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Local variations in species composition on *Laminaria hyperborea* stipes as response of degree of exposure and impact by aquaculture

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Abstract

Laminaria hyperborea kelp forest is a vitally important habitat that provides feeding ground, hiding space and habitat for numerous other species of both fauna and flora. Approximately 80 % of the total Norwegian algae biomass of 30 million tons is kelp forest build up by this species. *L. hyperborea* develops on hard-bottom substrates and covers in-between 5,000 to 10,000 km^2 of the Norwegian coast. *L. hyperborea* is suitable for attachment of epigrowth, because of its rough and perennial stipe. Farming of *Salmo salar* in open net cages at sea has had a rapid increase in production size since the breakthrough in the late 1970s. Yearly production in Norway is over 1.2 million tons, which results in annually release of approximately 34,000 tons nitrogen and 9,750 tons phosphorus. The Norwegian Standard NS 9410 describes methods for risk based environmental monitoring of benthic impact in soft-sediment areas under the cages, while areas with hard-sediments around the fish farms or the dissolved nutrients in the water column are not under any form of monitoring.

L. hyperborea and its species composition from several locations have been examined to have a closer look at the possibility of creating a new indicator for impact by emissions from fish farming activity. The locations were either impacted or not by fish farming activity and positioned in an exposed or protected area. From each location both sampling rounds, ten individuals of *L. hyperborea* were collected. Age was determined for all ten individuals, along with measurements of total length and stipe length. Five individuals, the oldest, the youngest and three in-between were chosen to be examined for immobile fauna and flora. The results shows that there are a difference in the species composition between locations. In addition, a seasonal variation in the species composition and a difference in the number of attached taxa depending on the kelp individuals age were detected. It were also observed that the protected impacted locations lacked kelp. It can seem like the distance from the cages for the exposed impacted locations have had an effect on the species composition. The missing kelp can be an indicator of elevated levels of emissions from fish farming, based on the high aggregations of *Echinus esculentus* and *Ophiocomina nigra*. Based on the results it is recommended to preform further investigation to see if *L. hyperborea* and its attached fauna and flora can function as an indicator for elevated emission loads. The method applied for this project has several holes, and suggestions for improvements are to focus on certain species, sample at specific times, increase sample size and to use sample quadrates. In addition, a suggestion of using methods described in this project together with important element from NS 9410 is presented, as an adaptation of hard-bottom monitoring directly beneath and in surrounding areas near fish farm cages.

Samandrag

Laminaria hyperborea (Stortare) som tareskog er eit særdeles viktig habitat som gjev mattilgang, oppvekststad og gøymestad for mange andre artar, av både fauna og flora. Om lag 80 % av den totale norske algebiomassa på 30 millionar tonn er tareskog bygd opp av denne arten. *L. hyperborea* veks på hardbotnsubstrat og dekker ein stad mellom 5000 og 10.000 km^2 av Noregs kystlinje. Sidan *L. hyperborea* har ein rugla og fleirårig stipes er denne taren godt egna for påvekstorganismar å feste seg. Oppdrett av *Salmo salar* (Atlanterhavslaks) i opne merdar i sjøen har hatt ei rask produksjonsauking sidan gjennombrotet seint på 1970-talet. Produksjonen i Noreg er over 1,2 millionar tonn per år, noko som resultera i årleg utslepp av om lag 34.000 tonn nitrogen og 9750 tonn fosfor. Den Norske Standarden NS 9410 beskriv metodikk for risikobasert miljøovervaking av botnpåverknad i områder med blautbotnsediment like under merdane, medan områda med hardbotnsediment rundt oppdrettsanlegga og dei oppløyste næringsstoffa ikkje vert overvaka.

L. hyperborea med fastsitjande påvekst frå fleire lokalitetar har blitt undersøkt for å sjå nærare på moglegheita av å danne ein ny indikator. Lokalitetane var anten påverka eller upåverka av oppdrettsaktivitet og plassert i eit eksponert eller beskytta område. Frå kvar lokalitet, begge innsamlingsrundane, vart ti individ av *L. hyperborea* henta opp frå sjøen. Alle vart aldersbestemt, samt totallengda og stipeslengda vart målt. Fem av desse ti; den eldste, den yngste og tre i mellom vart valt ut for å studere samansetjinga av den immobile faunaen og floraen. Resultat viser at der er ein ulikskap i artssamansetjinga mellom dei ulike lokalitetane. I tillegg vart ein sesongvariasjon i artssamansetjinga, samt ein ulikskap i mengde fastsitjande taxa avhengig av tareindividet sin alder oppdaga. Det vart òg observert at dei beskytta påverka lokalitetane mangla tare. Det kan verke som at avstand frå merdane for dei eksponerte påverka lokalitetane har hatt ein innverknad på artssamansetjinga. Den manglande taren kan vere ein indikator på auka nivå av avfallsstoff frå oppdrettsaktivitet, grunna høg førekomst av både *Echinus esculentus* (Svabergsjøpiggsvin) og *Ophiocomina nigra* (Svartslangestjerne). Basert på resultata er det anbefalt å gjennomføre vidare undersøkingar for å sjå om *L. hyperborea* med fastsitjande påvekst kan fungere som ein indikator på auka belastning av avfallsstoff. Metoden nytta i dette prosjektet har fleire svakheiter, forslag til forbetringar er å fokusere på nokre få artar, ha innsamling til gitte tider av året, auke mengda innsamla tare og nytte innsamlingsrammer. I tillegg er eit forslag om å nytte metoden beskriven i dette prosjektet saman med viktige element frå NS 9410 lagt fram, som ei tillemping av hardbotnovervaking like under merdane, men òg i områda rundt.

Contents

Abbreviations

DOM	Dissolved organic material
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
FAO	Food and Agriculture Organization of the United Nations
IMR	Institute of Marine Research
MOM	Monitoring – Ongrowing fish farms – Modelling
NS 4745 (NSO, 1975b)	Norwegian Standard: Water analysis – Determination of the sum of nitrite nitrogen and nitrate nitrogen
NS 9410:2016	Norwegian Standard: Environmental monitoring of benthic impact from marine fish farms
NS-EN ISO 19493:2007	International Standard: Water quality – Guidance on marine biological surveys of hard-substrate communities
NS-EN ISO 6878 (2004)	International Standard: Water quality – Determination of phosphorus, Ammonium molybdate spectrometric method
NTNU	Norwegian University of Science and Technology
POM	Particulate organic material
PON	Particulate organic nitrogen
POP	Particulate organic phosphorus
TBS	Trondheim Biological Station

1 Introduction

The Norwegian coastline is 100,915 km (Statistics Norway, 2013) long with all the fjords, islands, islets and skerries. Norway is the European country with the largest population of kelp and wrack, with approximately 10,000 km^2 of the coast populated with macroalgae (Fosså, 1999; Indregaard, 2010). *Laminaria hyperborea* ((Gunnerus) Foslie), *Laminaria digitata* ((Hudson) J.V. Lamouroux), *Saccharina latissima* ((Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders) and *Saccorhiza polyschides* ((Lightfoot) Batters) are all common macroalgae species along the Norwegian coast (Indergaard, 2010) (Figure 1-1). Kelp forests have a high primary production (Steneck et al, 2002) and measurements from Scotland shows a yearly production of 1,000 g $C•m^{-2}$ (Christie and Rueness, 1998). Light is the main limitation for distribution of kelp at high latitudes (Steneck et al, 2002). Along the coast of Norway, kelp is expected to be found from the low tide mark and down to at least 20 meters depth, and the area outside Møre has the best developed kelp forest (Christie and Rueness, 1998).

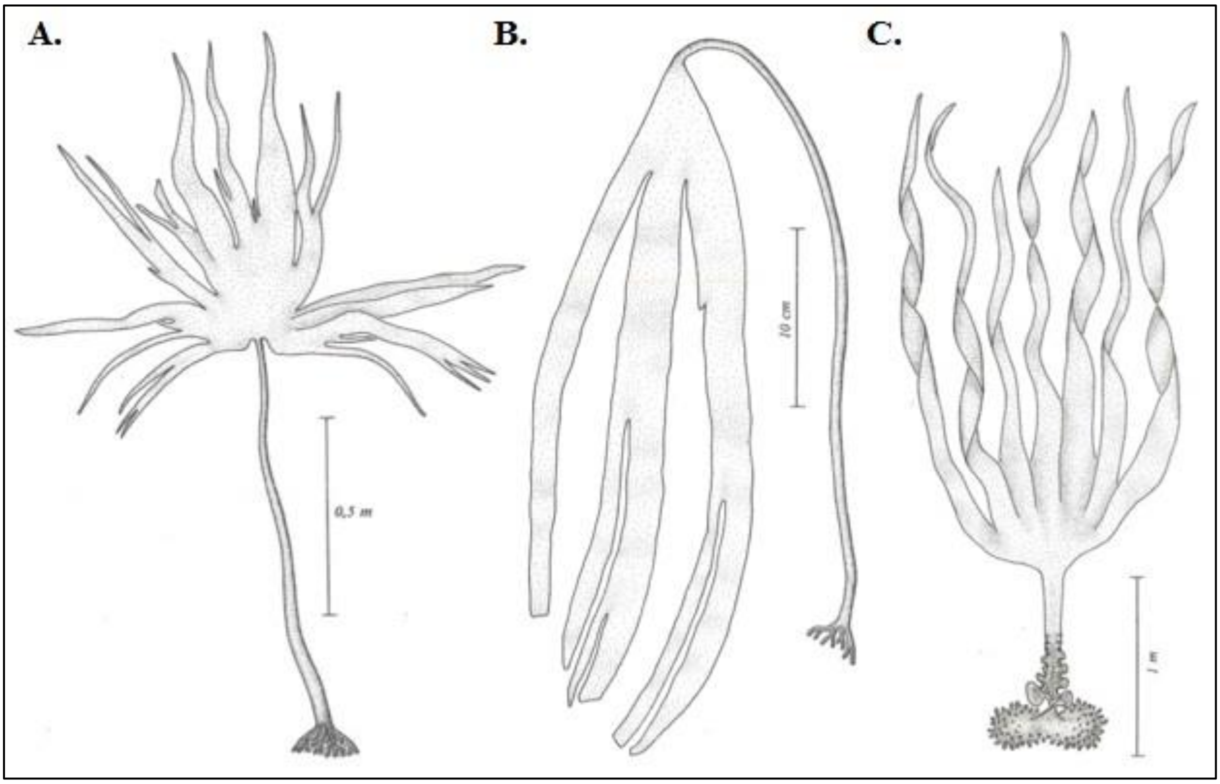

Figure 1-1: Illustrations of common macroalgae species at the Norwegian coast. **A**. *Laminaria hyperborea*. **B**. *Laminaria digitata*. **C**. *Saccorhiza polyschides* (Modified Åsen, 1980).

1.1 Kelp forest build up by *Laminaria hyperborea*

One important alga species along the coast of Norway is *Laminaria hyperborea*. The forest build up by this kelp corresponds to 80 % of the total Norwegian estimated algae biomass of 30 million tons (Jensen, 1998; Indergaard, 2010). It is often stated that *L. hyperborea* is a keystone species, because it provides shelter, food and habitat for many organisms (Rinde, 2007). According to Power et al. (1996), however, a keystone species is a species that has an impact on the ecosystem that is disproportionally large relative to its abundance. *L. hyperborea* has high abundance along the Norwegian coast, according to Fosså (1999) and Jensen (1999, as cited in Christie et al 2003) it is estimated to cover between 5,000 to 10,000 km^2 of the Norwegian shallow hard-bottom substrate. Because of the extensive distribution along the coast, it is not a keystone species by this definition (Power et al, 1996). From this point on it will be referred to as a fundamental species, as it is needed for the kelp forest to occur and develop.

L. hyperborea is a brown alga growing in the sublittoral zone (Jensen, 1998). The sublittoral zone is the zone that is covered of water at all time and is below the supralittoral and the eulittoral zone. *L. hyperborea* consist of three parts; holdfast, stipe and lamina. These three parts makes the kelp forest a three-dimensional habitat, vitally important for several organisms. Holdfast attaches the kelp to the seafloor. It is branched, which makes it a good hiding space for many juvenile individuals of different species. The stipe is round, strong and it elongates so the kelp can reach for the light. The surface is rough, making it easier for epigrowth to attach. Lamina is two-dimensional and makes up the largest surface area, and a new lamina is developed every winter, which makes it annual. Epigrowth attach to lamina as well as stipes, but it is smoother, so the number is lower (Christie et al, 2003).

There are three major factors disturbing *L. hyperborea* kelp forests: Grazing by sea urchins (Fosså, 1999; Bekkby et al, 2009), kelp trawling (Christie et al, 1998) and global climate changes (Moy et al, 2009). (1) In Norway the green sea urchin *Strongylocentrotus droebachiensis* (O.F.Müller), the common sea urchin *Echinus esculentus* (Linnaeus) and the white sea urchin *Gracilechinus acutus* (Lamarck) are examples of species normally found in all life stages in the kelp forest, where they graze on the degraded parts of *L. hyperborea* (Christie and Rueness, 1998; White, 2017). The problem arise when the populations reach densities that no longer can be supported by the available food. When the density of *S. droebachiensis* exceed 40-50 individuals per m^2, also the living parts of the kelp will be grazed on (Christie and Rueness, 1998). This can result in areas without kelp forest (Bekkby et al, 2009). (2) Kelp trawling has been common along the coast of Norway since the 1970s (Jensen, 1998; Bekkby et al, 2009). Based on the time it takes for the kelp forest to develop, the Government has decided that an area can be trawled with a frequency of minimum five years (four years in Rogaland) (Christie et al, 1998). The kelp biomass is regrown in five years, but the biodiversity needs more time to re-establish (Christie et al, 1998; Steen et al, 2016a). Kelp trawling removes potential habitats for many juvenile species, as the edible crab *Cancer pagurus* (Linnaeus), which lives inside holdfast during their juvenile stages of approximately 2 years. (3) Increased emissions of greenhouse gasses are one of the reasons why the earth is facing global climate changes. One of these changes is elevated sea temperatures, which is a severe challenge for *L. hyperborea*. *L. hyperborea* acquires a temperature between 0-20^{o}C in order to grow, however, the optimum growth temperature is 15^{o}C (Christie and Rueness, 1998). An increase in the mean sea temperature can lead to a change in the distribution (Christie and Rueness, 1998), as a shift towards northern areas were the ocean temperatures are colder.

1.2 Aquaculture: Farming of the Atlantic salmon *Salmo salar* in Norway

The Norwegian aquaculture industry has since the end of the 1970s had a rapid growth in production and size (Svåsand et al, 2017; Husa et al, 2014a). Statistics from FAO (2018a) (Food and Agriculture Organization of the United Nations) shows that the total production volume from Norwegian aquaculture in 2016 was 1,326,216 tons. Of this production, 1,233,619 tons was of the Atlantic salmon *Salmo salar* (Linnaeus) in seawater (FAO, 2018b). Over the years, open net cages used for fish farming in semi-exposed waters have increased in size. Cages at 500 m^3 that could hold 10-15 tons fish have developed into cages at 80,000 m^3 which is allowed to hold 200,000 individuals/1,000 tons (Svåsand et al, 2017). Most fish farms have a lower standing biomass in each cage, than the maximum allowed.

In Northern Europe there is four major environmental concerns related to aquaculture. They are (1) escapes of *S. salar* (interactions with wild stocks), (2) parasites and disease (etc. salmon lice), (3) chemical pollution (treatments for parasites and diseases) and (4) organic waste and nutrient accumulation. The Pollution Control Authority has made the system "*classification of environmental quality in fjord and coastal waters*" for Norway in order to help control these conditions (Maroni, 2000). To classify the environmental state of an area several measurements and tests have to be conducted. These are degree of pollution measured by Secchi depth, nutrient- and oxygen concentration, soft bottom fauna classification, amount of organic content in sediments and organic poisons and faecal bacteria (Maroni, 2000). All users of near-shore areas have to follow these standards in adapting their activities.

Growth in fish farming industry results in an increased amount of waste products from the cages (Figure 1-2). The main emissions are PON (particular organic nitrogen) and POP (particular organic phosphorous) in form of faeces and feed spill (Husa et al, 2010). DIN (dissolved inorganic nitrogen) and DIP (dissolved inorganic phosphorous) are also waste products released from fish farming, they are dissolved in the water masses and a result of the metabolism of the fishes (Svåsand et al, 2017).

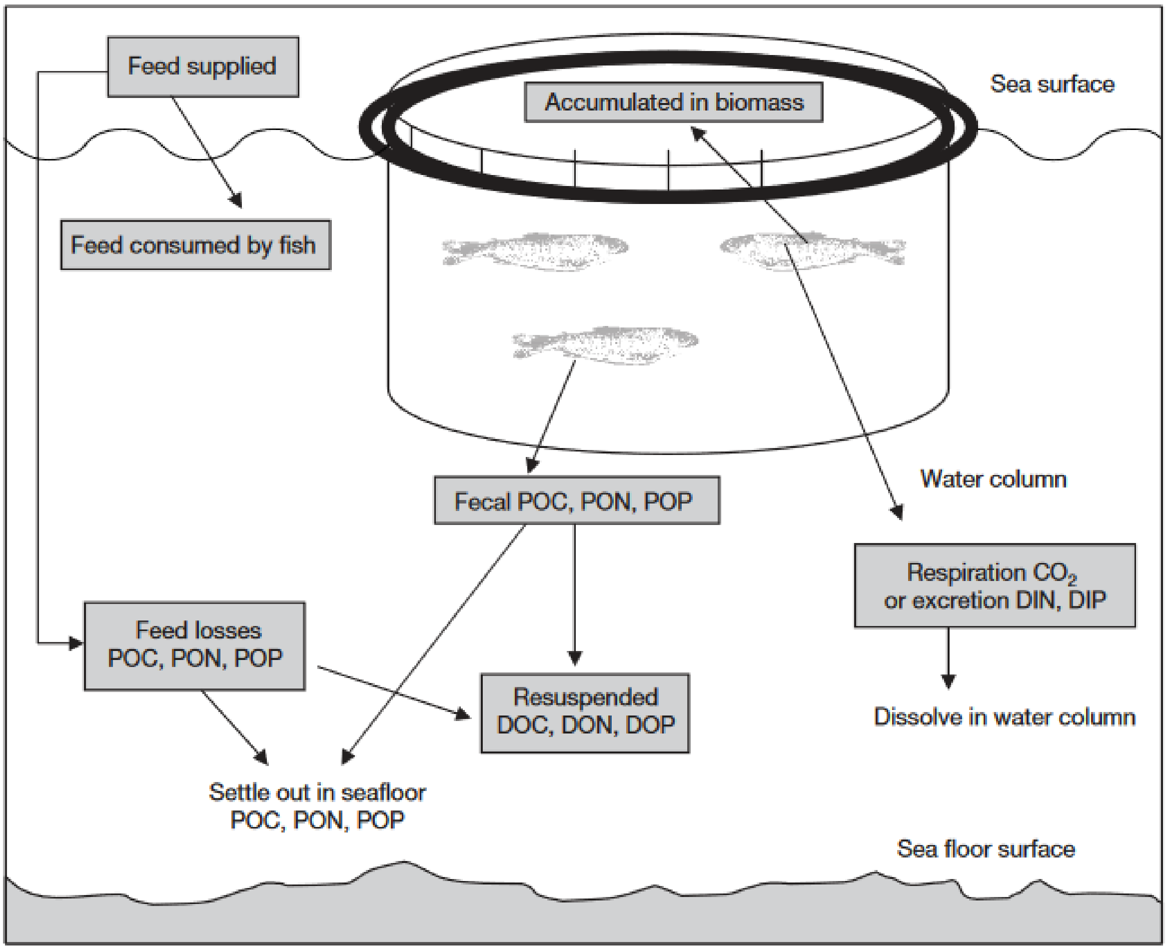

Figure 1-2: Emissions related to farming of *Salmo salar*. Particulate organic nitrogen (PON), particulate organic carbon (POC) and particulate organic phosphorus (POP) settles at the seafloor and are monitored through NS 9410: *Environmental monitoring of benthic impact from marine fish farms*. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) are dissolved in the water column and the levels are not monitored (From Wang et al, 2012).

There are several ways to calculate and/or modulate the annual release of emissions from fish farming activity, but none of the models are verified (Svåsand et al, 2017). Taranger et al. (2015) calculated that production levels in Norwegian Salmonid farming results in a release of approximately 34,000 tons of N and 9,750 tons of P, annually (Numbers calculated from 2012 production levels). Others have calculated and/or modulated the amount of DIN and DIP released, based on the 2016 production level (Table 1-1) (Svåsand et al, 2017).

Table 1-1: Calculated/modulated amount of released DIN (Dissolved inorganic nitrogen) and DIP (Dissolved inorganic phosphorus) based on 2016 production level of Salmonid fish farming in Norway (Svåsand et al, 2017).

	Lower amount	**Upper amount**
DIN	25,194 tons (Svåsand et al, 2016)	48,372 tons (Norderhaug et al, 2016)
DIP	-1,930 (Torrissen et al, 2016)	6,424 tons (Norderhaug et al, 2016)

It is known that emissions from fish farming have an impact on the benthic environment, where the result may be a succession (Carroll et al, 2003; Svåsand et al, 2017). A succession starts with an increase in biodiversity, until the system is saturated and a few dominant species are left, often polychaetes (Carroll et al, 2003). In 2017, 1,744,991 tons feed were used for consumption in Salmonid fish farming (Directorate of Fisheries, 2018). Because of the change in the feed content, from a marine protein source to almost 70 % terrestrial ingredients, the feed has become less digestible for the fish. This gives higher emissions of particulate matter as faeces and makes it possible to track the feed through the marine food webs and in the sediments/ecosystem (Svåsand et al, 2017). The Norwegian Standard 9410: *Environmental monitoring of benthic impact from marine fish farms,* is a sustainable indicator made to model and measure the impact on the benthic environment by fish farms (Standard Norge, 2016). This standard mainly monitor areas with soft-sediments. In cases where the area directly beneath the cages are hard-substrate, accumulation of organic material are attempted to be sampled by adapting the soft-sediment methodology (Standard Norge, 2016). As it is today, there are no environmental goals, indicators or thresholds to control emissions of nutrients in near vicinity of fish farming activity (Svåsand et al, 2017). The International Standard NS-EN ISO 19493: *Water quality – Guidance on marine biological surveys of hard-substrate communities* monitor benthic marine algae and fauna on hard-substrate (Standard Norge, 2007). However, this standard do not control for impact on hard-substrate areas on the grounds of elevated nutrient and particulate loads from aquaculture activity (Standard Norge, 2007). Therefore, monitoring of areas with hard-bottom substrate are not sufficiently developed either, when it comes to emission from fish farming activity.

Farming of *S. salar* has had a rapid growth in Norway and is an important industry. Aquaculture in Norway provides many jobs, and *S. salar* is one of Norway's biggest export product. With an increasing world population, aquaculture will be an important protein source and production will increase as long as possible. It is believed that this increase will lead to a higher amount of output of excess nutrient and particles, which can harm the surrounding ecosystem. Today these outputs are only controlled in soft-bottom sediments by established environmental monitoring (NS 9410). Based on this, farming of *S. salar* is chosen as a potential harmful source because of emissions of waste products from aquaculture activity.

1.3 Epigrowth

Epigrowth are all associated organisms of fauna and flora that can live on or be attached to another organism. There among, all organisms that can be found on a kelp species. *Laminaria hyperborea* has more epigrowth than for example *Saccorhiza polyschides* and *L. digitata* (Schultze et al, 1990). This is because *S. polyschides* has annual lamina and stipe and only holdfast that is perennial, while *L. hyperborea* has only annual lamina (Rueness, 1977). *L. digitata* has a much smoother stipe than *L. hyperborea*, which makes it more difficult for epigrowth to attach (Rueness, 1977).

The upper and younger part of *L. hyperborea* stipe is smoother than the lower and older part. This makes the part closer to holdfast more suitable for epigrowth and this rough part of the stipe is often overgrown with other organisms, both other algae and animals (Rinde et al, 1992). When it comes to only macrofaunal species, this trend is not always shown. *L. hyperborea* holdfast is branched, which forms a number of holes and crevices (Christie et al, 2003). Studies show that holdfast can accommodate hundreds of individuals of amphipods, polychaetes, snails, echinoderms and isopoda (Rinde et al, 1992). In a healthy and well developed kelp forest there is on average 3,000-10,000 mobile animals living on one kelp individual, divided between 200 species (Norderhaug and Christie, 2007). Samples from six *L. hyperborea* collected at the Norwegian coast (at 63^{o}N) in September 1996 show that the number of species and individuals are highest in the mid part of stipes, closely followed by the upper part and least in the lower part (Christie et al, 2003). During the whole study (1993-1997), 56 individuals of *L. hyperborea* were collected and 425,897 macrofaunal specimens were recorded from 238 fauna species/taxa (Christie et al, 2003). This indicates that the kelp forest not only has high productivity by itself, but is also a habitat with a high biodiversity (Norderhaug and Christie, 2007). Different species of red algae, Bryozoa, Hydrozoa, Polychaeta, Ascidiacea and Mollusca are often represented as epigrowth (Rinde et al, 1992).

It is believed that coverage of epigrowth and number of species increases with the age of the kelp (Rinde et al, 1992). In one study where individuals of *L. hyperborea* were collected, from August 1995 to September 1996 along the Norwegian coast, Christie et al. (2003) found that the number of macrofaunal individuals on stipes fluctuate throughout the seasons. With the lowest number from October to March. The same trend was not found for macrofaunal individuals attached to holdfast, there the number was relatively stable for all seasons (Christie et al, 2003). Schultze et al. (1990) studied the differences in macrofauna and macroflora composition on *L. hyperborea* in areas with different protection, and the distribution of the animals on the kelp. The exposed area had higher wave action and high turbidity, while the sheltered area was sheltered from most wave action and had low turbidity (Schultze et al, 1990). The results were in total 125 macrofaunal and 29 macrofloral species, dominated by Rhodophyceae (50 % of flora), Polychaeta (20 % of fauna), Bryozoa (14 % of fauna), Amphipoda (11 % of fauna) and Hydrozoa (8 % of fauna) (Schultze et al, 1990). The protected and unprotected sampling area also showed differences in species composition. Species richness and abundance were higher on stipes and holdfast in the unprotected area with more current and turbulent water (Schultze et al, 1990). The same trend was not shown for lamina, where more species and individuals were found in the protected area (Schultze et al, 1990). The species that were most abundant in the protected area were calcified and sensitive species, as some bryozoans (Schultze et al, 1990).

1.4 Fouling plates

Over the years there have been many studies of species composition on algae and one good tool in these kinds of studies are the use of artificial substratum, also called kelp mimics. Artificial substrates can vary in shape and material, but they all have in common that a closer investigation of the species composition is provided. Garcìa and Salzwedel (1993) used plates of asbestos in a PVC frame attached to the bottom (approximately 9 meters depth). Norderhaug et al. (2002) used a funnel with eight slits filled with hemp rope to mimic holdfast, and a brush of textile and plastic of 15 cm height to mimic epiphytes on stipes (Figure 1-3). Kraufvelin et al. (2002) used a grid system in mesocosms to count and determine large motile and all sessile animals. Artificial substrate, mimicking green-, red- and brown algae, composed of three ropes, one stone and one petri dish tied closely together, gave estimates of the number of smaller mobile fauna (Kraufvelin et al, 2002). Waage-Nielsen et al. (2003) studied previous experiments that had showed good results and chose to use untwisted hemp rope to mimic kelp habitats, both holdfast and epiphytic algae.

The exposure time of the fouling plates vary between different studies. Garcìa and Salzwedel (1993) collected two plates every second week in the experiment time of one year, one that had been emerged from start and one that had been emerged for two weeks. While Norderhaug et al. (2002) exposed the kelp mimics for 2 and 7 days, and Waage-Nielsen et al. (2003) exposed the kelp mimics for 3 and 35 days before sampling.

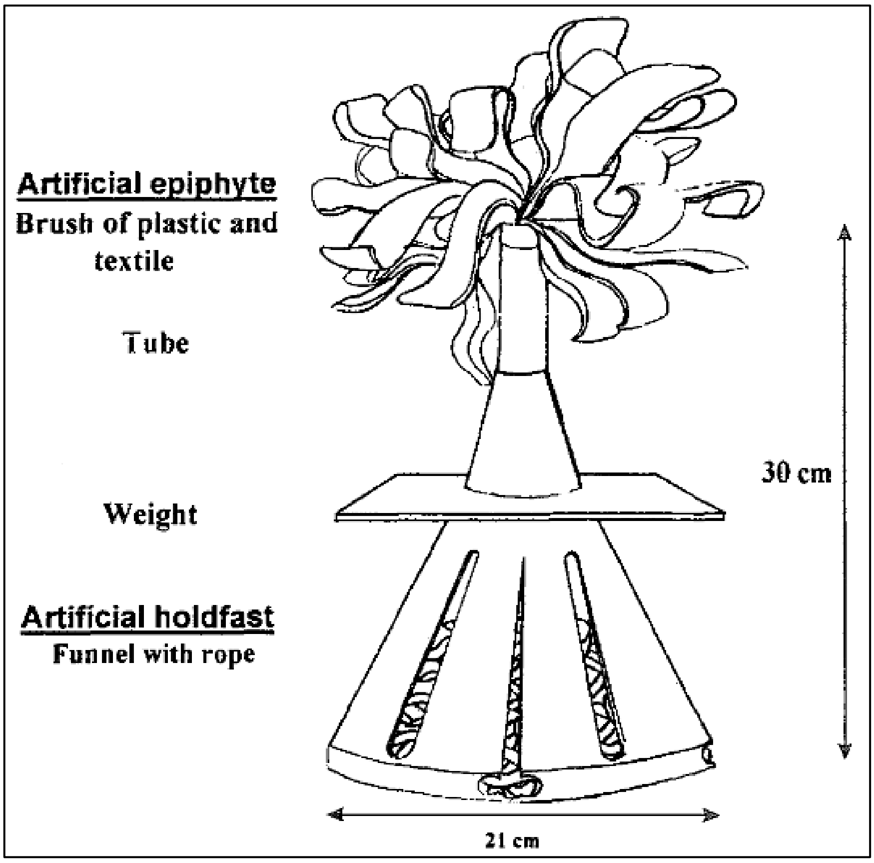

Figure 1-3: Artificial substratum designed to mimic kelp holdfast and epiphytic growth on kelp stipe (From Norderhaug et al, 2002).

It is well known that most spawning and recruitment in temperate water occur during the warmest months of the year, and this correlates with the season variation in primary production (Garcìa and Salzwedel, 1993). Therefore, new attached species can be investigated after the settling period during the summer months. The timeline of the study conducted by Waage-Nielsen et al. (2003) consisted of sampling in September (3 days experiment) and November (3 days and 35 days experiments). The experiment conducted by Norderhaug et al. (2002) had sampling in August and November (both 2 and 7 days). By doing it this way, both summer and late autumn were covered. It is expected that the biomass of epiphytes on kelp decreases during the winter, which also can reduce the number of fauna attached to it (Norderhaug et al, 2002).

The flora and fauna abundance, and numbers increased with increased exposure time of the artificial substratum (Norderhaug et al, 2002; Waage-Nielsen et al, 2003). Despite that some species/taxa that normally would colonise kelp were missing, most of them were described also on the mimics. After 2 days 99 taxa (85 % of taxa found on natural kelp) were described on the mimic applied by Norderhaug et al. (2002) and for all three experiments carried out by Waage-Nielsen et al. (2003) a total of 111 of the mobile taxa (86.7 % of taxa found on natural kelp) were described on the mimics. The missing species/taxa could be explained by the absence of some specified habitat properties in the artificial substratum, or that the specific species/taxon has a low dispersal ability (Waage-Nielsen et al, 2003).

1.5 Age determination of *Laminaria hyperborea*

By determining the age of *Laminaria hyperborea* it is possible to see if the age structure from the different locations are the same, which gives an anticipated equal prerequisite for the epigrowth. Kain and Jones (1963) described two methods to determine the age of *L. hyperborea*. The first method is to take a central longitudinal section at the base of the stipes and count all the dark lines. With this method, it is important to take into account if the individuals are collected in the slow-growing or fast-growing period, July-December and January-July respectively (Kain and Jones, 1963). The other method is to take a cross section of the stipes and count the rings. Kain and Jones (1963) described two errors by using the second method; these are the difficulty of differentiating primary and secondary tissue and the risk of not including the newest ring. To be sure to include the latest growth, the Institute of Marine Research has described an improved method (Steen et al, 2016a; Steen et al, 2016b). The cross section of the stipes should be made approximately 1 cm over holdfast, by doing this, the latest growth in the oldest individuals will more likely be included (Steen et al, 2016a).

By applying the transversely section method, the light areas between the darker division lines are the areas that should be counted, to set the age (Figure 1-4) (Steen et al, 2016a; Steen et al, 2016b). The size of a light area can say something about the growth conditions that year (Kain and Jones, 1963; Steen et al, 2016a; Steen et al, 2016b). It is expected that the thickness of a growth zone varies with age and that it is widest between 3-6 years, and after that decrease with increasing age (Steen et al, 2016a).

Figure 1-4: Transversely section of *L. hyperborea* stipes, approximately 1 cm over holdfast. The light areas between the darker division lines are to be counted to determine the age. *L. hyperborea* age 6 years (From Sauholmen, south side of Frøya, Trøndelag) (Photo: Albertine Rekdal Havnegjerde).

When age is to be set, it will be an advantage to do it right after the stipe is cut off and before it has been out of saltwater too long. It may be a possibility to cut off slices of the stipes and put them back in saltwater before determining the age, but for how long a slice will give exact information is not known. When the stipe is cut, alginate will start to ooze out and this may interfere with a correct age determination.

1.6 Study aim and approach

Laminaria hyperborea was chosen as a study organism for several reasons. Primarily because of its importance as a fundamental species along the Norwegian coast, but also because of the fact that it has perennial stipe and that the stipe is rough. *Saccorhiza polyschides* has an annual stipe and *L. digitata* is much smoother than *L. hyperborea*. Based on this, they are not thought to have as high an number of epigrowth as *L. hyperborea* and that makes them less relevant for this study.

Only immobile organisms living on the stipe will be identified. This is because of the sampling method without use of cloth bags. It is also reasoned by the fact that most studies of epigrowth on algae have been on mobile fauna and not immobile fauna. The sampling method include among others a variety of red and green algae, Bryozoa, Hydrozoa and Annelida.

The aim of this study is to describe immobile fauna and flora attached to *Laminaria hyperborea* stipes collected at different locations, impacted or not impacted by emissions from farming of *Salmo salar*, outside Frøya, Trøndelag.

Research questions to answer the aim:

- Are there differences in the species composition between the different locations?
- Will water samples support results found in previous research question?
- Will settling of immobile fauna and flora during the summer period result in a new or different species composition?
- Are the results sufficient enough to suggest further investigation to find out if epigrowth on *L. hyperborea* can be an indicator for elevated levels of emissions from fish farming activity?

2 Methods

2.1 Locations

The fieldwork was conducted in the coastal area outside Frøya in Trøndelag (Figure 2-1, 2-2 and 2-3). Four location categories were set and these were the starting point for the sampling of *Laminaria hyperborea* (Table 2-1). The location categories were exposed and protected not impacted by fish farming activity, and exposed and protected impacted by fish farming activity. Further, the different locations that were visited will be described based on observations at the locations from the divers and GoPro videos from each location.

Figure 2-1: Overview map of Frøya, Trøndelag, with all locations visited marked with small circles. Red circles represent fish farms with their respective identification number and farm name. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen, BPB: Bukkholmen, VLPB: Vestre Lamøyskjæret, KPB: Kollskjæra (Map retrieved from Directorate of Fishery).

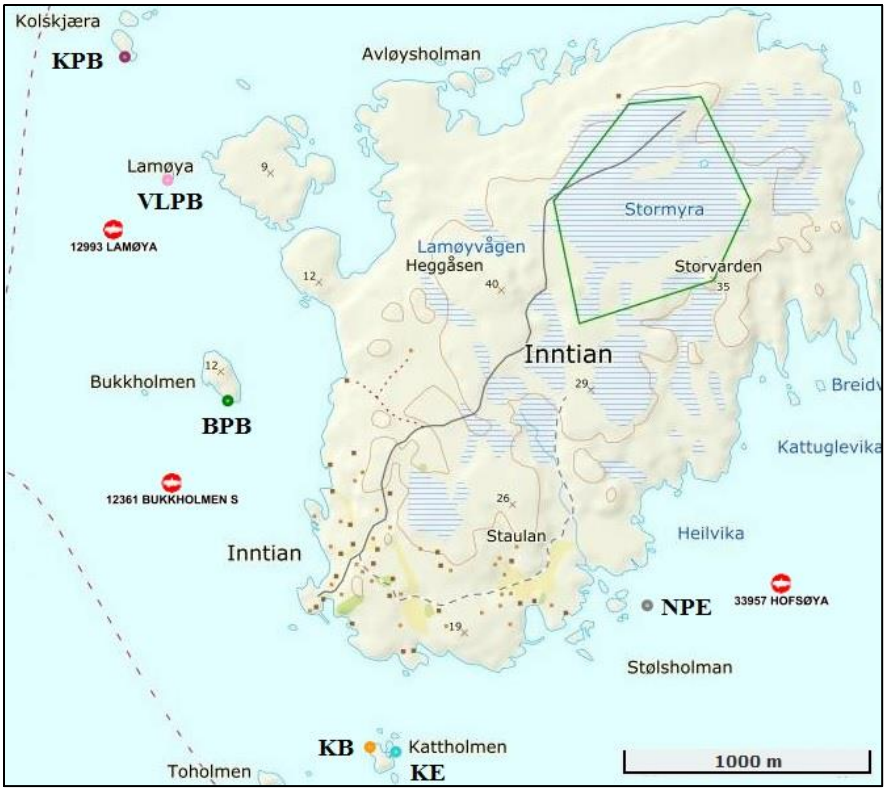

Figure 2-2: Overview map of the locations visited outside Sistranda, Frøya. Each location is marked with a circle in a specific colour. Red circles represent fish farms with their respective identification number and farm name. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, BPB: Bukkholmen, VLPB: Vestre Lamøyskjæret, KPB: Kollskjæra (Map retrieved from Directorate of Fishery).

Figure 2-3: Overview map of the location visited at the south side of Frøya, Trøndelag. The location is marked with a circle. Red circle represent the fish farm with identification number and farm name. SFE: Sauholmen (Map retrieved from Directorate of Fishery).

2.1.1 Kattholmen south (KE)

The location is located approximately 50 meters south from Kattholmen outside Sistranda, Frøya. This location is in an area with distance to fish farming activity and it is described as exposed, based on the direction of currents coming in from the open ocean. The characterization of the location is dense kelp forest build up by *L. hyperborea*. Lamina was overgrown with Bryozoa. Some *Echinus esculentus* could be observed, but not in numbers. Observation of *L. hyperborea* from GoPro video implies that the individuals are of size (> 150 cm), related to the other locations investigated.

2.1.2 Kattholmen north (KB)

This location is approximately 50 meters north from Kattholmen outside Sistranda, Frøya and in a distance from fish farming activity. The location is characterized by a larger distance between the kelp individuals, than at the exposed side. There is also individuals of *Saccorhiza polyschides* present and thereby indicates that the location has lower currents, and therefore is a protected location (Rueness, 1977). Lamina was overgrown with Bryozoa. *E. esculentus* was observed in a higher number than at the exposed side of the islet. Observation of *L. hyperborea* from GoPro video implies that the individuals were not that big (~ 100 cm), related to the other locations investigated.

2.1.3 Nordstøylholmen (NPE)

This location is approximately 300 meters downstream from Hofsøya fish farm, and approximately 30 meters from the coastline of the island Inntian, Frøya. The characterization of the location is that there was a dense kelp forest build up by *L. hyperborea*. *S. polyschides* was not observed, which substantiate that the location is exposed (Rueness, 1977). *L. hyperborea* lamina had some Bryozoa, but were not overgrown, as at the two Kattholmen locations. There was observed more *E. esculentus* than at Kattholmen south, but not in numbers. Observation of *L. hyperborea* from GoPro video implies that the individuals are of size (> 200 cm), relative to the other locations investigated.

2.1.4 Sauholmen (SFE)

This locations is approximately 150 meters downstream from Måøydraga fish farm, South of the island Frøya. The characterization of the location is that there was a kelp forest build up by *L. hyperborea* and that the outer areas had some individuals of *Saccharina latissima* in addition. The kelp forest was not as dense as at Nordstøylholmen. There was observed more *E. esculentus* than at Kattholmen south and approximately the same amount as at Nordstøylholmen. The kelp forest ended at some point closer to the fish farm, individuals was more dispersed and there were a higher number of *E. esculentus* and degraded kelp. *S. latissima* can be found both at sheltered and in some exposed areas, while *S. polyschides* is present at sheltered areas (Rueness, 1977). Since *S. polyschides* was not observed at this location, it is characterized as exposed. Observation of *L. hyperborea* from GoPro video implies that the individuals are a bit smaller (< 150 cm) than at the other exposed locations. It was also observed that the stipes had less epigrowth.

2.1.5 Bukkholmen (BPB), Vestre Lamøyskjæret (VLPB) and Kollskjæra (KPB)

Bukkholmen is located approximately 300 meters downstream from Bukkholmen S fish farm, outside Sistranda, Frøya. The area is protected, because of all the small islets around and the island of Inntian. The location was supposed to be the sampling area described as protected and impacted. The condition on the location was not as expected. No kelp was observed on the seafloor, but many *E. esculentus* and even more of the black brittle star *Ophiocomina nigra* (Abildgaard in O.F. Müller). The divers thought small black stones covered the seabed, until they got near enough to see all the *O. nigra* (Figure 2-4). *O. nigra* is a common brittle star species in sheltered areas (Moen and Svensen, 1999).

Figure 2-4: Close up of the condition at the location Bukkholmen. *E. esculentus* and *O. nigra* present. From GoPro video at the location.

Evidence that a kelp forest had been present earlier was observed by some parts of holdfast and stipes, that were half-eaten by *E. esculentus*. These kelp parts belonged to both *L. hyperborea* and *S. polyschides*. *O. nigra* was also observed with arms up in a waving position, which they do when they are catching small organic food particles (Figure 2-4) (Moen and Svensen, 1999).

Since Bukkholmen lacked kelp, two new locations with the same characterization were investigated. These two locations are situated north of Bukkholmen and downstream from Lamøya fish farm. Vestre Lamøyskjæret is 300 meters downstream the fish farm, while Kollskjæra is 600 meters downstream, both approximately measures. Neither south of Vestre Lamøyskjæret or south of Kollskjæra could be used to collect kelp, since the same conditions as at Bukkholmen were observed at those locations (Figure 2-5, 2-6 and 2-7). Because of this, *L. hyperborea* has not been collected at a location that is protected and impacted by emissions from fish farm activity.

Figure 2-5: Location Vestre Lamøyskjæret. *E. esculentus* grazing on the remains of algae. From GoPro video at the location.

Figure 2-6: Location Vestre Lamøyskjæret. *E. esculentus* grassing on the remains of a kelp forest build up by *L. hyperborea*. From GoPro video at the location.

Figure 2-7: Location Kollskjæra. *E. esculentus* and *O. nigra* (all black marks on the seabed) can be observed in numbers, including some half-eaten algae. From GoPro video at the location.

Table 2-1: The different locations visited with location category and description, geographical position, date of *Laminaria hyperborea* sampling, water sampling, date of CD-disc collection from the fouling plates and fish farm identification for samplings nearby a fish farm.

Location name	Location category	Location description	Position	Sampling date	Water sample	CD-disc	Fish farm **
Kattholmen south	Exposed not impacted	Location with stronger current more than 1,500 meters downstream from fish farm activity	N 63.71484 E 8.88327	19.11.16 13.09.17	Yes Yes	13.09.17	
Kattholmen north	Protected not impacted	Location with weaker current more than 1,500 meters downstream from fish farm activity	N 63.71492 E 8.88114	19.11.16 13.09.17	Yes Yes	13.09.17	
Nordstøylholmen	Exposed impacted	Location with stronger current closer than 1,500 meters downstream from fish farm activity	N 63.72097 E 8.90252	20.11.16 11.09.17	Yes Yes	11.09.17	Hofsøya, 33957
Sauholmen	Exposed impacted	Location with stronger current closer than 1,500 meters downstream from fish farm activity	N 63.67857 E 8.75892	21.03.17 19.02.18	Yes Yes	19.02.18	Måøydraga, 12370
Bukkholmen	Protected impacted	Location with weaker current closer than 1,500 meters downstream from fish farm activity	N 63.72688 E 8.86691	19.11.16*			Bukkholmen S, 12361
Vestre Lamøyskjæret	Protected impacted	Location with weaker current closer than 1,500 meters downstream from fish farm activity	N 63.73460 E 8.86031	20.11.16*			Lamøya, 12993
Kollskjæra	Protected impacted	Location with weaker current closer than 1,500 meters downstream from fish farm activity	N 63.73888 E 8.85587	20.11.16*			Lamøya, 12993

* *Laminaria hyperborea* not present at location ** Fish farm name and ID

2.2 Sampling of *Laminaria hyperborea*

Laminaria hyperborea was sampled twice from four different locations, during four field trips, in the coastal area outside Frøya, Trøndelag. The wet lab at Guri Kunna High School Department Frøya was used to set age, measure the kelp and identify the immobile species living on stipes (For additional information: Table 2-1).

Before sampling, fouling plates for each location were made. These are made of rope with 10 CD-discs separated by plastic pipes at 10 cm (Figure 2-8). The date the fouling plates were set out was written on each CD-disc. The fouling plates had extra lengths of rope measuring 1 meter and 2 meters from the CD-discs to the ends. A floating element was attached to the shorter end of the rope, the end with 2 meters extra length was used to attach to stones on the seabed.

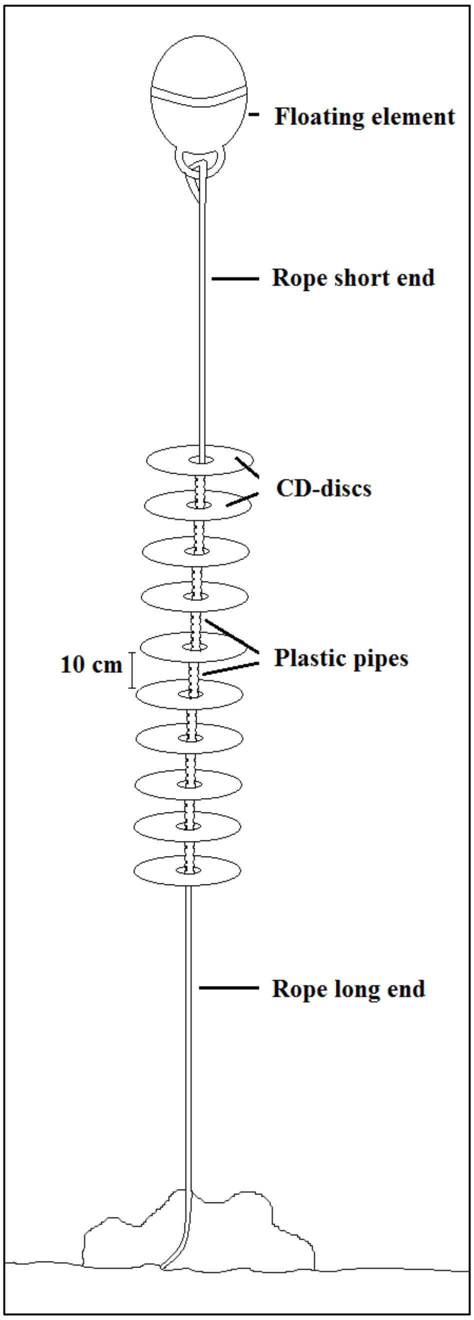

Figure 2-8: Illustration of the kelp mimic made for this project, including the floating element, rope, plastic pipes and CD-discs.

Boats were used to reach the desired locations and SCUBA divers collected the kelp. The kelp was collected at 5 meters depth at each location and chart datum were used to ensure correct depth at the different times and days. 10 individuals of *L. hyperborea* were collected from each location. Because of time and the fact that it is difficult for divers to handle, special made cloth bags were not used during the collection. This excludes the opportunity to study the mobile fauna living on the kelp. The kelp was held in tanks of seawater until the work was done to prevent them from drying out. All of the 10 individuals of *L. hyperborea* were length measured, both stipes alone and from holdfast to the longest part of lamina. The age of all individuals were determined by counting growth lines. Five individuals from each location: the oldest, the youngest and three in-between were chosen to study closer (Figure 2-9). By using stereomicroscope and literature, all immobile taxa of fauna and flora living on stipes were identified. To be sure to detect all taxa, at least five random incisions were made on each *L. hyperborea* after every taxa were thought to be covered. If a new taxon were discovered, five new random incisions were made. All individuals of *L. hyperborea* were also examined twice. A specific taxon were only registered once per individual of *L. hyperborea*. Therefore, the results will show if a taxon is present or not on the different individuals and such shows the richness as a measure of diversity. The degree of coverage or number of individuals of a specific taxon were not registered. This method gives results were a specific taxon can have up to 5 registrations for each location per sampling. Those taxa that were not able to be identify certainly, were picked off and put in small glasses with 96 % ethanol and brought back to Trondheim for closer examination.

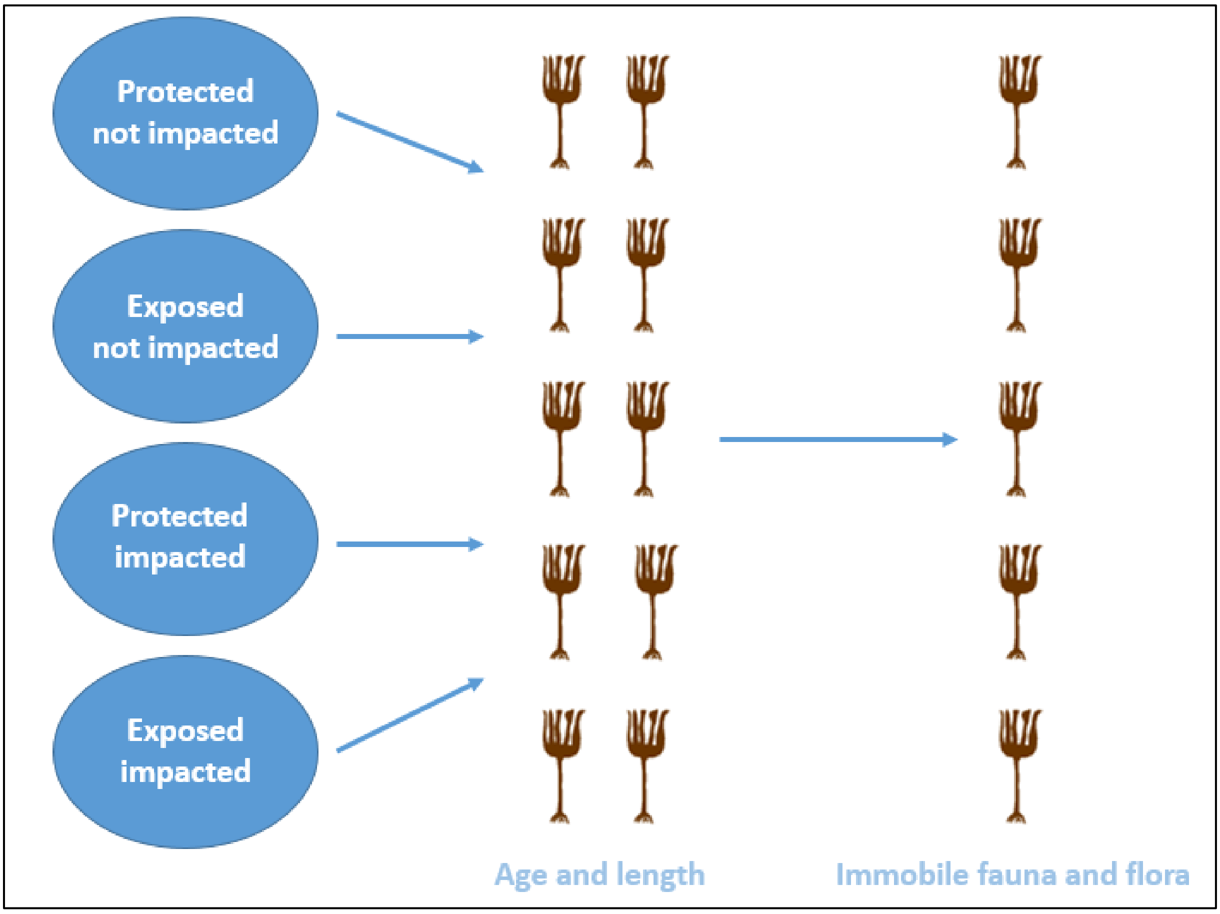

Figure 2-9: Schematically overview of project set up, with location categories, amount of collected kelp and amount of processed kelp.

A water sample of surface water was taken at every location kelp were collected. 50 mL lightproof brown bottles were used to bring the saltwater back at the wet lab at Guri Kunna High School Department Frøya. In the lab, 10 mL of the saltwater from every location were filtered by using a 50 mL syringe with a 0.45 µm, 25 mm diameter filter. To ensure correct data, two parallels of both ammonium (NH_4^+) and nitrite (NO_2^-) + nitrate (NO_3^-) + phosphate (PO_4^{3-}) were filtered and prepared for analysis from each location. The saltwater were filtered to 15 mL centrifuge tubes, frozen and held dark, until analysis. A zero sample was also taken. This sample was collected at Frohavet 19.11. 2016.

At the first fieldtrip the age was provisionally set by counting the growth lines, before a piece of stipes, from holdfast and 10 cm up, was cut off and frozen (-20°C). This was done to be able to bring the piece back to Trondheim and use a better compound microscope with possibility to take pictures and measure growth zones. Unfortunately, kelp and raw vegetables react at the same way when deep-frozen. The stipe tissue burst at several points and it was not possible to see the growth lines and set the age correctly. At the second, third and fourth fieldtrip were a light table used as a light source under the slice of the stipe and the growth lines could be counted correctly. A SLR camera (Nikon) were used to take pictures of every slice with a caliper. The pictures were later analysed in Image J to set the diameter.

2.3 Taxonomic determination

The taxonomic identification of fauna and flora attached to *Laminaria hyperborea* stipes were carried out by using renowned literature (Table 2-2). In addition to literature, collections of Bryozoa identified by Imanuel Vigeland and Carl Dons at NTNU University Museum were used to identify some bryozoans. Kjersti Sjøtun at the University Museum in Bergen help with identification of two algae. Torkild Bakken at NTNU University Museum help with identification of some annelids, bryozoans and chordates. Jussi Evertsen at Guri Kunna High School Department Frøya help with identification of some taxa within all phyla during the field trips. All specimens were identified to the lowest taxonomic level possible.

Table 2-2: Overview of the literature used for identification of the epigrowth specimens attached to *L. hyperborea* stipes.

Phylum/Division	**Literature**
Bryozoa	Danmarks fauna 46. Mosdyr (Marcus, 1940)
	Handbook of the marine fauna of North-West Europe (Hayward and Ryland, 1995)
	Ctenostome Bryozoans (Hayward, 1985)
	Cyclostome Bryozoans (Hayward and Ryland, 1985)
	The marine fauna of the British Isle and the North-West Europe: Vol II: Molluscs to Chordates (Hayward and Ryland, 1990b)
	Seasearch guide to bryozoans and hydroides of Britain and Ireland (Porter, 2012)
Chlorophyta, Ochrophyta and Rhodophyta	Norsk Algeflora (Rueness, 1977)
	Alger i farger. En felthåndbok om kystens makroalger (Rueness, 1998)
Cnidaria	Seasearch guide to bryozoans and hydroides of Britain and Ireland (Porter, 2012)
	The marine fauna of the British Isle and North-West Europe: Vol I: Introduction and Protozoans to Arthropods (Hayward and Ryland, 1990a)
	Handbook of the marine fauna of North-West Europe (Hayward and Ryland, 1995)
Chordata	The marine fauna of the British Isle and the North-West Europe: Vol II: Molluscs to Chordates (Hayward and Ryland, 1990b)
	Handbook of the marine fauna of North-West Europe (Hayward and Ryland, 1995) Danmarks fauna 75. Sækdyr. (Lützen, 1967)
Other: Annelida, Arthropoda, Porifera and Mollusca	The marine fauna of the British Isle and North-West Europe: Vol I: Introduction and Protozoans to Arthropods (Hayward and Ryland, 1990a)
	The marine fauna of the British Isle and the North-West Europe: Vol II: Molluscs to Chordates (Hayward and Ryland, 1990b)
	Dyreliv i havet. Håndbok i norsk marin fauna. (Moen and Svensen, 1999)

2.4 Laboratory and analysis

The laboratory at NTNU University Museum was used to identify the species that could not be identified at Frøya.

Kjersti Andresen, at Trondheim biological station (TBS), analysed the water samples collected at Frøya. She run some tests to find the level of ammonium (NH_4^+), nitrite (NO_2^-) + nitrate (NO_3^-) and phosphate (PO_4^{3-}) in the samples. The samples were autoclaved before they were analysed photometrical in an I.O. Analytical Flow Solution IV System. All analyses for nitrite + nitrate and phosphate were conducted after Norwegian Standards. NS 4745 (NSO, 1975b): *Water analysis – Determination of the sum of nitrite nitrogen and nitrate nitrogen* for nitrite + nitrate. NS-EN ISO 6878 (2004): *Water quality – Determination of phosphorus*, *Ammonium molybdate spectrometric method* for phosphate. Ammonium analysis were conducted after Kérouel and Aminot (1997).

2.5 Fouling plates

After the fouling period during the summer (2017) were two CD-discs on the artificial fouling plates from every location collected (Table 2-1). The fouling plates from Kattholmen south, Kattholmen north and Nordstøylholmen had been in the ocean for approximately 10 months, while the one at Sauholmen had been in the ocean for approximately 11 months. The date of collection were the same as the second sampling of *Laminaria hyperborea*, for each location. The divers had to break the discs, to get them of the rope. All the pieces were put in a cloth bag and brought to the boat. The discs were examined in the wet lab at Guri Kunna High School Department Frøya and all immobile fauna and flora settled were identified to the lowest taxonomic level possible.

2.6 Excluded taxa and possible wrong identification

Because of the method used and the process of learning during the project, some taxa have been excluded from the results. Algae from Rhodophyta and Ochrophyta that only were registered as *thread algae* and not put into any lower taxonomic unit than the phylum are excluded. There are several reasons. (1) They were present on all individuals of *Laminaria hyperborea,* but not listed before the second sampling round. (2) The specimens present were very small, supposedly under 1 mm of height and an optical microscope would have had to be used to differentiate the cells. (3) The specimens present might have been young individuals of taxa already identified from the specific individual of *L. hyperborea* for that location.

One taxon from the phylum Annelida, that is included in the results, might have been wrongly identified. This is the species *Spirorbis (Spirorbis) spirorbis* (Linnaeus, 1758). The species is only noted in the list during the first sampling round and at high numbers, except at Sauholmen. At Sauholmen it is listed once, in addition a new taxon is identified. This is the taxon *Circeis* sp. (Saint-Joseph, 1894). *Circeis* sp. is registered at all locations, in high numbers, during second sampling round. *S. (Spirorbis) spirorbis* and *Circeis* sp. are two taxa that can be confused with each other at first sight. They are both members of the subfamily Spirorbinae and to separate them the direction of the tube coil has to be investigated. Most certainly some of the registrations of *S. (Spirorbis) spirorbis* are *Circeis* sp. instead. Since *S. (Spirorbis) spirorbis* was registered 5 times at Sauholmen at the same time as 1 registration of *Circeis* sp., it has clearly been present, but it is not sure at how many stipes. Because of this, it cannot be excluded from the results, but it might be vice to have in mind that the number of registrations of both *S. (Spirorbis) spirorbis* and *Circeis* sp. probably are wrong.

3 Results

Results from four locations: Kattholmen south, Kattholmen north, Nordstøylholmen and Sauholmen, are used in this section (Figure 2-1, Table 2-1 and Section 2.1). Bukkholmen, Vestre Lamøyskjæret and Kollskjæra, all protected impacted locations, are not included in this section due to lack of *Laminaria hyperborea*.

Taxa from 7 phyla and 3 divisions were registered on *L. hyperborea* stipes as immobile fauna and flora (Table 3-2). These were Annelida, Arthropoda, Porifera, Bryozoa, Chlorophyta, Chordata, Cnidaria, Mollusca, Ochrophyta and Rhodophyta. One taxon, named “unidentified species”, was also registered but not included in any phylum (Appendix B.). In total were 669 specimens of immobile epigrowth registered from all locations, and these are identified to lowest taxonomic level possible. All identified specimens can be placed in 102 different taxa at species, genus, family, order, class or phylum level. 302 specimens were registered during the first sampling round and 367 were registered during the second sampling round. Nordstøylholmen had most registrations of the location both sampling rounds (Table 3-1). Bryozoa (N=35) is the phylum with most registered taxa, followed by the division Rhodophyta (N=24) (Table 3-2). A complete list of the 102 identified taxa with location and sampling time can be viewed in Appendix B..

Table 3-1: Overview of the registrations of taxa at the different location separated by sampling round. *Average* is the number of registered epigrowth specimens divided by five individuals of *L. hyperborea.*

	Taxa registered			
Location	First sampling	Average	Second sampling	Average
Kattholmen south	81	16.2	79	15.8
Kattholmen north	61	12.2	76	15.2
Nordstøylholmen	87	17.4	143	28.6
Sauholmen	73	14.6	69	13.8

Table 3-2: Overview of number of registered taxa on *L. hyperborea* stipes per phylum/division and location. Total number of taxa per phylum/division is noted in the column *Taxa*. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

	Taxa	**Taxa registered per location**			
Phylum		KE	KB	NPE	SFE
Annelida	5	4	4	5	5
Arthropoda	2	1	1	2	1
Porifera	3		1	2	1
Bryozoa	35	19	15	23	12
Mollusca	1	1	1	1	1
Chordata	8	4	2	5	
Cnidaria	8	4	3	5	2
Division					
Chlorophyta	5	2	2	3	3
Orchrophyta	10	4	4	6	9
Rhodophyta	24	15	12	19	11

3.1 Species distribution

A heat map visualization of the number of epigrowth specimens identified per individual of *Laminaria hyperborea* shows how specimen richness to a degree increases with age of the kelp, but that it also varies with locations (Table 3-3A.). Results also indicates that taxa richness varies with season (Table 3-3B.). By separating the two samplings and organizing after increasing kelp age, this new trend can be viewed, where Nordstøylholmen and Kattholmen north had an increase in richness. Kattholmen south had more or less the same number of taxa present, and Sauholmen had a small increase. Notice that even though *L. hyperborea* individuals from the second sampling at Nordstøylholmen in general are older than those at the first sampling, those that are at same age still have a higher number of taxa present (Table 3-3A.). The seasonal trend is also documented as average taxa numbers from the different locations and samplings (Table 3-1). One individual from Kattholmen north had a higher taxa diversity than the others (age: 8 year, taxa: 25). This is a result of a fully-grown *L. hyperborea*, with lamina, attached to the stipe and supplying with extra taxa that normally are found on lamina. The individual can also be observed as an outlier in Figure 3-1 and 3-2A.

Number	Colour
0-5	
6-10	
11-15	
16-20	
21-25	
26-30	
31-35	

Table 3-3: Heat map showing number of taxa identified on each individual of *L. hyperborea* separated by location (N=10 per location). Both first and second sampling are included. Interval is set to 5 and the column *Number* shows the number of taxa for each interval. **A.**: *L. hyperborea* individuals from each location are organized after increasing age. **B.**: *L. hyperborea* individuals are organized after increasing age within first and second sampling, respectively.

A.

Kattholmen south		**Kattholmen north**		**Nordstøylholmen**		**Sauholmen**	
Age	Taxa	Age	Taxa	Age	Taxa	Age	Taxa
3	11	5	12	4	11	4	7
5	15	5	13	6	17	4	11
6	12	5	15	7	16	5	9
6	13	6	9	8	21	5	16
7	20	6	10	8	30	6	15
8	14	7	10	9	22	7	14
8	23	7	13	9	27	7	17
9	14	7	17	9	28	8	18
9	20	8	13	10	31	9	16
11	18	8	25	11	22	10	20

B.

Kattholmen south			**Kattholmen north**			**Nordstøylholmen**			**Sauholmen**		
Time	Age	Taxa	Time	Age	Taxa	Time	Age	Taxa	Time	Age	Taxa
1.	5	15	1.	5	15	1.	4	11	1.	4	7
1.	6	12	1.	6	10	1.	6	17	1.	5	16
1.	7	20	1.	7	10	1.	7	16	1.	7	14
1.	8	14	1.	7	13	1.	8	21	1.	9	16
1.	9	20	1.	8	13	1.	9	22	1.	10	20
2.	3	11	2.	5	12	2.	8	30	2.	4	11
2.	6	13	2.	5	13	2.	9	27	2.	5	9
2.	8	23	2.	6	9	2.	9	28	2.	6	15
2.	9	14	2.	7	17	2.	10	31	2.	7	17
2.	11	18	2.	8	25	2.	11	22	2.	8	18

Results from taxa counts, both first and second sampling (N=40), showed that the number of identified epigrowth taxa varied between the locations (Figure 3-1, Table C-1 Appendix C.). Where Nordstøylholmen's lower quartile is greater than both Kattholmen north and Sauholmen's upper quartile (Figure 3-1, Table C-1 Appendix C.). There were also differences in distribution of taxa between exposed and protected locations, and impacted and not impacted locations (Figure 3-2A. and B., Table C-2 Appendix C.). Since none of the protected impacted locations had *L. hyperborea* to sample, only Kattholmen north is representing protected locations (Figure 3-2A). The impacted and not impacted locations have *L. hyperborea* individuals with almost the same average age, 7.3 years for impacted locations and 6.8 years for not impacted locations (Table 3-3). Still, the lower quartile of impacted locations are greater than the median value of not impacted locations (Figure 3-2, Table C-2 Appendix C.). Almost 46 % of the variance in number of taxa can be explained by the age (year) of the kelp individuals (R^2=0.4595, p<0.001, Figure 3-3).

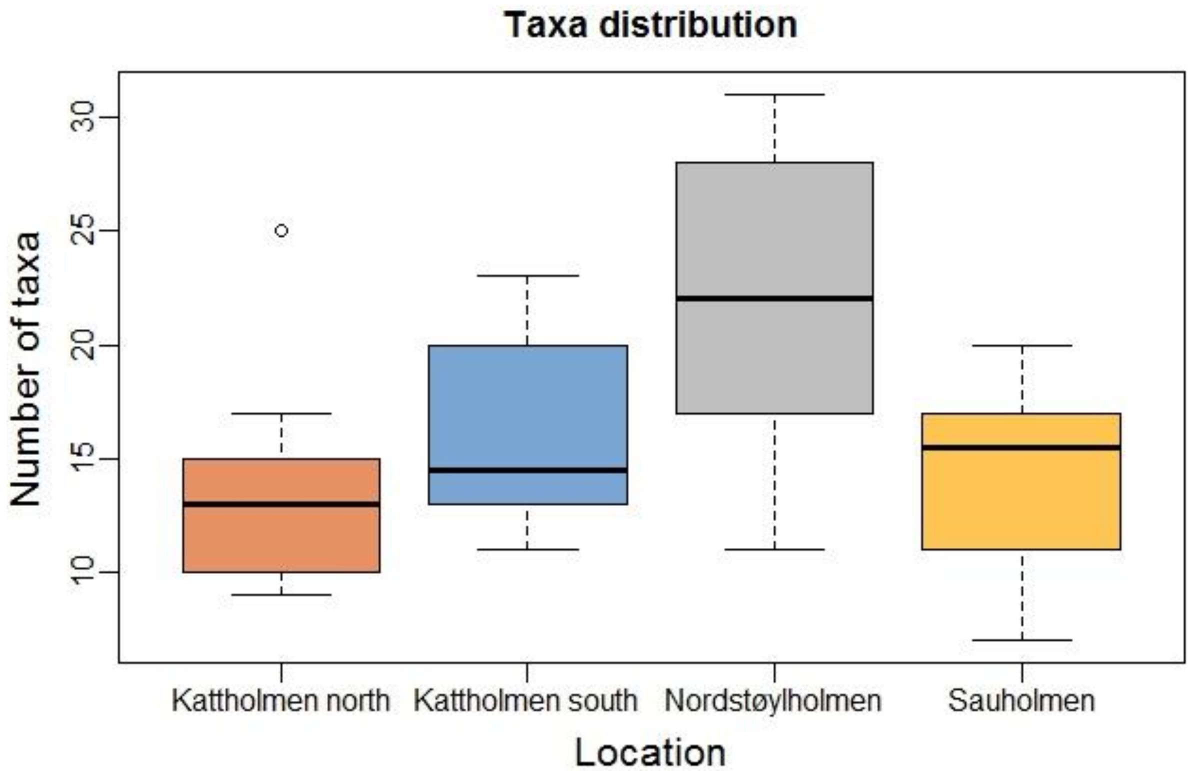

Figure 3-1: Specimen identification from the different locations (N=4). Individuals of *L. hyperborea* (N=10 per location) from first and second sampling are included. The boxes represent the interquartile range, whiskers represent the smallest and largest values, the line represent the median specimen number and the circle represent an outliner. Values are shown in Table C-1 in Appendix C..

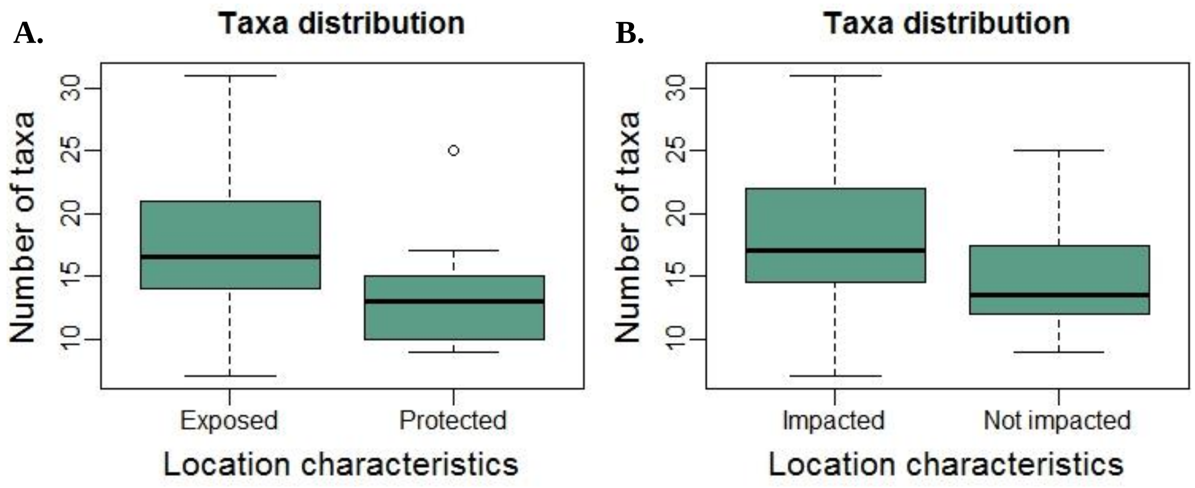

Figure 3-2: Relationship between number of identified taxa on individuals of *L. hyperborea* and location characteristics. **A.**: Location characteristics exposed (N=3) or protected (N=1) by waves and current. **B.**: Location characteristics impacted (N=2) or not impacted (N=2) by output of emissions from fish farms. All individuals for identification of taxa (N=40) from each location are included. The boxes represent the interquartile range, whiskers represent the smallest and largest values, the line represent the median specimen number and the circle represent an outliner. Values are shown in Table C-2 in Appendix C..

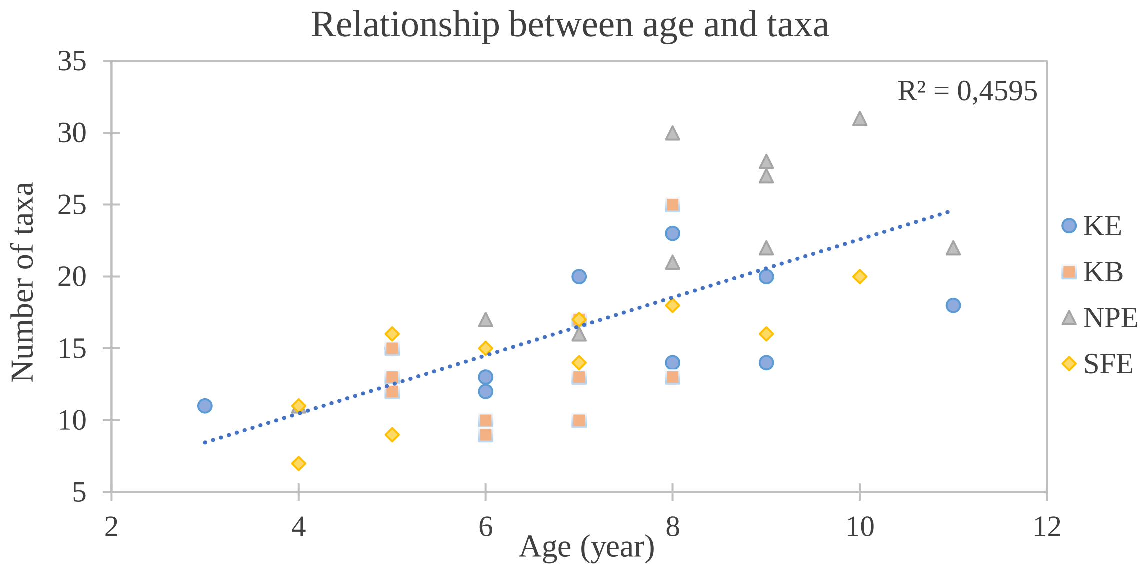

Figure 3-3: Relationship between number of specimens detected at each individual of *L. hyperborea* (N=40), and age (year). All individuals used for species composition are included. R^2=0.4595, p<0.001. Linear regression is added. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

3.2 Relationships with stipes length (cm)

Results shows 44.5 % of the variance in stipes length (cm) can be explained by the age (year) of *Laminaria hyperborea* individuals, where stipes length increase with age (R^2=0.4452, p<0.001, Figure 3-4, N=40). Stipe measurements and taxa identification shows that approximately 70 % of the variance in number of taxa can be explained by stipe length (cm), with all locations included (R^2=0.6968, p<0.001, Figure 3-5, N=40). Separation by location (Figure 3-6, N=10 per location) indicate that ~38 %, ~19.5 %, ~87 % and ~42 % of the variance in taxa numbers can be explained by the stipe length (cm) at Kattholmen south (R^2=0.3836, p=0.033), Kattholmen north (R^2=0.1958, p=0.112), Nordstøylholmen (R^2=0.8666, p<0.001) and Sauholmen (R^2=0.4213, p=0.025), respectively. Results shows that Nordstøylholmen had individuals with the longest stipes and most taxa, while Kattholmen north had individuals with shortest stipes and fewest taxa (Figure 3-5). Kattholmen north and Sauholmen had more scattered results (Figure 3-5). By compering Figure 3-4 and 3-5 it can be observed that the four individuals that had most taxa and longest stipes, is not the oldest ones.

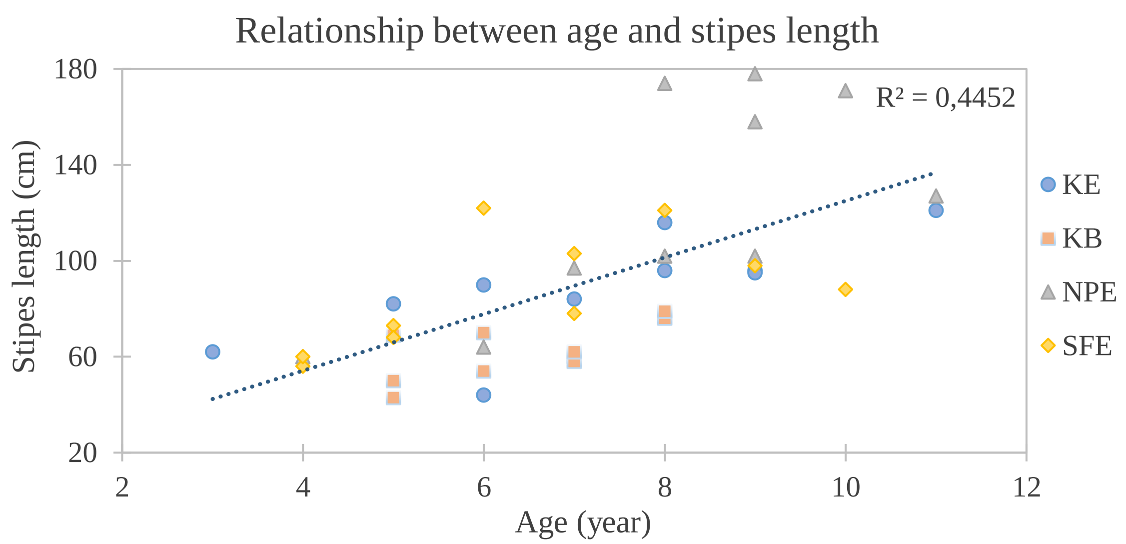

Figure 3-4: Relationship between stipes length (cm) and age (year). Only individuals of *L. hyperborea* used for identification of species composition are included (N=40). Individuals from both first and second sampling. R^2=0.4452, p<0.001. Linear regression is added. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

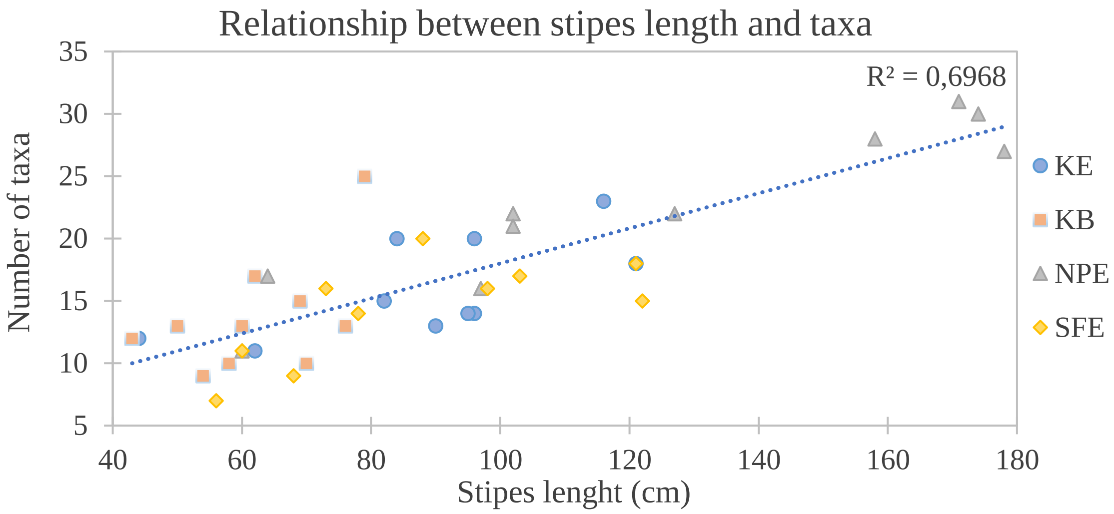

Figure 3-5: Relationship between stipes length (cm) and specimens. Only the individuals of *L. hyperborea* that epigrowth specimens were identified from are included (N=40). Individuals from both first and second sampling at each location. R^2=0.6968, p<0.001. Linear regression is added.KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

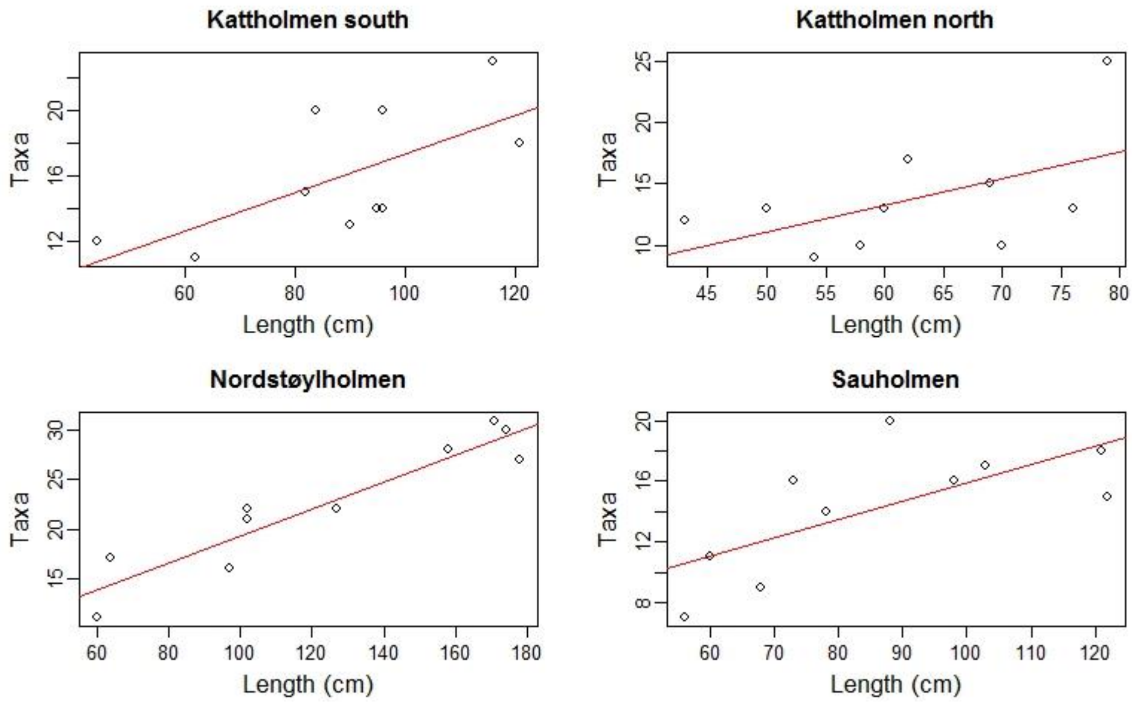

Figure 3-6: Relationship between stipes length (cm) and specimens, separated by location. Only the individuals of *L. hyperborea* that epigrowth specimens were identified from are included (N=10 per location). Individuals from both first and second sampling at each location. KE: R^2=0.3836, p=0.033, KB: R^2=0.1958, p=0.112, NPE: R^2= 0.8666, p<0.001, SFE: R^2= 0.4213, p=0.025. Linear regressions are added. Note that the scale of the axis are not the same

3.3 Relationship with stipes diameter (cm)

Results from stipes diameter measurements and age determinations of *Laminaria hyperborea* individuals, only second sampling (N=40), shows that ~59 % of variance in stipe diameter (cm) can be explained by the age (year) of the kelp (R^2=0.589, $p<0.001$, Figure 3-7). Separation by location shows that at both Kattholmen south and Kattholmen north, >60 % of the variance in stipe diameter (cm) can be explained by the age (year) of *L. hyperborea* individuals (KE: R^2=0.6328, p=0.004, KB: R^2=0.6173, p=0.004, Figure 3-8, N=10 per loaction). For Nordstøylholmen and Sauholmen 10 % or less of the variance in stipe diameter (cm) can be explained by kelp age (year) (NPE: R^2= 0.101, p=0.687, SFE: R^2=0.0762, p=0.223, Figure 3-8, N=10 per location).

Results only including *L. hyperborea* individuals used for epigrowth taxa composition from second sampling shows that ~ 66 % of the variance in stipe diameter (cm) can be explained by *L. hyperborea* age (year) (R^2=0.6607, $p<0.001$, Figure 3-9, N=20). Separation by locations also shows that ~61 %, ~56 %, ~33 % and ~47 % of the variance in stipe diameter (cm) can be explained by kelp age (year), for Kattholmen south (R^2=0.6132, p=0.073), Kattholmen north (R^2=0.5582, p=0.091), Nordstøylholmen (R^2= 0.3305, p=0.942) and Sauholmen (R^2=0.4762, p=0.122), respectively (Figure 3-10, N=5 per location). By studying Figure 3-10 it can be observed that the smallest stipe diameter at Nordstøylholmen is larger than most of the stipes diameters at the other locations. Attempt to strengthen the results by using a compound microscope in the lab at NTNU University Museum shows that deep freezing of *L. hyperborea* results in bursting of the tissue and makes it impossible to determine age or measure stipe diameter (Figure 3-11) (For comparison: Figure 1-4). Because of this, only individuals from second sampling have stipe diameter measurements.

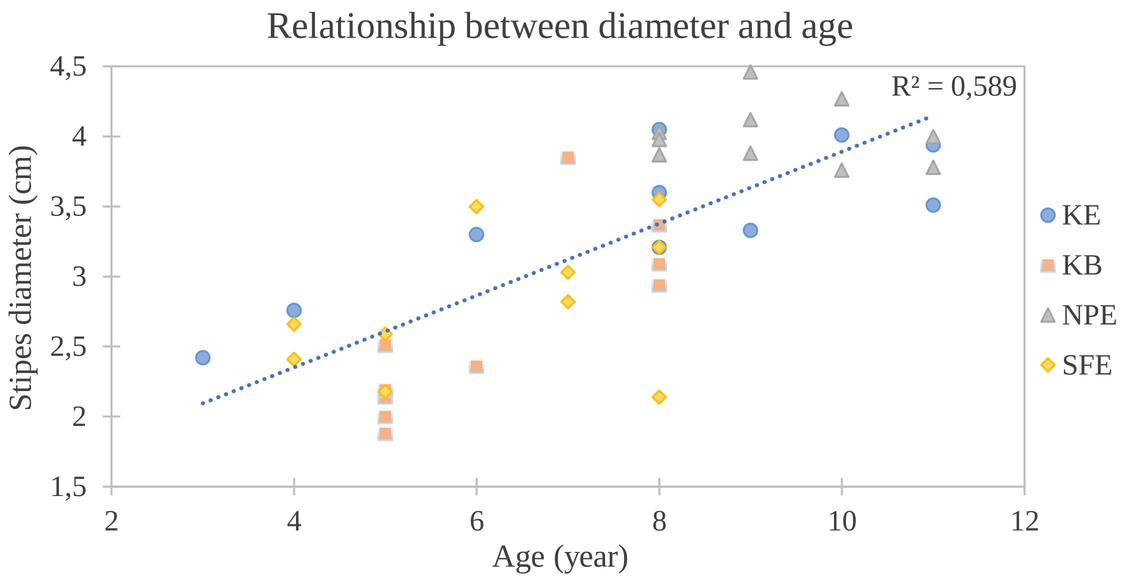

Figure 3-7: Stipes diameter (cm) relative to age (year). All individuals of *L. hyperborea* are from the second sampling at each location (N=40). R^2=0.589, p<0.001. Linear regression is added. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

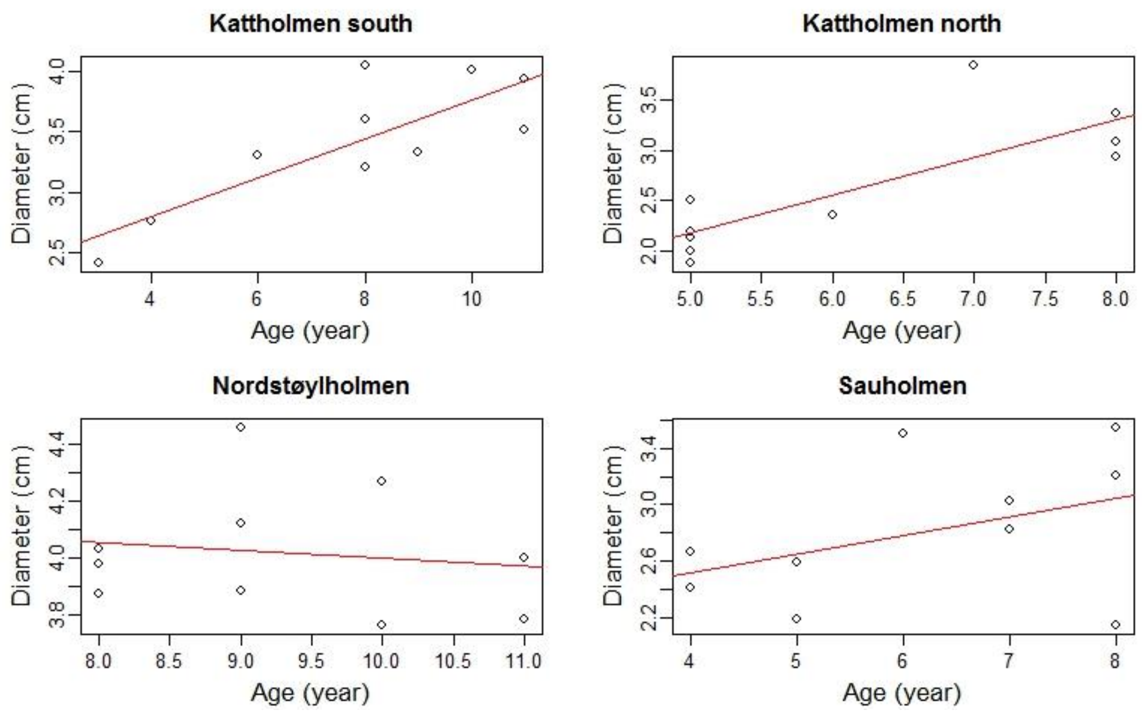

Figure 3-8: Stipes diameter (cm) relative to age (year), seperated by location. All individuals of *L. hyperborea* are from the second sampling at each location (N=10 per location). Linear regressions are added. KE: R^2=0.6328, p=0.589, KB: R^2=0.6173, p=0.004, NPE: R^2= 0.101, p=0.687, SFE: R^2= 0.0762, p=0.223. Note that the scale of the axis are not the same.

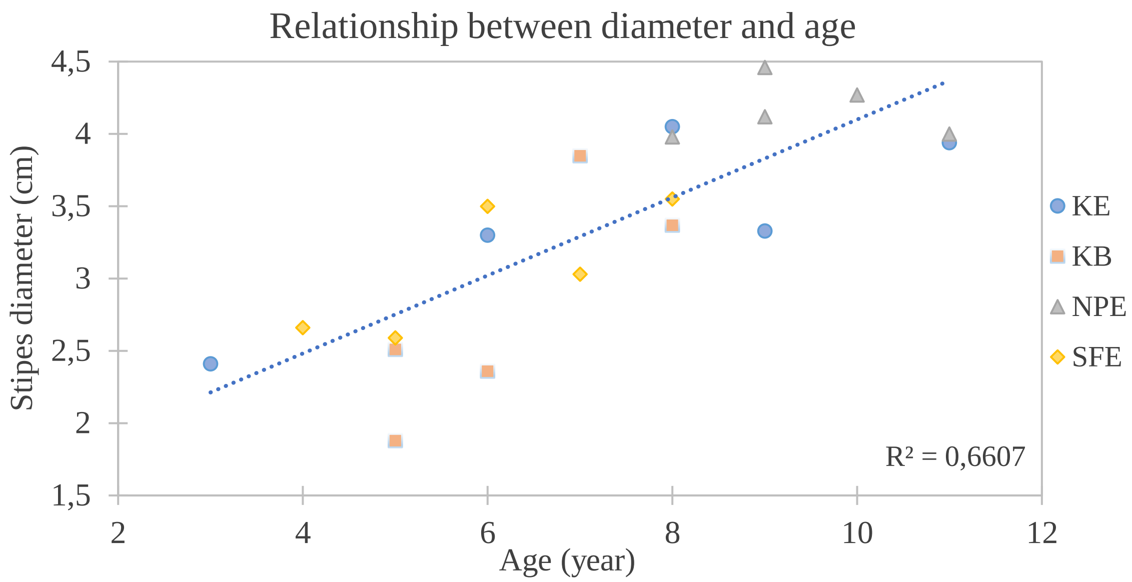

Figure 3-9: Stipes diameter (cm) relative to age (year). Only the individuals of *L. hyperborea* that epigrowth specimens were identified from are included (N=20). R^2=0.6607, $p<0.001$. Linear regression is added. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

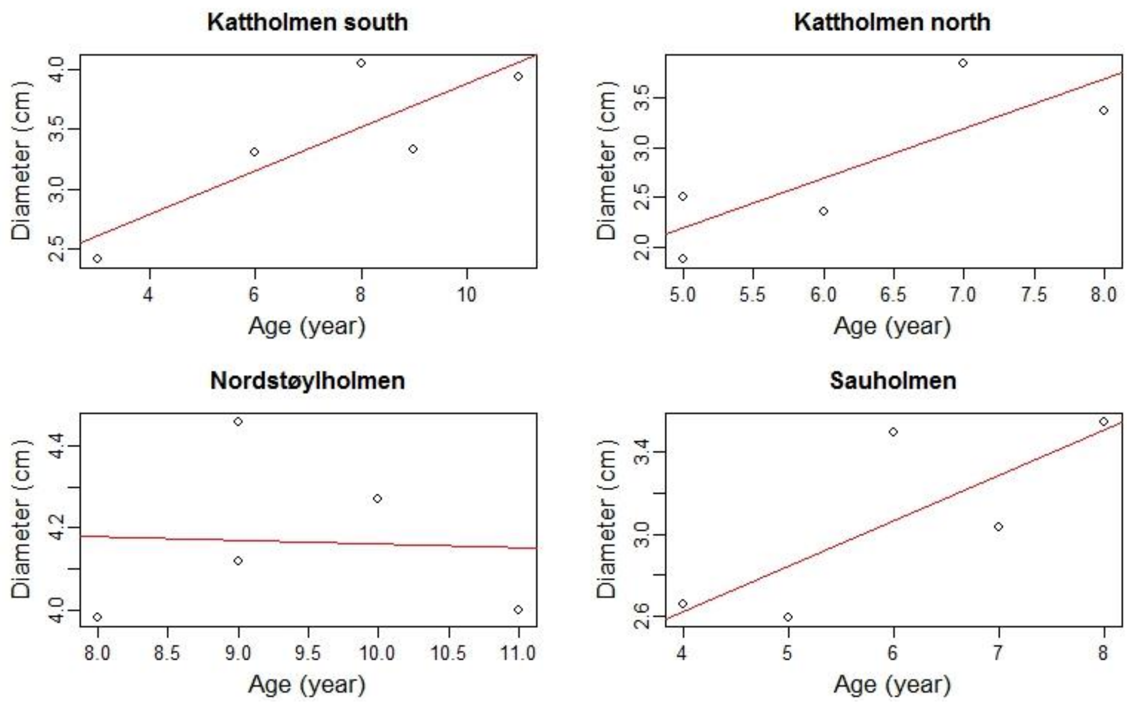

Figure 3-10: Stipes diameter (cm) relative to age (year), seperated by location. Only the individuals of *L. hyperborea* used for identification of epigrowth specimens are included (N=5 per location). Linear regressions are added. KE: R^2=0.6132, p=0.073, KB: R^2=0.5582, p=0.091, NPE: R^2= 0.3305, p=0.942, SFE: R^2= 0.4726, p=0.122. Note that the scale of the axis are not the same.

Figure 3-11: Transversely section of *L. hyperborea* stipe, approximately 1 cm over holdfast, after deep frezing and thawning. *L. hyperborea* individual from first sampling at Kattholmen south. Picture taken in compound microscope (Photo: Albertine Rekdal Havnegjerde).

3.4 Identified taxa

Registrations of the identified epigrowth taxa, found on *Laminaria hyperborea* stipes, shows that there are a diverse composition, that some taxa are more diffucult to give a certain identification and that kelp as a habitat is complex (Figure 3-12, 3-13 and 3-14). *Membranipora membranacea* (Linnaeus) (Figure 3-12A:) is an example of a taxon that had high registration number for all locations, except at Sauholmen. At this location it only were registered three times during second sampling, and they were all one small colony attached to the upper part of the stipe. One unindentified species and Cyclostomatida indet B are examples on taxa that are diffucult to identify (Figure 3-12B. and C.). While the unidentified species not even could be assigned to a phylum, individuals of Cyclostomatida indet B could be identified with certainty to the taxonomic level order. Since it were decided to identify every taxa on each individual of *L. hyperborea*, results shows complexity in the kelp forest with epigrowth on epigrowth (Figure 3-13). In addition, results shows that there were a large varity of epiphytes (Figure 3-14). Kjersti Sjøtun at the University in Bergen help with identification of two taxa, belonging to Orchrophyta and Rhodophyta, respectively (Figure 3-14).

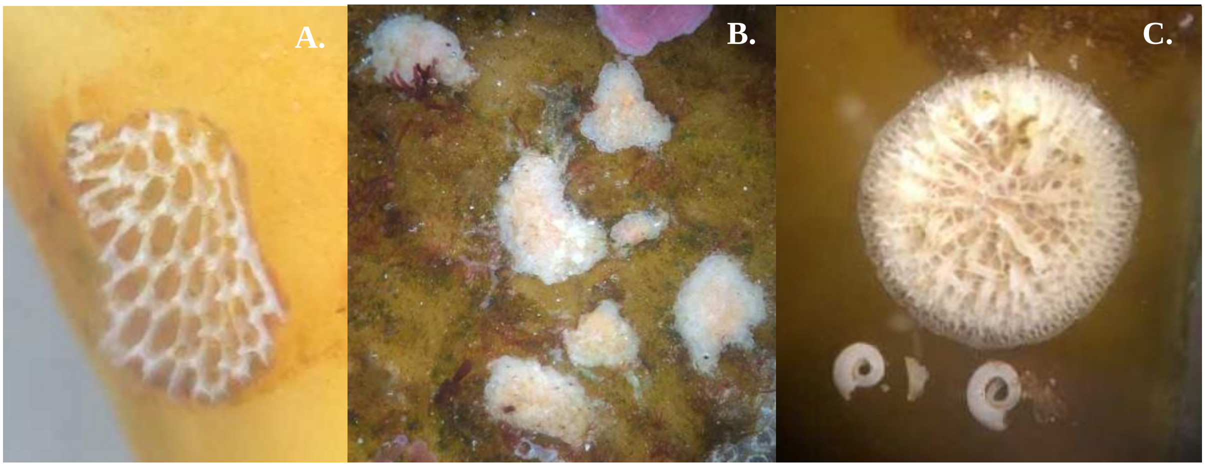

Figure 3-12: Registered taxa from the location Sauholmen. **A.**: Small colony of *Membranipora membranacea* in the upper part of stipe (growth zone). From the second sampling. **B.**: Several patches of the unidentified species, from second sampling at Sauholmen. **C.**: Cyclostomatida indet B and *Circeis* sp. From first sampling (Photo: Albertine Rekdal Havnegjerde).

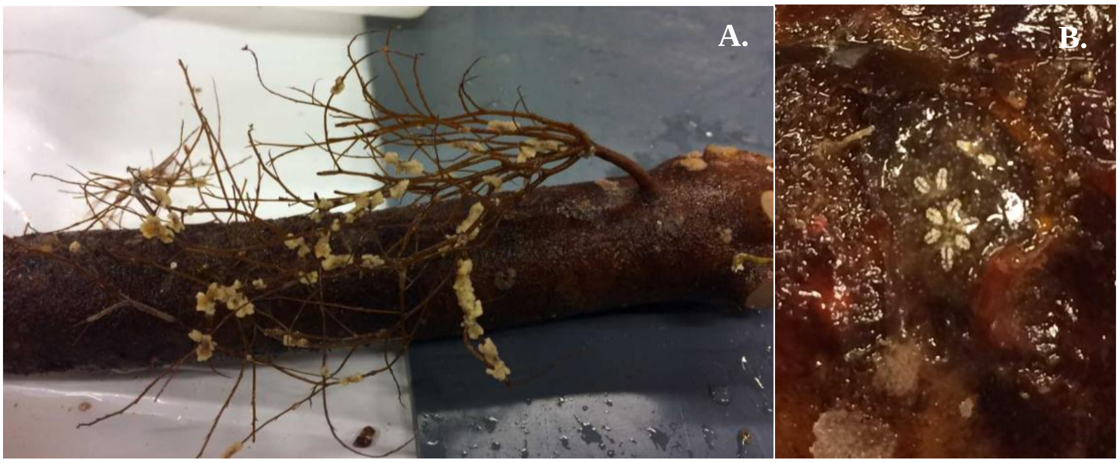

Figure 3-13: **A.**: Epigrowth on epigrowth are a common sight. Here *Tubulipora liliacea* (Pallas) on *Desmarestia aculeata* ((Linnaeus) J.V.Lamouroux) attatched to *Laminaria hyperborea* stipe. From first sampling at Nordsøylholmen. **B.**: Small colony of *Botryllus schlosseri* (Pallas) on top of a Bryozoa colony. From first sampling at Kattholmen south (Photo: Albertine Rekdal Havnegjerde).

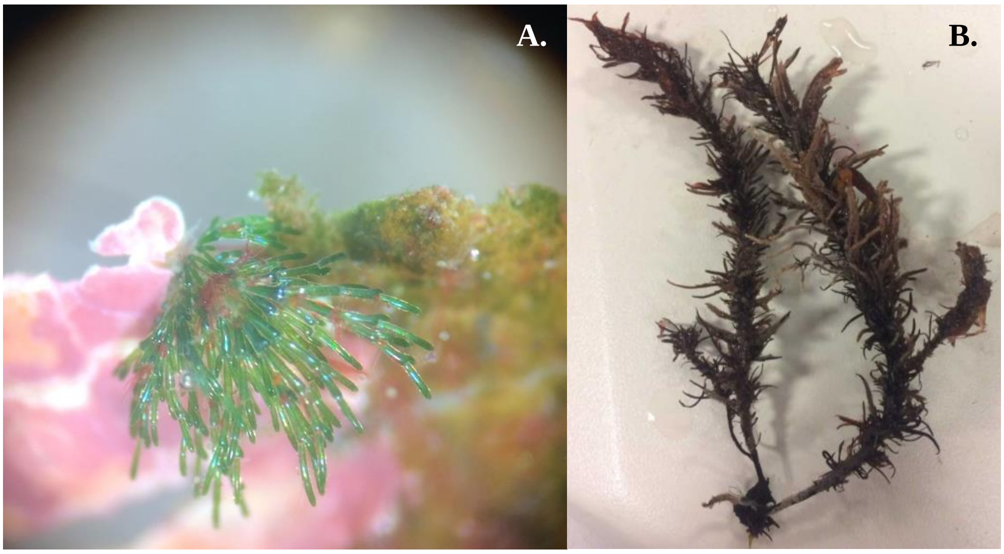

Figure 3-14: Two of the algae attached to *Laminaria hyperborea* stipes. **A.**: *Cladophora* sp. (Kützing) with *Phymatolithon* sp. (Foslie) in the background. From second sampling at Sauholmen. **B.**: *Rhodomela lycopodioides* ((Linnaeus) C.Agardh) overgrown with Bryozoa. From second sampling at Nordstøylholmen (Photo: Albertine Rekdal Havnegjerde).

The results are complicated to interpret if taxa diversity of a phylum/division is high and only displayed in a table, as in Table 3-2. This has resulted in further investigation of one phylum and one division, Bryozoa and Rhodophyta respectivily (Figure 3-15 and 3-16). A complete taxa list for all phyla/divisions can be found in Appendix B.. Results shows that 35 taxa of Bryozoa and 24 taxa of Rhodophyta were identified from all locations. Including all in one graph makes the graph more difficult to interpret. Since some taxa are more common to find, some shows a clearer difference between locations and some are time consuming to indentify, a selection of taxa are chosen to be presented. This resulted in one graph for Bryozoa with 19 taxa included and one graph for Rhodophyta with 18 taxa included (Figure 3-15 and 3-16). Graphs with all taxa of Bryozoa and Rhodophyta can be viewed in Appendix A. Figure A-1 and A-2, respectivily.

Results shows that the distribution of Bryozoa taxa varies with location category (Figure 3-15). In total were 35 different taxa of Bryozoa identified (Table 3-2). 19 at Kattholmen south, 15 at Kattholmen north, 23 at Nordstøylholmen and 12 at Sauholmen (Table 3-2). Of these species/genus/family/order a total of 65, 51, 82 and 39 specimens were registered at Kattholmen south, Kattholmen north, Nordstøylholmen and Sauholmen, respectively (Table 3-1). Notice that *M. membranacea* is present in a high number (N=9-10) at all locations, except at Sauholmen (N=3). Observations also shows that species in the family Crisidae (*Crisia aculata*

(Hassall), *C. eburnea* (Linneaus), *C. ramosa* (Harmer), *Crisidia cornuta* (Linneaus) and *Filicrisia geniculata* (d'Orbigny)) and the family Scrupariidae (*Scruparia ambigua* (d'Orbigny) and *S. chelata* (Linneaus)) only are registered at exposed locations, as Kattholmen south and Nordstøylholmen. There is also one registration of *Crisia* sp. (Lamouroux) at Sauholmen, which also is an exposed location. *Celleporina caliciformis* (Lamouroux) is also one speceis that shows a clear difference between locations. The species has 1-2 registrations for all locations, except at Nordstøylholmen were it has 8 registrations.

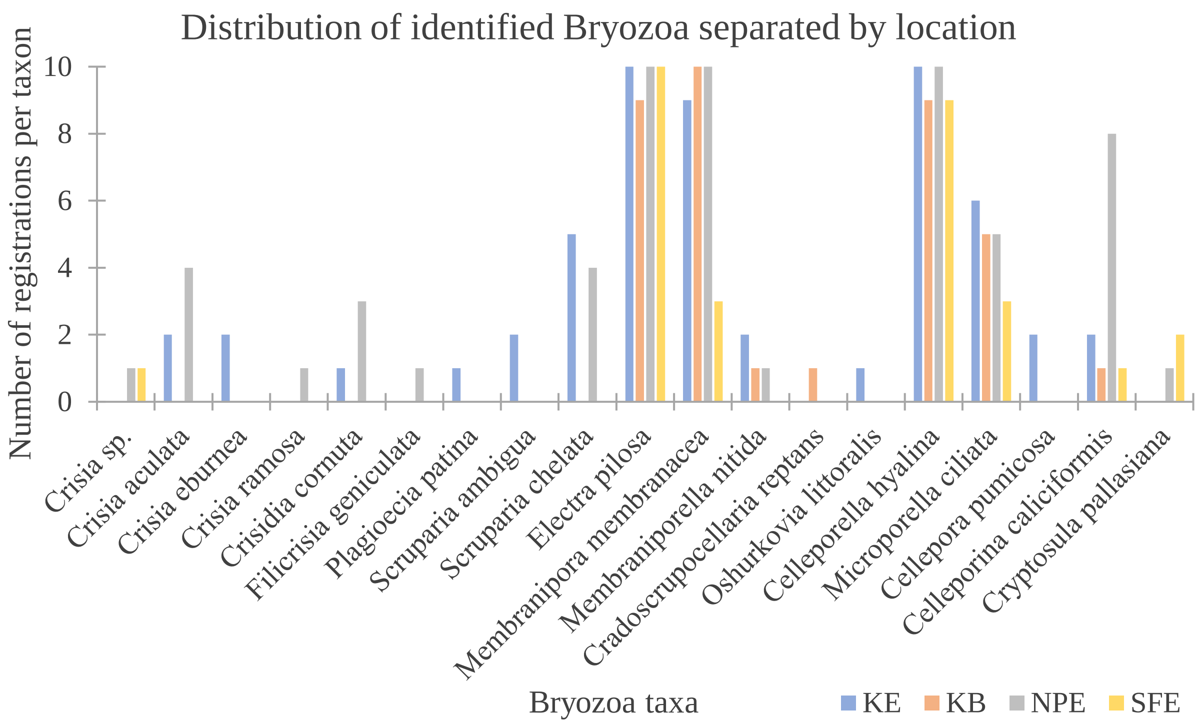

Figure 3-15: Number of registered Bryozoa specimens on *L. hyperborea* stipes, separated by location. Only taxa that showed a clear difference in the composition between the locations and the most common ones are included. *L. hyperborea* from both first and second sampling (N=10 per location). KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

Results of Rhodophyta taxa distribution shows that there is a difference, both when it comes to diversity and number of registrations (Figure 3-16). In total were 24 different taxa of Rhodophyta identified (Table 3-2). 15 at Kattholmen south, 12 at Kattholmen north, 29 at Nordstøylholmen and 11 at Sauholmen (Table 3-2). Of these a total of 46, 38, 61 and 47 specimens were registered at Kattholmen south, Kattholmen north, Nordstøylholmen and

Sauholmen, respectively (Table 3-1). Results can indicate that epiphytes of Rhodophyta has better conditions for setteling at the impacted locations, based on registered taxa at the locations (Figure 3-16). Nordstøylholmen clearly has highest diversity and Sauholmen has most registrations of many taxa (Figure 3-16).

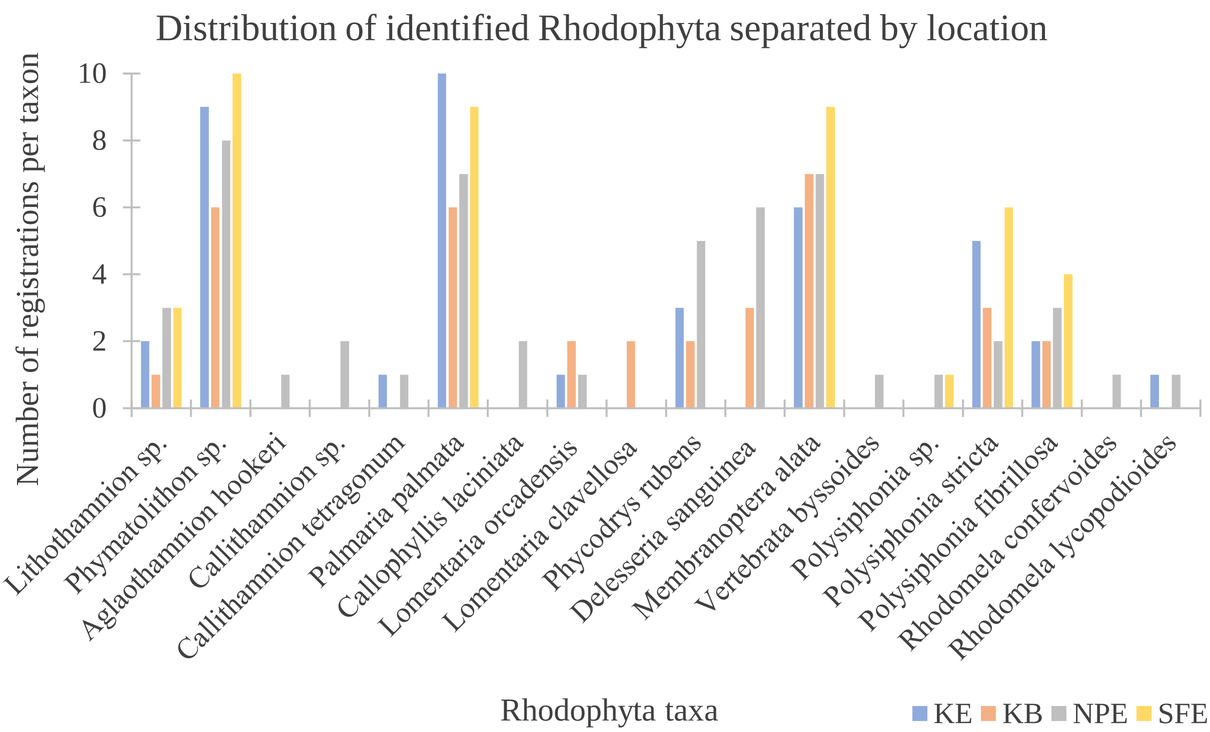

Figure 3-16: Number of registered Rhodophyta specimen on *L. hyperborea* stipes, separated by location. Only taxa that showed a clear difference in the composition between the locations and the most common ones are included. *L. hyperborea* from both first and second sampling (N=10 per location). KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

3.5 Water samples

Results shows that overall there is not detected noteworthy elevated levels of nutrients at the locations, of neither phosphorus, nitrite + nitrate or ammonia, compared to each other or Frohavet (Figure 3-17, 3-18 and 3-19). The last ten water samples for ammonium, has not been analysed (Figure 3-19). This includes all water samples from Sauholmen, and samples from the second sampling round at Kattholmen south, Kattholmen north and Nordstøylholmen.

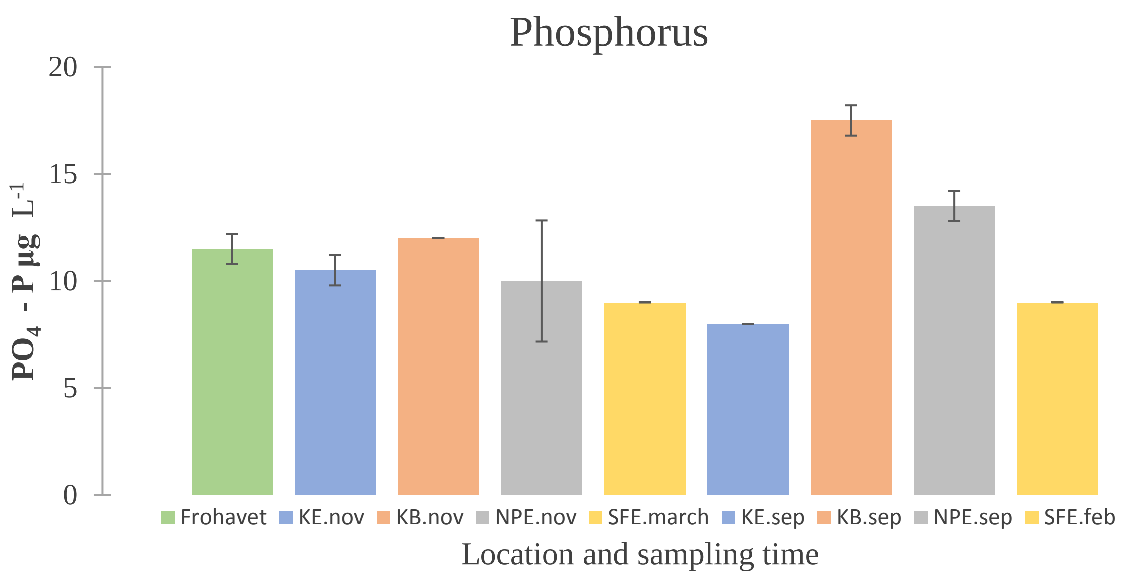

Figure 3-17: Level of phosphorus (PO_4^{3-}) in water samples taken at the locations during each collection. Frohavet (November) is the zero sample and is taken in open ocean, away from fish farming activity. Standard deviations are included. N=2 per sampling. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen. Nov: November, Sep: September, Feb: February.

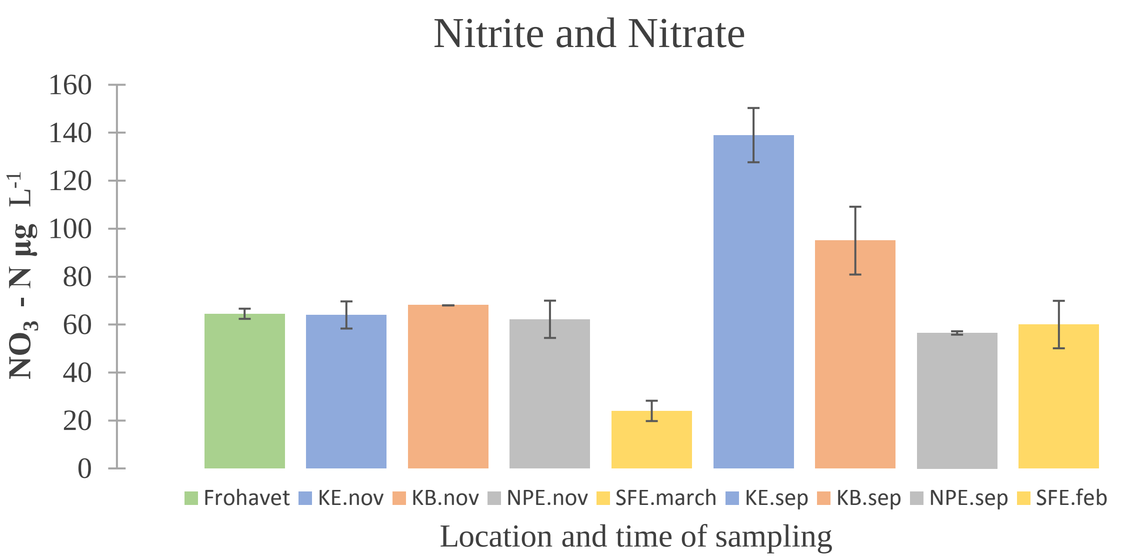

Figure 3-18: Level of nitrite (NO_2-) and nitrate (NO_3-) in water samples taken at the locations during each collection. Frohavet (November) is the zero sample and is taken in open ocean, away from fish farming activity. Standard deviations are included. N=2 per sampling. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen. Nov: November, Sep: September, Feb: February.

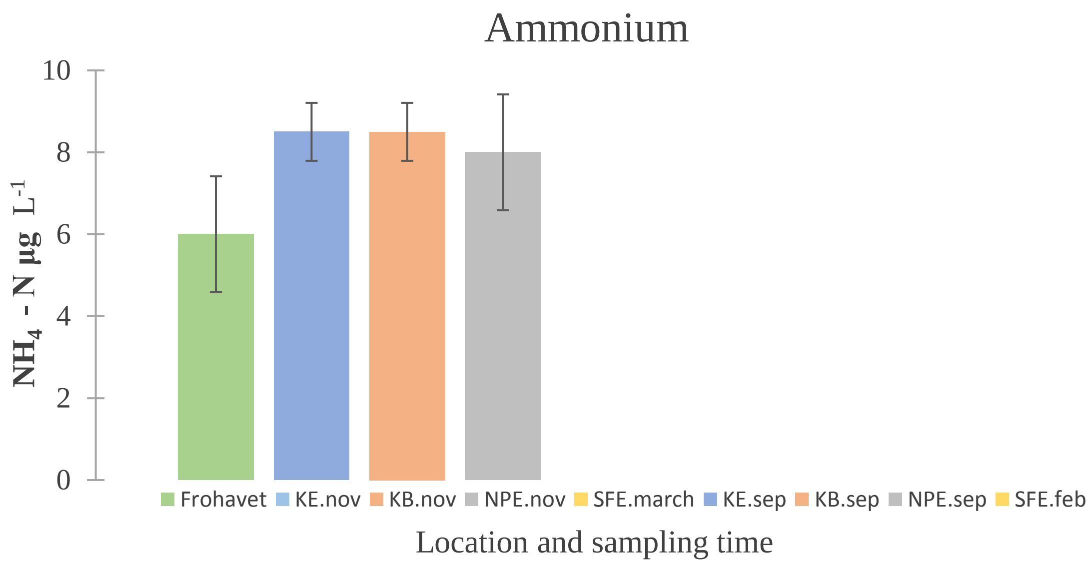

Figure 3-19: Level of ammonium (NH_4^+) in water samples taken at the locations during each collection. Frohavet (November) is the zero sample and is taken in open ocean, away from fish farming activity. Standard deviations are included. N=2 per sampling. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen. Nov: November, Sep: September, Feb: February.

3.6 Fouling plates

Identification of specimens on the CD-discs shows that there are a varying degree of fouling, in both diversity and abundance (Figure 3-20). Notice that the silver coating on the CD-discs has disappeared (Figure 3-20). All artificial fouling plates were deployed in the collection area during the first sampling at 5 meters depth (cart datum), but variations in location characteristics, conditions and time of deployment have not been equal for the locations. This might have resulted in the difference in registered taxa and richness (Figure 3-21). Off all taxa found on *Laminaria hyperborea*, only ~7 % (KE and KB), ~1 % (NPE) and ~3 % (SFE) were recovered on the kelp mimics. Nordstøylholmen had only one genus present, *Phymatolithon* sp. (Foslie), distributed as 8 small colonies. Because of this, it is excluded from the venn diagram (Figure 3-21). Kattholmen south had four species, two genus and one family present. Were *Campanularia volubilis* (Linneaus), *Heteranomia squamula* (Linneaus), *Tubulipora* sp. (Lamarck) and *Celleporella hyalina* (Linneaus) had only one individual/colony present, respectively. *Electra pilosa* (Linneaus), *Circeis* sp. and *Phymatolithon* sp. had more than one individual/colony present. Kattholmen north had four species and three genus present. Were *Membranipora membranacea*, *Phymatolithon* sp., *Lithothamnion* sp. (Heydrich), *Microporella* *ciliata* (Pallas) and *C. hyalina* had more than one colony present. One individual of *Ulva lactuca* (Linneaus) and *Polysiphonia* sp. (Greville) were observed. Sauholmen had one species, two genus and one phylum present. Of those, two colonies of *C. hyalina* were observed, while *Phymatolithon* sp., *Lithothamnion* sp. and a microalga from Orchrophyta had several colonies/individuals present.

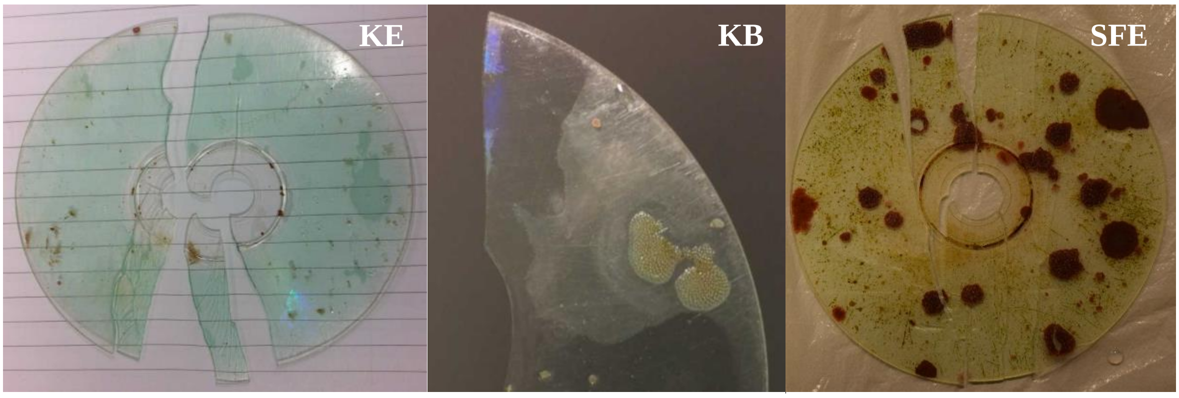

Figure 3-20: Artificial fouling plates made of CD-discs after approximately one year in sea. Left: CD-disc from Kattholmen south. Some fouling can be observed. Middle: Parts of a CD-disc from Kattholmen north. One colony of *M. membranacea* can be observed. Right: CD-disc from Sauholmen. Several colonies of *Phymatolithon* sp. and *Lithothamnion* sp. can be observed (Photo: Albertine Rekdal Havnegjerde).

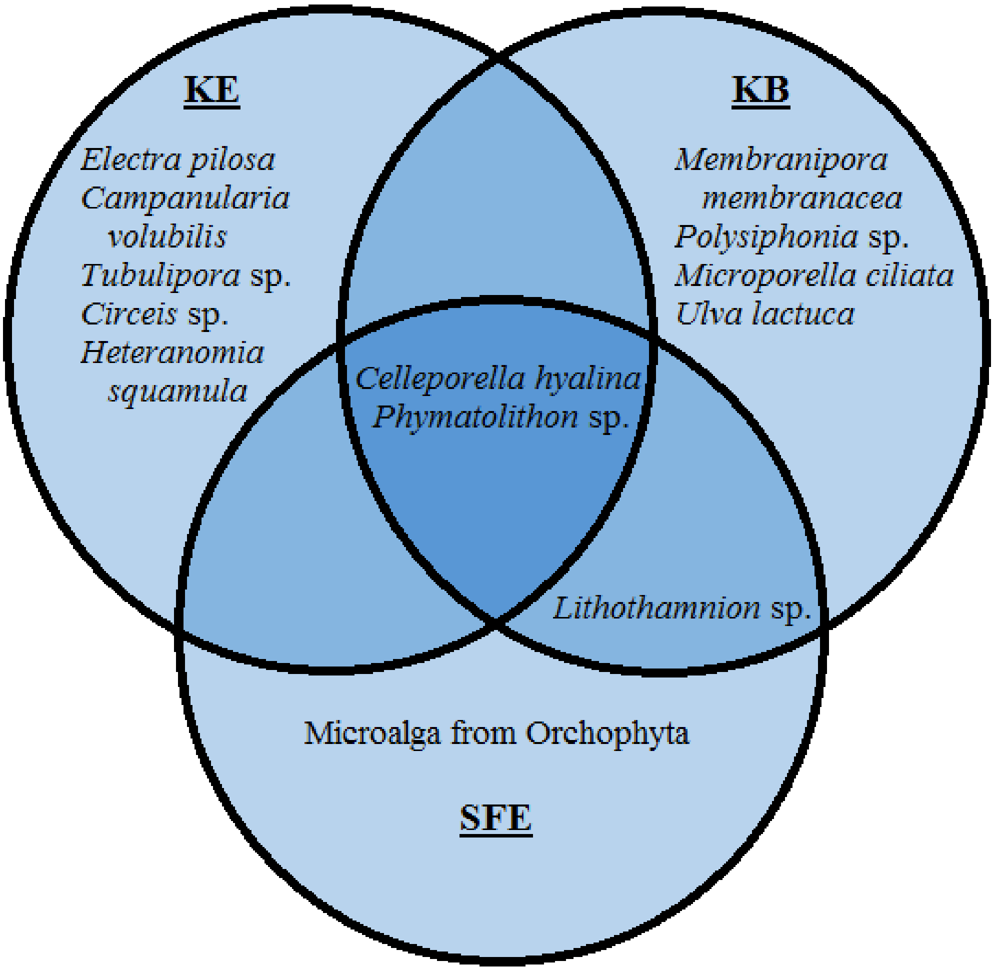

Figure 3-21: Venn diagram visualizing the taxa registered from the CD-discs on the artificial fouling plates, separated by location. Nordstøylholmen is excluded, because *Phymatolithon* sp. was the only registered taxa at this location. KE: Kattholmen south, KB: Kattholmen north, SFE: Sauholmen.

4 Discussion

The most important findings are that there are differences in the species composition between the locations. These differences can be seen in the lack of *Membranipora membranacea* at Sauholmen, species only found at exposed locations and in the distribution of Rhodophyta taxa. In addition, the learning process of understanding what works and do not work when it comes to development of a new method has been essential for this project. Missing kelp forest at the protected impacted locations, which results in the disappearance of a whole community, are also an important finding.

4.1 Justification for the location categories

The locations were classified into different categories based on several factors. The degree of enclosure of skerries and islands, or how open the area is towards the open ocean is one factor (Figure 2-1, 2-2 and 2-3). Another factor is distance from fish farm facilities. Other studies have used 1,500-2,000 meters distance from nearest fish cage as an approved distance for reference locations, by showing that the diffuse deposition zone is from around 250 meter to between 500-1,000 meters (Kutti et al, 2007; Kutti and Olsen, 2007; Svåsand et al, 2017; White, 2017) and that the acute deposition zone is up to around 50 meters (Carroll et al, 2003; Mayor et al, 2010; Mayor and Solan, 2011; White, 2017). Therefore, all locations are outside the acute deposition zone, and since the intention with this project was to examine conditions at locations in near vicinity of fish farming activity, this was desired. All impacted locations are positioned in the diffuse deposition zone, except Sauholmen that is positioned in-between the acute and diffuse zones. Kattholmen south is in a distance from the nearest fish farm approved for reference locations. Kattholmen north has a distance of ~1,200 meters from its nearest fish farm. The direction from this farm is upstream, and by this assumed to be in an accepted distance for reference sites after all. This is based on that the studies showing that the diffuse deposition zone ends within 1,000 meters, have studied a downstream transect from the fish farms (Kutti et al, 2007) and that the detection of aquaculture waste beyond 500 meters from a farm is unusual (White, 2017). It is also based on NS 9410 statement that the facility zone and the transition zone normally ends within 25-30 meters and 500 meters form the fish farm, respectively (Standard Norge, 2016). A third factor are information given in reports for the different fish farms. Particulars about sediment types, strength and directions of currents, and direction of particle movement gave information that laid foundation for deciding if a location were protected, or exposed (Måsøval, 2015; Skottene, 2016; Tunheim, 2017, Wahlvåg, 2017a; Wahlvåg, 2017b). Bekkby et al. (2014) showed that stipes length and stipes diameter for

Laminaria hyperborea increased with wave exposure and degree of water flow, respectively. This supports that locations used in this project are exposed or protected. As stipes length and stipes diameter, with increasing age, generally are higher for the exposed locations, than for Kattholmen north (Figure 3-4, 3-7 and 3-9). The last factor was registrations of lice counts at the different fish farms, which indicated if the farm had standing fish or not (Barentswatch, 2018). All fish farms had standing fish during sampling, except during first sampling at Sauholmen and second sampling at Nordstøylholmen. The miss were 18 weeks at Sauholmen and 5 weeks at Nordstøylholmen (Barentswatch, 2018). This miss do not affect the results to a large degree. Overall, impact by emissions harm in a long-term scale, and affected organisms will suffer from the alterations until normal conditions are restored.

4.2 Bukkholmen, Vestre Lamøyskjæret and Kollskjæra

As mentioned in the introduction (Section 1.2), *Laminaria hyperborea* is a fundamental species and the kelp forest build up by this species is vitally important for many other organisms. The kelp forest is often referred to as the oceans equivalence to the terrestrial rain forest, because of its importance, productivity and support of a high species diversity (Fosså, 1999; Christie et al, 2003; Lorentsen et al, 2010). If the kelp forest disappears, it will not only effect one species, but the whole community. Therefore, it should be of concern that *L. hyperborea* are missing from these three locations characterized as protected impacted, especially since *L. hyperborea* shows signs of earlier presence. The locations were clearly heavily impacted from grazing, with half-eaten stipes, hundreds of *Echinus esculentus* and thousands of *Ophiocomina nigra* (Figure 2-4 to 2-7). Bekkby et al. (2015) observed high densities of *E. esculentus* grazing on *L. hyperborea*, and assumed that it were a result of insufficient food supplies due to low coverage of epiphytes on kelp stipes. This supports that lack of *L. hyperborea* at the protected impacted locations are a result of the high densities observed of *E. esculentus*. White (2017) has detected aggregations of the white sea urchin *Gracilechinus acutus* (Lamarck) grazing under *Salmo salar* fish farms in Norway. This species is in the same family as *E. esculentus*, and it was observed a few individuals of it at these protected impacted locations (Figure 4-1). *G. acutus* exploit aquaculture waste as an energy-rich trophic subsidy (White, 2017), and can like many other sea urchin species drive ecosystem changes through overgrazing on kelp when in high population densities (Filbee-Dexter and Scheibling, 2014; White, 2017). *E. esculentus* is assumed to be one of those other sea urchin species, and this is supported by the findings in Bekkby et al.

(2015). In addition, the high abundance of *O. nigra* with their arms up in a waving position indicates that there probably are high amounts of particles in the water masses (Moen and Svensen, 1999). The three locations are all positioned within the diffuse deposition zone from their respective fish farms (White, 2017). With approximate distance of 300 meters, 300 meters and 600 meters from the fish farm for Bukkholmen, Vestre Lamøyskjæret and Kollskjæra, respectively. Kattholmen north, which is situated in-between the same islets and island act as a reference site. This location is ~ 1,200 meters from the fish farm Bukkholmen S, an accepted distance based on the upstream direction from the fish farm and findings in other studies (Standard Norge, 2016; White, 2017). If the reason for absences of the kelp forest was a result of something else than emissions from fish farming activity, Kattholmen north should lack *L. hyperborea* as well. Therefore, it is reasonable to draw the conclusion that the conditions at Bukkholmen, Vestre Lamøyskjæret and Kollskjæra at least partly are a result of aquaculture activity. It is not possible to state that the conditions only are a result of this activity, but it is assumed that a complex of factors, which are driven by elevated levels of emissions, have caused the conditions. Other environmental factors that may cause stress for *L. hyperborea* are changes in currents or salinity, extreme temperature events and sedimentation (Husa et al, 2014b).

Figure 4-1: Seabed at the protected impacted location Kollskjæra. In the lower right corner one individual of the white sea urchin *G. acutus* can be observed, in addition to several individuals of *O. nigra* and *E. esculentus*. From GoPro videos at the location.

4.3 Bryozoa and Rhodophyta

The total number of both Bryozoa and Rhodophyta taxa had to be reduced and the result can be viewed in Figure 3-15 and 3-16, respectively. Taxa that are expected to be found at all locations are included, as their absence should raise a warning sign. Taxa that shows a clear difference are also included, because they are thought to be consistent with a red thread within location categories. Taxa that are time consuming to identify are excluded, because a new method supplementing already established monitoring (NS 9410) cannot be based on taxa that takes hours to identify or that are not possible to identify with certainty. Based on this, one graph of Bryozoa and one of Rhodophyta with 19 and 18 taxa, respectively were created (Figure 3-15 and 3-16). Graphs with all taxa included can be viewed in Appendix A. (Figure A-1 and A-2).

4.3.1 Bryozoa

Three species considered common in the North-East Atlantic Ocean are *Membranipora membranacea*, *Electra pilosa* and *Celleporella hyalina* (Schultze et al, 1990; Hayward and Ryland, 1995; Hayward Ryland, 1999). It is expected to find them at all locations on their suitable habitat. Both *M. membranacea* and *C. hyalina* has *Laminaria* as their preferred substrate, while *E. pilosa* can be found on various substrata, including *L. hyperborea* (Hayward and Ryland, 1995; Hayward and Ryland, 1998; Hayward and Ryland, 1999). They are all common in the sublittoral zone, and *C. hyalina* can reach grate abundance in sheltered areas on *Laminaria* (Hayward and Ryland, 1998; Hayward and Ryland, 1999). *E. pilosa* and *C. hyalina* had 9-10 registrations at all locations. *M. membranacea* also had 9-10 registrations at all locations, except at Sauholmen, where there were only 3 registrations (Figure 3-15). Those three registrations were all one small colony at the upper part of stipes from the second sampling round (Figure 3-12). Because all registrations of *M. membranacea* at Sauholmen were in February 2018, one year after the first sampling, it may indicate that conditions at this location are facing a recovery. Seeing that both Sauholmen and Nordstøylholmen are characterised with the same location category, it is attention worthy that Nordstøylholmen is not missing this species. However, the low attendance at Sauholmen compared to Nordstøylholmen can possibly be explained by the distance from the fish farms, which are approximately 150 and 300 meters, respectively. This difference in distance might be enough to reduce the living conditions at Sauholmen to a degree where colonising of *M. membranacea* cannot be supported. In addition, the fish farm at Sauholmen, Måøydraga, had a MOM-B (Monitoring – Ongrowing fish farm - Modelling) monitoring in April 2016 (Skottene, 2016). The fish farm got the best rating and it were assumed that the location would tolerate high production load (Skottene, 2016), still the location only had fish for four months before slaughtering (Barentswatch, 2018). This possibly indicates, what earlier argued, that the locations conditions not were that good.

Species of the family Crisidae and the family Scrupariidae were only found on the exposed locations, and with both a higher number and more taxa at Nordstøylholmen, than at Kattholmen south and Sauholmen (Figure 3-15). Species from both Crisidae and Scrupariidae are common in shallow water in the sublittoral zone (Hayward and Ryland, 1995). Nordstøylholmen had 14 specimens from six taxa, Kattholmen south had 12 specimens from five taxa and Sauholmen had 1 specimen from one taxon, of a total of eight taxa. The high attendance of these families at Nordstøylholmen and Kattholmen south might indicate that environmental conditions with stronger currents are favourable. The fact that Sauholmen only had one registration of these families, can again, possibly be explained by the shorter distance from the fish farm, which assumable will lead to larger impact from aquaculture activity.

Celleporina caliciformis was identified at all locations, but the number of registrations varied. It is a common species on kelp, especially holdfast, and it is distributed in the temperate Northeast Atlantic Ocean, including western Norway (Hayward and Ryland, 1999). This species had 1-2 registrations on *L. hyperborea* stipes at Kattholmen south, Kattholmen north and Sauholmen, while at Nordstøylholmen the registered number was eight (Figure 3-15). The explanation why this species had higher registrations at Nordstøylholmen might be factors as condition, tolerance and predation. The impacted locations may have had conditions not suitable for predators. If *C. caliciformis* has better tolerance than its predators, it could explain the presence and the high registration as a response of lacking grazing pressure. Hayward and Ryland (1999) states that predation pressure on bryozoans will have an impact on their presence, and that, common predators are sea slugs (Heterobranchia). In addition, Schultze et al. (1990) showed that mobile macrofauna had lower attendance at the exposed sites, which includes sea slugs. Nevertheless, if predation was the only factor, it does not explain the low number at Sauholmen. Again, a possible explanation can be distance from the fish farm. *C. caliciformis* might be a tolerant species, but not tolerant enough to stand the conditions of assumed higher emission load at Sauholmen.

Several species/taxa were only found at Kattholmen south. These are *Crisia eburnea*, *Plagioecia patina* (Lamarck), *Scruparia ambigua*, *Oshurkovia littoralis* (Hastings) and *Celleporella pumicosa* (Pallas) (Figure 3-15). *Tubulipora liliacea* (Pallas), *Shizomavella (Schizomavella) linearis* (Hassall) and *Alcyonidium mamillatum* (Alder) were only registered at this location as well, but are excluded from Figure 3-15 due to time consuming identification

(Appendix A. Figure A-1). Kattholmen south is an exposed location, as Nordstøylholmen and Sauholmen, but not impacted. The occurrence of these species only at Kattholmen south can be a response of disadvantageous conditions caused by fish farming activity at the other exposed locations. It is important to mention that all of these species had 1-2 registrations only, and can by this be species that have settled randomly. However, if it is assumed that the settling are not random, they might occur as a response of distance from a fish farm. Kattholmen south is outside the diffuse deposition zone, and should not be affected by elevated emission loads from fish farming.

4.3.2 Rhodophyta

As results shows, epiphytes of Rhodophyta might have better living and settling conditions at impacted locations. This is based on the selected taxa (N=18) for Figure 3-16. Nordstøylholmen had 17 of the selected taxa, while Kattholmen south, Kattholmen north and Sauholmen had 10, 10 and 7 taxa present, respectively (Figure 3-16). This indicates that Nordstøylholmen has good conditions for settling of Rhodophyta taxa. On the other hand, despite only having seven taxa present, Sauholmen had 42 registrations in total. Which indicates that this location also have favourable conditions for Rhodophyta taxa, as Nordstøylholmen. This is reasoned in that ammonium outputs from aquaculture easily are utilised by algae, and that elevated levels are favourable for fast-growing macroalgae (Svåsand et al, 2017). Which can result in higher numbers of epiphytes that are thin, foliaceous and thread shaped (Svåsand et al, 2017), as shown in the results for this project. Over time, the consequence might be a reduction of perennial, slow growing species (Worm and Sommer, 2000), like *L. hyperborea*. The latter might also have been the precursor for the conditions observed at the protected impacted locations, where kelp were missing.

It is assumed that Nordstøylholmen and Sauholmen have altered conditions by elevated levels of waste products due to their position nearby fish farming activity. Since both locations are described as exposed impacted it can be argued why Nordstøylholmen has so many more taxa present and why Sauholmen has high registration numbers for the few taxa present. Once again, the distance from the fish farms might be the reason. Studies done on aquaculture inputs in the sediments has revealed that inputs will appear further out than the acute deposition zone, albeit at decreasing concentrations with distance (Carroll et al, 2003; Kutti et al, 2007; White, 2017). Neither Nordstøylholmen nor Sauholmen are located in the acute deposition zone (White, 2017), however, exact distance from the fish farm can be an important factor, as nutrients will dilute with distance. Currents, topography and amount of filtering organisms etc. are factors that will affect dilution and it is therefore no definitive answer on how rapid the dilution will occur. Overall, it can be assumed that Sauholmen has a higher degree of impact, than Nordstøylholmen, and has therefore Rhodophyta taxa with higher tolerance present. The Rhodophyta taxa only present at Nordstøylholmen are likely tolerant too, but not to the same degree as those present at Sauholmen.

4.4 Fouling plates

As mention in the introduction (section 1.4), many other studies concerning kelp forest have used artificial fouling plates to determine settling time of epigrowth. There have been variation in applied substratum and number of rediscovered taxa compared to natural kelp. Norderhaug et al. (2012) rediscovered 85 % and Waage-Nilsen et al. (2003) rediscovered 86.7 % of the taxa found on natural kelp. Results from this project indicates that kelp mimics made by CD-discs has its disadvantages. Only ~7 % of the taxa found on stipes were rediscovered on the CD-discs from the two Kattholmen locations, ~1 % from Nordstøylholmen and ~3 % from Sauholmen. The low percentage can probably be explained by the silver coating on the CD-discs, because it had disappeared when the CD-discs were collected. How long it has taken the coating to disappear is unknown and specimens that attached before the disappearing are lost too. Therefore, only specimens that attached after the coating had disappeared would be registered as epigrowth during examination of the CD-discs. However, only one of the sides of the CD-disc had coating in the first place, so it do not explain why there were low number of epigrowth on both sides. The method were recommended by others, which have had great success, so it is possible that the specific CD-discs that were used in this experiment are not suited for attaching. The CD-discs might have had a smoother surface, in contrast to the successful ones in other studies. As mentioned in the introduction (Section 1.4) absence of some specific habitat properties in the artificial substratum might explain the missing species/taxa (Waage-Nielsen et al, 2003). By studying videos of the fouling plates at the locations, it is clear that the CD-discs are the largest problem, based on that epigrowth have attached to other parts of the fouling plates. Epigrowth, especially epiphytes, had attached on both the plastic pipes and the rope above the CD-discs (Figure 4-2). For better results, components with more structure should be applied. Like the rope and the plastic pipes used in this project or material used for the kelp mimic applied by Norderhaug et al. (2002) (Figure 1-3). Overall, if a component with some sort of coating are used, the coating should be sanded off before deployment to prevent epigrowth to be lost when the coating disappears.

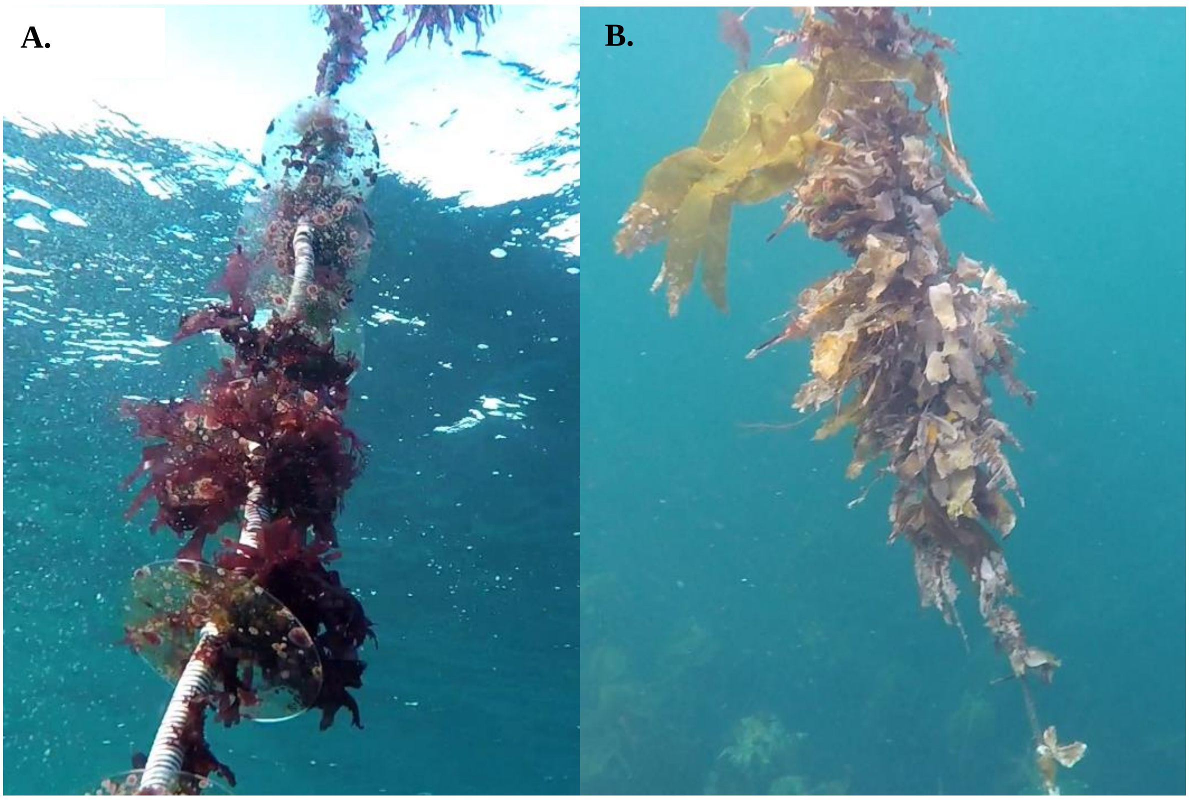

Figure 4-2: Artificial fouling plates after almost one year in the ocean. From GoPro videos at the locations. **A.**: Epiphytes attached to the plastic pipes between the CD-discs. At Sauholmen. **B.**: Epiphytes with epigrowth attached to the rope above the CD-discs. At Nordstøylholmen.

4.5 Length, age and diameter

Results have shown that stipes length, stipes diameter and number of identified taxa increases with increasing age of *Laminaria hyperborea* (Figure 3-3 to 3-10). There are a few exceptions, were the percentage of the described variance are low. At Nordstøylholmen low percentage of stipe diameter could be explained by age of the kelp, both with all individuals of *L. hyperborea* included (R^2= 0.101) and with only those used for species composition (R^2= 0.3305) (Figure 3-8 and 3-10). This are expected based on the age of the individuals, since *L. hyperborea* normally have less stipe diameter growth after 6 years of age (Steen et al, 2016a). At Sauholmen, a low percentage of stipe diameter could be explained by the age with all individuals of *L. hyperborea* included (R^2=0.0762, Figure 3-8). This also counted for number of taxa explained by stipe length at Kattholmen south (R^2=0.3836) and Kattholmen north (R^2=0.01958) (Figure 3-6). Nevertheless, with almost 50 % or more of the variations of stipes length, stipes Diadiameter and number of taxa explained by age, it is considered to be a good variance explanation. This means that there overall are a connection between those parameters. A longer and thicker stipe increases the surface area of the kelp, which is a result of age, and so increase the area for settling of epigrowth, as shown in Rinde et al. (1992) and Steen et al. (2016b). It is important to have in mind, when comparing species composition attached to different individuals of kelp, that there are a connection between age, length, diameter and taxa. Because an individual at three years will not have the same foundation for number of attached taxa as an individual at eight years (Table 3-3A.). By such comparison, a difference in taxa number would be present anyways.

As mentioned in the introduction (Section 1.3), number of macrofaunal specimens on stipes fluctuates between seasons, with the lowest number from October to March (Christie et al, 2003). Results from this project also indicates a fluctuation throughout seasons (Table 3-3B.). The difference is that the specimens in this project are all sessile organisms, in contrast to Christie et al. (2003) were only mobile fauna were studied. Table 3-3B. reviles the differences in number of taxa related to season. Kattholmen north and Nordstøylholmen had increasing number of taxa, with age in consideration, for second sampling compared to the first sampling. Sauholmen only showed a small increase and Kattholmen south did not show this trend. These findings can be explained by the sampling time. First sampling at Kattholmen north, Kattholmen south, Nordstøylholmen and both sampling at Sauholmen were conducted in the period when the lowest numbers of epigrowth are expected (Christie et al, 2003). It would therefore be interesting to conduct a sampling at Sauholmen in-between April to September, preferably in September as the other locations. Why Kattholmen south did not show this seasonal trend is not known, and further investigations are needed.

4.6 Dominating fauna and flora

Schultze et al. (1990) showed, as mentioned in the introduction (Section 1.3), that Rhodophyta was the dominating epiphytes on *Laminaria hyperborea* stipes, with 50 % of attached flora. This coincide with results from this project, were Rhodophyta dominated with ~60 % of the floral taxa. When it comes to the dominating fauna groups, results from Schulze et al. (1990)

and this project do not show that much similarity. In their results Bryozoa only dominated with 14 % of the fauna (Schultze et al, 1990), while in this project, Bryozoa dominated with ~55 %. Chordata, Cnidaria and sessile Polychaeta are the following dominating fauna groups in this project, with ~13 %, ~13 % and ~8 %, respectively. Nevertheless, these two studies cannot completely be compared, because Schultze et al. (1990) included mobile fauna as well as the immobile, which resulted in Polychaeta dominating the fauna species composition (20 %).

Results showed that exposed locations had higher number of taxa, than protected locations (Figure 3-2A.). With a few exemptions, all phyla had equal or more taxa present at the exposed locations, than at the protected location (Table 3-2). In addition, the total number of specimens registered at the different locations where highest at Nordstøylholmen (N=230), followed by Kattholmen south (N=160), Sauholmen (N=142) and Kattholmen north (N=137) (Table 3-1). Schultze et al. (1990) showed that species richness on stipes are higher in unprotected areas with stronger currents and turbulent water, which gives results as expected.

4.7 Water samples

Variations in nutrient level for phosphorus, nitrite + nitrate, and ammonia were low (Figure 3-17, 3-18 and 3-19). Based on the water samples, it cannot be explained why there are a difference in the species composition at the different locations. The results are as expected and it is the reason why dissolved nutrients are not monitored. The nutrients dilutes as they are transported, and are difficult to detect (Svåsand et al, 2017). It is also difficult to set an expected nutrient level, since it varies with geographical position, *Salmo salar* biomass at the fish farms, time of year, water exchange and currents etc. (Svåsand et al, 2017). An increase by 50 % in phytoplankton biomass relative to natural values are defined as eutrophication (Svåsand et al, 2017) and can be used as an indicator of elevated nutrient loads. Due to nitrogen's property as a limiting nutrient for phytoplankton growth, eutrophication normally happens during the summer months when nitrogen is scarce, and is a result of input of inorganic nitrogen from fish farming activity (Svåsand et al, 2017). Multiple studies have tried to detect elevated levels of phytoplankton, as a response value of elevated nutrient load, but few have detected a difference (Taylor et al, 1992; Pitta et al, 1999; Price et al, 2015 and references therein). This can be based on the fact that phytoplankton are grazed on and such can disappear. However, several studies have detected increased zooplankton levels nearby fish farming facilities, which can indicate a rapid utilisation of phytoplankton by zooplankton (Pitta et al, 2009). This is also a complicated method to perform and therefore not standardised for monitoring. Instead of water samples, sediment samples might be a good alternative. Nutrients can be stored in the sediments, and a difference in the nutrient levels could have been detected. Kutti et al. (2007) found higher concentrations of phosphorus in bottom sediments within 250 meters from a fish farm, compared to larger distances. High concentrations of phosphates have been found to be a useful indicator that the sediments are affected by aquaculture activity (Schaanning, 1994; Smith et al, 2005). Alternatively, a sediment sample, as applied in the NS9410 for soft- sediments can be adopted. In this standard, a score for impact on sediments by organic material are calculated based on faunal-, chemical- and sensory examination (Standard Norge, 2016). Since the exact position of the locations are in areas with hard-bottom substrate, the sediment samples would have had to be collected in the nearest area with soft-bottom sediments.

It was decided to not analyse the last ten samples of ammonia (Figure 3-19). A request of dropping the last analysis were suggested, because of low capacity on the lab at TBS. Since there could not be detected any difference in the water samples of phosphorus or nitrite + nitrate, it was assumed that a difference in the ammonium samples would fail to appear as well.

4.8 Today's missing monitoring and new methods

When it comes to emissions from production of *Salmo salar* in open net cages, only areas with soft-bottom sediments close to the fish farms are monitored (Svåsand et al, 2017) (Section 1.2). For nutrients and particles from this production, there are no demarcation for not affecting areas with hard-bottom substrates. Currents, topography, winds etc. are factors that decides where these emissions will have an impact (Svåsand et al, 2017). In addition, organisms do not only live in areas with soft-sediments. Therefore, it is needed to get some kind of monitoring of the areas not covered by the Norwegian Standard 9410 or the International Standard NS-EN ISO 19493. Based on the rapid increase of production, it is assumed that the impact of emissions will increase along with it, and for each day monitoring are absent, alteration of the ecosystem might become more severe. Hard-bottom substrates are, as mentioned in the introduction (section 1.1), habitat for *Laminaria hyperborea* kelp forest. The kelp forest is in itself an important habitat for several other species, and by this important to monitor.

If a new indicator for elevated levels of waste product outputs from fish farming activity, monitored by *L. hyperborea* with epigrowth, were to be applied, some changes from this project should be done. First of all, time of sampling has a lot to say for the results. The seasonally variations in the epigrowth species composition is unavoidable. Either one standard for each season has to be designed, or monitoring of hard-bottom substrate can be conducted during one specific season only. Recommendations for the least is that monitoring should be conducted during late summer to early autumn. This is reasoned in the fact that this is the period with highest number of epigrowth, both showed in Christie et al. (2003) and results from this project (Table 3-3B.).

The International Standard for hard-bottom surveys NS-EN ISO 19493 request use of sample quadrates of 50 x 50 cm for surveys in the sublittoral zone (Standard Norge, 2007). This might improve the method in this project also, and so give an advantage when selecting individuals of *L. hyperborea* to examine closer. In the method used in this project the divers could, even though they should not, be selective in selection of *L. hyperborea* individuals. An individual with high visible number of epigrowth might be appealing to choose. If sample quadrates were used the divers selectivity will be decreased. However, it has come to mind that the sample quadrat often is deployed after the diver has gone out in the water and had an overview of the kelp forest and so can be selective to a degree after all. Therefore, it is recommended that the sample quadrat is deployed by a person in the boat, to get an entirely random selection. It is stated in the International Standard NS-EN ISO 19493 that sample quadrates are not applicable for kelp forest (Standard Norge, 2007). This might be because the sample quadrat most likely will by stuck at the top of the kelp were lamina is. A suggestion for adapting this method too also include the kelp forest is that the divers should force it to the seabed. Not move it to any side, only in a horizontal line. Quadrat survey in the sublittoral zone, following NS-EN ISO 19493, record all species inside the quadrate area *in situ* (Standard Norge, 2007). This means that kelp are not collected for thorough examination of its attached immobile fauna and flora, but specimens observed in field are either recorded by percentage coverage or number of individuals per taxon. This excludes most of the Bryozoa, since they often are too small to notice at first, and examination of the stipe with a compound microscope is needed. As seen in results for this project (Figure 3-15, Appendix A. and B.), species of Bryozoa are a large part of the immobile fauna and by this important to include in an investigation of the species composition. Therefore, collection of *L. hyperborea* for thorough examination will be an addition to the survey in NS-EN ISO 19493, which can function as an improvement when it comes to monitoring of emissions from fish farming activity.

This study include a variety of Bryozoa, Rhodophyta, Chordata, Hydrozoa and Annelida etc. distributed in 102 taxa of immobile fauna and flora (Appendix B.). There can be no room for incorrect species identification if a new environmental monitoring method based on identification were to be implemented. Many of the taxa identified are difficult to place in the correct taxonomic unit at the lowest levels. This has resulted in that many of the taxa have been placed at either order, family or genus level. Chordata is one of these phyla (Appendix B.). In total 8 taxa were identified, but only three were placed in the taxonomic unit species. Different species of tunicates can be difficult to separate only based on outer morphological traits. It is often needed to dissect the specimens to have a closer examination of the internal organization, e.g. position of the gut and gonads, and structure of the branchial sac (Hayward and Ryland, 1995). Effective and systematic identification methods will be obstructed by these kinds of specimens. It is therefore, recommended to limit the extent of species for identification. Based on results from this project a variety of Bryozoa, Rhodophyta, Annelida and Arthropoda should be chosen to represent immobile fauna and flora on *L. hyperborea* stipes in the new developed method for monitoring. Examples are species from the Bryozoa families Crisiidae, Scrupariidae, Membraniporidae, Hippothoidae, Microporellidae and Celleporidae. Rhodophyta species/taxa examples are *Palmaria palmata* ((Linnaeus) Weber & Mohr), *Membranoptera alata* ((Hudson) Stackhouse), *Phymatolithon* sp., *Rhodomela lycopodioides* ((Linnaeus) C.Agardh), *Phycodrys rubens* ((Linnaeus) Batters), *Delesseria sanguinea* ((Hudson) J.V.Lamouroux) *Lithothamnion* sp., *Vertebrata fucoides* ((Hudson) Kuntze), *Polysiphonia elongata* ((Hudson) Sprengel), *P. stricta* ((Dillwyn) Greville) and *P. fibrillosa* ((Dillwyn) Sprengel). In addition, all species/taxa of Annelida and Arthropoda identified during this project may be included, with one exemption. *S. (Spirorbis) spirorbis* should be excluded based on its preferred substrata, which is algae species from the genus *Fucus* (Hayward and Ryland, 1995). It is of course open for an extension of the list and/or removing some of those mentioned above. There might be geographical variations along the Norwegian coast that influences the species composition and so have to be taken in consideration. It is therefore, recommended to create standards based on intervals of investigations on a transect, that follows the coastline. The checkpoints that shows the same immobile fauna and flora composition can be monitored by the same standards.

With fewer species to identify, there is time to increase the sample size. This project had a limited time aspect (weather conditions etc.) and only one person to identify. The sample size of 10 individuals *L. hyperborea* for measuring and five for species identification, for each location and sampling round, was equal a workload for one person. However, with a limited species selection to focus on, sample size should increase to give a more thorough foundation for statements about the condition at a location. Exactly how much the sample size should increase has to be investigated further, but a reasonable suggestion is to deploy 3-4 sample quadrats per location.

Instead of attempting to detect elevated nutrient levels in the water masses, sediment samples might be an alternative (as mentioned in Section 4.7). By adopting the sediment samples from NS 9410, organic load from fish farming activity will be included as well. In addition, the new method described in this project can act as an improvement of the not sufficient method applied for hard-substrate areas directly beneath the cages. This might be important for future monitoring of aquaculture emissions, based on increasing farm sizes and movement of farms to locations in more open water with stronger currents and higher wave activity. As currents get stronger, fine-grained sediments disappear and hard-substrate are left (Buhl-Mortensen et al, 2015). This means that the future fish farms will be positioned in areas were todays monitoring by NS 9410 are not doable. By combining important elements from NS 9410 and the methods presented in this project, monitoring of hard-bottom areas by examination of *L. hyperborea* and its immobile epigrowth might function as a good indicator for elevated levels of emissions from fish farming activity.

5 Conclusion

This study has documented that there are a difference in the species composition between the different locations. Both when it comes to protected and exposed locations, and between the impacted and not impacted locations. This is shown in that *Membranipora membranacea* lacked at Sauholmen during the first sampling, and only had three colonies present during the second sampling. The location might be facing a recovery based on the observations during the second sampling, but overall, absence of *M. membranacea* should raise a warning flag. The findings of species from the families Crisidae and Scrupariidae are consistent with a red thread between the exposed locations. Higher registration number of *Celleporina caliciformis* at Nordstøylholmen might be a response of lacking grazing pressure, which might be a response of exposure and tolerance. Higher taxa number and specimen registrations of Rhodophyta at Nordstøylholmen and Sauholmen, respectively, can be explained by ammonium outputs from aquaculture activity that favour short-lived epiphytes. The high registration number of Rhodophyta specimens at Sauholmen are possible caused by those taxa's tolerance. Overall, difference in species composition between Nordstøylholmen and Sauholmen are explained by Sauholmen's shorter distance to its fish farm, compared to Nordstøylholmen.

It has also been documented that protected impacted locations suffer from alternations supposedly caused by elevated levels of waste products from aquaculture activity. The missing kelp at Bukkholmen, Vestre Lamøyskjæret and Kollskjæra are at least partly due to emissions from farming of *Salmo salar*. This is based on high aggregations of *Echinus esculentus* and *Ophiocomina nigra* at those locations and that Kattholmen north, which act as the reference site, had a kelp forest build up by *Laminaria hyperborea*.

Water samples could not support the findings mentioned above, since elevated levels for neither ammonium, phosphate nor nitrite + nitrate were detected. It is not possible to say if settling of immobile fauna and flora during the summer period resulted in a new or different species composition, either. This is because the CD-discs used in the artificial fouling plates did not work as expected, and almost no epigrowth had attached. Some changes might improve the kelp mimic, as CD-discs without silver coating or substratum with a rougher surface.

The results from this project in itself are not sufficient to propose a new indicator for elevated levels of emissions from farming of *S. salar*. However, by combining important elements from NS 9410 and the methods presented in this project, environmental monitoring of hard-bottom areas might be feasible. Based on results from this project, further investigations for deciding if *L. hyperborea* with immobile epigrowth can be an indicator are suggested and some improvements are recommended. These are to focus on some species, as a selection of certain bryozoans, rhodophytes, annelids and arthropods. Sampling of *L. hyperborea* should be carried out at specific times of the year, preferably in late summer. The sample size should be increase and sample quadrates should be utilize. It is recommended to deploy 3-4 sample quadrate for all locations.

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Appendix A.

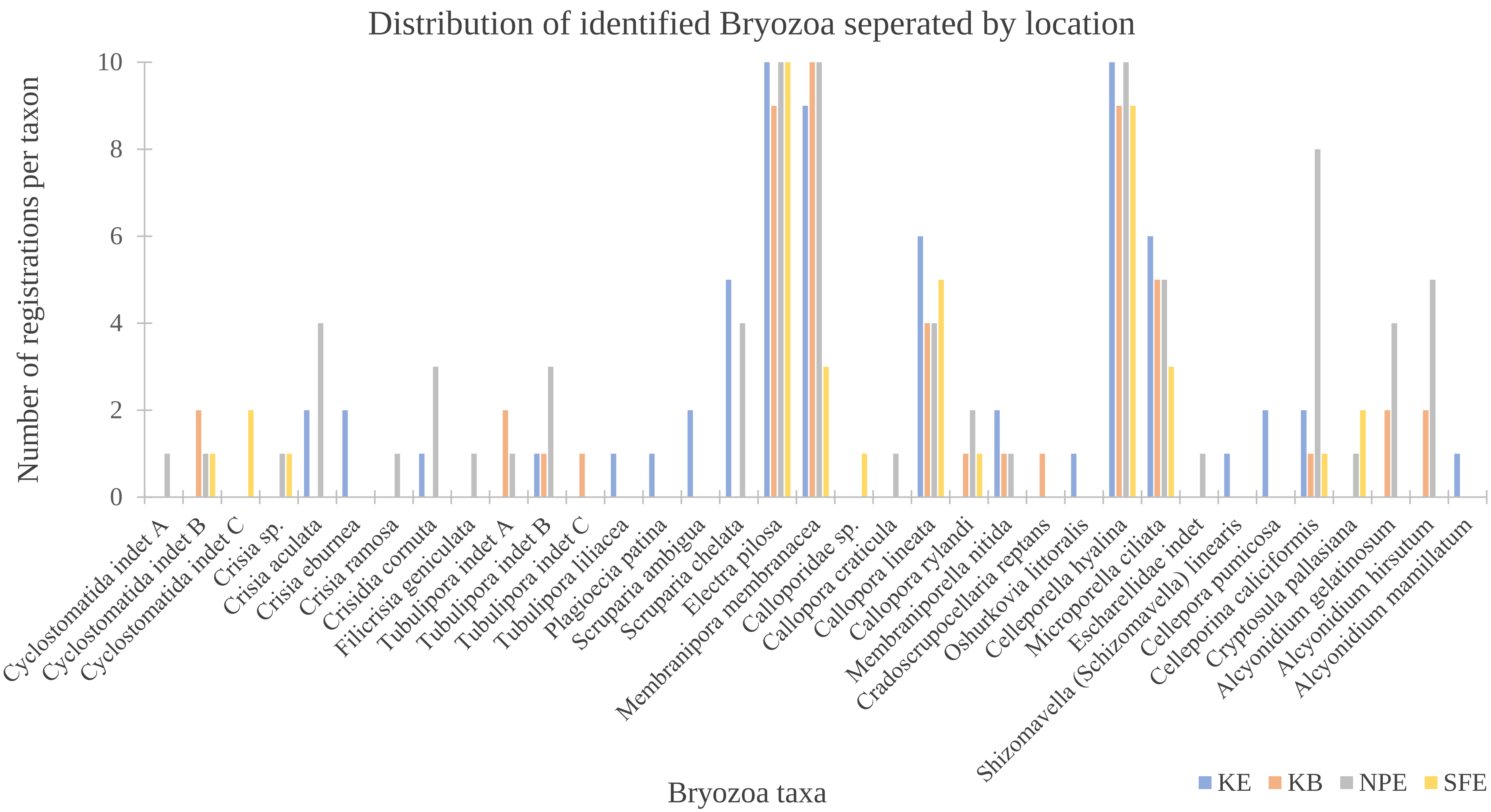

Figure A-1: Number of registered Bryozoa specimens on *Laminaria hyperborea* stipes, separated by location. *L. hyperborea* from both first and second sampling (N=10 per location). KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

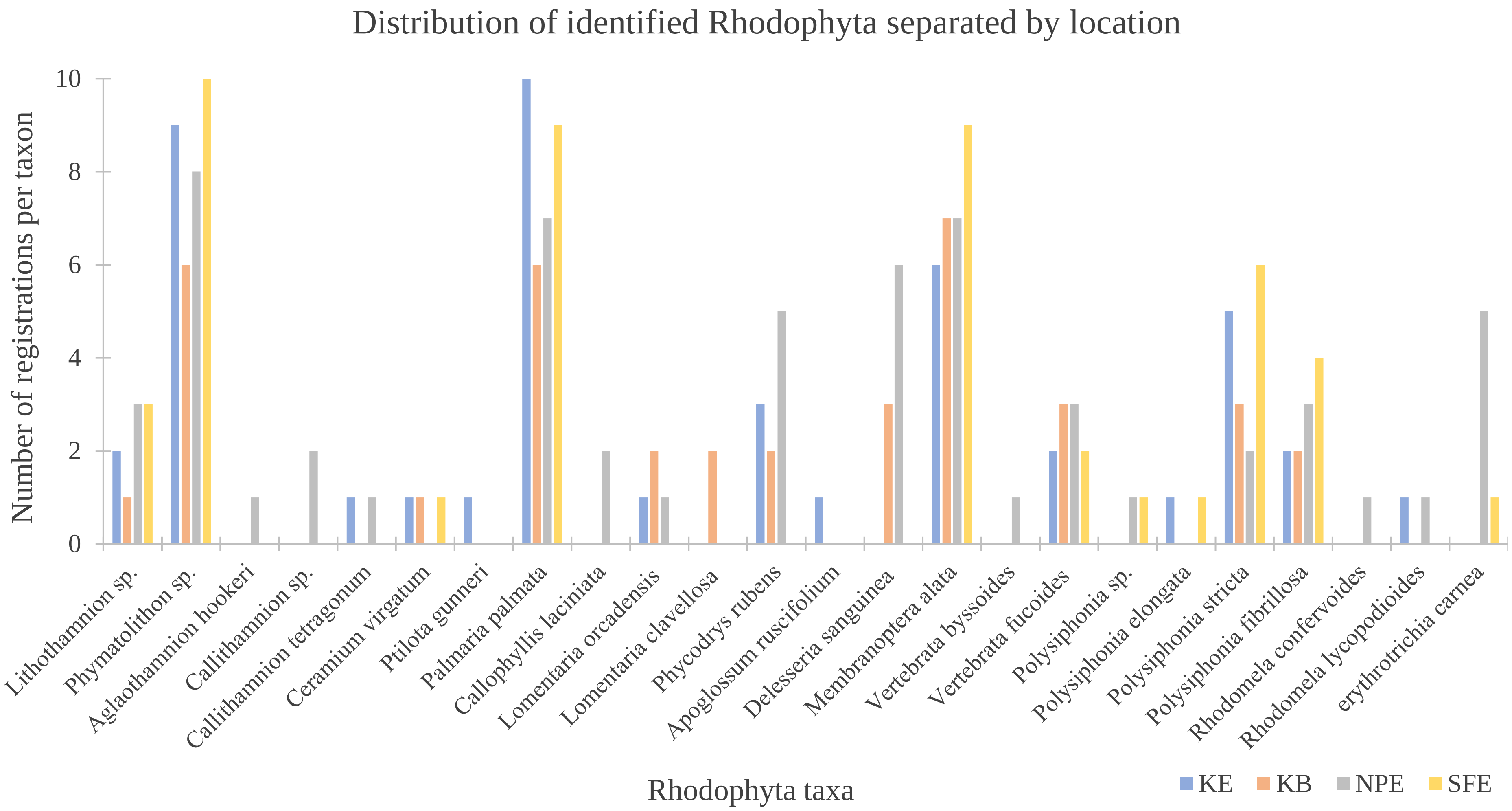

Figure A-2: Number of registered Rhodophyta specimens on *Laminaria hyperborea* stipes, separated by location. *L. hyperborea* from both first and second sampling (N=10 per location). KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen

Appendix B.

Table B-1: Overview of all identified taxa sorted by phyla, sampling round and location. Number indicates on how many of the five examined *Laminaria hyperborea* stipes the specific taxon were registered. KE: Kattholmen south, KB: Kattholmen north, NPE: Nordstøylholmen, SFE: Sauholmen.

PHYLUM	**TAXON**	**NUMBER OF TAXA ON *L. HYPERBOREA*, 1. SAMPLING**				**NUMBER OF TAXA ON *L. HYPERBOREA*, 2. SAMPLING**			
		KE	KB	NPE	SFE	KE	KB	NPE	SFE
Annelida	*Spirorbis (Spirorbis) spirorbis* (Linnaeus, 1758)	5	5	4	1				
Annelida	*Filograna implexa* (Berkeley, 1835)	2	1	2	1	1	1	3	
Annelida	*Hydroides norvegica* (Gunnerus, 1768)				1			1	3
Annelida	*Spirobranchus triqueter* (Linnaeus, 1758)	3	2	3	1	2	3	4	1
Annelida	*Circeis* sp. (Saint-Joseph, 1894)				5	2	5	5	5
Arthropoda	*Verruca stroemia* (O.F. Müller, 1776)			1				1	2
Arthropoda	*Balanus balanus* (Linnaeus, 1758)			1		1	1	4	
Porifera	*Porifera indet* (Grant, 1836)								1
Porifera	*Demospongiae* indet (Sollas, 1885)							1	
Porifera	*Sycon ciliatum* (Fabricius, 1780)						1	4	
Bryozoa	*Schizomavella (Schizomavella) linearis* (Hassall, 1841)	1							
Bryozoa	*Plagioecia patina* (Lamarck, 1816)	1							
Bryozoa	*Cryptosula pallasiana* (Moll, 1803)			1	2				
Bryozoa	*Crisia* sp. (Lamouroux, 1812)				1			1	
Bryozoa	*Scruparia ambigua* (d'Orbigny, 1841)	1				1			
Bryozoa	*Tubulipora liliacea* (Pallas, 1766)	1							
Bryozoa	*Cyclostomatida* indet A (Busk, 1852)							1	

PHYLUM	TAXON	NUMBER OF TAXA ON *L. HYPERBOREA*, 1. SAMPLING				NUMBER OF TAXA ON *L. HYPERBOREA*, 2. SAMPLING			
		KE	KB	NPE	SFE	KE	KB	NPE	SFE
Bryozoa	*Cyclostomatida* indet B (Busk, 1852)				1		2	1	
Bryozoa	*Cyclostomatida* indet C (Busk, 1852)				2				
Bryozoa	*Tubulipora* indet A (Lamarck, 1816)		2					1	
Bryozoa	*Tubulipora* indet B (Lamarck, 1816)	1	1	1				2	
Bryozoa	*Tubulipora* indet C (Lamarck, 1816)		1						
Bryozoa	*Membranipora membranacea* (Linnaeus, 1767)	4	5	5		5	5	5	3
Bryozoa	*Alcyonidium mamillatum* (Alder, 1857)	1							
Bryozoa	*Electra pilosa* (Linnaeus, 1767)	5	4	5	5	5	5	5	5
Bryozoa	*Cellepora pumicosa* (Pallas, 1766)	2							
Bryozoa	*Celleporella hyalina* (Linnaeus, 1767)	5	4	5	4	5	5	5	5
Bryozoa	*Calloporidae* sp. (Norman, 1903)								1
Bryozoa	*Callopora rylandi* (Bobin & Prenant, 1965)				1		1	2	
Bryozoa	*Callopora lineata* (Linnaeus, 1767)	3	1		1	3	3	4	4
Bryozoa	*Callopora craticula* (Alder, 1856)							1	
Bryozoa	*Alcyonidium gelatinosum* (Linnaeus, 1761)						2	4	
Bryozoa	*Alcyonidium hirsutum* (Fleming, 1828)		2	5					
Bryozoa	*Celleporina caliciformis* (Lamouroux, 1816)			3	1	2	1	5	
Bryozoa	*Crisia aculeata* (Hassall, 1841)	1		1		1		3	
Bryozoa	*Scruparia chelata* (Linnaeus, 1758)					5		4	
Bryozoa	*Crisia eburnea* (Linnaeus, 1758)					2			
Bryozoa	*Microporella ciliata* (Pallas, 1766)	2			1	4	5	5	3
Bryozoa	*Membraniporella nitida* (Johnston, 1838)					2	1	1	
Bryozoa	*Oshurkovia littoralis* (Hastings, 1944)					1			

PHYLUM	TAXON	NUMBER OF TAXA ON *L. HYPERBOREA*, 1. SAMPLING				NUMBER OF TAXA ON *L. HYPERBOREA*, 2. SAMPLING			
		KE	KB	NPE	SFE	KE	KB	NPE	SFE
Bryozoa	*Escharellidae* indet (Levinsen, 1909)							1	
Bryozoa	*Crisia ramosa* (Harmer, 1891)							1	
Bryozoa	*Crisidia cornuta* (Linnaeus, 1758)					1		3	
Bryozoa	*Cradoscrupocellaria reptans* (Linnaeus, 1758)						1		
Bryozoa	*Filicrisia geniculata* (d'Orbigny, 1853)							1	
Chlorophyta	*Cladophora rupestris* ((Linnaeus) Kützing, 1843)	1	2	2		2		4	
Chlorophyta	*Bryopsis plumosa* ((Hudson) C.Agardh, 1823)					2		1	
Chlorophyta	*Ulva lactuca* (Linnaeus, 1753)		2	1	3			1	2
Chlorophyta	*Cladophora* sp. (Kützing, 1843)								3
Chlorophyta	*Codium fragile* ((Suringar) Hariot, 1889)								1
Chordata	*Botryllus schlosseri* (Pallas, 1766)	2		2				2	
Chordata	*Botrylloides leachii* (Savigny, 1816)	3	1				1		
Chordata	*Ascidia mentula* (Müller, 1776)					1	2	3	
Chordata	*Molgulidae* sp. (Lacaze-Duthiers, 1877)							1	
Chordata	*Ascidiidae* indet A (Herdman, 1882)	1							
Chordata	*Polyclinidae* indet B (Milne Edwards, 1841)							1	
Chordata	*Polyclinidae* indet A (Milne Edwards, 1841)							1	
Chordata	*Ascidiidae* indet B (Herdman, 1882)			2					
Cnidaria	*Obelia geniculata* (Linnaeus, 1758)			3		1		2	
Cnidaria	*Campanularia volubilis* (Linnaeus, 1758)		2	2		3	2		
Cnidaria	*Hydroidolina* indet (Collins, 2000)			1	1	1			
Cnidaria	*Clytia hemisphaerica* (Linnaeus, 1767)					1	1		

PHYLUM	**TAXON**	**NUMBER OF TAXA ON *L. HYPERBOREA*, 1. SAMPLING**				**NUMBER OF TAXA ON *L. HYPERBOREA*, 2. SAMPLING**			
		KE	KB	NPE	SFE	KE	KB	NPE	SFE
Cnidaria	*Clytia paulensis* (Vanhöffen, 1910)				1				
Cnidaria	*Gonothyraea loveni* (Allman, 1859)						1		
Cnidaria	*Diphasia attenuata* (Hincks, 1866)							1	
Cnidaria	*Orthopyxis integra* (MacGillivray, 1842)			1					
Mollusca	*Heteranomia squamula* (Linnaeus, 1758)	4	2	1	3		2	3	
Ochrophyta	*Ochrophyta* sp. A (Cavalier-Smith, 1995)		1	1	2				
Ochrophyta	*Ectocarpales* sp. (Besey, 1907)	1	1	2	1		1	1	
Ochrophyta	*Laminaria* sp. (J.V. Lamouroux, 1813)		1		1	2	3	4	2
Ochrophyta	*Saccorhiza polyschides* ((Lightfoot) Batters, 1902)				2				
Ochrophyta	*Desmarestia viridis* ((O.F.Müller) J.V.Lamouroux, 1813)				1				
Ochrophyta	*Saccharina latissima* ((Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, 2006)				1				
Ochrophyta	*Laminaria hyperborea* ((Gunnerus) Foslie, 1884)	1		1	3		1	2	
Ochrophyta	*Sphacelaria* indet (Lyngbye, 1818)							1	
Ochrophyta	*Desmarestia aculeata* ((Linnaeus) J.V.Lamouroux, 1813)	4		3	2	2		4	
Ochrophyta	*Laminaria digitata* ((Hudson) J.V. Lamouroux, 1813)		1						
Rhodophyta	*Phycodrys rubens* ((Linnaeus) Batters, 1902)	3	2	1				4	
Rhodophyta	*Delesseria sanguinea* ((Hudson) J.V.Lamouroux, 1813)		1	2			2	4	
Rhodophyta	*Membranoptera alata* ((Hudson) Stackhouse, 1809)	4	3	5	5	2	4	2	4
Rhodophyta	*Palmaria palmata* ((Linnaeus) Weber & Mohr, 1805)	5	2	5	4	5	4	2	5
Rhodophyta	*Vertebrata fucoides* ((Hudson) Kuntze, 1891)	2	3	2				1	2
Rhodophyta	*Phymatolithon* sp. (Foslie, 1898)	5	3	4	5	4	3	4	5
Rhodophyta	*Polysiphonia stricta* ((Dillwyn) Greville, 1824)	4	3	2	1	1			5

PHYLUM	TAXON	NUMBER OF TAXA ON *L. HYPERBOREA*, 1. SAMPLING				NUMBER OF TAXA ON *L. HYPERBOREA*, 2. SAMPLING			
		KE	KB	NPE	SFE	KE	KB	NPE	SFE
Rhodophyta	*Polysiphonia fibrillosa* ((Dillwyn) Sprengel, 1827)	1	1	2	4	1	1	1	
Rhodophyta	*Ptilota gunneri* (P.C.Silva, Maggs & L.M.Irvine, 1993)					1			
Rhodophyta	*Ceramium virgatum* (Roth, 1797)				1	1	1		
Rhodophyta	*Apoglossum ruscifolium* ((Turner) J.Agardh, 1898)	1							
Rhodophyta	*Polysiphonia elongata* ((Hudson) Sprengel, 1827)	1							1
Rhodophyta	*Lithothamnion* sp. (Heydrich, 1897)		1	1	1	2		2	2
Rhodophyta	*Erythrotrichia carnea* ((Dillwyn) J.Agardh, 1883)				1			5	
Rhodophyta	*Callithamnion* sp. (Lyngbye, 1819)							2	
Rhodophyta	*Aglaothamnion hookeri* ((Dillwyn) Maggs & Hommersand, 1993)							1	
Rhodophyta	*Callithamnion tetragonum* ((Withering) S.F.Gray, 1821)					1		1	
Rhodophyta	*Callophyllis laciniata* ((Hudson) Kützing, 1843)			2					
Rhodophyta	*Lomentaria orcadensis* ((Harvey) F.S.Collins, 1937)					1	2	1	
Rhodophyta	*Lomentaria clavellosa* ((Lightfoot ex Turner) Gaillon, 1828)						2		
Rhodophyta	*Polysiphonia* sp. (Greville, 1823)			1					1
Rhodophyta	*Rhodomela confervoides* ((Hudson) P.C.Silva, 1952)							1	
Rhodophyta	*Vertebrata byssoides* ((Goodenough & Woodward) Kuntze, 1891)			1					
Rhodophyta	*Rhodomela lycopodioides* ((Linnaeus) C.Agardh, 1822)					1		1	
Unknown	Unidentified species		1		3	1	1		3
	Total number of taxa at location	81	61	87	73	79	76	143	69
	Average number of taxa on *Laminaria hyperborea* stipe	16.2	12.2	17.4	14.6	15.8	15.2	28.6	13.8

Appendix C.

Table C-1: Boxplot values of the number of taxa on *Laminaria hyperborea* stipe at the different locations. KB: Kattholmen north, KE: Kattholmen south, NPE: Nordstøylholmen, SFE: Sauholmen.

Location	KB	KE	NPE	SFE
Sample size	10	10	10	10
Min	9.00	11.00	11.00	7.00
Lower quartile	10.50	13.25	18.00	11.75
Median	13.00	14.50	22.00	15.50
Upper quartile	14.50	19.50	27.75	16.75
Max	25.00	23.00	31.00	20.00

Table C-2: Boxplot values of the number of taxa on *Laminaria hyperborea* stipe at different location characteristics; exposed vs. protected and impacted vs. not impacted. Exposed includes Kattholmen south, Nordstøylholmen and Sauholmen, protected includes Kattholmen north. Impacted includes Nordstøylholmen and Sauholmen, not impacted includes Kattholmen north and Kattholmen south.

Location characteristics	Exposed	Protected	Impacted	Not impacted
Sample size	30	10	20	20
Min	7.00	9.00	7.00	9.00
Lower quartile	14.00	10.50	14.75	12.00
Median	16.00	13.00	17.00	13.50
Upper quartile	20.75	14.50	22.00	17.25
Max	31.00	25.00	31.00	25.00