysis on the samples.

The diet consisting of detritus from *Laminaria hyperborea* had a higher C:N ratio than the diet of phytoplankton (Figure 11). The sample of *L. hyperborea* detritus had an average carbon nitrogen ratio of 17:1 compared with the average ratio of 3:0 for the phytoplankton samples. These values are in contrast to the typical Redfield ratio for carbon : nitrogen which is 6 : 6 (Geider & La Roche, 2002). The Redfield ratio is important in determining from what source an element originated, for example land-based or marine-based. Alfred Redfield equated the elemental composition of plankton with that of inorganic C, N, and P in seawater and that variations in inorganic nutrients were a result of the decomposition of organic matter (Geider & La Roche, 2002).

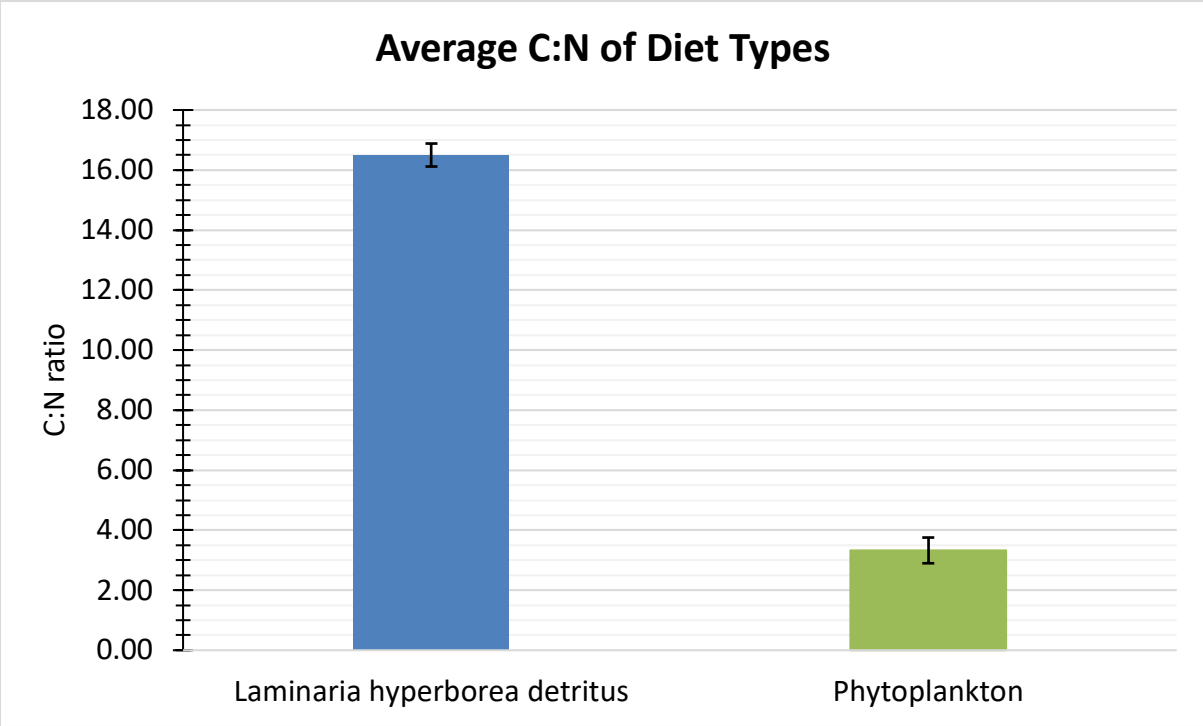

Figure 11: Comparison of carbon:nitrogen content between the two diet groups. Detritus from Laminaria hyperborea *had a C:N ratio of 17:1 while the phytoplankton mixture had a C:N ratio of 3:0.*

The kelp detritus from *Laminaria hyperborea* contained around ten times more nitrogen and carbon than the phytoplankton mixture (Table 1). This means that the *Mytilus edulis* organisms which were fed detritus received a higher amount of carbon and nitrogen than the phytoplankton group for every time they were fed.

Table 1: Comparison of average % nitrogen and average % carbon in detritus from Laminaria hyperborea *and the phytoplankton diet. The detritus from* Laminaria hyperborea *had a higher percentage of carbon and nitrogen than the phytoplankton blend.*

	Average	
Diet	% Nitrogen	% Carbon
Laminaria hyperborea	2.14 ± 0.06	32.76 ± 0.19
Phytoplankton	0.36 ± 0.02	3.33 ± 0.06

2.3 Measurements

Mytilus edulis shell length measurements

The blue mussels were measured once a month for four months from February to May 2017. The first measurement was on the day of set-up, 15 February. Measurements of shell length were performed by taking the plates out of the aquaria and placing on a flat surface. A digital caliper was used (Cocraft Digital Caliper 0-150mm) to measure from the anterior to posterior position on the shell. The shell length measurements were recorded for every individual according to group (small or large) and aquarium type/diet. The final measurements were taken on 2nd May.

Semibalanus balanoides shell diameter measurements

The *Semibalanus balanoides* stone plates were moved from the chain at Drøbak to the cellar at the Department of Biosciences, University of Oslo on 1st of May. The plates were then taken into the Algae Lab for their initial measurement on 3rd of May. Each plate was placed in a square plastic box filled with water from its respective aquarium and a stereo microscope (Nikon SMZ-U) was used to measure the shell diameter of 20 randomly chosen living individuals from each plate (Figure 12). Individual *S. balanoides* organisms were determined to be living if their cirri were beating/filtering water. The microscope (0,75X) was connected to a computer monitor (Dell) where a digital camera system for microscopy (Nikon Digital Sight DS-L1) was used to measure the shell lengths on each plate. These measurements were then photographed (Figure 13) with a microscope camera head (Nikon Digital Sight DS-5M) and organized according to plate number/diet/date.

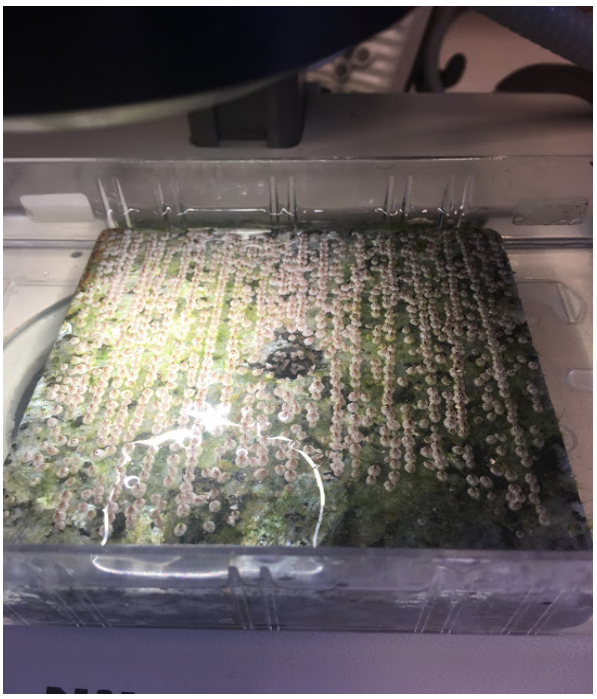

Figure 12: Initial measurements ready to be taken of Semibalanus balanoides *organisms just moved from Drøbak. The plate is placed on the base of the stereo microscope.*

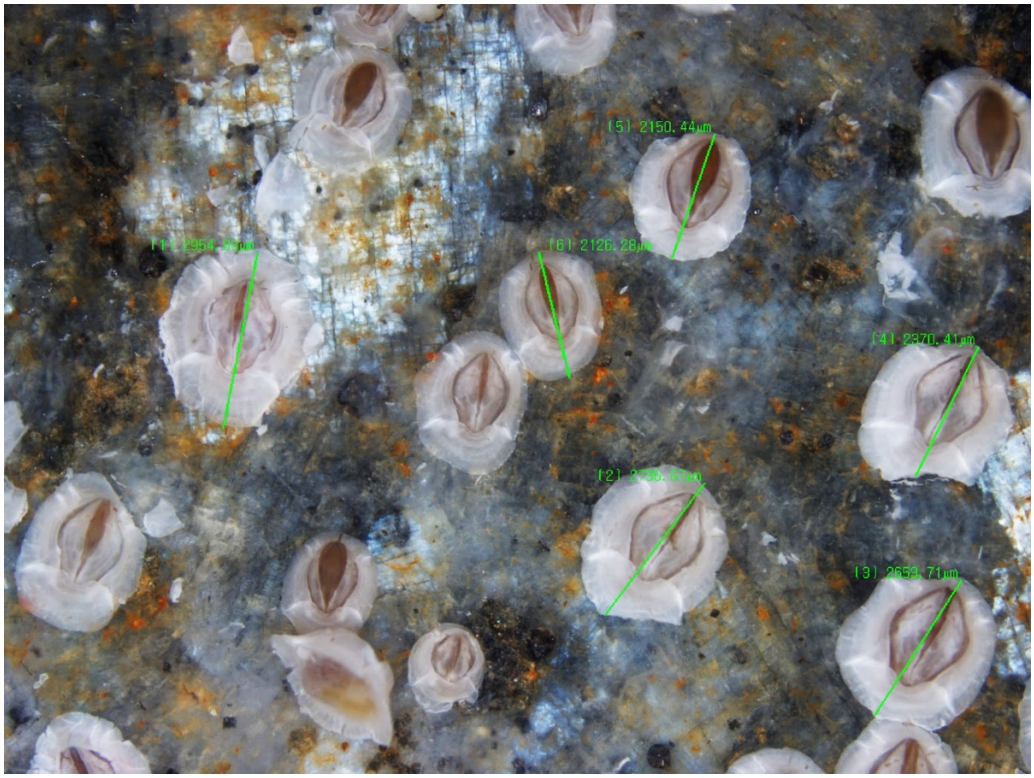

Figure 13: First photo of plate 4 of the Semibalanus balanoides *organisms which would be receiving the phytoplankton diet. Shell diameter measurements are in micrometers.*

The last planned measurements were taken on 28th August. However, since the barnacles had not grown as much as expected, their test period was extended by one month. They were fed during this time to see if their growth would increase much more. Therefore, the fourth and final measurements were taken 17th of October.

Control measurements

Control organisms were measured identically to the corresponding test organisms.

Mytilus edulis control

The control individuals were measured once a month with the first measurement taken on the day of setup. In March 2017, all of the control mussels were alive and appeared to be filtering. The water was replaced in the tanks as there was a buildup of feces and particles. None of these mussels had moved from their original glued positions. This control experiment was mistakenly taken down in early April 2017 before the test period conclusion and a new one had to be set up on 10 May 2017.

The second control had the same set-up as the first control the only difference being that the mussels used were taken from outside Tollboden in Drøbak along the Oslofjord. This group were measured from May to August 2017. Aquaria were cleaned every 2-3 weeks and the mussels measured once a month from the day of set-up. The shell length was measured as described previously. The first measurements were taken on 10th of May.

When taking the second set of measurements on 15th of June, it was discovered that some of the mussels had moved from their glued positions. They were identified and re-glued using a new super glue (Loctite Power Glue Repair Extreme). The final measurements of the control were taken on 15th September.

The natural seawater varied between temperatures of 1°C in February 2017 to 5°C in March 2017 and finally 12°C in September. The PSU increased from 32 in February 2017 to 34 in May 2017.

Semibalanus balanoides control

The control organisms were measured identically to the *Semibalanus balanoides* test organisms. The control was transported back and forth between the university's biological station at Drøbak and the Department of Biosciences at the University of Oslo. The same microscope and camera settings were used so the test and control measurements could be compared precisely. The first measurements were taken in May 2017 and the last measurements taken in August 2017.

2.4 Lipid analysis

A lipid analysis of the *Mytilus edulis* samples was performed by ALS Laboratory Group Norway AS. The NMR method was used to determine the amount of lipids in the samples. The measurement uncertainty was 6.0% and the units were reported in 0.1g/100g. A minimum of 30g was required per sample and mussels from each group were combined into one sample to meet this minimum. For example, the five large *M. edulis* individuals which received the phytoplankton diet were submitted as one sample for analysis and are labeled the large *M. edulis* phytoplankton group.

2.5 Statistical analysis

2.5.1 Mann-Whitney U test on *Mytilus edulis* data

Data were analyzed in Microsoft Excel using the XLSTAT extension package. Summary statistics were performed for each group of data and normality of each group was determined using the Shapiro-Wilk normality test. The Mann-Whitney U test was used to determine the significance of difference between data groups for *M. edulis* shell length measurements. This test was also used to determine the statistical significance of daily growth rates between the test groups and control. The Mann-Whitney U test is a non-parametric test which is used for a dataset with an ordinal dependent variable and an unpaired independent variable (Neely et al., 2003). A non-parametric test was chosen as not all of the data could be confirmed to have a normal distribution. This test considers differences between two populations (Quinn & Keough, 2002). The H_0 being tested is that the two samples come from populations with identical distributions against the H_A that the samples come from populations which differ only in location (mean or median) (Quinn & Keough, 2002). The alpha was set at 0.05, meaning any p-value less than this would result in rejection of the null hypothesis and the data would be considered significantly different.

2.5.2 Two-factor ANOVA on *Semibalanus balanoides* data

Data for *Semibalanus balanoides* was analyzed in Microsoft Excel. A single-factor ANOVA (analysis of variance) was first performed to analyze differences between aquaria in the same diet group as all measurements were random. ANOVA is used for partitioning the variation in a response variable into that explained and that unexplained by one or more factors (Quinn & Keough, 2002). The aims of ANOVA are to examine the relative contribution of different sources of variation to the total amount of variability in the response variable and to test the null hypothesis that population group or treatment means are equal (Quinn & Keough, 2002). As the mean of each aquarium was considered to be significantly different from each other within each diet group, a two-factor ANOVA was used to compare between both diet groups and identify which factor was contributing most to the amount of variability. Diet and aquarium were the two factors used in the analysis. The alpha was set at 0.05, with any p-value below this indicating that the data are significantly different. This would result in a rejection of the null hypothesis.

3 Results

3.1 Statistical analysis of shell growth

3.1.1 Mann-Whitney U test on *Mytilus edulis*

Mytilus edulis large group

Values for total amount grown for each organism over the experimental period were used in the statistical test. The average amount grown for each diet group was used for comparison and a Mann-Whitney U test was performed to determine the significance between these two diet groups. There is a significant difference ($p = 0.011$) between the growth of large *Mytilus edulis* individuals fed a diet of *Laminaria hyperborea* detritus and the growth of *M. edulis* individuals fed a diet of phytoplankton (Table 2).

Table 2: Mann-Whitney U test for the comparison of averages in shell length growth between the two diet groups for the large Mytilus edulis *organisms.*

Mann-Whitney test / Two-tailed test:	
U	35
U (standardized)	0.000
Expected value	84.000
Variance (U)	378.000
p-value (Two-tailed)	0.011
alpha	0.05

The average difference in growth for each diet group was used for visual comparison of the data. When looking at these two values (Figure 14), it can be seen that the large mussels receiving the phytoplankton diet grew more in terms of shell length compared to the mussels receiving the *L. hyperborea* detritus diet. The phytoplankton large mussel group (n=13) had an average growth of 9.58mm (SD = ± 0.81mm) while the *L. hyperborea* detritus large mussel group (n=15) had an average growth of 8.87mm (SD = ± 0.39mm) over the study period of 76 days. This was determined by subtracting the first measurement from the final measurement to get the difference in growth for each individual. The differences from each organism were averaged to get one value for the group and then the standard deviation was calculated for the

average. The large mussels which were fed phytoplankton grew on average 0.71mm ± 0.25mm more than the large mussels fed kelp detritus.

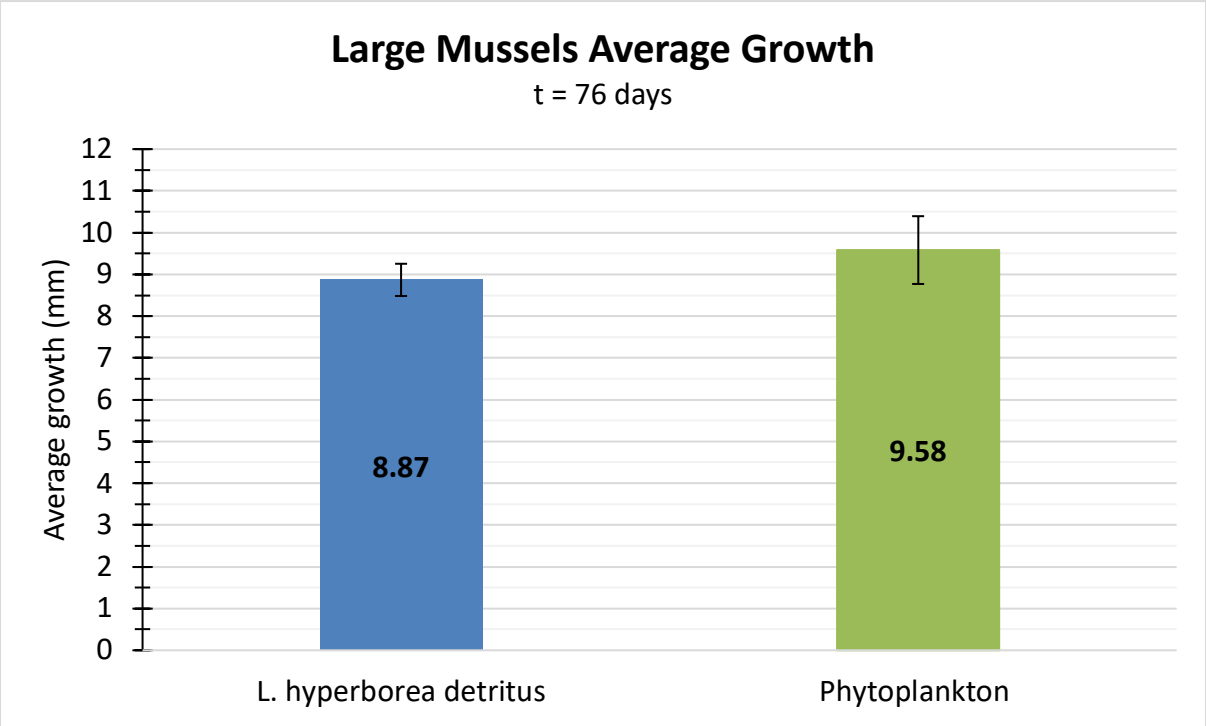

Figure 14: Comparison of average difference in growth for the large Mytilus edulis *diet groups. The* Mytilus edulis *organisms in the group which were fed with detritus from* Laminaria hyperborea *grew 8.87mm (SD = ± 0.39mm) on average, compared to the average growth of 9.58mm (SD = ± 0.81mm) for the phytoplankton* Mytilus edulis *group.*

Mytilus edulis large group control

The large *Mytilus edulis* control was tested against the *Laminaria hyperborea* detritus group and the phytoplankton group. The data used in the tests was the average shell growth per day for each group during the experiment period. Large *M. edulis* which received detritus from *L. hyperborea* grew significantly more ($p < 0.0001$) per day than the control group (Table 3).

Table 3: The Laminaria hyperborea *detritus group tested against the control group for the large* Mytilus edulis *organisms. A Mann-Whitney U test was performed to determine the significance of difference between average growth rate per day between the two groups.*

Mann-Whitney test / Two-tailed test:	
U	196
U (standardized)	4.481
Expected value	98.000
Variance (U)	473.537
p-value (Two-tailed)	< 0.0001
alpha	0.05

When comparing between the large *Mytilus edulis* organisms on the phytoplankton diet and the large control group, the phytoplankton-fed group had a significantly higher ($p = 0.001$) growth rate (Table 4).

Table 4: The phytoplankton diet group tested against the control group for the large Mytilus edulis *group. A Mann-Whitney U test was performed to determine the significance of difference between average growth rate per day between the two groups.*

Mann-Whitney test / Two-tailed test:	
U	168
U (standardized)	0.000
Expected value	98.000
Variance (U)	473.407
p-value (Two-tailed)	0.001
alpha	0.05

The control for the large mussels (n=15) had an average daily growth rate of 0.08mm/day. The control fed on phytoplankton species naturally found in the seawater from Oslofjord. When comparing the test groups with the control group, the phytoplankton diet group grew the most per day with the *Laminaria hyperborea* detritus group having the second highest growth rate per day and the control group grew the least per day (Figure 15).

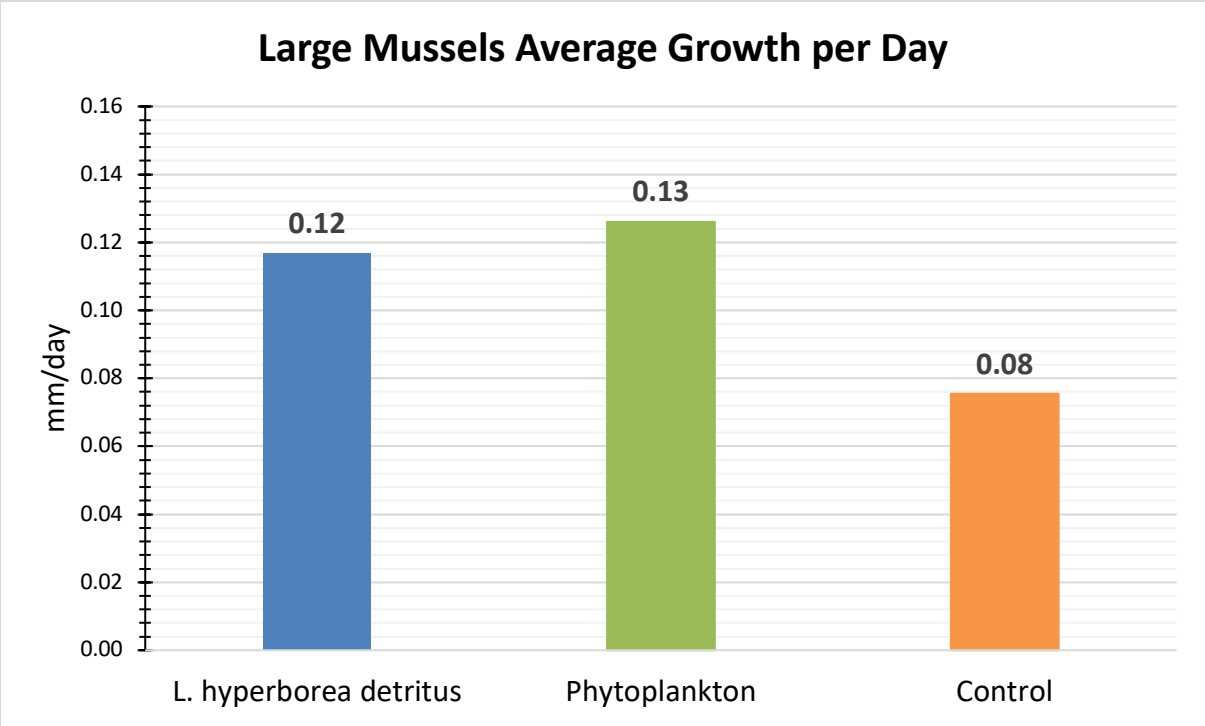

Figure 15: Comparison of daily growth rate between the two test groups and control group for the large Mytilus edulis *organisms. The highest growth rate was the phytoplankton group with an average of 0.13mm/day, then the* Laminaria hyperborea *detritus group with 0.12mm/day and the lowest daily growth rate, the control with 0.08mm/day.*

Mytilus edulis small group

As with the *Mytilus edulis* large group, a Mann-Whitney U test was used to analyze the significance of difference in growth between the two diet groups. The values for amount of growth for each organism from the phytoplankton group were compared to all the values for amount of growth for each organism from the detritus group. There is not a significant difference ($p = 0.434$; $\alpha = 0.05$) between the growth of small *M. edulis* individuals fed a diet of *Laminaria hyperborea* and the growth of *M. edulis* individuals fed a diet of phytoplankton (Table 5).

Table 5: Mann-Whitney U test for the comparison of averages in shell length growth for the two diet groups for the small Mytilus edulis *organisms. There is less than a 5% chance that there is a significant difference in growth between the two diet groups.*

Mann-Whitney test / Two-tailed test:	
U	80.500
U (standardized)	0.000
Expected value	98.000
Variance (U)	473.537
p-value (Two-tailed)	0.434
alpha	0.05

In addition, the average difference in growth for each diet group was calculated for visual comparison of the data. The small mussels which received phytoplankton (n=15) grew on average 9.54mm (SD = ± 0.82mm) compared to the average growth of 9.29mm (SD = ± 0.67mm) for the mussel group that fed on *Laminaria hyperborea* detritus (n=15) (Figure 16). These numbers were calculated the same as described earlier for the large mussels. The difference between the averages for the small mussel groups is 0.25mm (SD = ± 0.27mm).

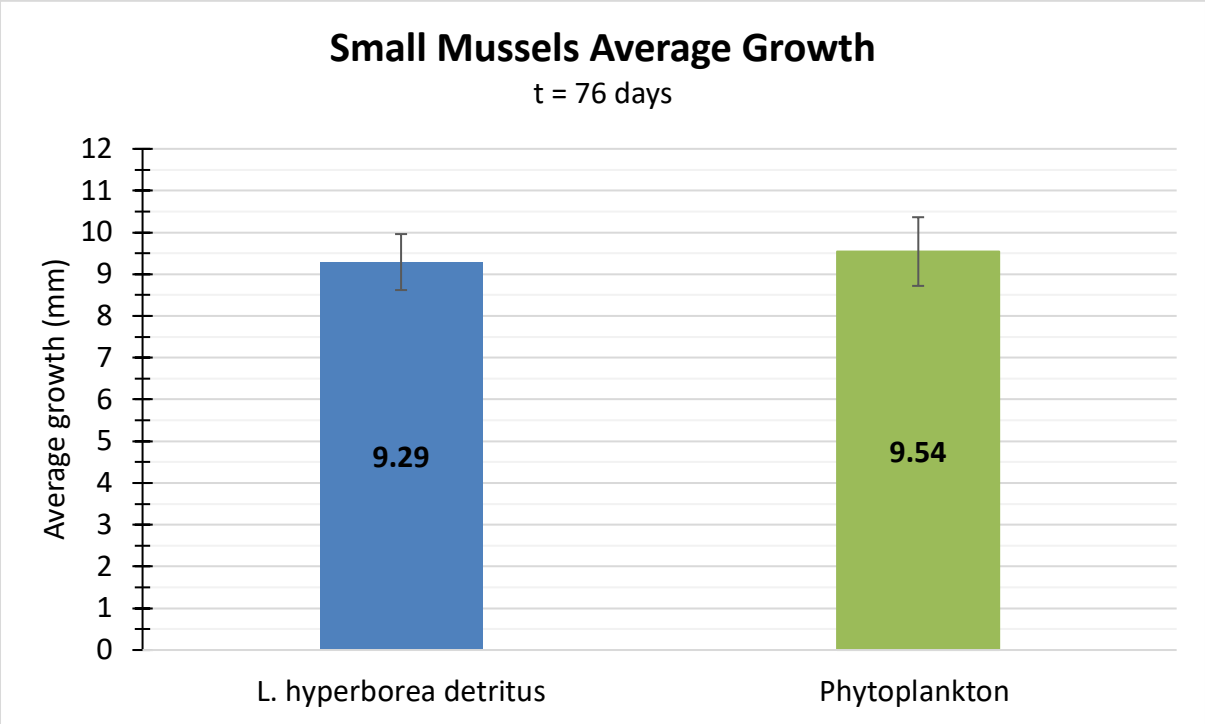

Figure 16: Comparison of average small Mytilus edulis *growth between the two diet groups. The* Mytilus edulis *organisms which were fed detritus from* Laminaria hyperborea *grew 9.29mm (SD = ± 0.67mm) and the* Mytilus edulis *group which received the phytoplankton diet grew 9.54mm (SD = ± 0.82mm).*

Mytilus edulis small group control

The control for the small *Mytilus edulis* group was tested against the diet groups which were fed with *Laminaria hyperborea* detritus or the blend of phytoplankton. The small *M. edulis* organisms which received detritus from *L. hyperborea* had a significantly higher ($p < 0.0001$) growth rate per day than the control group (Table 6).

Table 6: The Laminaria hyperborea *detritus group tested against the control group for the small* Mytilus edulis *organisms. A Mann-Whitney U test was performed to determine the significance of difference of the growth rate per day between the two groups.*

Mann-Whitney test / Two-tailed test:	
U	196
U (standardized)	4.482
Expected value	98.000
Variance (U)	473.148
p-value (Two-tailed)	< 0.0001
alpha	0.05

The small *M. edulis* organisms fed the phytoplankton blend had a significantly higher ($p < 0.0001$) daily growth rate than the control group (Table 7).

Table 7: The phytoplankton diet group tested against the control group for the small Mytilus edulis *organisms. A Mann-Whitney U test was performed to determine the significance of difference between the two groups average growth rate per day.*

Mann-Whitney test / Two-tailed test:	
U	196
U (standardized)	4.482
Expected value	98.000
Variance (U)	473.148
p-value (Two-tailed)	< 0.0001
alpha	0.05

The control group for the small mussels (n=11) had an average daily growth rate of 0.07mm/day. This was a significantly lower growth rate than both the test groups. The phytoplankton diet group had the highest growth per day with 0.13mm/day and the *L. hyperborea* detritus group grew on average 0.12mm/day (Figure 17). The large and small groups of *Mytilus edulis* on the *Laminaria hyperborea* detritus diet had a daily growth rate of 0.12mm/day.

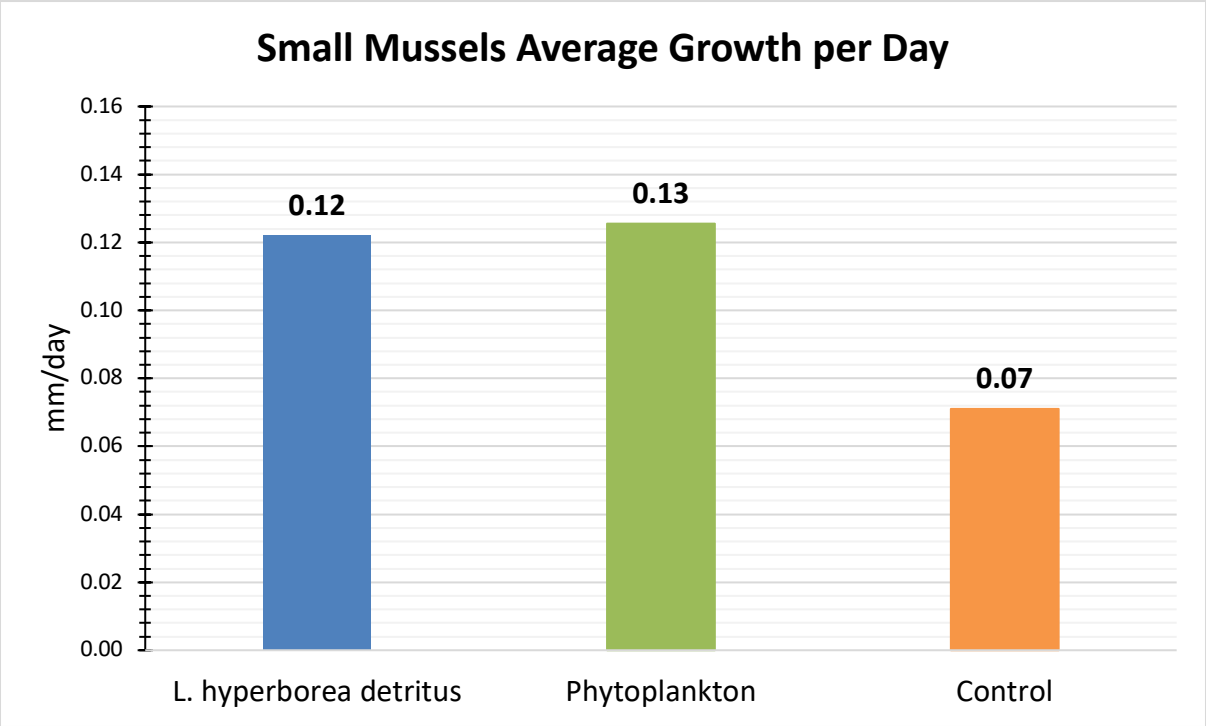

Figure 17: Comparison of daily growth rate between the two test groups and control group for the small Mytilus edulis *organisms. The phytoplankton group grew on average 0.13mm/day. The small mussels which were fed* Laminaria hyperborea *detritus had a daily growth rate of 0.12mm/day and as with the large mussels the control had the lowest growth rate of 0.07mm/day.*

3.1.2 ANOVA test on *Semibalanus balanoides*

Test groups

A two-factor ANOVA test was used to determine the significance of the *Semibalanus balanoides* test groups growth. The shell diameter measurements were compared based on diet type and aquarium. When looking only at the diet factor, there was no significant difference ($p = 0.756$) between the *Laminaria hyperborea* group and the phytoplankton group (Table 8). However, the aquarium factor resulted in a rejection of the null hypothesis ($p < 0.0001$) as there was found to a be significant difference between the size of organisms between aquaria. The null hypothesis was rejected ($p = 0.012$) for the interaction between diet type and aquarium. There was a significant difference in the size of the organisms when considering the combination of diet and aquarium number.

Table 8: A two-way ANOVA test with replication was done on the final shell diameter measurements for both Semibalanus balanoides *diet groups, phytoplankton and* Laminaria hyperborea *detritus.*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Diet	0.016487696	1	0.016487696	0.096244294	0.756950485	3.924330485
Aquaria	7.923577225	2	3.961788613	23.12630835	3.71265E-09	3.075852636
Interaction	1.559070848	2	0.779535424	4.550413548	0.012551661	3.075852636
Within	19.52944219	114	0.171310896			
Total	29.02857796	119				

Control group

Each diet group was analyzed against the control in order to compare the size of *Semibalanus balanoides* between the two groups. A two-factor ANOVA was done on the shell diameter data for the *Laminaria hyperborea* detritus group and the control (Table 9). There was a significant difference ($p < 0.0001$) in the means between the two groups when considering the factor of diet. The aquaria factor had a p-value of 0.002, therefore the alternative hypothesis of significant difference in means between aquaria is accepted. The interaction between diet type and aquarium was significantly different ($p = 0.001$) and the null hypothesis is rejected.

Table 9: The results of the two-factor ANOVA performed on the data from the Laminaria hyperborea *group and the control group for* Semibalanus balanoides.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Diet	8.407449053	1	8.407449053	22.09522686	7.31611E-06	3.924330485
Aquaria	4.815206356	2	2.407603178	6.327310232	0.002478434	3.075852636
Interaction	5.025942091	2	2.512971046	6.604222637	0.001932714	3.075852636
Within	43.37811048	114	0.380509741			
Total	61.62670798	119				

The two-factor ANOVA shows the results of comparing the data from the phytoplankton group and the control group for *Semibalanus balanoides* (Table 10). The null hypothesis was rejected ($p < 0.0001$) when only looking at the influence of diet. There was a significant difference in means between the diet groups. There was no significant difference ($p = 0.065$) in means of *S. balanoides* organisms between aquaria. The interaction between aquarium

number and diet was significant ($p < 0.0001$) and therefore the null hypothesis was rejected for this combination of factors.

Table 10: The results of the two-factor ANOVA performed on the data from the phytoplankton group and the control group for Semibalanus balanoides.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Diet	7.679304043	1	7.679304043	18.97559105	2.90892E-05	3.924330485
Aquaria	2.265719776	2	1.132859888	2.799301322	0.065041195	3.075852636
Interaction	11.82384546	2	5.911922729	14.60838475	2.24867E-06	3.075852636
Within	46.13509315	114	0.4046938			
Total	67.90396243	119				

When looking at the two diet groups average size, there was no difference whether the *Semibalanus balanoides* organisms received detritus from *Laminaria hyperborea* or the phytoplankton mixture. A difference can be seen when comparing between the aquaria of each diet group (Figure 18). The control organisms were significantly bigger than the organisms fed the *L. hyperborea* diet. The same pattern can be seen when looking at the phytoplankton data. The control organisms were significantly larger than the *S. balanoides* group given the phytoplankton mixture.

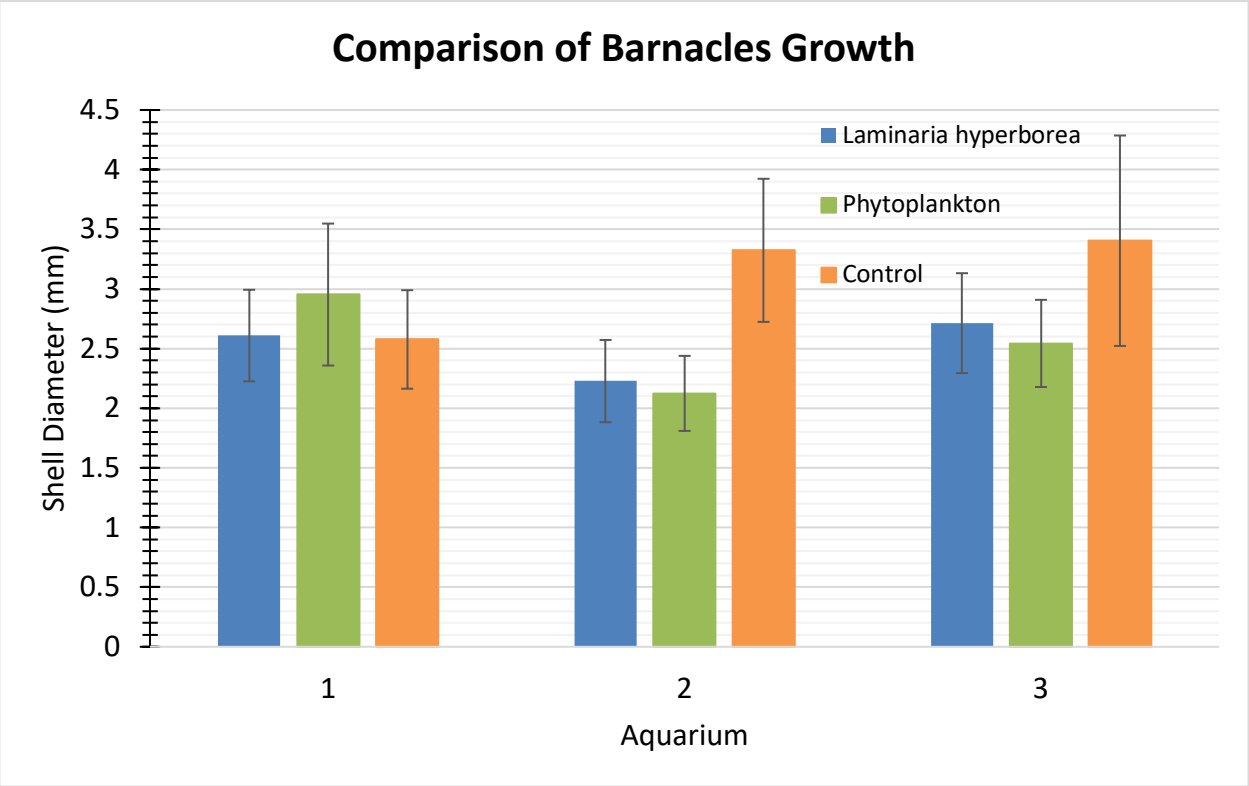

Figure 18: Comparison of average size of the Semibalanus balanoides *organisms from both test groups and the control. The group given* Laminaria hyperborea *detritus had an average final size of 2.52mm (SD = ± 0.43mm). The phytoplankton group had an average final size of 2.54mm (SD = ± 0.55mm). In comparison, the* Semibalanus balanoides *organisms belonging to the control group had a final average size of 3.05mm (SD = ± 0.85mm).*

3.1.3 Summary of statistical results

The null hypothesis of no difference between the means of groups was rejected for the large *Mytilus edulis* group (Table 11). There was no significant difference between the means of the small *M. edulis* groups and the null hypothesis was accepted. There was not a significant difference in the size of *Semibalanus balanoides* between diet groups and therefore the null hypothesis was accepted.

Table 11: Summary of the statistical results for each organism in the experiment. The null hypothesis is rejected for the large Mytilus edulis *organisms. The null hypothesis is accepted for both the small* Mytilus edulis *organisms and the* Semibalanus balanoides *organisms.*

Null hypothesis		
Organism group	Acceptance	Rejection
Large *Mytilus edulis*		✓
Small *Mytilus edulis*	✓	
*Semibalanus balanoides**	✓	
*when considering the diet factor alone.		

3.2 Lipid analysis of tissues

3.2.1 *Mytilus edulis* large test groups and control

A lipid analysis was performed on the two different diet groups and the control group for the large *Mytilus edulis* organisms. The *Laminaria hyperborea* detritus group had the highest fat content of the three, 0.6g/100g (Figure 19). The control had a fat content of 0.5g/100g and the phytoplankton diet group had the lowest amount of lipids with 0.2g/100g.

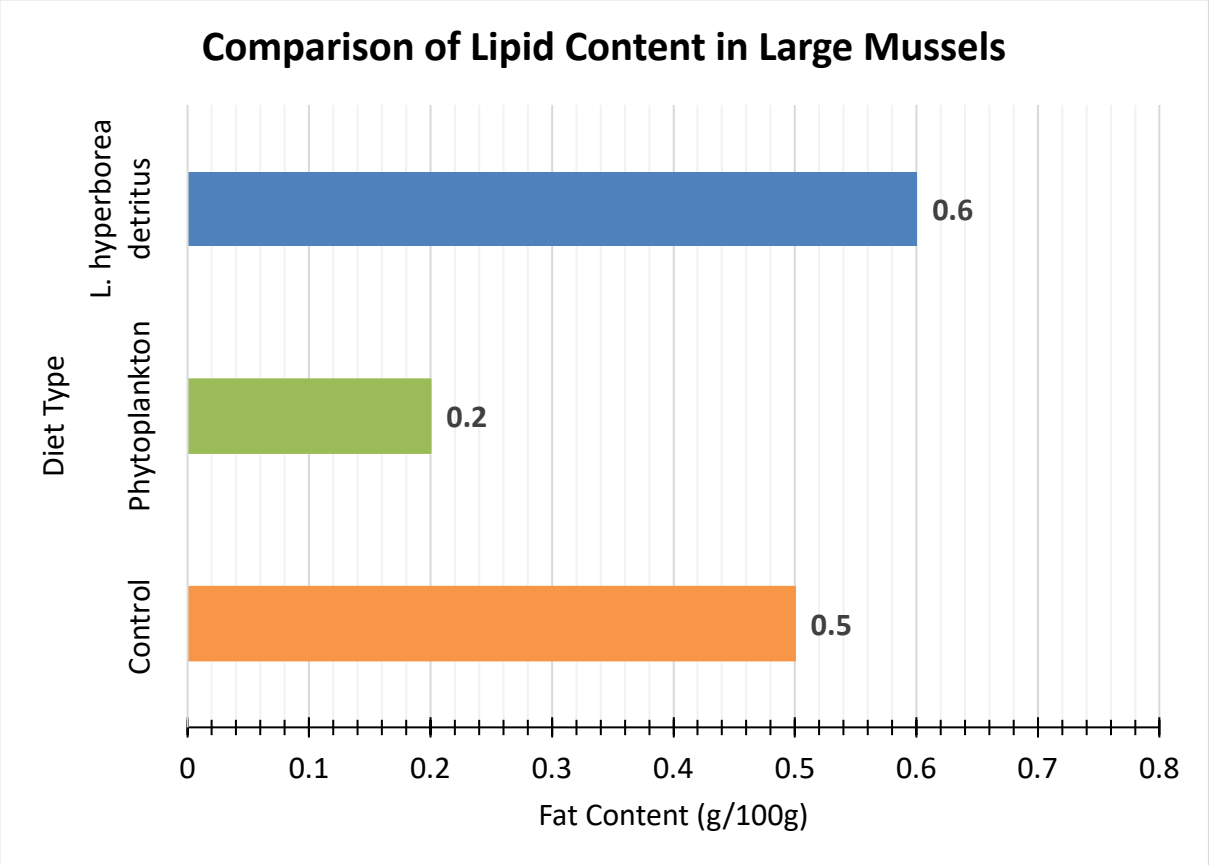

Figure 19: Comparison of fat content between the three groups of large Mytilus edulis *organisms. The highest lipid content of 0.6g/100g belonged to the large* Mytilus edulis *group fed with* Laminaria hyperborea *detritus. The control had a fat content of 0.5g/100g and the phytoplankton diet group had the lowest fat content of 0.2g/100g.*

3.2.2 *Mytilus edulis* small test groups and control

A lipid analysis was also performed on the small *Mytilus edulis* organisms from both diet groups and the control group. The control group had the highest amount of lipids with a value of 0.6g/100g (Figure 20). The *L. hyperborea* detritus group had a result of 0.3g/100g, while the phytoplankton diet group had the lowest amount of fat at 0.2g/100g.

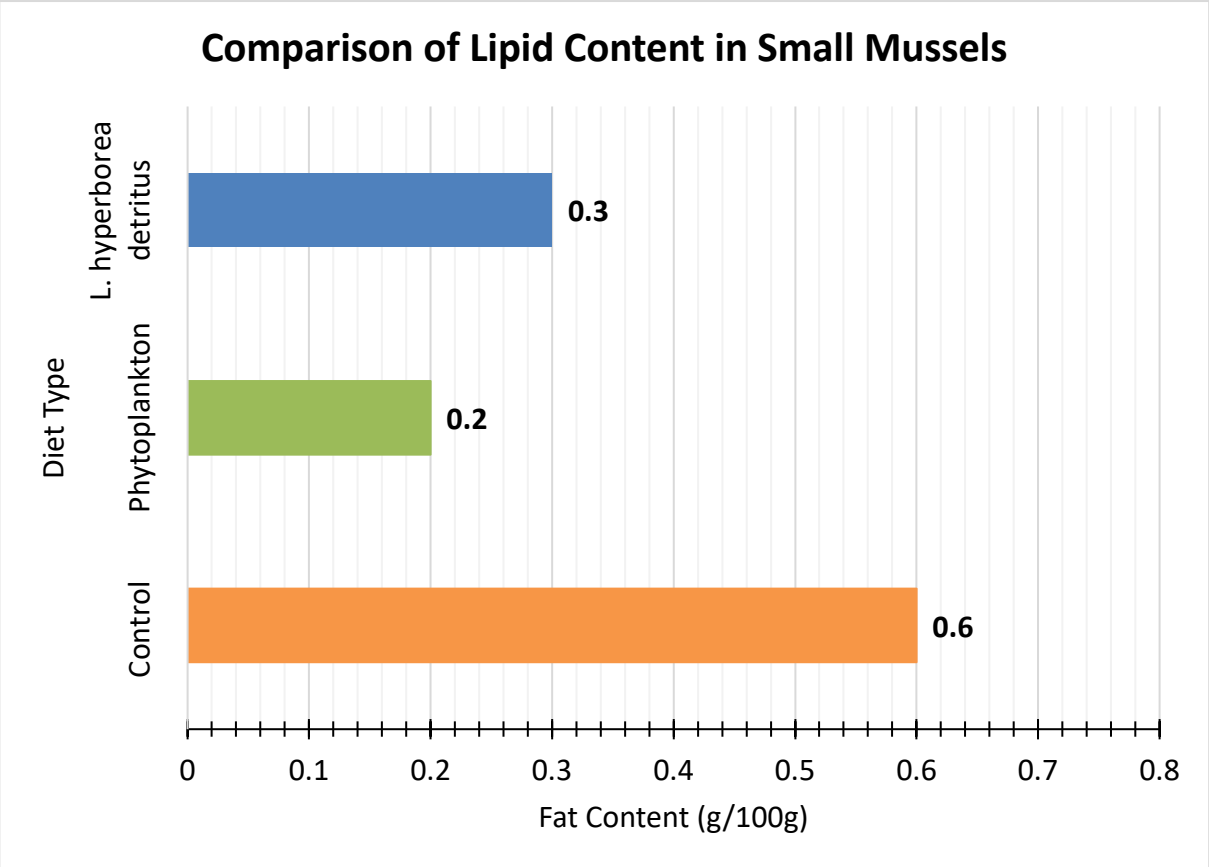

Figure 20: Comparison of fat content between the three groups of small Mytilus edulis *organisms. The control group had the highest fat content of 0.6g/100g. The test group which received* Laminaria hyperborea *detritus had a fat content of 0.3g/100g, followed by the phytoplankton group with the lowest lipid amount of 0.2g/100g.*

3.2.3 *Semibalanus balanoides*

The *Semibalanus balanoides* organisms did not have enough tissue in order to perform a lipid analysis. A minimum of 30g per sample was required to do a lipid analysis with the NMR method. Most of the organisms had died by the time the last measurements were taken.

3.3 Response to study aims

Mytilus edulis

There was a significant difference between the two diet groups growth for the large *Mytilus edulis* organisms. This means the null hypothesis can be rejected for the large size group. The large *M. edulis* organisms on the phytoplankton diet grew more than the organisms on the *Laminaria hyperborea* detritus diet.

There was not a significant difference between the two diet groups growth for the small *Mytilus edulis* organisms. The null hypothesis is accepted for the small size group.

Semibalanus balanoides

There was no significant difference between the two diet groups average size for the *Semibalanus balanoides* organisms when considering only the factor of diet. The null hypothesis is accepted for the *S. balanoides* organisms. The interaction between the aquaria and diet factors was significant.

4 Discussion

4.1 Different growth results based on size group of *Mytilus edulis*

As stated in the Results section, there was a significant difference in growth between the *Laminaria hyperborea* diet group and the phytoplankton diet group for the **large** *Mytilus edulis* organisms. The large mussel group which received *L. hyperborea* detritus grew less on average than the large mussel group which received phytoplankton. The result of the Mann-Whitney U test computed this difference to be significant. Both of the large *M. edulis* test groups were also compared against the average growth of the control. The control grew significantly less on average than both the *L. hyperborea* detritus group and the phytoplankton group. When comparing the daily average growth rate for all three, the phytoplankton diet group had the highest growth rate, followed by the *L. hyperborea* detritus group and the lowest growth rate per day belonged to the control group for the large *M. edulis* organisms. In a growth experiment by Stromgren and Cary (1984), it was found that *M. edulis* individuals grew better on a multispecies diet compared to individuals fed on single species diets and that this could be due to a shortage of vital nutrients. With regards to this thesis, this does not explain why the control had the lowest growth rate compared to the two diet groups when these organisms probably had the most varied diet.

There was not a significant difference in growth between the *Laminaria hyperborea* diet group and the phytoplankton diet group for the **small** *Mytilus edulis* organisms. This means that small *M. edulis* organisms fed with *L. hyperborea* detritus grew just as well as the group fed with the phytoplankton mixture. The average growth for the two test groups was significantly higher than the control group belonging to the small *M. edulis* organisms. As with the large *M. edulis* group, the small *M. edulis* on the phytoplankton diet had the highest growth rate when compared to the detritus group and control. Similarly, the *L. hyperborea* detritus group had the next highest daily growth rate with the control growing the least per day on average.

When considering the reasons why the large *Mytilus edulis* group did not grow as well as the small *M. edulis* group, it is useful to look at the results of other growth experiments on this species. Small *M. edulis* organisms grew significantly more in mm than large *M. edulis*

organisms in an study done on the effects of increased concentrations of CO_2 in seawater on the shell growth of blue mussels (Berge et al., 2006). The authors were unsure if this difference in growth was due to a systematic interaction effect of size and pH or a random effect of sampling variance. The small group of mussels had a mean shell length of 11 mm and the large group had an average shell length of 21 mm, which is similar to the size groups for the mussels used in this experiment.

In a study done on *Mytilus edulis* organisms growth in the Gulf of Maine, the shell lengths of mussels at one particular location were significantly larger than the shell lengths at other tested locations (Lesser et al., 2010). The authors speculated that the location with greater shell growth had the benefits of increased availability of food, longer emersion times and also an increased seasonal sea surface temperature (≈ 20°) which would increase metabolic rates. The *M. edulis* organisms in this thesis experiment were constantly emerged and received a steady food supply, however temperature was maintained at 10°C in the artificial seawater system. In Oslofjord, peak summer sea surface temperatures can be up to 20°C (Baalsrud & Magnusson, 2002), however this is not average for the year. A consideration for future work could be to test *M. edulis* growth on a diet of detritus from *Laminaria hyperborea* and use different temperatures to see if that factor had a significant effect on the results.

The results from the lipid analysis of the *Mytilus edulis* samples show that the large group fed detritus from *Laminaria hyperborea* had the highest lipid content. This is unusual because kelps are generally quite low in lipid content (Maehre et al., 2014). *L. hyperborea* had a lipid content of 1.14 g/100g DW when analyzed with ether extraction and 1.42 g/100g DW with dichloromethane/methanol extraction (Maehre et al., 2014). The control group had the second highest amount of fat in their tissue. The large *M. edulis* organisms on the phytoplankton diet had the lowest amount of fat. A contrasting pattern is seen in the results of the lipid analysis on the small *M. edulis* organisms. The highest amount of fats in their tissue belonged to the control group, followed by the *L. hyperborea* detritus group and again the lowest amount of lipids found in the tissue of the phytoplankton diet group.

Detritus from *L. hyperborea* had a higher amount of carbon and nitrogen than the phytoplankton mixture. It would be valuable to perform an isotope analysis on both the test and control organism's tissues to determine the major source of carbon and nitrogen for each group in this experiment. Bustamante and Branch (1996) conducted a study to determine the

trophic relationship between sessile filter-feeders and kelp detritus using stable isotope analyses in South Africa. One of the filter-feeders used was of the genus *Mytilus* and the kelp-derived particles from the *Laminaria* genus. It was found that the filter-feeders used particulate kelp detritus as their major source of organic carbon and nitrogen. They also discovered that kelp particles dominated the composition of suspended POC in the intertidal zone. In another example of stable isotope analysis, kelp-derived detritus was found not to significantly contribute to the carbon content of *M. edulis*. Duggins et al. (1989) evaluated $\delta^{13}C$ from samples of kelp species belonging to *Laminaria* and *Alaria* and compared these to isotopic values of dominant phytoplankton in the Aleutian Islands. When looking at consumers $\delta^{13}C$ values in kelp-dominated islands, all species had significantly more kelp-derived carbon in their tissues except *M. edulis*. Lesser et al. (2010) also used stable isotope analyses and determined that phytoplankton dominated the diets of all *M. edulis* organisms tested, although there was evidence that diets were a mix of phytoplankton and detritus originating in the intertidal. While detritus from kelp can certainly supplement *M. edulis* nutritional needs, it is certainly not the primary food source for this species.

In this experiment, the detritus from *Laminaria hyperborea* had a mean C:N ratio of 15.29 ± 0.38 and most animals require food sources to have a C:N ratio below 17 to meet their nitrogen requirements (Russell-Hunter, 1970). Norderhaug et al. (2006) used fresh and degraded *L. hyperborea* in a feeding experiment with amphipods. The fresh kelp harvested in March had a C:N ratio of 12 with the most degraded kelp having a value of less than 2. Norderhaug et al. (2006) found in this experiment that the amphipods did not grow on kelp collected in August when the C:N ratio of the kelp tissue was > 30. The authors concluded that kelp can be used as a food source if the C:N ratio is not too high, resulting in nitrogen storage, or too low, causing carbon storage, as the growth of the amphipods was best on the fresh kelp from March with a low C:N ratio and low-degraded kelp diets. A potential further study with this thesis could be to harvest *L. hyperborea* at different seasons as they store nitrogen from January to April (Sjøtun et al., 1996) and therefore have a different C:N composition year round. The effects of feeding filter-feeders detritus from *L. hyperborea* of varying carbon-nitrogen content could then be tested.

This experiment demonstrates that *M. edulis* can survive on kelp detritus from *L. hyperborea*. In the case of the small *M. edulis* individuals, they can even grow as well as on a phytoplankton diet. Considering that the mussel shell is approximately 20% of the total

organic content of the whole mussel, increase in shell length can be a useful indicator of mussel growth (Stromgren & Cary, 1984).

4.2 *Semibalanus balanoides* test groups smaller than control

The results from the two-way ANOVA showed that the interaction between aquaria and diet type was significant, however when looking at each factor individually there is no significant difference in average size of *Semibalanus balanoides* based on diet. There was a significant difference in organism size between aquaria. When comparing the *Laminaria hyperborea* detritus group and the control, the interaction was determined to be significantly different between diet and aquaria. The factor of diet alone resulted in a significant difference between the *L. hyperborea* and control group's average organism size. The average size between aquaria was also significantly different. The control had a higher mean size of *S. balanoides* when compared to the *L. hyperborea* detritus group. The interaction between diet and aquaria was significant when comparing phytoplankton and control groups. The control group for *Semibalanus balanoides* had a greater average size than the group on the phytoplankton diet.

While there have been numerous experiments involving *Semibalanus balanoides*, more research is needed focusing on the relationship between this filter-feeder and kelp derived detritus. There are various studies done on the growth and settlement of this species when looking at other parameters. When examining the relationship between *Semibalanus balanoides* and fucoid canopy algae, it was found that *S. balanoides* organisms can be deterred from settling in heavily canopied algal forests (Jenkins et al., 1999). Some macroalgae species can act as a barrier to *S. balanoides* settlement and increase post-settlement mortality in the intertidal. The characteristics of barnacle feeding are of particular interest to researchers. For example, *S. balanoides* increases feeding activity with high flow speeds and food concentrations meaning that they feed when food availability is high (Sanford et al., 1994). Also, larger body size and higher temperatures resulted in a decrease in barnacle feeding. In this thesis experiment, the temperature of the artificial seawater was 10°C and while the flow was not measured, water pumps in the aquaria ensured that there was proper circulation. As an extension to this study, flow speeds and food concentrations could be monitored to see the effects on *S. balanoides* growth while comparing growth on a kelp detritus and phytoplankton diet. Bertness et al. (1991) also found that *S. balanoides* growth is

a direct function of water column food concentrations and the flow rate of food to organisms. The authors also determined that barnacles in exposed areas grew faster than those at sheltered areas.

Sanford et al. (1994) measured *Semibalanus balanoides* monthly in a field location with high flow and found that they grew from 2.5mm to more than 10mm in diameter over a 6-month period. In contrast, *S. balanoides* in the lab grew from 2.25mm ± 0.42mm to 2.52mm ± 0.43mm on the *L. hyperborea* detritus diet and 2.18mm ± 0.42 mm to 2.54mm ± 0.55mm on the phytoplankton diet over a 4-month period. Though the control did grow significantly better than both diet groups, the average final size was equally unimpressive at 3.05mm ± 0.85mm. Monitoring the flow rate and concentration of food might give a better explanation of why *S. balanoides* grew so poorly.

4.3 Conclusion

The *Mytilus edulis* organisms used in this experiment grew differently based on size group. While the large group fed the phytoplankton mixture experienced significantly higher growth than the *Laminaria hyperborea* detritus group, the smaller organisms grew equally well on the *L. hyperborea* diet. It is unknown why the smaller *M. edulis* organisms grew better in shell length than the large individuals. One would think that the smaller individuals would have to devote more energy to developmental processes than to secondary production. It can be that the larger organisms had a higher metabolic requirement and were not given an adequate amount of food to invest supplemental energy in shell growth. The results from the lipid analysis showed that *M. edulis* on the *L. hyperborea* detritus diet had a higher fat content in their tissues than the organisms on the phytoplankton diet. As mentioned earlier, *L. hyperborea* is relatively low in lipids so this was unexpected. When looking at the two size groups on the *L. hyperborea* diet, the larger *M. edulis* organisms had a higher fat content than the small *M. edulis* group. It could be that the small *M. edulis* organisms used most of their energy towards growth and therefore have a smaller storage capacity. A fatty acid profile should be used in future studies to confirm diet sources consumed by test organisms. Detritus from *L. hyperborea* had a higher carbon and nitrogen content than the phytoplankton mixture, which meant the organisms on the detritus diet were getting more nutrition at every feeding in comparison to the phytoplankton group. This could influence the results.

The *Semibalanus balanoides* test groups did not grow differently from each other when considering only the factor of diet. Nonetheless, the control had a significantly larger average size than both diet groups. Reasoning for the modest growth of the *S. balanoides* organisms could be that food concentration was too low, circulation in the aquaria weak or both factors (Bertness et al., 1991; Sanford et al., 1994). It could also be that detritus derived from *Laminaria hyperborea* does not provide this species with the nutrients required to thrive.

The results from this thesis show that there may be a stronger connection between *Mytilus edulis* and detritus derived from *Laminaria hyperborea* than with *Semibalanus balanoides* and this kelp species. More research needs to be done to fully understand this relationship. As kelp forests come under threat due to warming sea surface temperatures (Filbee-Dexter & Wernberg, 2018), dependent species may suffer if these forests continue to decrease worldwide. As these trophic relationships can be quite complex, more factors should be taken into consideration with future experiments.

4.4 Improvements and future work

As discussed throughout the thesis, there are a few areas of improvement to this experiment. The first problem encountered was the mistaken dismantling of the *Mytilus edulis* control. While a second control was set up with similarly sized organisms, this was done in May and the experiment began in February. This means that the control organisms were not monitored during the spring bloom and may have had different growth results. There were also problems with the artificial seawater system at the university. Despite replacement of the water in the system, there were growth of various organisms in the water, such as nematodes. Lastly the PSU took some time to adjust to a stable level as stated earlier.

Some further considerations for future work on a diet-growth experiment involving kelp detritus include a stable isotope analysis on the filter-feeder species to confirm the source of those species carbon and nitrogen. Temperature could be incorporated to see how much of an effect, warmer or colder temperatures could have on the filter-feeders growth while on different diets. Flow rate and concentration of particles are two factors which may play an important role on feeding behaviors and could be investigated.

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Appendix 1

First glue - Clas Ohlson Universal Super Glue Water-resistant (3g)

Second glue - Loctite Power Glue Repair Extreme (20g)

Caliper model - Cocraft Digital Caliper 0-150mm (Serial number: C1604130215)

Water pump model - Fluval Nano Aquarium Filter (up to 55L) 6.8W Flow rate: 307 LPH (UPC: 015561104555)

Appendix 2

Table 12: Raw data from carbon nitrogen analysis performed by the Toxicology lab at the Department of Biosciences. 'Phyto' refers to each of the samples of the phytoplankton blend (n = 5) and 'hyp' refers to the samples of detritus (n = 5) from Laminaria hyperborea.

Raw Data from Lab					
sample			**weight mg**	**N %**	**C %**
phyto001	ph001		1.748	0.360	3.307
phyto002	ph002		1.700	0.374	3.379
phyto003	ph003		1.576	0.389	3.381
phyto004	ph004		2.329	0.344	3.209
phyto005	ph005		2.668	0.336	3.358
hyp006	hy006		5.788	2.070	32.605
hyp007	hy007		8.047	2.205	32.917
hyp008	hy008		5.573	2.213	32.946
hyp009	hy009		4.339	2.085	33.854
hyp010	hy010		5.825	2.137	33.460

Table 13: Percent nitrogen and percent carbon of the Laminaria hyperborea *detritus used as food in the experiment.*

Hyperborea			
Replicates	% Nitrogen	% Carbon	C:N ratio
1	2.070	32.605	15.75121
2	2.205	32.917	14.92834
3	2.213	32.946	14.88748
4	2.085	32.854	15.75731
5	2.137	32.459	15.18905
Average	2.142	32.756	15.29234
		STDEV	0.382961

Table 14: Percent nitrogen and percent carbon of the phytoplankton mixture as a food source in the experiment.

Phytoplankton			
Replicates	% Nitrogen	% Carbon	C:N ratio
1	0.360	3.307	9.186111
2	0.374	3.379	9.034759
3	0.389	3.381	8.691517
4	0.345	3.209	9.301449
5	0.336	3.358	9.994048
Average	0.361	3.327	9.220621
		STDEV	0.428535

Appendix 3

Table 15: Data from the large Mytilus edulis *group that was fed detritus from* Laminaria hyperborea*. These measurements were used for analysis in the Mann-Whitney U test.*

Kelp Large Group (n =15)			t = 76 days
First Measurement	Final Measurement	Kelp Difference in Growth	Growth rate/day
23.97	32.55	8.58	0.112894737
21.49	30.22	8.73	0.114868421
18.16	27.08	8.92	0.117368421
20.94	29.79	8.85	0.116447368
22.13	30.85	8.72	0.114736842
17.36	27.08	9.72	0.127894737
25.05	34.4	9.35	0.123026316
21.54	29.93	8.39	0.110394737
18.14	26.44	8.30	0.11
23.05	32.24	9.19	0.120921053
16.27	25.55	9.28	0.122105263
26.27	34.83	8.56	0.112631579
21.94	30.45	8.51	0.111973684
18.58	27.35	8.77	0.115394737
22.08	31.25	9.17	0.120657895
Average difference		8.869333333	0.116701754
STDEV		0.385875052	0.005077303

Table 16: Data from the large Mytilus edulis *group that was fed the phytoplankton mixture. These measurements were used for analysis in the Mann-Whitney U test.*

Phytoplankton Large Group (n = 13)			t = 76 days
First Measurement	Final Measurement	Phytoplankton Difference in Growth	Growth rate/day
22.49	31.4	8.91	0.117236842
19.14	29.18	10.04	0.132105263
20.73	Dead		0
17.18	28.76	11.58	0.152368421
23.31	33.22	9.91	0.130394737
25.82	35.22	9.4	0.123684211
19.75	29.1	9.35	0.123026316
23.73	32.81	9.08	0.119473684
21.09	31.3	10.21	0.134342105
20.69	29.28	8.59	0.113026316
24.88	34.44	9.56	0.125789474
25.48	Died		0
21.35	30.49	9.14	0.120263158
19.89	30.25	10.36	0.136315789
22	30.45	8.45	0.111184211
Average difference		9.583076923	0.109280702
STDEV		0.811497994	0.044000936

Table 17: Control measurements for the large Mytilus edulis *group.*

Control Large Group (n = 15)			t = 128 days
First Measurement	Final Measurement	Difference in Growth	Growth rate/day
28.83	41.37	12.54	0.09796875
25.75	34.79	9.04	0.070625
28.71	37.91	9.2	0.071875
24.74	31.62	6.88	0.05375
22.53	31.17	8.64	0.0675
25.55	36.09	10.54	0.08234375
26.5	35.57	9.07	0.070859375
27.99	37.31	9.32	0.0728125
24.21	33.77	9.56	0.0746875
23.86	32.9	9.04	0.070625
26.43	34.52	8.09	0.063203125
31.88	44.59	12.71	0.099296875
27.78	40.03	12.25	0.095703125
32.7	44.45	11.75	0.091796875
23.06	29.37	6.31	0.049296875
Average difference		9.662666667	0.075489583
STDEV		1.886048662	0.014734755

Table 18: Summary data for the large Mytilus edulis *test groups and control group growth rate.*

Diet	Average difference in growth (mm)	Average growth/day (mm/day)
L. hyperborea detritus	8.87	0.12
Phytoplankton	9.58	0.13
Control	9.66	0.08

Table 19: Measurement data for the small Mytilus edulis *group which received detritus from* Laminaria hyperborea.

Kelp Small Group (n = 15)			t = 76 days
First Measurement	Final Measurement	Kelp Difference in Growth	Growth rate/day
7.43	16.83	9.4	0.123684211
10.58	19.13	8.55	0.1125
6.34	15.17	8.83	0.116184211
10.69	20.4	9.71	0.127763158
11.45	20.84	9.39	0.123552632
12.03	20.64	8.61	0.113289474
9.37	19.63	10.26	0.135
9.11	17.56	8.45	0.111184211
7.94	17.36	9.42	0.123947368
7.95	17.52	9.57	0.125921053
13.58	22.38	8.8	0.115789474
12.61	22.04	9.43	0.124078947
8.87	19.91	11.04	0.145263158
8.48	17.35	8.87	0.116710526
10.92	19.93	9.01	0.118552632
Average difference		9.289333333	0.12222807
STDEV		0.670268271	0.008819319

Table 20: Measurement data for the small Mytilus edulis *group which received the phytoplankton mixture.*

Phytoplankton Small Group (n = 15)			t = 76 days
First Measurement	Final Measurement	Phytoplankton Difference in Growth	Growth rate/day
10.2	19.45	9.25	0.121710526
11.64	20.94	9.3	0.122368421
11.99	21.35	9.36	0.123157895
10.06	19.44	9.38	0.123421053
9.71	19.66	9.95	0.130921053
9.29	18.16	8.87	0.116710526
10.17	18.6	8.43	0.110921053
8.02	16.83	8.81	0.115921053
14.08	23.4	9.32	0.122631579
12.1	21.67	9.57	0.125921053
12.33	22.55	10.22	0.134473684
8.83	18.32	9.49	0.124868421
9.59	19.17	9.58	0.126052632
11.17	23.36	12.19	0.160394737
11.18	20.58	9.4	0.123684211
Average difference		9.541333333	0.12554386
STDEV		0.822174894	0.010818091

Table 21: Measurement data for the small Mytilus edulis *control group.*

Control Small Group (n = 11)			t = 128 days
First Measurement	Final Measurement	Difference in Growth	Growth rate/day
9.92	missing		0
11.45	20.76	9.31	0.072734375
10.16	18.31	8.15	0.063671875
14.38	26.39	12.01	0.093828125
10.35	20.15	9.8	0.0765625
14.22	26.09	11.87	0.092734375
11.48	missing		0
13.39	died		0
11.18	15.11	3.93	0.030703125
13	died		0
13.08	25.69	12.61	0.098515625
17.41	21.91	4.5	0.03515625
11.69	23.53	11.84	0.0925
13.19	19.57	6.38	0.04984375
14.71	24.35	9.64	0.0753125
Average difference		9.094545455	0.052104167
STDEV		2.907417985	0.036953598

Table 22: Summary data for the small Mytilus edulis *test groups and control group growth rate.*

Small Mussels			
Diet	Average difference in growth (mm)	Average growth/day (mm/day)	STDEV
L. hyperborea detritus	9.29	0.12	
Phytoplankton	9.54	0.13	
Control	9.09	0.07	

Appendix 4

Table 23: Two-factor ANOVA table for the Semibalanus balanoides *test groups.*

Anova: Two-Factor With Replication				
SUMMARY	Aquarium 1	Aquarium 2	Aquarium 3	Total
Laminaria hyperborea				
Count	20	20	20	60
Sum	52.18639	44.55004	54.26551	151.00194
Average	2.6093195	2.227502	2.7132755	2.52
Variance	0.147278785	0.118882484	0.175219245	0.18649738
Standard deviation	0.383769182	0.344793393	0.418591979	0.43
Phytoplankton				
Count	20	20	20	60
Sum	59.05691	42.48557	50.86606	152.40854
Average	2.9528455	2.1242785	2.543303	2.54
Variance	0.354052783	0.098925392	0.13350669	0.305232964
Standard deviation	0.595023346	0.314524073	0.365385672	0.55
Total				
Count	40	40	40	
Sum	111.2433	87.03561	105.13157	
Average	2.7810825	2.17589025	2.62828925	
Variance	0.274497459	0.108843604	0.157812801	

Table 24: Two-factor ANOVA table for the Semibalanus balanoides *test group on the* Laminaria hyperborea *detritus diet and the control group.*

Anova: Two-Factor With Replication				
SUMMARY	Aquarium 1	Aquarium 2	Aquarium 3	Total
Laminaria hyperborea				
Count	20	20	20	60
Sum	52.18639	44.55004	54.26551	151.00194
Average	2.6093195	2.227502	2.7132755	2.516699
Variance	0.147278785	0.118882484	0.175219245	0.18649738
Control				
Count	20	20	20	60
Sum	51.53057	63.14829	68.08617	182.76503
Average	2.5765285	3.1574145	3.4043085	3.05
Variance	0.170523586	0.893119055	0.778035292	0.715523958
Standard deviation				0.85
Total				
Count	40	40	40	
Sum	103.71696	107.69833	122.35168	
Average	2.592924	2.69245825	3.058792	
Variance	0.155102501	0.714753893	0.586848776	

Table 25: Two-factor ANOVA table for the Semibalanus balanoides *test group on the phytoplankton diet and the control group.*

Anova: Two-Factor With Replication				
SUMMARY	Aquarium 1	Aquarium 2	Aquarium 3	Total
Phytoplankton				
Count	20	20	20	60
Sum	59.05691	42.48557	50.86606	152.40854
Average	2.9528455	2.1242785	2.543303	2.540142333
Variance	0.354052783	0.098925392	0.13350669	0.305232964
Control				
Count	20	20	20	60
Sum	51.53057	63.14829	68.08617	182.76503
Average	2.5765285	3.1574145	3.4043085	3.046083833
Variance	0.170523586	0.893119055	0.778035292	0.715523958
Total				
Count	40	40	40	
Sum	110.58748	105.63386	118.95223	
Average	2.764687	2.6408465	2.97380575	
Variance	0.291874252	0.756988319	0.634169291	

Appendix 5

Table 26: The lipid analysis results for the large Mytilus edulis *organisms.*

Large Mussel Groups	
Diet	Fat content (g/100g)
Control	0.5
Phytoplankton	0.2
L. hyperborea detritus	0.6

Table 27: The lipid analysis results for the small Mytilus edulis *organisms.*

Small Mussel Groups	
Diet	Fat content (g/100g)
Control	0.6
Phytoplankton	0.2
L. hyperborea detritus	0.3