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Macronutrient Distribution in Relation to Waste Emission from Aquaculture Activities

A field study in Trondheimsfjorden

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Sammendrag

Den globale produksjonen av oppdrettsfisk har siden 1980-tallet nesten 12-doblet seg. I 2010, nådde produksjonen av oppdrettsfisk en ”all-time high” med 60 millioner tonn matfisk. Under produksjonen frigjøres store mengder ammonium (NH_4) og fosfat (PO_4) til det marine kystvannet i den grad at det kan være til skade for omgivelsene. Den økte tilførselen av makronæringsstoffer til systemet kan føre til overgjødsling (eutrofiering) og endringer i forholdet mellom næringsstoffer. Dette kan igjen føre til forandringer i planteplankton sammensetningen som kan lede til endringer i næringskjedens struktur. Dessverre er kunnskapen og forståelsen for hvordan næringsstoffer og organisk avfall fra akvakultur distribueres og påvirker økosystemer fortsatt dårlig.

Målet med dette arbeidet har vært å få en bedre forståelse for hvordan makronæringsstoffer distribueres i sjøvannet rundt fiskemerder. Dette er gjort ved å sammenligne konsentrasjonen av NH_4, PO_4, NO_3 og SiO_3 målt på stasjoner som ligger nær oppdrettsanlegg med referanse konsentrasjoner målt på stasjoner som har liten eller ingen påvirking av oppdrettsanleggene. Prøvene ble samlet inn i Trondheimsfjorden i løpet av senvinteren (februar) og tidlig på våren (april), for å kunne sammenligne sesongvariasjoner i makronæringsstoffkonsentrasjoner. Prøvene ble analysert ved hjelp av en Auto-Analyser for NH_4, PO_4 og NO_3 konsentrasjoner, og manuelt for SiO_3 konsentrasjon.

Resultatene viser en betydelig økning i NH_4 konsentrasjon om våren. PO_4, NO_3 og SiO_3 konsentrasjonene avtar som forventet, som følge av den målbare økningen i klorofyll konsentrasjon. I løpet av det andre toktet (vårsesongen), ble ammonium konsentrasjoner opp til 18μg L^{-1} målt, noe som er en betydelig økning sammenlignet med første tokt (vintersesongen) hvor alle målte ammonium konsentrasjoner var lavere enn 10μg L^{-1}. Ammoniumkonsentrasjonene i disse farvannene kan derfor betraktes som betydelige, og utslipp fra akvakultur kan være en potensiell kilde til ammoniumet. Men på dette stadiet i prosjektet, er det ikke mulig å gjøre noen konklusjoner med hensyn til hvorvidt disse ammoniumkonsentrasjonene er et resultat av akvakultur.

Abstract

The production of farmed fish has increased globally almost 12-fold since the 1980s. And in 2010, aquaculture production food fish reached an all-time high at 60 million tonnes. This activity releases large amounts of ammonium (NH_4) and phosphate (PO_4) in marine waters, to the extent that it could be endangering the surrounding environment. The increase in the supply of macronutrients to the system could lead to eutrophication and changes in nutrient ratios. This could in turn cause a shift in phytoplankton composition and changes in the food web structure. However, at present, the understanding of how nutrients and organic waste from aquaculture systems are distributed and influence the ecosystems is still poor.

The aim of this work has been to gain a better understanding of the distribution of macronutrients around aquaculture cages, by comparing the concentration of NH_4, PO_4, NO_3 and SiO_3 measured at stations located near aquaculture with reference concentrations measured at stations where no or very little aquaculture activities are present. Samples were collected in Trondheimsfjorden during late winter (February) and early spring (April), to compare seasonal variations in macronutrient concentrations. The samples were analysed with an Auto-Analyser for NH_4, PO_4 and NO_3 concentrations, and manually for SiO_3 concentration.

Results show a considerable increase in NH_4 concentration in spring. PO_4, NO_3 and SiO_3 concentrations decreases as expected, due to a measurable increase in phytoplankton concentration. During the second cruise (spring season), ammonium concentrations up to 18µg L^{-1} were measured, which is a significant increase compared to the first cruise (winter season) when all ammonium concentrations measured were lower than 10µg L^{-1}. The ammonium concentrations in these waters can thus be regarded as considerable, and a potential source of the ammonium could be emissions from aquaculture. However, at this stage in the project, it is not possible to make any conclusions as to whether or not these ammonium concentrations are a result of aquaculture activities.

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Table of Contents

1. Introduction

Norway's long coastline with its cold, fresh seawater provides excellent conditions for aquaculture activities. Since the establishment of the modern fish farming industry in the 1970s the production of farmed fish has risen steeply, and today, Norway is one of the largest producers and exporters of farmed fish worldwide (Ministry of Fisheries and Coastal Affairs, 2013). This has led to an extensive use of farming locations along the coast. Maintaining a sustainable industry and a healthy environment will require identification and monitoring of areas that can have negative effects on the local environment (Maroni, 2000, Ministry of Fisheries and Coastal Affairs, 2009).

Waste from marine aquaculture cages is released directly into the environment. This waste contains large amounts of dissolved inorganic nutrients (DIN) from fish excreta (NH_4 and PO_4), particulate organic nutrients from feces, and dissolved organic nutrients from resuspension of the particulate fractions. The majority of the nitrogen wastes are released to the open waters in the form of NH_4, whereas the majority of phosphorus accumulates in sediments (Olsen and Olsen, 2008). Release of the dissolved inorganic nutrients has been of a particular concern because of their ability to cause changes and fluctuations in seawater nutrient concentrations. This can produce undesirable effects in the ecosystem (Iriarte et al., 2010), such as eutrophication and changes in the stoichiometric ratio of nutrients, which can alter the structure of phytoplankton communities. These changes can have further consequences for both ecosystem structure and function, e.g. the food chain (Iriarte et al., 2010, Justić et al., 1995, Mente et al., 2006, Olsen and Olsen, 2008) and the biogeochemical cycling of elements (Moore et al., 2013).

Microorganisms are responsible for approximately half of earth's primary production. The majority of this is accounted for by phytoplankton, which assimilate macronutrients to organic molecules through photosynthesis. The oceanic cycles of nutrients such as nitrogen and phosphorus (and carbon) are thus closely coupled through the metabolic requirements of marine phytoplankton (Arrigo, 2005, Moore et al., 2013, Morel and Price, 2003, Weber and Deutsch, 2010). There are, essentially, two types of primary production. The first is referred to as "regenerated production". This type of primary production is fuelled by ammonium, which is returned to the water column as nitrogenous organic molecules are metabolized end excreted by marine by organisms. However, due to nutrient losses, the regenerated nitrogen is not sufficient to support primary production alone. The remaining nutrient supplies are termed "new", and this second type of primary production is therefore referred to as "new production". The new production takes place through upward fluxes of nitrate from deeper water and by nitrogen input from terrestrial and atmospheric sources (Eppley and Peterson, 1979, Sakshaug et al., 2009a). It is this "new production" which can potentially be affected by the high nutrient emissions of aquaculture activities.

For marine and estuarine phytoplankton, nitrogen is often considered as the limiting nutrient in production of organic matter (Kennish, 2001, Libes, 2009, Zehr and Ward, 2002). Most microorganisms are able to use nitrogen in the form of both nitrate, nitrite, and ammonium.

However, studies have shown that some species will prefer one nitrogen source over the other (Zehr and Ward, 2002)), and many species prefer the less energetically costly ammonium (Dortch, 1990). Aquaculture cages actually release a majority of the nitrogen as ammonium, leading to a possible shift in available nitrogen in the surrounding waters from nitrate to ammonium. This change in the NH_4:NO_3 ratio can be expected to lead to changes in species composition of the phytoplankton communities, and possibly increased algae growth and biomass production (Olsen and Olsen, 2008).

Phytoplankton can use nitrate as a nitrogen source through a sequential reduction from nitrate to nitrite (NO_2) to ammonium (NH_4). This reduction, like all nitrogen transformations, involves the use of metalloenzymes. The metal availability in the seawater can thus limit crucial steps in the nitrogen cycle and affect all general metabolic processes of phytoplankton. When excess ammonium is released into the environment the need for and thereby the uptake of trace metals may therefore be affected (Morel and Price, 2003, Stumm and Morgan, 1996).

Nutrient discharges in Norwegian coastal waters have increased significantly since the 1990s. Much of this increase is due to the growth of the aquaculture industry, and there is reason to believe that this increase will continue unless more environmentally friendly methods of operation are developed and used (Klima- og forurensningsdirektoratet, 2012). A concern is that, at present, there is not an agreement on how nutrients and organic wastes from aquaculture systems are distributed and influence ecosystems. There is also limited knowledge of how these nutrients and organic matter affect the structure and function of the ecosystem (CINTERA, 2011).

The work presented in this thesis is part of the ongoing CINTERA project – *a Cross-disciplinary Integrated Eco-systemic Eutrophication Research and Management Approach*. The project aims to improve our knowledge of ecosystem responses to eutrophication caused by aquaculture activities. This cross-disciplinary project will study marine fjord ecosystems in both Norway and Chile (CINTERA, 2011). The research began with the WAFOW project "*Can Waste Emission from Fish Farms Change the Structure of Marine Food Webs? A comparative study of coastal ecosystems in Norway and Chile*". During this project, three mesocosm experiments were carried out, two in Chile and one in Norway (Olsen et al., 2006). The objective of the WAFOW project was to create conditions simulating the nutrient enrichment occurring in fjord ecosystems caused by aquaculture, in order to evaluate the capacity of the marine community to assimilate the incoming nutrient waste (Hunnestad, 2012). The CINTERA project is a continuation of this research. The next step for the project is the study of real life conditions. The work presented in this thesis is the result of two cruises carried out in fjord systems outside of Trøndelag. In these fjords, aquaculture is a well established industry and can thus provide good research locations. The first cruise was conducted in February 2013, collecting water samples from winter conditions, and the second one in April 2013, collecting water samples from early spring conditions.

1.1 Objective

The main objective of this master thesis has been to gain a better understanding of the distribution of macronutrients around aquaculture cages, by comparing the concentration of NH_4, PO_4, NO_3 and SiO_3 measured at stations located near aquaculture with reference concentrations measured at stations where no or very little aquaculture activities are present. Samples were collected during late winter (February) and early spring (April), to compare seasonal variations in macronutrient concentrations

While the focus of this work has been the distribution of macronutrients, it is part of a larger project. I have therefore worked closely together with two other master students whose focus has been the distribution of bioactive micronutrients (Horgheim, 2013) and the biological aspect of the ecosystem (Skrove, Unpublished).

2. Background and theory

2.1 Aquaculture

Definition of aquaculture used by FAO (Food and Agriculture Organization of the United Nations) is

"Aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture"(FAO, 2012).

Aquaculture in Norway has an extensive history, dating back to the 1850s. But the technological breakthrough for salmonid farming came first in the 1970s when sea-based cages were introduced. Growing salmon in these cages proved to be successful already within the first year, and they were quickly spread along the entire coast (Gjedrem, 1993). The cages, which have a continuous water exchange with the surrounding waters, continued to expand significantly over the next decades, causing the production of farmed fish to rise steeply (Skogen et al., 2009, Wang et al., 2012). Today, Norway is a leading producer and exporter of farmed fish worldwide (Ministry of Fisheries and Coastal Affairs, 2013).

The main areas for marine aquaculture in Norway are the many fjords along the coast (Skogen et al., 2009). The environmental conditions these fjords provide, along with good seawater quality, have been important in managing a successful industry (Ministry of Fisheries and Coastal Affairs, 2009). However, the extensive use of farming locations has led to a concern regarding the environmental impacts of fish farms (Buschmann et al., 2006), and the concerns increasing as the global aquaculture is developing rapidly (Troell et al., 2009). Fish production in cages is shown to have a measurable impact on the water column, which is caused by the release of organic waste and inorganic nutrients that are generated in the production process (Soto and Norambuena, 2004, Wang et al., 2012). The ecological impact of aquaculture is, however, dependent on the recipient waters capacity to assimilate the nutrients which are released (Wang et al., 2012), the general characteristics of the surrounding environment and of course the operation of the site. Farms located at sites with good water circulation will have reduced risk of accumulating waste below the cages (Soto and Norambuena, 2004), and thus exert less effect on the environment.

One of the major challenges aquaculture faces today is sustaining a continued increase in fish production while minimizing the environmental impact (Cheshuk et al., 2003, Navarrete-Mier et al., 2010, Sugiura et al., 2006, Wang et al., 2012). In the course of half a century aquaculture has expanded from being almost negligible to fully comparable with capture production when it comes to feeding people. World food fish production by aquaculture has expanded almost 12-fold since the 1980s, and in 2010 aquaculture production reached an all-time high at 60 million tonnes (excluding plants and non-food products). The global capture

fisheries production has been kept stable since 2006 at about 90 million tonnes per year (FAO, 2012).

In 2010, FAO recorded 181 countries and territories with aquaculture production (FAO, 2012). This rapid growth of aquaculture globally may face limitations in both availability of suitable sites and in the ecological carrying capacity of already existing sites (Troell et al., 2009). But the reduction or preventing of aquaculture production is no option as long as the demand for aquaculture products is increasing (Sugiura et al., 2006) and the livelihoods of many million people are depending on it. Fish and fish products represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health, and in 2009, fish accounted for 16,6 percent of the world populations intake of animal protein. (FAO, 2012).

The aquaculture industry has taken measures to reduce the release of nutrient waste and its impacts on the local environment, including improving feed composition and digestibility, improved feeding technology and site rotation (Cheshuk et al., 2003, Wang et al., 2012). But are these efforts enough? Even though less nutrients are released per kilo fish produced, the overall discharges have increased due to increase in total fish production (Skogen et al., 2009).

In Europe, Norway is the top aquaculture producer, responsible for about 40 % of the production. This makes Norway the seventh largest producer in the world. In addition, Norway is the second largest exporter. China tops both of these lists (FAO, 2012).

2.1.1 Environmental impacts of aquaculture

The production of farmed fish involves more and more the use of water-based enclosures. These cage aquaculture systems (CAS) are essentially open systems and are thus characterized by a high degree of interaction with the surrounding environment. Unlike the conventional land-based aquaculture systems, they discharge their waste directly into the environment (Islam, 2005). With aquaculture being the major source of anthropogenic nutrients to the Norwegian coastal waters (Skogen et al., 2009), there has been an increased awareness that this industry may have a considerable impact on the marine and nearshore ecosystems (Islam, 2005). This includes changes in the benthic communities, increased nutrient loads in coastal waters and the associated problems of algal blooms (Buschmann et al., 2006), oxygen depletion and silting (OSPAR, 2000). The pelagic ecosystems have an inherent capacity of persistence. Smaller changes in nutrient input are moderated through adaptive responses. There is, however, an upper assimilation capacity above which pelagic ecosystems lose integrity. This capacity is mediated by two mechanisms: the incorporation of nutrients in organisms and a dilution process driven by hydrodynamics (Olsen and Olsen, 2008). At present there is no scientific concept agreed upon for understanding how nutrients and organic waste from aquaculture systems distribute and accumulate in ecosystems (CINTERA, 2011).

2.1.2 The release of nutrients

The quantities of nutrients discharged from aquaculture are often calculated as the difference between feed used and the estimated production of fish biomass. Such data can however only provide an indication of the scale of nutrients released (Klima- og forurensningsdirektoratet, 2012, OSPAR, 2000). Wang et al (2012) quantified the release rates of carbon (C), nitrogen (N) and phosphorus (P) waste from Norwegian salmon farms in 2009. Of the total feed input, 70% C, 62% N and 70% P were released back to the environment as inorganic and organic waste, corresponding to 397, 50 and 9.3 kg C, N and P, respectively, t^{-1} WW of fish produced. With a total salmon production 1.02 x 10^6 t in 2009, the annual discharge of C, N and P is equivalent to about 404 000, 50 600 and 9 400 t, respectively (Wang et al., 2012). This is a substantially increase from 1990 when the annual discharge of N and P were about 7000 and 1500 t, respectively (Klima- og forurensningsdirektoratet, 2012).

In Figure 1, the different sources responsible for nitrogen and phosphorus emissions in Norwegian waters are shown.

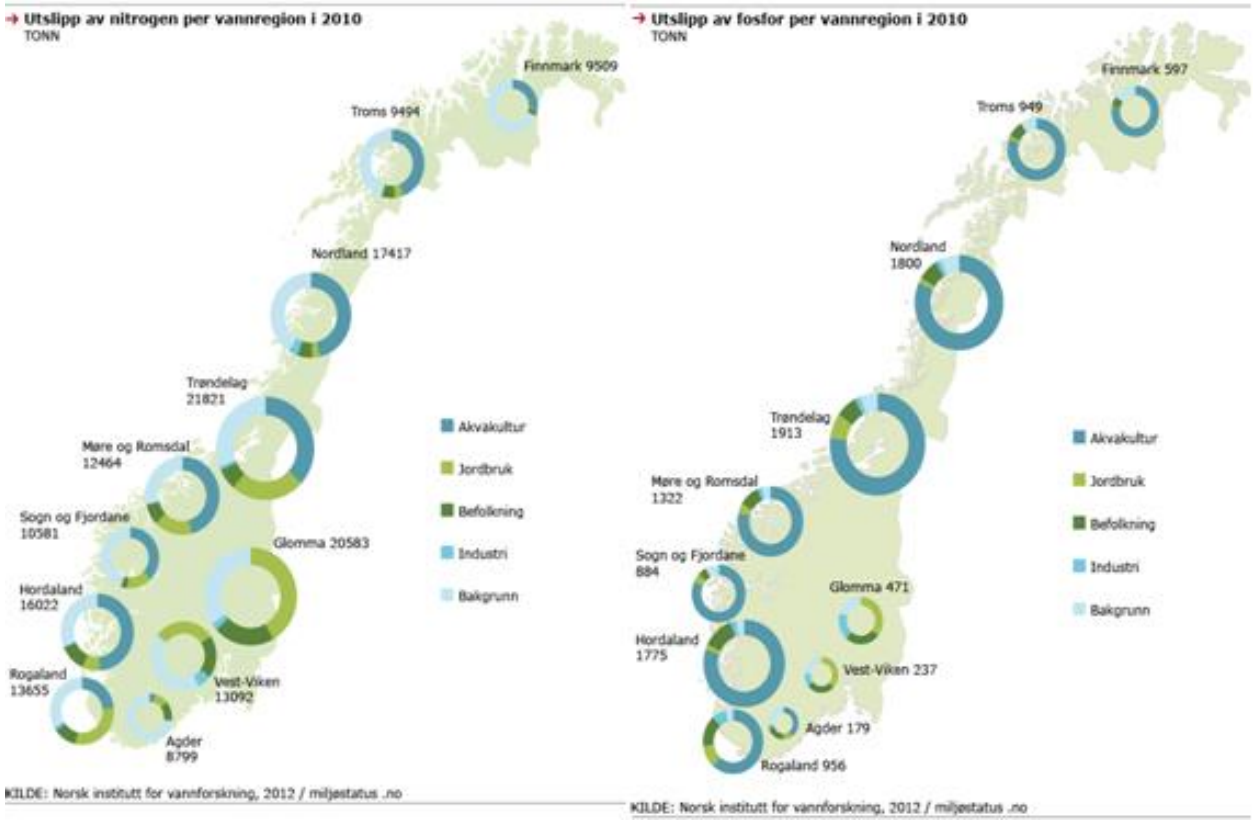

Figure 1. Sources responsible for nitrogen and phosphate emissions in Norwegian waters (Klima- og forurensningsdirektoratet, 2012)

Cage systems will discharge high organic and nutrient loadings generated from feed waste, excretion and faecal productions directly into the environment. The amount of waste will depend on factors such as stocking density, feeding regime and feeding rate (Islam, 2005), and time of year. The fish will grow most during summer, and that is also when we will get the highest emissions (Havforskningsinstituttet, 2012, Wang et al., 2012). The discharged nutrients are spread in the direction of the current and taken up biologically within a few hundred metres to a few kilometres of the point of release, to the extent than an increase in concentration are no longer detected. In large areas of water there is also a powerful dilution effect, and a further reduction is possible through the binding of phosphorus to sediment (OSPAR, 2000). During feeding, pulses of nitrogenous compounds, mainly ammonium, are detected near the cages (Havforskningsinstituttet, 2012).

2.1.3 Eutrophication

In many parts of the world, high levels of nutrients such as nitrate, nitrite, ammonia and reactive phosphate have been reported near aquaculture zones (Islam, 2005). These nutrients, although essential for microalgae growth, can result in undesirable effects for the ecosystems when changes and fluctuations in concentrations occurs (Iriarte et al., 2010). Some waters, including fjords, are often nutrient poor and low-productive. Significant inputs of nutrients from fish farming activities can lead to eutrophication of the ecosystem (Havforskningsinstituttet, 2012, Mente et al., 2006). Eutrophication results from excessive enrichment of the water with nutrients which may cause an accelerated growth of algae in the water column. This may result in disturbances in the marine ecosystems, including a shift in the composition of the flora and fauna communities, affecting the waters biodiversity, and the depletion of oxygen, causing death of fish and other species (OSPAR, 2000). This depletion of oxygen happens because oxygen is utilized during algae decomposition. During algae blooms the oxygen level can become so low that the sea floor is left dead and the livelihoods of many species lost, resulting in a reduced biodiversity (Miljødirektoratet, 2012).

Fertilization of coastal ecosystems caused by salmon farming and other human activities is now a serious environmental problem as it stimulates plant growth and disrupts the balance between the production and metabolism of organic matter in the coastal zone (Cloern, 2001).

2.1.4 Stoichiometric changes

Increased concentrations of nutrients are not the only result of aquaculture. The stoichiometric ratios of nutrients, N:P, Si:N, and Si:P, can also be changed (Justić et al., 1995). Fish farms contribute dissolved N and P to the environment, but not silicic acid (Mente et al., 2006). This can lead to stoichiometric changes in the surrounding water. The atomic Si:N:P ratio of marine diatoms, which are abundant constituents of coastal phytoplankton, is about 16:16:1, when nutrient levels are sufficient. Deviations from this ratio in nutrients have been used to explain shifts in the composition of phytoplankton assemblage. The new conditions favouring the growth of certain phytoplankton whilst limiting the growth of others. For example may silicic acid limitation result in a shift towards high flagellate to diatoms ratios (Iriarte et al.,

2010, Justić et al., 1995, Mente et al., 2006). This reduction of diatom growth in favour of the noxious flagellates may exacerbate eutrophication. Long term silic acid limitations are also associated with significant blooms of non-siliceous algae in coastal waters (Justić et al., 1995).

The release of ammonium through excretion (ammonium being a natural byproduct of fish metabolism) and the decay of uneaten feed can even lead to changes in the NH4:NO3 ratio. Phytoplankton which are important regulators of ammonium concentrations through nitrogen uptake, are affected by these fluctuations (Hargreaves, 1998) through species preferences (Dortch, 1990). Although it is assumed that most microorganisms can use inorganic nitrogen in the form of nitrate, nitrite, and ammonium, studies have shown that some species prefer one nitrogen source over the other (Zehr and Ward, 2002). For example will some phytoplankton prefer the less energetically costly ammonium over nitrate (Dortch, 1990) which has to be enzymatically reduced to ammonium within the cell (Hargreaves, 1998, Zehr and Ward, 2002). This preference means that ammonium is more readily utilized than nitrate, and this preference is independent of the ammonium concentration. Although uptake or growth on the preferred nitrogen source would be expected to be greater, uptake and growth on other nitrogen sources still occur for most phytoplankton, sometimes at rapid rates and independent of the concentration of the preferred nitrogen source (Dortch, 1990).

Still, changes in the NH4:NO3 ratio can be expected to lead to changes in the species composition of phytoplankton communities due to this initial preference. Phytoplankton in the smaller size fractions (nano- and picoplankton) often have a higher preference for ammonium over nitrate than the larger fractions (macroplankton) (Dortch, 1990, Stolte et al., 1994, Wafar et al., 2004). The phytoplankton size distribution in a population will thus, to some degree, be dependent on the nitrogen source available and the preferences of the phytoplankton (Stolte et al., 1994).

2.2 Seawater chemistry - Oceanic nutrients

All living organisms require a wide range of nutrients for growth and maintenance. Phytoplankton, which are responsible for the vast majority of primary production in marine waters, will take up both macronutrients and micronutrients during photosynthesis and assimilate them into macromolecules, resulting in the formation of organic matter. These nutrients are important drivers of microbial activity, but at the same time, microorganisms play a major role in cycling nutrients in the oceanic system (Moore et al., 2013, Morel and Price, 2003).

2.2.1 Macronutrients

Macronutrients play an important role in controlling the growth of phytoplankton and marine plants. They are usually present in low concentrations in oceanic surface waters, but show increasing concentrations with depth. The macronutrients most often referred to are nitrogen, phosphorus and silicon (Brügmann and Kremling, 1999, Kennish, 2001). These nutrients all follow a seasonal cycle (Clarke and Leakey, 1996), but microorganisms also play an important role in the global cycling of the nutrients (Arrigo, 2005). Nitrate will most often play the role of the limiting nutrient, but sometimes phosphate can also limit production. Although other elements are needed as well, they will usually not limit the growth to a great extent (Kennish, 2001).

Silicon in seawater is present in both dissolved and particulate forms. The concentrations of both these forms vary with depth and location. Silicon is utilized by some phytoplankton for skeleton work. For example is silicon a major constituent of diatoms, which form a large proportion of the marine phytoplankton community (Hansen and Koroleff, 1999, Kennish, 2001). Silica fluxes have during the last decades remained rather constant, or even decreased, due to eutrophication. This has led to lower DSi:DIN and DSi:P ratios in estuaries and coastal regions. This can have consequences for the phytoplankton community structure, and have major impacts on the water quality (Voss et al., 2011).

Phosphorus is one of the key nutrient elements that, together with nitrogen and iron (Fe) can limit phytoplankton growth in marine environments. On a geological time scale, phosphorous is actually considered to be the ultimate limiting nutrient. The availability of phosphorus in the oceans depends on the balance between the input of biological availability P from rivers, sediments and the recycling in the system. Atmospheric inputs are generally unimportant. The distribution of dissolved inorganic phosphorus (DIP) in the water column is mainly determined by oceanic circulation patterns, temporal and spatial variability in biological activity and the rate of recycling (Voss et al., 2011). Phosphorus exists in the sea as ionized products of the phosphoric acid, H_3PO_4. Of these fractions, PO4 accounts for about 10% of the total inorganic phosphate (Hansen and Koroleff, 1999, Kennish, 2001).

Nitrogen occurs in the ocean in several bio-available forms. This includes simple ionic forms such as nitrate (NO3), nitrite (NO2) and ammonium (NH4), and more complex organic forms such as urea. Out of the three main macronutrients, nitrogen is usually thought of as *the* key nutrient limiting biological production of organic matter (Kennish, 2001, Libes, 2009, Zehr and Ward, 2002). The main source of nitrogen is the upward fluxes of rich deep water. During these fluxes there will also be an upwelling of phosphate and silicate. Physical forces and biological control are involved in this moving of nitrogen (Zehr and Ward, 2002). A considerable part of the nitrogen also enters seawater from the atmosphere, but the anthropogenic inputs in coastal waters are becoming increasingly significant (Kennish 2001, Libes, 2009).

Most of the nitrogen in seawater is in the form of N_2 (Hansen and Koroleff, 1999, Kennish, 2001, Libes, 2009). This nitrogen is biologically inaccessible except to some few microbes,

nitrogen fixers, which are able to assimilate and convert N_2 into more reactive compounds (Libes, 2009). About 10% of the total nitrogen in the ocean exists as inorganic and organic compounds (Hansen and Koroleff, 1999, Kennish, 2001). The dissolved inorganic ions, nitrate, nitrite, and ammonium, are commonly referred to as DIN (dissolved inorganic nitrogen) (Libes, 2009).

Off all the essential nutrients, nitrogen is the only one whose seawater concentration is clearly controlled biologically (Morel et al., 2006). Nitrogen is cycled through several oxidation-reduction reactions of nitrogenous compounds, primarily mediated by microorganisms. The result of these transformations is that nitrogen has a large number of naturally occurring oxidation states (Libes, 2009, Zehr and Ward, 2002). The availability of nitrogenous nutrients and biological productivity in the marine system will be controlled by this cycle (Zehr and Ward, 2002), but the cycle is itself affected by the availability of micronutrients. Low micronutrient concentration can limit critical steps in the cycle because all nitrogen transformations involve metalloenzymes (Morel and Price, 2003). Iron and molybdenum are essential metals in enzymes that mediate the reduction of nitrate and nitrite in phytoplankton, as well as the fixation of molecular nitrogen in some microorganism. Particularly iron has been recognized as a potential limiting element (Morel and Price, 2003).

2.2.2 Macronutrients and the state of the ecosystem

The ecosystems chemical condition is evaluated on the basis of background values for well established indicators, including nutrient concentrations. The Norwegian criteria for marine water quality related to nutrient concentrations are shown in Table 1. This Norwegian classification system (NCS) is based on nutrient concentration ("normalised" for salinity between 0-20) for winter and summer. Some fjords and coastal areas along the Norwegian coast have been classified according to this system (NCS) (Molvær et al., 2007), however, there have so far been little systematic long-term measurements of nutrient concentrations in Norwegian fjords from Rogaland and further north (Havforskningsinstituttet, 2012).

Table 1. The Norwegian classification critera for nutrients. Surface water have different summer and winter values (Molvær et al., 2007)

		Classes				
	Parameters	**I**	**II**	**III**	**IV**	**V**
		Very good	**Good**	**Fair**	**Bad**	**Bad**
Summer	PO4 µg P/L	<4	4-7	7-16	16-50	>50
(jun-aug)	NO3 µg N/L	<12	12-23	23-65	65-250	>250
	NH4 µg N/L	<19	19-50	50-200	200-325	>325
Winter	PO4 µg P/L	<16	16-21	21-34	34-50	>50
(des-feb)	NO3 µg N/L	<90	90-125	125-225	225-350	>350
	NH4 µg N/L	<33	33-75	75-155	155-325	>325

The environmental authority's standard values for DIN (NH4+NO3+NO2) and DIP (PO4) in Norway are set to 140 and 19 μg L^{-1}, respectively (Olsen et al., 2012).

2.2.3 Macronutrient concentrations in Norwegian studies

Nutrient concentrations measured in two studies are presented in Table. These studies can provide some data on the macronutrient concentrations measured in Norwegian coastal waters. The first study was conducted in 1997 in Hopavågen, a landlocked coastal embayment in central Norway (Öztürk 2003). The second study was conducted in Nordmøre during 2011-2012 (miljødokumentasjon nordmøre). Values provided from the studies are converted to μg L^{-1} where necessary.

Table 2. Macronutrient concentrations obtained during two studies conducted in central Norway. Values are converted to μg L^{-1} where necessary, and some are rendered as less then (<) where exact values are not provided in the studies (Öztürk et al., 2003, Olsen et al., 2012)

	Hopavågen		Nordmøre	
	Winter	Summer	Winter	Summer
NO3 (μg/L)	max 73	ca 14	ca 80	< 10
PO4 (μg/L)	max 15,5	< 1,3	ca 15	< 2,5
NH4 (μg/L)	max 20 (autumn)	1,4 - 10	< 10	< 10
SiO3 (μg/L)	max 140,5	< 2,8	-	-

2.2.4 Micronutrients – trace metal

Trace metals are present in seawater in extremely low concentrations. They are mostly metals and metalloids, and are found in dissolved, colloidal, and particulate forms (Kennish, 2001, Morel and Price, 2003). Some trace metals are micronutrients, or bioactive trace metals, and thus have the potential to control plankton species composition and productivity, and be bio limiting (Libes, 2009). They are essential nutrients.

The chemical behaviour of trace metals, and thus its bioavailability is strongly dependent on whether it is present as free metal ion or complexated (Stumm and Morgan, 1996) as the uptake of metal ions by cellular organisms is largely controlled by the free metal ion in solution (Hunter et al., 1997). Many trace metals will undergo biogeochemical cycling in seawater (Libes, 2009). As a result of the metal uptake by phytoplankton, most dissolved trace metals are depleted at the ocean surface. When phytoplankton die or are eaten by zooplankton, the metals will sink with the biomass, resulting in surface concentrations that are small fractions of those in the deep (Morel and Price, 2003).

Despite its low concentration, many trace metals are known to be critically important to the life processes of marine organisms (Kennish, 2001, Morel and Price, 2003). These

micronutrients act as cofactors or are part of cofactors in enzymes. They can also be structural elements in proteins (Morel and Price, 2003). Micronutrients are thus involved in all general metabolic processes in phytoplankton, including photosynthesis and respiration, and assimilation of macronutrients (Stumm and Morgan, 1996).

The question of what limits the productivity of the oceans has historically been debated among N and P partisans. But the acquisition of macronutrients is not independent of the availability of trace metals that catalyzes their transformations (Morel and Price, 2003). Trace metals can be limiting the productivity in waters where macronutrient supply is evidently sufficient. In Figure 2 the primary metal requirements for carbon, nitrogen and phosphorus acquisition and assimilation by marine phytoplankton are shown. A low concentration or availability of any of those metals can have an affect on the nutrient cycles. A low metal availability can, for example, limit critical steps in the nitrogen cycle because all nitrogen transformations involve metalloenzymes. Iron has particularly been recognized as a potential limiting element in the nitrogen cycle (Morel and Price, 2003). It is essential in enzyme mediated processes such as nitrogen fixation and nitrate and nitrite reduction (Sakshaug et al., 2009c). But molybdenum and copper are also important for critical steps in the cycle.

The way trace metals limit plankton growth generally involves co-limitation by more than one trace metal and/or macronutrient (Libes, 2009). Within the modern ocean there is no single nutrient that could be considered limiting in isolation (Moore et al., 2013). As shown in figure xx trace metals can influence the carbon and phosphorus cycling indirectly through their effects on the nitrogen cycle (Morel and Price, 2003). Discussion of nutrient limitation should therefore specify the process being considered given the range of usage for each nutrient (Moore et al., 2013). That being a macronutrient or a micronutrient.

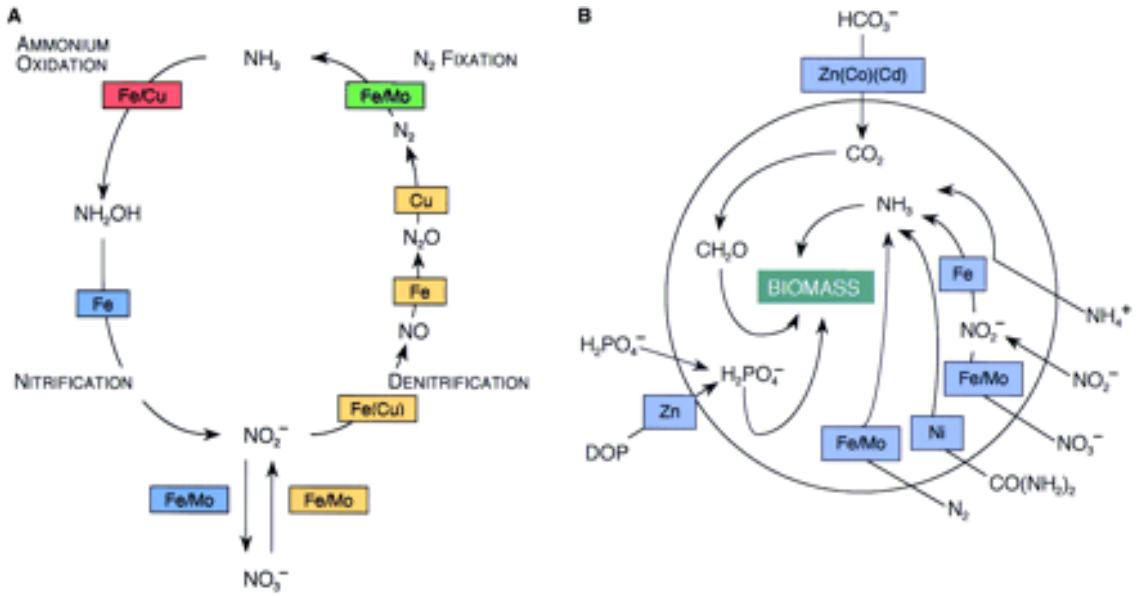

Figure 2. (A) Model of the nitrogen cycle showing how metals are involved in the enzymatically catalysed steps. The colours identify the reactions involved in nitrogen fixation (green), denitrification (yellow), nitrification (blue), and ammonium oxidation (red). (B) Shows the main metal requirements for nitrogen, carbon and phosphorous acquisition and assimilation by marine phytoplankton (Morel and Price, 2003)

Even though many micronutrients are essential to the growth of marine organisms, they may also be toxic at elevated concentrations. This toxicity will vary greatly among organism due to different uptake, storage and removal strategies (Kennish, 2001).

2.3 Phytoplankton – the foundation of the marine ecosystem

2.3.1 Plankton

Plankton refers to organisms located in the water column that are too small and/or weak to move long distances against the ocean currents. They represent the first step in the marine food chain and play a major role in the cycling of chemical elements in the ocean (Steele, 2009). The plankton community forms a dynamic system where interactions among the different components occur all the time. The different components range from tiny single-celled organisms such as bacteria and phytoplankton to zooplankton and large predators of more than 1 m in diameter (Steele, 2009).

2.3.2 Phytoplankton

Photosynthetic microbes, collectively termed phytoplankton (Moore et al., 2013), are unicellular organisms that carry out oxygenic photosynthesis. They live in the upper illuminated waters of all aquatic ecosystems where they drift with the currents (Steele, 2009). All phytoplankton species are capable of photosynthesis (Cloern, 1996), and this diverse group make up the base of the food chain in most marine ecosystems (Libes, 2009, Steele, 2009). They are responsible for almost half of earth's net primary production (Arrigo, 2005, Moore et al., 2013, Morel and Price, 2003, Steele, 2009).

Phytoplankton depends on sunlight for photosynthesis and occurs therefore almost entirely in the upper sunlit layer of the ocean (Steele, 2009). There are several thousand known species of phytoplankton, many of which are diatoms or flagellates (Rinde et al., 1998, Steele, 2009). Although microscopic, they cover a vast size range, from 0.4μm to 2mm (Sakshaug et al., 2009b). Their cell size will affect many aspects of phytoplankton physiology, including nutrient uptake. The uptake of nitrate can particularly be related to cell size. More so than ammonium uptake. The hypothesis is that small phytoplankton prefer ammonium, whilst larger phytoplankton are better at taking up nitrate (Steele, 2009, Stolte and Riegman, 1995). Initial nitrate uptake rates between small and large phytoplankton may, however, not differ significantly. But larger phytoplankton can maintain a higher uptake rate for a longer time due to better storage capacity in the vacuole. Ammonium, which is assimilated more rapidly than nitrate, is not accumulated in the same way. The intracellular ammonium pools are never very high. The availability of ammonium versus nitrate is therefore believed to influence the phytoplankton size in a population (Stolte and Riegman, 1995).

The phytoplankton communities are locally diverse, and in a liter of seawater several hundred species may be found (Steele, 2009). The composition of the communities will also contininuosly changes such that different species become abundant at different times. This process of continuous reorganization is often termed "succession". For phytoplankton communities this succession is often quite predictable, so that the same species often dominates in the same water at the same time year after year (Carlsson and Graneli, 1999, Sakshaug et al., 2009b, Skoog et al., 2004). Many species can even be classified according to which season they are predominant, but a few species are ubiquitous year round (Sakshaug et al., 2009b). The succession of phytoplankton can be caused by physical, chemical or biological changes. The availability of nutrients and the competitive abilities of the different phytoplankton species are often thought to be important factors leading to succession (Carlsson 1999).

During winter the phytoplankton population is small in number and generally consist of small flagellates and diatoms. By the end of Mars the cell count starts to increase, and the spring blooms usually sets in sometime in April (kystøkologi s89). Phytoplankton blooms are prominent features of the biological variability in coastal ecosystems. These episodic population increases is a fundamental part of the phytoplankton dynamics. The phytoplankton populations often exist in a static "quasi-equilibrium" in which the primary production is balanced by the phytoplankton losses and transport. Phytoplankton blooms are departures from this quasi-equilibrium when the primary productivity temporarily exceeds the losses and transports. During blooms measurable geochemical changes occur, and more and more evidence suggest that these natural cycles of bloom variability are being altered by human activities, including the input of contaminants and nutrients (Cloern, 1996).

The biomass of algae in a system can be measured as the concentration of chlorophyll a content. This is done by filtering exact volumes of seawater on GF/F filter (Wathman) and extracting in acetone. The content of chlorophyll a can then be quantified by fluorometry, using a Turner fluorometer (Reitan et al., 2002). The OSPAR-commission has sat values for chlorophyll a concentrations in Norwegian waters to be within normal at 2-4µg chl a L^{-1}, and at elevated levels at >4,5µg chl a L^{-1} (Olsen et al., 2012).

2.3.3 Phytoplankton and oceanic nutrients

To keep up with the grazing of zooplankton, phytoplankton must continue to divide every day or every week (Morel and Price, 2003). Many species even have the capacity for rapid growth with several doublings each day (Cloern, 1996). The phytoplankton community are thus important contributors of biomass to the marine food chain (Libes, 2009, Morel and Price, 2003). However, they are at the same time depleting their own milieu, the surface waters, of nutrients as these are needed for growth, and are continuously being exported out as settling biomass (Morel and Price, 2003). Nutrients are returned to dissolved form again by excretion or remineralization of dead organic matter. This uptake of nutrient and their regeneration are somewhat separated vertically (Steele, 2009), and these vertical concentration profiles are

characteristic of many algal nutrients, both macronutrients and micronutrients. This cycle depletes the concentration of nutrients in surface waters in the ratios that they occur in phytoplankton and enriches them in deeper waters by the same ratios (Morel and Price, 2003, Redfield, 1958, Stumm and Morgan, 1996). These ratios are referred to as Redfield ratios. During photosynthesis nitrogen and phosphate are taken up together with carbon in the atomic ratio C:N:P ≈ 106:16:1. Respiration of these organic particles after settling releases these elements in approximately the same proportions (Arrigo, 2005, Redfield, 1958, Stumm and Morgan, 1996). The Redfield model can be extended to many micronutrients as well, as these are present in phytoplankton in relatively constant proportions (Stumm and Morgan, 1996).

There are however variations in the phytoplankton nutrient stoichiometry. The actual chemical species that are taken up by the phytoplankton depend on biological species, their physiological state, and environmental conditions (Arrigo, 2005, Libes 2009). The Redfield ratio is therefore not a universally optimal value, it only represents an average for the oceanic phytoplankton growing under different conditions and employing a range of growth strategies. The deep-sea ratio is thus a reflection of the stoichiometry of the current global phytoplankton community (Arrigo, 2005). As the environmental conditions changes, this observed nutrient stoichiometry can be altered, and current nutrient inventories will change (Arrigo, 2005, Libes 2009).

Phytoplankton have a great influence on the cycling of nutrients in the ocean. But the phytoplankton community are also very much a reflection of the nutrient composition in the water (Arrigo, 2005). This reciprocal relationship between organisms and their environment are important when trying to understand the chemistry of an aquatic habitat (Stumm and Morgan, 1996). The phytoplankton community can be affected on both short and long term as a result of this interaction, and as environmental conditions changes, the composition of the phytoplankton community could be markedly altered (Arrigo, 2005, Stumm and Morgan, 1996).

2.4 The project's relevance

In coastal waters, the growth of phytoplankton is often limited by the availability of nutrients. The emissions of nutrients from fish farms in eutrophied coastal areas will therefore enhance the negative effects of eutrophication (Dalsgaard and Krause-Jensen, 2006). However, coastal eutrophication problems are not caused by the increased nutrients loads alone, but rather by the unbalance in the delivery of nitrogen and phosphorus with respect to silica. Hence, undesirable coastal eutrophication often occurs with the development of non-siliceous algae which are responding to the new sources of nitrogen and phosphorus (Voss et al., 2011).

The unbalance in nutrient delivery to the system can thus lead to structural changes in the algae community. For example, studies have shown that ammonium stimulates the growth of fast-growing macro-algae with high volume to surface ratio. These species, often thin leaf-like and filamentous, can reduce light conditions and effectively compete for nutrients. Over time this can lead to a reduction of the perennial, slow-growing species, such as seaweed (Havforskningsinstituttet, 2012).

Nutrient discharges from marine aquaculture are of great significance in Norwegian coastal waters (OSPAR, 2000). The industry has taken measures to reduce the release of nutrient waste (Cheshuk et al., 2003, Wang et al., 2012), but the discharges has still increased over the last years due to the increase in total fish production (Skogen et al., 2009). It is therefore important to gain better understanding for how nutrients from aquaculture distributes and accumulates in ecosystems. This can be achieved through studies and measurement of the concentration and distribution of macronutrients in areas with aquaculture activities and compare these values with reference data collected from locations where no, or very little, aquaculture are present. Or maybe even better, compare the measured concentrations with data collected systematically over several seasons and years.

2.5 The project so far

The work presented in this thesis is part of the ongoing CINTERA project. The research started back in 2009 with the WAFOW project "*Can Waste Emission from Fish Farms Change the Structure of Marine Food Webs? A comparative study of coastal ecosystems in Norway and Chile*". During the WAFOW project 3 mesocosm experiment was conducted, two in Chile and one in Norway. The experiments were designed to maintain closed environments for longer periods of time with conditions simulating the nutrient enrichment occurring in fjord ecosystems with aquaculture.

A few master students have already been involved in the WAFOW project. In general, all the experiments conducted during the WAFOW project show a response in phytoplankton (chlorophyll and cell counts) and particulate organic carbon (POC). These parameters showed a linear relation with ammonium loading rate, however the slope differed in Norway compared to Chile. There was a generally lower response to the nutrient addition in the biomass of phytoplankton in Norway. The experiments in Chile also reviled a shift in the species composition of phytoplankton that was caused by the nutrient loading. The nitrate-ammonium shift that aquaculture waste may bring on had a strong effect on the availability of micronutrients in the sea water and uptake in the biota. With ammonium as the main nitrogen source, the reduction process of nitrate-nitrite to ammonium is reduced or eliminated, causing a change in micronutrients necessity (WAFOW, Unpublished). The project so far has shown that aquaculture waste may cause indirectly changes in the ecosystem caused by changes in macro- and micronutrient availability.

3. Material and Methods

3.1 Introduction

Two cruises were performed during winter/spring of 2013. The first cruise took place during 12th-13th of February collecting water samples from winter conditions and the second cruise took place during 16th-18th of April collecting water samples from early spring conditions. The two cruises were carried out in the fjord systems outside of Trøndelag, situated in central Norway.

Figure 3. Norway

During the first cruise water samples were collected at 8 different stations. During the second cruise, 15 stations were visited, but due to large waves, the CTD-rosette could only collect water samples for macronutrient analyses at 14 stations.

3.2 Study area

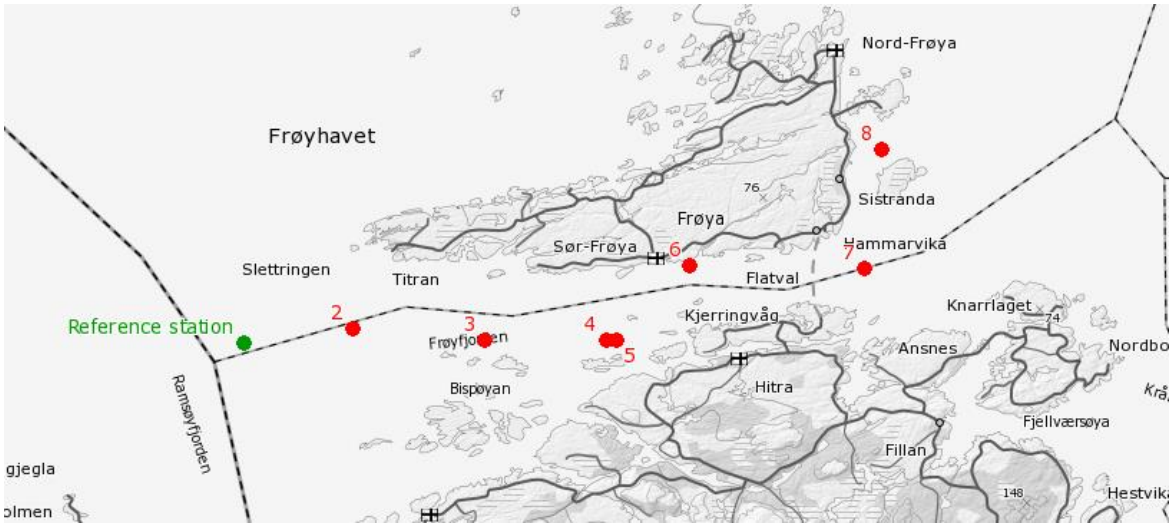

Figure 4. Sampling stations during the first cruise (12-13 February). Primarily located in Frøyfjorden which lies between Hitra and Frøya.
(2) Vest Frøyfjorden, (3) Vest Torsøya, (4) Vest Langøya, (5) Øst Langøya, (6) Storhallaren, (7) Øst Frøyfjorden, (8) Inntian Nord Frøya

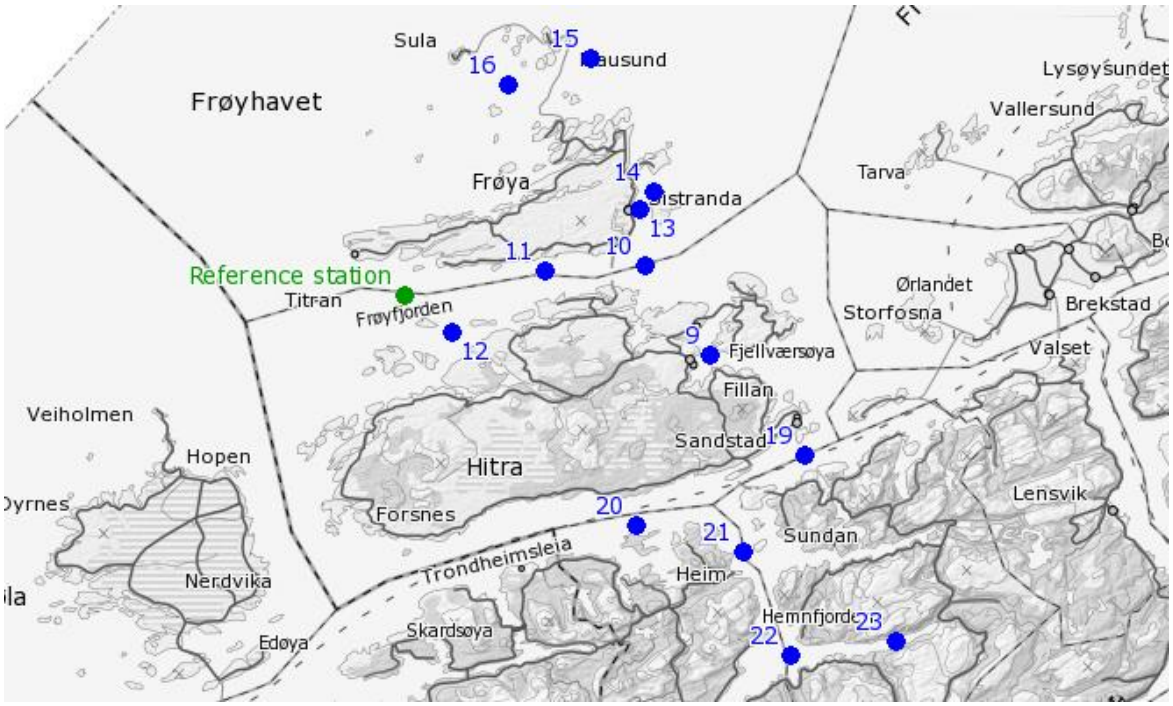

Figure 5. Sampling stations during the second cruise (16-18 April).
(9) Fillfjorden, (10) Øst Frøyfjorden, (11) Midt. Frøyfjorden, (12) Øst Torsøya, (13) Inntian Frøya, (14) Inntian Nord Frøya, (15) Øst Mausen, (16) Sørvest Mausen, (19) Nordøst Hemskjel, (20) Nord Røstøya, (21) Vest Jamtøya/Hemnefjorden, (22) Midt. Snillfjord, (23) Snillfjorden

3.2.1 Aquaculture in the area

This following map is taken from Fiskeridirektoratet. It shows the aquaculture activity in the study area as it was reported at the end of May.

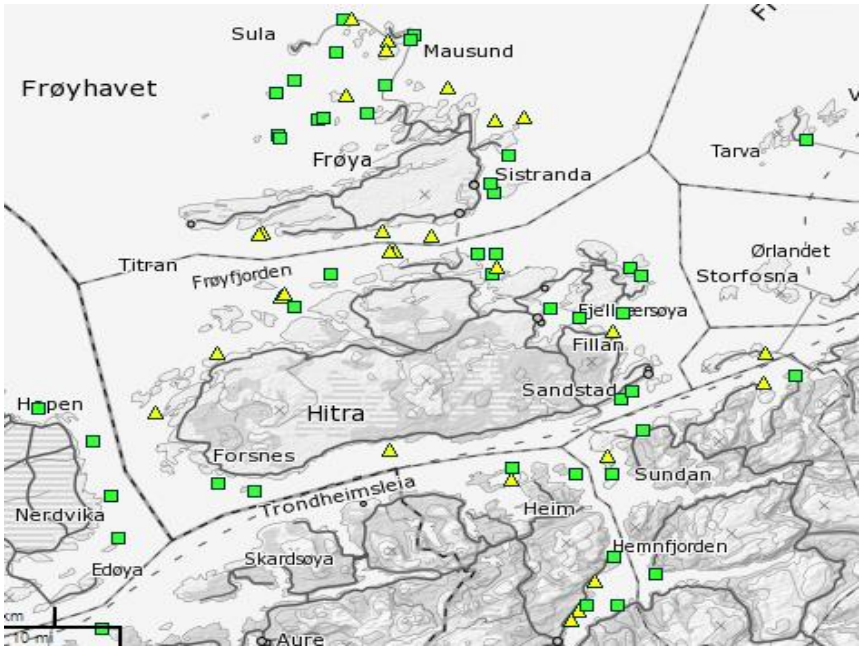

Figure 6. Aquaculture activity reported in the area at the end of May 2013. Green: fish at reporting. Yellow: no fish at reporting (Fiskeridirektoratet, 2013)

3.3 Analytical methods

Dissolved nutrient concentration of nitrate/nitrite, ammonium, phosphate and silicate was determined using standard methods (Grasshoff et al., 1999, Strickland and Parsons, 1972). These methods are based on colorimetric techniques where the concentration of a coloured compound in solution is measured by its absorbance of a specific wavelength of light (Skoog et al., 2004).

These analytical methods can be performed manually, automated or with the use of sensors. The measurement step is usually accomplished by spectrophotometry for all three methods. With the use of sensors, direct detection of nutrients can be achieved. On contact with the seawater these sensors can send physical signals representing the nutrient concentration. A direct detection is not possible with manual or automated methods. These methods are however good representatives of the situation at sampling. The samples can be analysed on board the vessel or stored for analysis on a later time. The manual methods require the samples to be treated individually and manually for each variable one whish to measure. The automated methods are in all practice automated versions of the manual methods. However, these methods can perform several analyses simultaneously with very little human interference (Hansen and Koroleff, 1999).

Two analytical methods were used during analyses of the collected water samples; a manual method for the determination of silicate concentration, and an automated method for the determination of ammonium, phosphate and nitrate/nitrite concentration.

3.3.1 Sampled water

When the samples are collected, the biological activity in seawater does not stop. The microorganisms naturally present in seawater can induce changes in nutrient concentration as bacteria and plankton continues to digest and excrete materials. Water samples should therefore not be exposed to too much light and preferably analysed within hours. If the samples have to be stored for weeks or months before analysis, freezing is the better method to preserve the samples (Kremling and Brügmann, 1999).

Seawater consists of many constituents. Some components are suspended particulate material whilst others are dissolved material. Filtration can to some extent differentiate between these phases. And it is often both reasonable and practical to do so. The term dissolved will then often refer to the fraction of seawater which passes through a 0.45 μm or 0.4 μm filter. During spectrophotometric determination of macronutrient concentration, high concentrations of solids can lead to analytical errors due to scattering of light (Kremling and Brügmann, 1999). A pre-treatment of the sample is therefore often favourable. However, any treatment of the sample, including filtration and the transfer of the sample from one container to another, is a contamination risk, and the sample is at risk of being modified/altered (Hansen and Koroleff, 1999).

There are many different filters and filter materials, and choosing which filter to use will depend on the analysis to be done. None of the existing filters can meet all the requirements necessary for a universal application. The choice of filter is therefore often a compromise between the different requirements of the analysis. Filtration for analysis of seawater nutrients will often use glass fibre filters. However, these filters could alter the samples silica concentration, due to the glass being made of pure borosilicate fibres. When silica concentrations are to be analysed, polycarbonate filters are the better choice (Kremling and Brügmann, 1999).

3.4 Analyses

3.4.1 Pretreatment of samples

Water samples were obtained using 12x2.5 litre Niskin bottles deployed on a CTD-rosette (se Figure 7. In addition to collecting water samples for measurements, this instrument measures conductivity, temperature, and density. The Niskin bottles were open in both ends when lowered in the water, and closed at predefined depths by signals from the ship. During the first cruise water samples were collected at 7m. During the second cruise water samples were collected at 4+6+8m. The collected water samples were filtered through a 200µm funnel before further treatment.

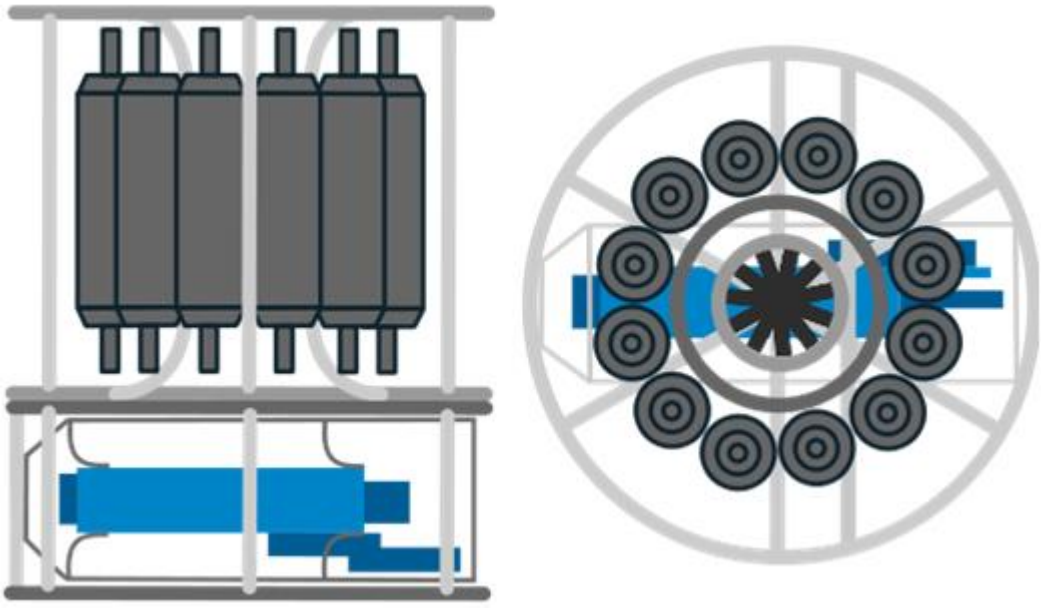

Figure 7. A CTD sampler (Conductivity, temperature, depth) mounted on a rosette with Niskin Bottles

Water samples for the macronutrients analyses were filtered through isopore membrane filters. These polycarbonate filters, 25 mm in diameter, had a pore size of 0.4μm. Filtration was carried out by suction under vacuum onboard the vessel to obtain relative clean water samples. Immediately after filtration the water samples were collected in 50mL centrifuge tubes (for analyses of NH4 and Si) and 100mL white plastic bottles (for analyses of PO4 and NO3) before being stored in the freezer awaiting analysis.

3.4.2 Determination of reactive silicate

The manual method for determination of silicate is based on Strickland and Parsons (1972) analytical procedure. The term "reactive silicate" is used when describing this method. Determination of silicate in seawater is based on the formation of yellow silicomolybdate complexes. The term reactive silicate comes from the fact that not all forms of silica in solution will react and form these desired coloured compounds. The reactive silicate may therefore not represent the total dissolved silica in solution, but it will nonetheless give a meaningful measure of the silicate available to organisms (Strickland and Parsons, 1972).

The silicomolybdate complex is formed when molybdate is allowed to react with seawater. However, other molybdate complexes may form simultaneously. The silicomolybdate complex has only a low intensity colour and the light absorption are often not satisfactory. A reducing solution of metol and oxalic acid will reduce the silicomolybdate complexes and form a more intensely coloured blue compound. At the same time any other molybdate complexes formed is decomposed (Strickland and Parson, 1972).

The silicate and molybdate must have time to combine before the reducing agent is added. Ten minutes is enough, and no more then thirty minutes should pass to avoid structurally changes in the silicomolybdate complexes. The time it takes to develop the more intensely coloured blue complexes varies with the amount of silicon. To be sure most of the silicomolybdate is reduced, 3 hours should be allowed, and the absorbance must be read within 24 hours. If left standing for any longer, the solution can no longer be considered stable and may give an inaccurate absorption reading (Strickland and Parson, 1972). The absorbance of the blue complex was measured at 810 nm with a 5 cm cell.

3.4.3 Determination of ammonium, phosphate and nitrate/nitrite

Determination of ammonium, phosphate and nitrate/nitrite was conducted using an automated analyser. The first fully automated instrument for chemical analysis was introduced on the marked already back in 1957. This first automated instrument, named the Auto-Analyzer, was originally designed for the clinical laboratories. However, automated systems for industrial chemical analysis followed soon after (Crandell, 1985, Skoog et al., 2004). The main advantages with these analytical systems are that several variables can be determined

simultaneously and with very little human involvement (Hansen and Koroleff, 1999, Skoog et al., 2004).

Methods for the determination of seawater compounds have been developed and modified since the introduction of the automated analysers. However, most of them are still based on the continuous flow-analysis (CFA) first introduced back in 1957 (Hansen and Koroleff, 1999). These automated analysers systems can do both the sample processing operations and the final measurement step. A continuous stream of water is pumped through a flowing stream, where a number of operations take place in a closed tubing system before it is transported to a flow-through detector. This detector is often a spectrophotometric cell that measures the absorbance of the then converted light absorbing compound. The addition of samples, standards and reagents to the stream are done at intervals and all operations necessary for the analysis take place between the sample introduction and detection (Crandell, 1985, Hansen and Koroleff, 1999, Skoog et al., 2004). The chemical reactions are based on those used in the manual methods. However, reaction time and sample volume necessary to convert the nutrient into a coloured compound are modified to save both time and chemicals (Hansen and Koroleff, 1999).

The automated analyses for the determination of ammonium, phosphate and nitrate/nitrite concentrations were performed at Trondhjem Biologiske Stasjon (TBS).

4 Results

Macronutrient concentrations measured during cruise 1 and cruise 2 are first presented. Then a seasonal comparison is done for NH4, DIP, DIN and SiO3 (from February to April) to illustrate changes in distribution. Further, the nutrient ratios N:P, N:Si and Si:P are calculated in a attempt to show any stoichiometric changes between cruise 1 and cruise 2. Lastly, chlorophyll a concentrations are compared to NH_4, DIN and DIP in an effort to gain better understanding of the nutrient concentrations measured.

4.1 Macronutrient concentration

Table and Table show the general distribution of macronutrients after Cruise 1 and Cruise 2, respectively. The reference stations represent data collected at an area with little or no aquaculture activity. The other stations all represent active zones with aquaculture activity to a greater or lesser extent.

During this first cruise there were minor differences between the reference station and the active zones. The ammonium concentration ranged from 5-10µg L^{-1} in all the active zones, whereas the reference station had an ammonium concentration of 9µg L^{-1}. The highest concentrations were registered at stations 4 and 5, Vest Langøya and Øst Langøya respectively. These two stations were located very close to an active cage system. The phosphate concentration is approximately the same for the active zones and the reference stations.

Table 3. Cruise 1, distributions of macronutrients in active zones compared to a reference station. The concentrations for NH4, PO4 and NO3 have an error margin of ±2 µg/L. The silicate concentration is determined manually and can therefore have a greater margin of error. Depth represents the total depth at station measured with the CTD, whereas sampling represent the depth at which samples were collected.

		Date	Depth (m)	Sampling (m)	NH4-N (µg/L)	PO4-P (µg/L)	NO3-N (µg/L)	SiO3-Si (µg/L)
2	**Vest Frøyfjorden**	12.02.	255	7	5	15	90	88
3	**Vest Torsøya**	12.02.	161	7	7	15	89	97
4	**Vest Langøya**	12.02.	131	7	10	16	89	99
5	**Øst Langøya**	12.02.	145	7	10	16	86	95
6	**Storhallaren**	12.02.	89	7	7	14	87	88
7	**Øst Frøyfjorden**	13.02.	102	7	8	16	99	102
8	**Inntian Nord Frøya**	13.02.	44	7	7	15	89	101
1	**Reference station**	12.02.	316	7	**9**	**15**	**90**	**97**

During the second cruise the distribution of macronutrients showed a greater variety within the active zones and the reference station. The ammonium concentration ranged from 1-18µg L^{-1} in the active zones, whereas the reference station had an ammonium concentration of 13µg L^{-1}. Stations 19-23, located in the more sheltered Trondheimsleia, Hemnefjorden and Snillfjorden (see Figure 5) showed the lowest values. These five stations also showed the lowest values for phosphate and nitrate concentration. The phosphate concentration did not otherwise show any considerable differences in the active zones. But they were a bit higher than that measured at the reference station.

Table 4. Cruise 2, distributions of macronutrients in active zones compared to a reference station. The concentrations for NH4, PO4 and NO3 have an error margin of ±2 µg/L. The silicate concentration is determined manually and can therefore have a greater margin of error. Depth represents the total depth at station measured with the CTD, whereas sampling represent the depth at which samples were collected.

		Date	Depth (m)	Sampling (m)	NH4-N (µg/L)	PO4-P (µg/L)	NO3-N (µg/L)	SiO3-N (µg/L)
9	**Fillfjorden**	16.04.	179	4+6+8	17	11	46	20
10	**Øst Frøyfjorden**	16.04.	103	4+6+8	18	9	31	25
11	**Midt. Frøyfjorden**	16.04.	108	4+6+8	15	7	19	30
12	**Øst Torsøya**	16,04.	82	4+6+8	14	7	13	27
13	**Inntian Frøya**	16.04.	30	4+6+8	17	8	24	23
14	**Inntian Nord Frøya**	16.04.	45	4+6+8	13	8	25	31
15	**Øst Mausen**	17.04.	132	4+6+8	16	7	19	33
16	**Sørvest Mausen**	17.04.	91	4+6+8	15	8	22	34
19	**Nordøst Hemnskjel**	18.04.	168	4+6+8	11	6	19	35
20	**Nord Røstøya**	18.04.	170	4+6+8	9	5	9	27
21	**Vest Jamtøya/ Hemnefjorden**	18.04.	207	4+6+8	9	5	9	30
22	**Midt. Snillfjord/ Hemnefjorden**	18.04.	400	4+6+8	4	3	3	19
23	**Snillfjorden**	18.04.	199	4+6+8	1	3	7	21
18	**Reference station**	17.04.	407	4+6+8	**13**	**5**	**6**	**20**

4.2 Seasonal distribution of macronutrients

The distribution of ammonium, DIP (dissolved inorganic phosphate), DIN (dissolved inorganic nitrogen) and silicate are presented in Figure 8, 9, 10 and 11, respectively. The concentrations for both cruises are shown in each figure in order to gain a better understanding of the seasonal changes from February to April.

Stations 7 and 10 and stations 8 and 14 are located at the same coordinates. Samples were taken from these locations during both cruises, and allows for a direct comparison between the two seasons.

4.2.1 Ammonium – NH_4

There is a general increase in ammonium concentration from the first cruise to the second cruise (see Figure 8). For stations 7 and 10, located at the same coordinates, the ammonium concentration increased with 10µg L^{-1} from February to April. For stations 8 and 14 the ammonium concentrations increased with 6µg L^{-1}. The stations located in Snillfjorden (stations 22 and 23) presents the lowest ammonium concentrations during both cruises, and clearly go against the general trend in increasing ammonium concentration for the second cruise.

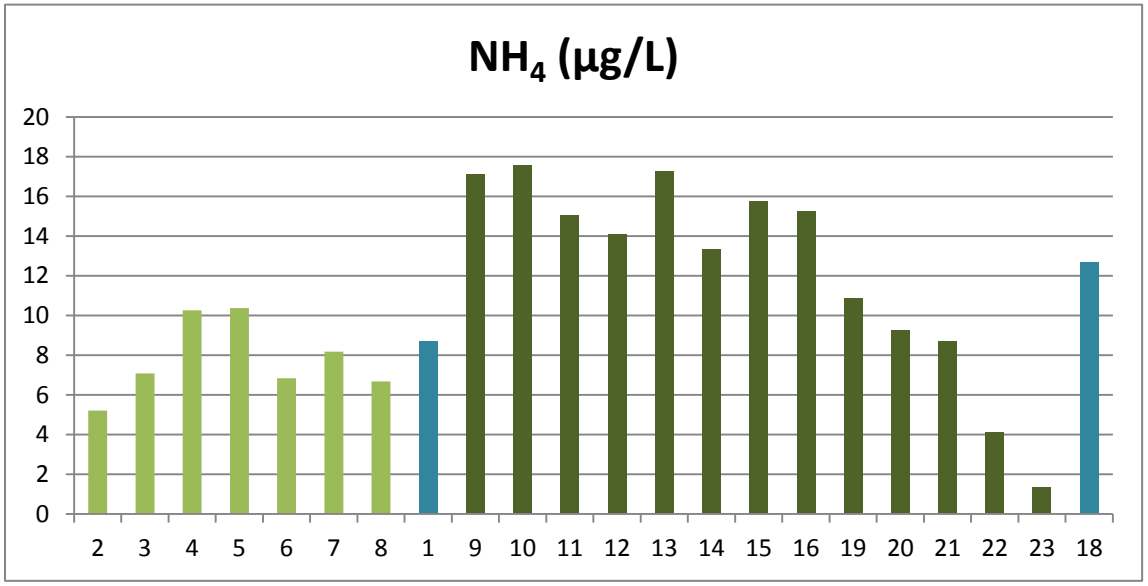

Figure 8. Concentration distribution of ammonium (Light green: Cruise 1. Dark green: Cruise 2. Blue: Reference station. NH4 data has an error margin of ±2 µg/L.)

4.2.2 Dissolved inorganic phosphate – DIP

The measured phosphate concentrations decreased from the first to the second cruise (see Figure 9). For stations 7 and 10 and stations 8 and 14, located at the same coordinates, the concentrations have decreased by 7µg L^{-1} for both locations. The stations in Snillfjorden again present the lowest concentrations values.

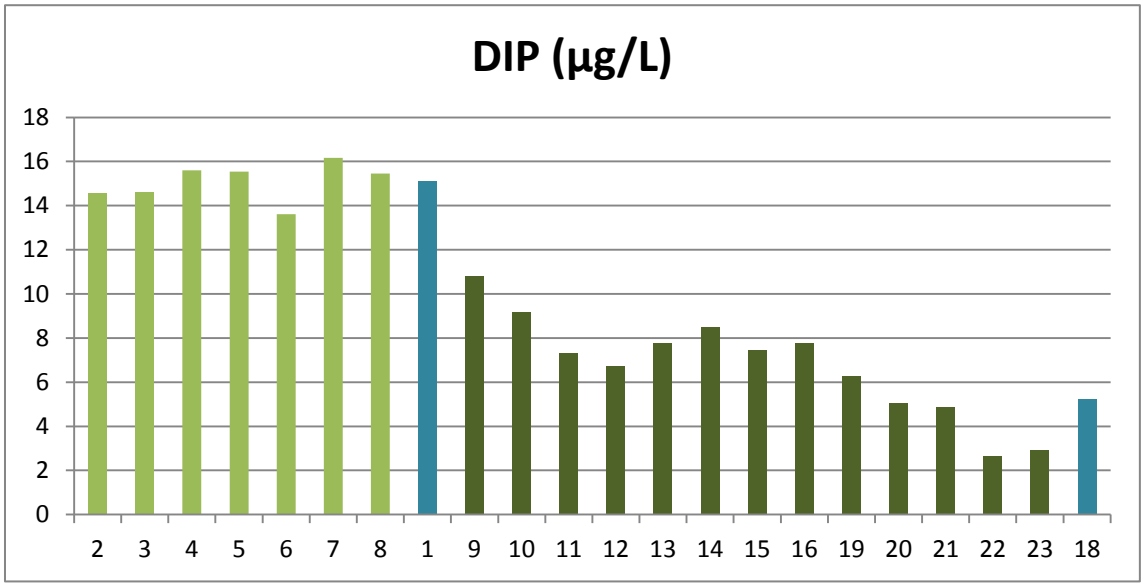

Figure 9. Concentration distribution of DIP (dissolved inorganic phosphate) (Light green: Cruise 1. Dark green: Cruise 2. Blue: Reference station. PO4 data has an error margin of ±2 µg/L.)

4.2.3 Dissolved inorganic nitrogen – DIN

There is a significant decrease in DIN concentration from the first cruise to the second cruise (see Figure 10). For stations 7 and 10 and stations 8 and 14, located at the same coordinates, the concentrations have decreased by 58µg L^{-1} for both locations. The two stations in Snillfjorden (22-23) display clearly the lowest concentrations.

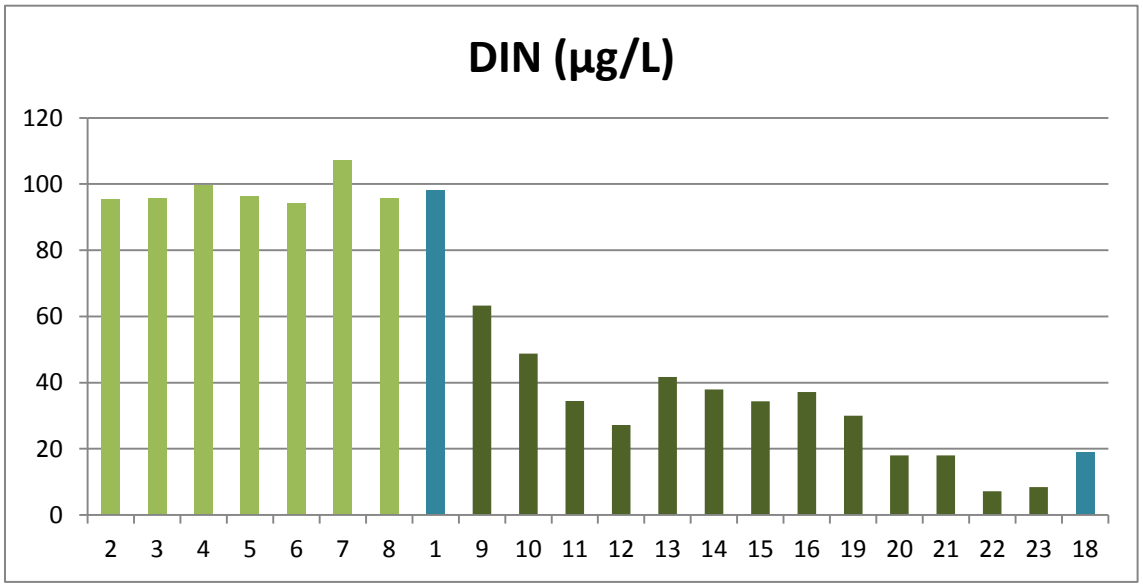

Figure 10. Concentration distribution of DIN (dissolved inorganic nitrogen = NH4+NO3+NO2) (Light green: Cruise 1. Dark green: Cruise 2. Blue: Reference station. PO4 data has an error margin of ±2 µg/L).

4.2.4 Silicate - SiO3

There is a significant decrease in silicate concentration from the first cruise to the second (see Figure 11). For stations 7 and 10 and stations 8 and 14, located at the same coordinates, the concentrations have decreased by 77 and 70µg L^{-1}, respectively.

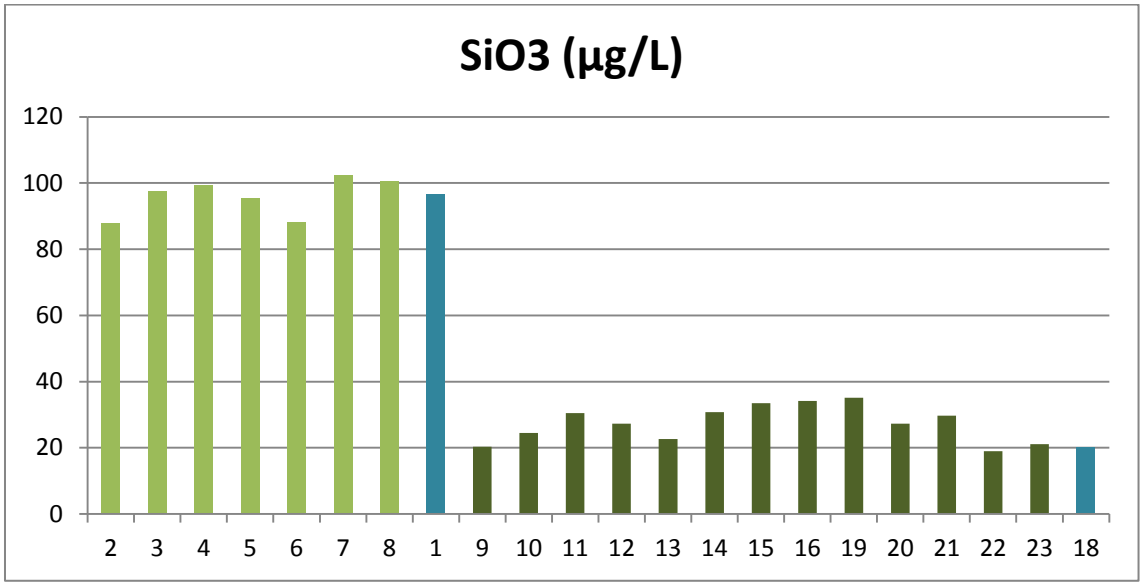

Figure 11. Concentration distribution of silicate (Light green: Cruise 1. Dark green: Cruise 2. Blue: Reference station. PO4 data has an error margin of ±2 µg/L.)

4.3 Nutrient ratio

In table 5 and 6 nutrient ratios are calculated for Cruise 1 and Cruise 2, respectively. The nutrient ratios are all given in molar units. Calculated values are compared with the respective Redfield ratios.

4.3.1 Cruise 1

N:P ratio is < 16 for all active zones and reference station. All ratios are thus lower than the Redfield ratio. However, with N:P ratios around 14-15 they do not differ much from the Redfield ratio. The active zones and reference station show no significant difference between them.

N:Si ratio is > 1 for all active zones and reference station. All ratios are thus higher than the Redfield ratio. The active zones and reference station show no significant difference between them, all displaying a N:Si ratio around 2.

Si:P ratio is <<16 for all active zones and reference station. All ratios are thus lower than the Redfield ratio. The active zones and reference station show no significant difference between them, all displaying a Si:P ratio around 7.

Table 5. Cruise 1, calculated molar ratios compared with the respective Redfield ratios (µmol µmol⁻)

		Calculated N:P	Redfield N:P	Calculated N:Si	Redfield N:Si	Calculated Si:P	Redfield Si:P
2	**Vest Frøyfjorden**	14,5	16	2,2	1	6,6	16
3	**Vest Torsøya**	14,5	16	2,0	1	7,3	16
4	**Vest Langøya**	14,1	16	2,0	1	7,0	16
5	**Øst Langøya**	13,7	16	2,0	1	6,8	16
6	**Storhallaren**	15,3	16	2,1	1	7,1	16
7	**Øst Frøyfjorden**	14,7	16	2,1	1	7,0	16
8	**Inntian Nord Frøya**	13,7	16	1,9	1	7,2	16
1	**Reference station**	**14,4**	16	**2,0**	1	**7,1**	16

4.3.2 Cruise 2

During the second cruise there is generally more variation in the nutrient ratios within the active zones.

N:P ratio is < 16 for all active zones and reference station (some <<16). All ratios are thus lower than the Redfield ratio. The active zones display some variations, with ratios ranging from 6 (around Snillfjorden) to around 13. The reference station has a N:P ratio of 8 (well under Redfields ratio of 16).

N:Si ratio is > 1 for all active zones, but two, and reference station. Most ratios are thus higher than the Redfield ratio. There are great variations within the active zones, with ratios ranging from around 1 to 6. The two stations with N:Si ratio <1 are located in Snillfjorden. The reference station has a N:Si ratio around 2.

Si:P ratio is <<16 for all active zones and reference station All ratios are thus lower than the Redfield ratio. There are variations within the active zones, with ratios ranging from around 2 to around 8. The stations with the highest ratios are located in Snillfjorden. The reference station has a Si:P ratio around 4 (well under the Redfield ratio of 16).

Table 6. Cruise 2. Calculated molar ratios compared with the respective Redfield ratios (µmol µmol^{-})

		Calculated N:P	Redfield N:P	Calculated N:Si	Redfield N:Si	Calculated Si:P	Redfield SI:P
9	**Fillfjorden**	13,0	16	6,2	1	2,1	16
10	**Øst Frøyfjorden**	11,8	16	4,0	1	3,0	16
11	**Midt. Frøyfjorden**	10,4	16	2,3	1	4,6	16
12	**Øst Torsøya**	8,9	16	2,0	1	4,5	16
13	**Inntian Frøya**	11,9	16	3,7	1	3,2	16
14	**Inntian Nord Frøya**	9,9	16	2,5	1	4,0	16
15	**Øst Mausen**	10,2	16	2,1	1	5,0	16
16	**Sørvest Mausen**	10,5	16	2,2	1	4,8	16
19	**Nordøst Hemnskjel**	10,6	16	1,7	1	6,2	16
20	**Nord Røstøya**	7,9	16	1,3	1	6,0	16
21	**Vest Jamtøya/ Hemnefjorden**	8,2	16	1,2	1	6,7	16
22	**Midt. Snillfjord/ Hemnefjorden**	6,1	16	0,8	1	7,9	16
23	**Snillfjorden**	6,4	16	0,8	1	7,9	16
18	**Reference station**	**8,0**	16	**1,9**	1	**4,2**	16

4.4 Chlorophyll a

The chlorophyll a (>200µm) concentrations show that there is an increase in chlorophyll a concentration from the first cruise to the second cruise (see Figure 12). The stations around Hemnefjorden and Snillfjorden (station 20-23) clearly present the highest chlorophyll a concentrations.

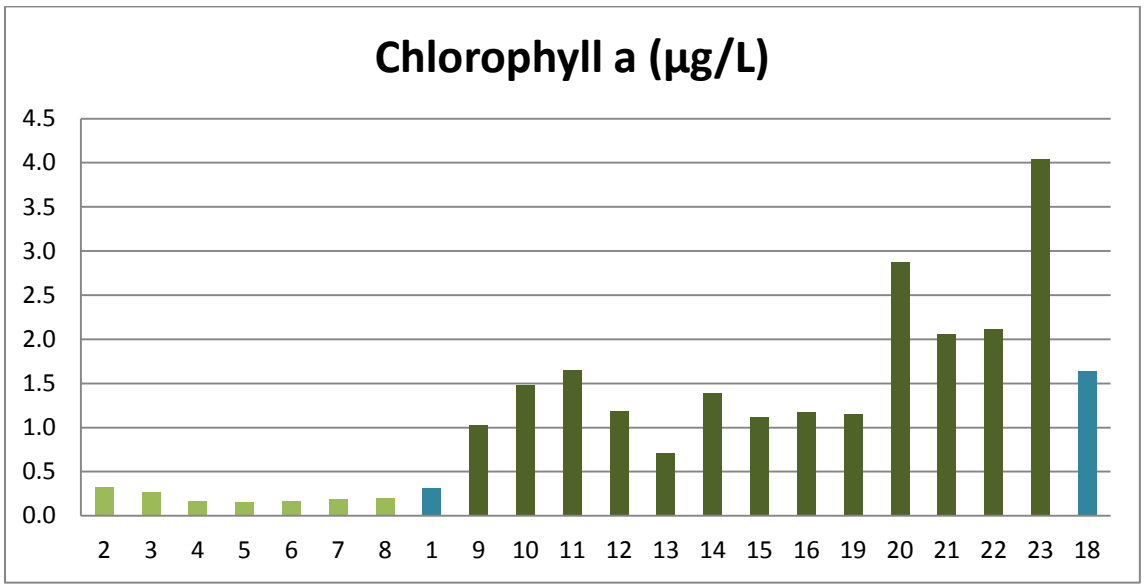

Figure 12. Chlorophyll a concentrations (Light green: Cruise 1. Dark green: Cruise 2. Blue: Reference station. (Chlorophyll a concentrations determined by Skrove, unpublished)

Figure 13, 14 and 15 show that the nutrient deficiency becomes more pronounced as the phytoplankton biomass (chlorophyll a concentration) increases during the second cruise. The trend is clear for NH4, DIN and DIP.

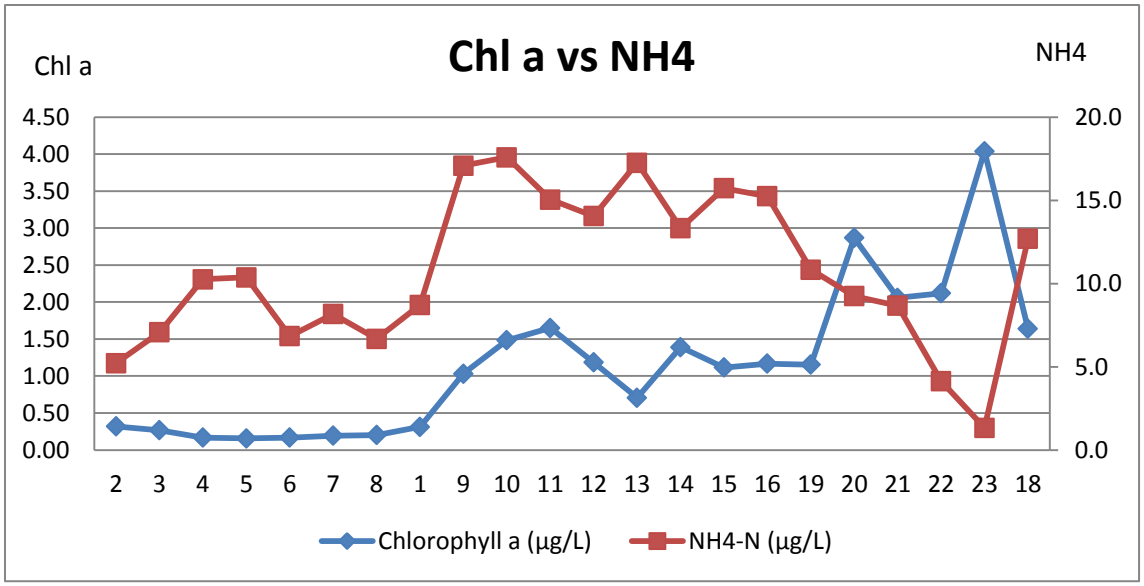

Figure 13. Chlorophyll a concentration compared to ammonium concentration for both cruise 1 (stations 2-8 + 1 (ref) and cruise 2 (stations 9-23 + 18(ref)). Blue, left axis: chlorophyll a. Red, right axis: ammonium

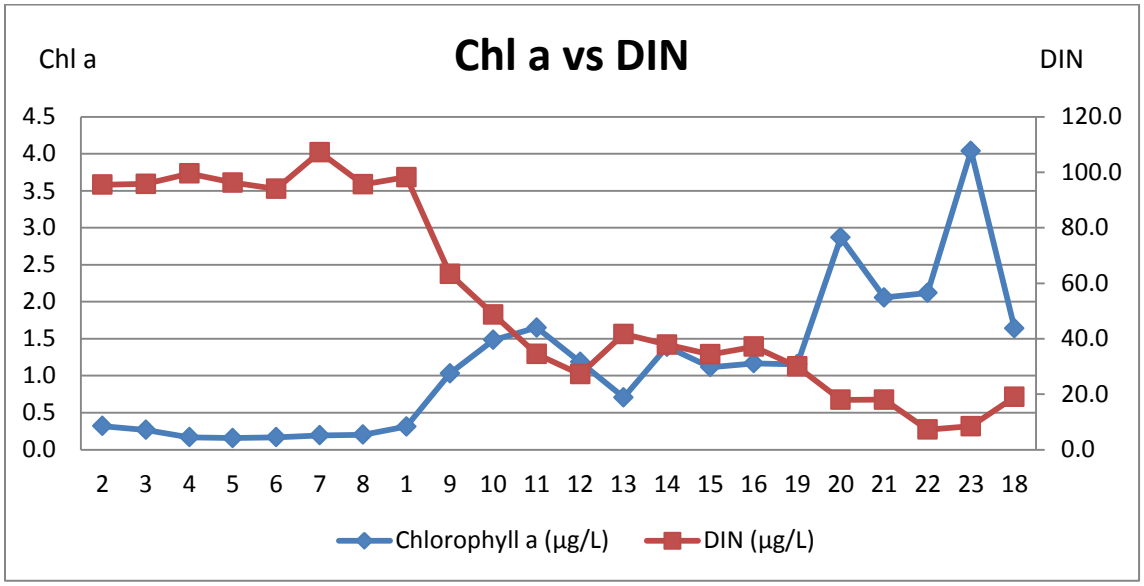

Figure 14. Chlorophyll a concentration compared to DIN (dissolved inorganic nitrogen) concentration for both cruise 1 (stations 2-8 + 1 (ref) and cruise 2 (stations 9-23 + 18(ref)). Blue, left axis: chlorophyll a. Red, right axis: ammonium

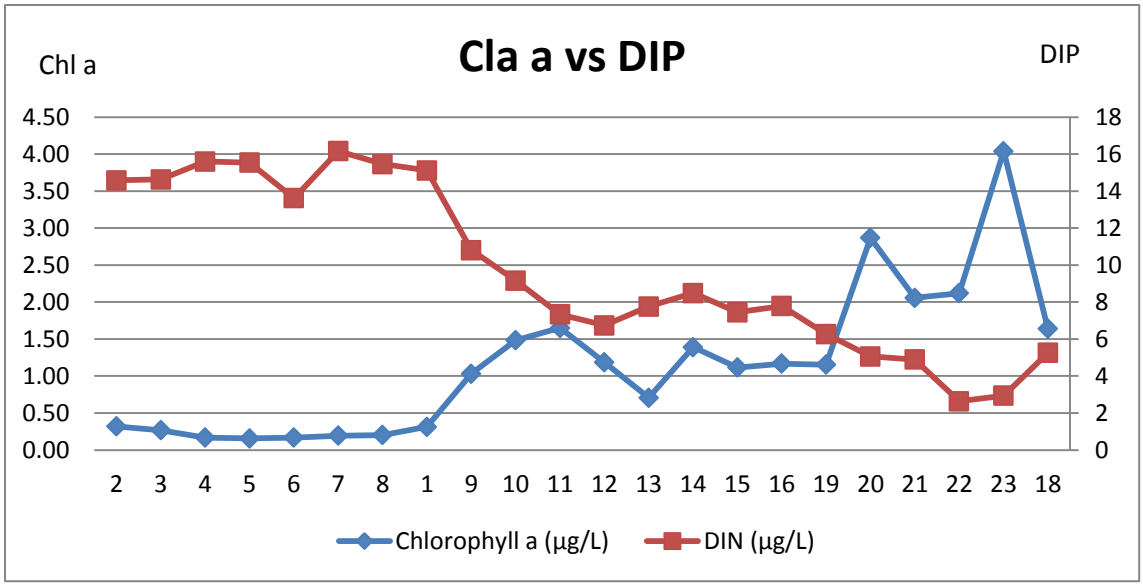

Figure 15. Chlorophyll a concentration compared to DIN (dissolved inorganic nitrogen) concentration for both cruise 1 (stations 2-8 + 1 (ref) and cruise 2 (stations 9-23 + 18(ref)). Blue, left axis: chlorophyll a. Red, right axis: ammonium

5 Discussion

5.1 Sampling

The intention for the two cruises conducted, was to collect water samples that would represent a transect line through the active aquaculture zones, from upstream to downstream, in an attempt to gain a better understanding of the distribution of the different macronutrients. However, the complex current patterns proved difficult to follow. In some areas the currents in the surface layer (0-5m) would go one way, and in the sub surface layer (5-10m) the currents would go in the complete opposite direction. Because of the unpredictability of the current system it proved difficult to decide witch stations were located upstream and which stations were located downstream of the aquaculture activities, and it became simply too much to take on for this thesis. The different stations are therefore only labelled as active zones, without any regards to the water currents.

During both cruises one station was chosen as reference station to which the active stations would be compared to. These reference stations should be affected as little as possible by aquaculture activities and/or coastal water with high nutrient content. Before setting out, the thought was that the current system brought water from west to east. The choice for reference stations were therefore that they would lay west of the active zones. But, because of the complexity of the current system, these stations might be contaminated by nutrients from aquaculture activities located east of the stations.

During the second cruise, the CTD-rosette could not be launched at the intended reference station due to bad weather. Consequently, water samples for macronutrient analyses were not collected at this location. Instead, a reference station was chosen in Frøyfjorden. This station was likely affected by aquaculture activities and the reference values probably reflect this. It is therefore to be expected that any interpretations and conclusions made based on these data alone will be affected by this choice of reference station. It will reduce our ability to say too much about the potential impact aquaculture can have on the distribution of macronutrients in the surrounding waters. Historical macronutrient values should therefore, where possible, be used in addition to indicate whether there is considerable increase in NH4, DIN and DIP concentration or not due to increasing aquaculture activities.

The water samples were filtered prior to freezing and storage. This pre-treatment, with the included transfers between several containers, is a contamination risk which could, to some degree, alter the samples (Hansen and Koroleff, 1999). However, the choice was taken to filter the samples during both cruises to avoid disturbances from any suspended particulates during analyses, and the risk of altering the samples in any large extent, seemed small. This would also allow all the samples to be treated in the same manner.

5.1.1 ...and analyses

The concentration of the dissolved nutrients was determined using standard methods (Grasshoff et al., 1999, Strickland and Parsons, 1972). These methods, based on colorimetric techniques which measure the absorbance of a coloured compound of a certain wavelength, are well known and widely used. Strengths and weaknesses of the methods are therefore not discussed in this thesis.

5.2 Macronutrient distribution

Ammonium and phosphate are the two most important nutrients to consider when assessing releases from aquaculture, as they can have the greatest impact on the surrounding waters and ecosystems. The emissions from the aquaculture activities will vary during the day, but also throughout the year. Because the fish grows most during summer, the release of nutrients is also expected to be the highest during this time.

5.2.1 Cruise 1 – February

During the first cruise the concentration of both NH4 and PO4 can be classified according to the NCS (the Norwegian classification system) as very good or good (for winter situation). They were also both in accordance with the concentrations measured in the Nordmøre study and Hopsjøen study, with NH4 concentrations less than, or around, 10µg L^{-1}, and PO4 concentrations around 15µg L^{-1}. Lastly, the measured concentrations, when comparing the reference station with the active zones, were generally similar. There did not seem to be any measurable difference between the reference station and the active zones. Based on these findings the release of NH4 and PO4 from aquaculture did not seem to contribute with any significant macronutrient enrichments to the study area at this time.

The concentration of both nutrients, NH4 and PO4, will decrease naturally as the spring blooms starts to set in and the phytoplankton communities increases in number, and the upwelling of nutrients from deeper waters are reduced. However, aquaculture can change these trends, and actually support the system with added nutrients. Problem arises if these nutrient inputs becomes too much for the system to handle.

5.2.2 Cruise 2 – April

The second cruise took place in April. During this time the state of the water chemistry is somewhat between winter and summer conditions, and thus somewhat between NCSs summer and winter values. However, when considering this, the NH4 and PO4 concentrations can be assumed to represent relatively good water quality for this time of year. Because this classification system can just say something about the general water quality, and consequently

can not take into account local differences, seasonable changes and historical data might provide better understanding of the possible implications of the measured concentrations.

A comparison between cruise 1 (February) and cruise 2 (April) shows that there are seasonally differences in the ammonium and phosphate distribution. The ammonium concentrations show a general increase from February to April, whereas the phosphate concentration decreases from February to April.

The ammonium concentrations measured in April show that there is considerable variation within the active zones during this cruise. With concentrations ranging from 1 to 18 µg NH4 L^{-1}, some stations have concentrations relatively higher than those measured in the Nordmøre study and Hopavågen study, while others have concentrations lower than those found in these studies. The stations with the highest measured concentrations, some up to 18µg NH4 L^{-1}, do indicate that the release of ammonium in April are considerable. Fish and other animals excrete inorganic nitrogen as ammonium/urea which can be traced in the upper water column. However, this ammonium is usually rapidly assimilated by phytoplankton, and high values are only measured when the supply is greater than the consumption. This can happen when non-natural inputs of ammonium is releases to the system. The general increase observed in ammonium concentration from February to April, despite the additional increase in measured phytoplankton mass, could therefore be the result of aquaculture emissions. If this is the case, these measured ammonium concentrations would be considered quite interesting.

However, the high release of ammonium to the system could also result from degradation of phytoplankton and natural excretion from the food chain. Determination of the phytoplankton assemblage would provide some information about the amount they might be contributing to this ammonium release. Because this is beyond the scope of this thesis, we can not exclude this release as a possible contributing factor for the high ammonium concentrations measured during the second cruise.

The measured concentrations of NH4 and PO4 in the active stations during this second cruise did not differ significantly from the reference station. The exception is a few stations in Hemnefjorden and Snillfjorden which generally expressed low concentrations. Because the reference station most likely is contaminated with nutrient inputs from aquaculture activities, this might not be a good indicator on whether or not aquaculture contributed with any significant macronutrient enrichment to the study area at this time. The reference station, if located somewhere more pristine, might have shown concentration values lower than those actually measured during this cruise. This means, the high values measured in our reference station might in fact be shielding the impact of aquaculture. Further studies in the area can give more information on the possible effect of aquaculture.

The phosphate concentration show the opposite trend of ammonium as it decreases from February to April. The concentration in the active zones range from 3 to 11µg PO4 L^{-1}. This is somewhat high for summer values and somewhat low for winter values when compared to the

Nordmøre study and Hopavågen study. However, in April, the system might be considered to be somewhat between these two seasons, and thus the concentrations could be regarded as fairly normal.

Phosphate is supplied to the upper water column through animal excretion and the upwelling of nutrient rich deep water. Via this upwelling, nitrate will also be supplied to the upper waters. The seasonal differences for nitrate, measured during cruise 1 and cruise 2, show that the concentration decreases from February to April, indicating that, at this time, there has yet to be a significant upwelling of water rich in nutrients. Although aquaculture activities releases a fairly amount of phosphate, it is not nearly as much as the ammonium being released, and much of the phosphate is accumulated in the sediments. Naturally increase in phytoplankton concentration and reduced upwelling of nutrients may therefore be the cause of the observed decrease in phosphate concentration.

Both nitrate (NO3) and silicate (SiO3) showed values within normal for both cruises when compared to the Nordmøre study and the Hopavågen study. During the first cruise the concentrations for NO3 and SiO3 were measured to be in average around 90 and 95 μg L^{-1}, respectively. During the second cruise, these concentrations decreased, as expected due to increased phytoplankton biomass, to around 18 and 27 μg L^{-1}, respectively.

5.3 Macronutrient distribution and chlorophyll a concentrations

The chlorophyll a concentration increases from the first cruise to the second. However, all concentrations measured are still within OSPAR-commission's standard value for the North Sea, sat to 2-4μg chlorophyll L^{-1}, with elevated levels at >4,5μg L^{-1}. This would indicate that the phytoplankton blooms has yet to set in, or, at least in some areas, are in the early stages. But, there is a significant increase in chlorophyll a concentration from February to April, meaning the primary production is higher during this second cruise. This increase in primary production would also indicate that the demand for nutrients would be greater. Emissions from aquaculture will support this need for nutrients, but, at this time, it does not seem to be doing so at the risk of eutrophication.

The chlorophyll a concentrations were generally highest in Hemnefjorden and Snillfjorden (stations 20 to 23). These stations did also systematically display the lowest macronutrient concentrations, including ammonium and phosphate concentrations. These stations are located in more sheltered areas, some can even be considered closed areas, and differs therefore from some of the other active stations. This could mean the dilution effect and the inflow of new fresh water are somewhat restricted in this area. The macronutrients could become concentrated as a result of the reduced circulation and give rise to increased primary production.

The other active stations sampled during the second cruise are located in areas with a (possible) higher degree of water exchange. They can be considered as more open locations. These stations do also show a higher chlorophyll a concentration compared to the first cruise, but these values, measured to be less than 2µg chlorophyll L^{-1}, are under the OSPAR commission`s standard value for the North Sea. This would indicate no harmful algae growth, and the nutrients released from aquaculture activities in the area seems only to support the primary production within reasonable limits.

5.4 Nutrient ratio

The relative concentration of N and P can be used to estimate which of the nutrients limits the growth of phytoplankton in the system. The Redfield ratios are widely used for this purpose, although they do have some limitations. They represent an average phytoplankton stoichiometry, and can therefore not be considered universally valid. Local differences in the phytoplankton communities may result in variations in the stoichiometry because the actual chemical species taken up by phytoplankton depends on the biological species present, their physiological state and environmental conditions (arrigo 2005 + libes 2009). This could result in ratios that are locally different from Redfield's ratios. Historical data or perennial measurements can provide better understanding of the expected nutrient ratio for the actual area. However, the ratios themselves and the seasonally differences can also provide information about the situation.

The N:P Redfield ratio is sat to 16. Measured N:P ratios less than 16 would indicate that the system is nitrogen limited, while N:P ratios higher than 16 would indicate that the system is leaning towards phosphate limitation. In marine waters, nitrogen is often identified as the growth limiting nutrient. Areas which experiences high inputs of nitrogenous compounds will have a N:P ratio closer to 16 or higher if the system is unable to assimilate the excess input of nitrogen. During the first cruise N:P ratios around 14-15 were calculated for all stations, including the reference station. This is close to the Redfield ratio, but still somewhat lower, indicating that nitrogen might still be limiting the production. But, overall, it seems the system, as measured in February, is well balanced.

During the second cruise the active zones showed a greater variation in the N:P ratios. The stations showing the highest chlorophyll a concentrations (stations 20-23 in Hemnefjorden and Snillfjorden), did also express the lowest N:P ratios (N:P between 6 to 8). The nutrient deficiency seemed do become more pronounced as the phytoplankton biomass increased. The reasonable high primary production measured in these stations appeared to be depleting the system for dissolved inorganic nitrogen (DIN). However, the system might not have reached this state of chlorophyll a concentrations at this time without the additional nutrient input from aquaculture. So, even though the N:P ratios calculated in these stations were quite low, indicating nitrogen limited systems, the emissions from aquaculture could be the underlying reason for the high primary production at this time.

The active stations located in more open areas did also have N:P ratios lower than the Redfield ratio. With N:P ratios around 10-13 they to are indicating a system that is nitrogen limited.

With N:P ratios less than 16 for all stations, it would seem the systems are still nitrogen limited despite the additional input from aquaculture. And with chlorophyll a concentrations in the range of 2-4μg L^{-1}, it appears that the systems are only supplied with nutrients in the range of what they can manage to assimilate without the risk of a harmful phytoplankton bloom.

For the first cruise, the N:Si and Si:P ratios were measured to be around 2 and 7, respectively. With a N:Si Redfield ratio of 1 and a Si:P Redfield ratio of 16, this indicates that the system is silicate limited. During the second cruise there were a general increase in N:Si ratio and a general decrease in Si:P ratio (although small). This would indicate a diatom growth from February to April which is further depleting the system for silicate.

The N:Si and Si:P ratios will have great affect on the phytoplankton community structure, especially the shift from diatoms to non-diatoms, or siliceous algae to non-siliceous algae. Silicic acid limitation could have major impacts on the water quality, as a reduction of diatom growth in favour of the noxious flagellates may exacerbate eutrophication. A long term silicic acid limitation can be associated with significant blooms of non-siliceous algae (Iriarte et al., 2010, Justić et al., 1995, Mente et al., 2006).

5.5 Possible consequences for the ecosystem

The growth of phytoplankton is often limited by the availability if nutrients in coastal waters. Emissions of ammonium and phosphate from aquaculture activity can therefore supply these waters with nutrients in volumes which could enhance the negative effect of eutrophication. However, it is not the increase in nutrient concentration alone that causes eutrophication problems, but also the unbalance of the delivery of nutrients. Especially the unbalance in the delivery in nitrogen and phosphate with respect to silica. Undesirable eutrophication in coastal waters often occurs with the development of non-siliceous algae which are responding to the new, increased sources of nitrogen and phosphate (Voss 2011). In addition, because aquaculture releases much of it nitrogen as ammonium, causing a possible shift in available nitrogen source from nitrate to ammonium, structurally changes in phytoplankton communities can occur (Olsen and Olsen, 2008).

6 Conclusion and further work

Aquaculture will continue to release inorganic nutrients such as ammonium and phosphate to coastal waters as long as the industry continues to make use of the many localities in fjords along the Norwegian coast. It will therefore be important to gain better understanding for how nutrients and organic wastes from the industry are distributed and influence ecosystem.

During this work, samples were collected in late winter (February) and early spring (April). The results show that there are seasonal changes in macronutrient concentrations. However, while most of these seasonal changes are expected, due to increased phytoplankton concentration, the changes in ammonium concentrations are of a greater interest. During the second cruise (spring season), ammonium concentrations up to 18µg L^{-1} were measured, which is a significant increase compared to the first cruise (winter season) when all ammonium concentrations measured were lower than 10µg L^{-1}. The ammonium concentrations measured in these waters could thus be regarded as considerable, and a potential source of the ammonium could be emissions from aquaculture.

Due to a contaminated reference station, lack of historical data for this exact area and missing information (at this time) about the composition of the phytoplankton communities, it is not possible, at this stage in the project, to make any conclusions as to whether or not these ammonium concentrations are a result of aquaculture activities. However, data obtained during this work can be used in further work on the CINTERA project, which will continue collecting samples through several seasons until 2015. Because the emissions from aquaculture are expected to be highest during summer, it will be interesting to see the results from the next stages in the project.

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Table of Contents

Appendix A. Auto-Analyser results

A.1 Ammonium – NH4

Table I. Ammonium concentration (µg (NH4)N / L

	Date	Station	Station	Coordinates	Depth (m)	Sampling (m)	Parallel 1 (µg/L)	Paralel 2 (µg/L)	Mean (µg/L)	SD (µg/L)
1 (February)	12.02.	2	Vest Frøyfjorden	N 63°37.427` E 8°15.977	255	7	4	7	5	2,2
	12.02.	3	Vest Torsøya	N 63°37.541` E 8°25.468	161	7	8	6	7	1,4
	12.02.	4	Vest Langøya	N 63°37.888` E 8°34.131	131	7	11	9	10	1,2
	12.02.	5	Øst Langøya	N 63°37.912` E 8°34.815	145	7	11	10	10	0,9
	12.02.	6	Storhallaren	N 63°40.544` E 8°39.562	89	7	9	5	7	2,9
	13.02.	7	Øst Frøyfjorden	N 63°40.979` E 8°52.059	102	7	9	7	8	1,7
	13.02.	8	Inntian Nord Frøya	N 63°44.771` E 8°52.412	44	7	6	8	7	1,2
	12.02.	1	Reference station 1	N 63°36.634` E 8°08.379	316	7	12	5	9	5,3
2 (April)	16.04.	9	Fillfjorden	N 63°36.605` E 9°00.709	179	4+6+8	18	16	17	1,2
	16.04.	10	Øst Frøyfjorden	N 63°40.979` E 8°52.059	103	4+6+8	18	17	18	0,2
	16.04.	11	Midt. Frøyfjorden	N 63°40.148` E 8°40.509	108	4+6+8	15	15	15	0,1
	16,04.	12	Øst Torsøya	N 63°36.524` E 8°30.403	82	4+6+8	14	14	14	0,3
	16.04.	13	Inntian Frøya	N 63°43.790` E 8°51.018	30	4+6+8	18	17	17	0,7
	16.04.	14	Inntian Nord Frøya	N 63°44.771` E 8°52.412	45	4+6+8	14	13	13	0,4
	17.04.	15	Øst Mausen	N 63°51.334` E 8°43.462	132	4+6+8	17	15	16	1,4
	17.04.	16	Sørvest Mausen	N 63°49.548` E 8°34.149	91	4+6+8	15	15	15	0,1
	18.04.	19	Nordøst Hemnskjel	N 63°31.856` E 9°12.735	168	4+6+8	11	10	11	0,6
	18.04.	20	Nord Røstøya	N 63°27.441` E 8°54.001	170	4+6+8	10	9	9	0,5
	18.04.	21	Vest Jamtøya/ Hemnefjorden	N 63°26.595` E 9°06.699	207	4+6+8	9	8	9	0,6
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	N 63°21.473` E 9°13.332	400	4+6+8	4	5	4	0,7
	18.04.	23	Snillfjorden	N 63°22.674` E 9°25.325	199	4+6+8	2	1	1	0,5
	17.04.	18	Refernce station 2	N 63°38.141` E 8°24.425	407	4+6+8	14	11	13	1,8

A.2 Phosphate – PO_4

Table II. Phosphate concentration (µg (PO4)P / L)

	Date	Station	Station	Coordinates	Depth (m)	Sampling (m)	Parallel 1 (µg/L)	Parallel 2 (µg/L)	Mean (µg/L)	SD (µg/L)
1 (February)	12.02.	2	Vest Frøyfjorden	N 63°37.427` E 8°15.977	255	7	14	15	15	0,3
	12.02.	3	Vest Torsøya	N 63°37.541` E 8°25.468	161	7	15	-	15	-
	12.02.	4	Vest Langøya	N 63°37.888` E 8°34.131	131	7	16	16	16	0,1
	12.02.	5	Øst Langøya	N 63°37.912` E 8°34.815	145	7	16	16	16	0,1
	12.02.	6	Storhallaren	N 63°40.544` E 8°39.562	89	7	10	17	14	4,7
	13.02.	7	Øst Frøyfjorden	N 63°40.979` E 8°52.059	102	7	15	17	16	1,0
	13.02.	8	Inntian Nord Frøya	N 63°44.771` E 8°52.412	44	7	16	15	15	0,2
	12.02.	1	Reference station 1	N 63°36.634` E 8°08.379	316	7	15	15	15	0,4
2 (April)	16.04.	9	Fillfjorden	N 63°36.605` E 9°00.709	179	4+6+8	11	11	11	0,1
	16.04.	10	Øst Frøyfjorden	N 63°40.979` E 8°52.059	103	4+6+8	9	9	9	0,0
	16.04.	11	Midt. Frøyfjorden	N 63°40.148` E 8°40.509	108	4+6+8	7	7	7	0,2
	16,04.	12	Øst Torsøya	N 63°36.524` E 8°30.403	82	4+6+8	7	7	7	0,1
	16.04.	13	Inntian Frøya	N 63°43.790` E 8°51.018	30	4+6+8	6	9	8	1,9
	16.04.	14	Inntian Nord Frøya	N 63°44.771` E 8°52.412	45	4+6+8	8	9	8	0,1
	17.04.	15	Øst Mausen	N 63°51.334` E 8°43.462	132	4+6+8	7	8	7	0,1
	17.04.	16	Sørvest Mausen	N 63°49.548` E 8°34.149	91	4+6+8	8	8	8	0,0
	18.04.	19	Nordøst Hemnskjel	N 63°31.856` E 9°12.735	168	4+6+8	6	6	6	0,1
	18.04.	20	Nord Røstøya	N 63°27.441` E 8°54.001	170	4+6+8	5	5	5	0,2
	18.04.	21	Vest Jamtøya/ Hemnefjorden	N 63°26.595` E 9°06.699	207	4+6+8	5	5	5	0,3
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	N 63°21.473` E 9°13.332	400	4+6+8	3	3	3	0,1
	18.04.	23	Snillfjorden	N 63°22.674` E 9°25.325	199	4+6+8	3	3	3	0,0
	17.04.	18	Reference station 2	N 63°38.141` E 8°24.425	407	4+6+8	5	5	5	0,1

A. 3 Nitrate – NO_3 (+NO_2)

Table III. Nitrate concentration (µg (NO3+NO2)N / L)

	Date	Station	Station	Coordinates	Depth (m)	Sampling (m)	Parallel 1 (µg/L)	Parallel 2 (µg/L)	Mean (µg/L)	SD (µg/L)
1 (February)	12.02.	2	Vest Frøyfjorden	N 63°37.427` E 8°15.977	255	7	89	91	90	1,6
	12.02.	3	Vest Torsøya	N 63°37.541` E 8°25.468	161	7	89	-	89	-
	12.02.	4	Vest Langøya	N 63°37.888` E 8°34.131	131	7	87	92	89	3,6
	12.02.	5	Øst Langøya	N 63°37.912` E 8°34.815	145	7	87	85	86	1,0
	12.02.	6	Storhallaren	N 63°40.544` E 8°39.562	89	7	77	98	87	14,7
	13.02.	7	Øst Frøyfjorden	N 63°40.979` E 8°52.059	102	7	98	100	99	1,0
	13.02.	8	Inntian Nord Frøya	N 63°44.771` E 8°52.412	44	7	88	89	89	0,8
	12.02.	1	Reference station 1	N 63°36.634` E 8°08.379	316	7	91	88	90	2,3
2 (April)	16.04.	9	Fillfjorden	N 63°36.605` E 9°00.709	179	4+6+8	48	45	46	2,0
	16.04.	10	Øst Frøyfjorden	N 63°40.979` E 8°52.059	103	4+6+8	31	32	31	0,8
	16.04.	11	Midt. Frøyfjorden	N 63°40.148` E 8°40.509	108	4+6+8	19	20	19	0,5
	16,04.	12	Øst Torsøya	N 63°36.524` E 8°30.403	82	4+6+8	13	14	13	0,5
	16.04.	13	Inntian Frøya	N 63°43.790` E 8°51.018	30	4+6+8	24	25	24	0,4
	16.04.	14	Inntian Nord Frøya	N 63°44.771` E 8°52.412	45	4+6+8	25	24	25	0,4
	17.04.	15	Øst Mausen	N 63°51.334` E 8°43.462	132	4+6+8	19	18	19	0,3
	17.04.	16	Sørvest Mausen	N 63°49.548` E 8°34.149	91	4+6+8	22	22	22	0,1
	18.04.	19	Nordøst Hemnskjel	N 63°31.856` E 9°12.735	168	4+6+8	19	19	19	0,2
	18.04.	20	Nord Røstøya	N 63°27.441` E 8°54.001	170	4+6+8	9	8	9	1,0
	18.04.	21	Vest Jamtøya/ Hemnefjorden	N 63°26.595` E 9°06.699	207	4+6+8	9	10	9	0,6
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	N 63°21.473` E 9°13.332	400	4+6+8	3	3	3	0,3
	18.04.	23	Snillfjorden	N 63°22.674` E 9°25.325	199	4+6+8	9	5	7	3,0
	17.04.	18	Reference station 2	N 63°38.141` E 8°24.425	407	4+6+8	7	5	6	1,3

Appendix B Determination of silicate

B.1 Standard curve

Table IV. Determination of standard curve

Si µM	Parallel 1 absorbans	Parallel 2 absorbans	Mean absorbance
0,2	0,043	0,045	0,044
0,5	0,061	0,066	0,064
1	0,087	0,092	0,090
5	0,323	0,324	0,324
10	0,615	0,611	0,613
20	1,238	1,243	1,241

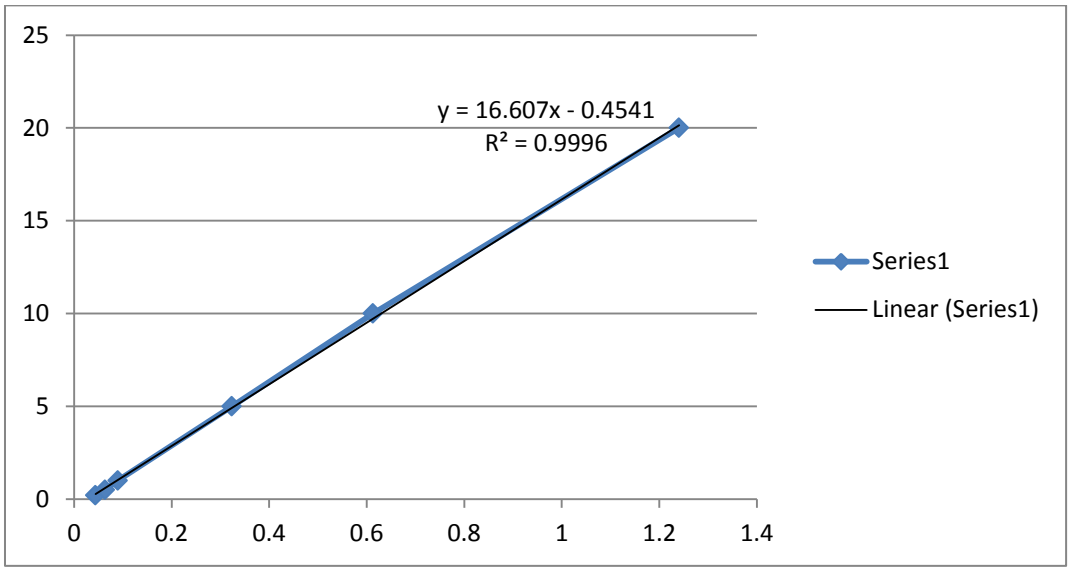

Figure I. Determination of standard curve

B.2 Measure absorbance

B.2.1 Cruise 1

Table V. Absorbance measured for samples collected during cruise 1

Station		Sample	Parallel 1 absorbance	Parallel 2 absorbance	Mean absorbance
2	Vest Frøyfjorden	3	0,2060	0,2070	0,2065
		4	0,1710	0,1700	0,1705
3	Vest Torsøya	5	0,2040	0,2030	0,2035
		6	0,2150	0,2160	0,2155
4	Vest Langøya	7	0,2140	0,2140	0,2140
		8	0,2140	0,2130	0,2135
5	Øst Langøya	9	0,2020	0,2010	0,2015
		10	0,2090	0,2090	0,2090
6	Storhallaren	11	0,1680	0,1670	0,1675
		12	0,2120	0,2120	0,2120
7	Øst Frøyfjorden	13	0,2270	0,2270	0,2270
		14	0,2130	0,2120	0,2125
8	Inntian Nord Frøyfjorden	15	0,2180	0,2160	0,2170
		16	0,2160	0,2160	0,2160
1	Reference station 1	1	0,2080	0,2040	0,2060
		2	0,2100	0,2100	0,2100

B.2.2 Cruise 2

Table VI. Absorbance measured for samples collected during cruise 1

Station		Sample	Parallel 1 absorbance	Parallel 2 absorbance	Mean absorbance
9	Fillfjorden	17	0,0450	0,0490	0,0470
		18	0,0410	0,0400	0,0405
10	Øst Frøyfjorden	19	0,0550	0,0540	0,0545
		20	0,0510	0,0510	0,0510
11	Midt. Frøyfjorden	21	0,0670	0,0660	0,0665
		22	0,0650	0,0640	0,0645
12	Øst Torsøya	23	0,0570	0,0560	0,0565
		24	0,0610	0,0610	0,0610
13	Inntian Frøya	25	0,0450	0,0440	0,0445
		26	0,0540	0,0520	0,0530
14	Inntian Nord Frøyfjorden	27	0,0590	0,0590	0,0590
		28	0,0760	0,0710	0,0735
15	Øst Mausen	29	0,0750	0,0750	0,0750
		30	0,0700	0,0680	0,0690
16	Sørsvest Mausen	31	0,0720	0,0710	0,0715
		32	0,0750	0,0760	0,0755
19	Nordøst Hemnskjel	35	0,0830	0,0810	0,0820
		36	0,0690	0,0690	0,0690
20	Nord Røstøya	37	0,0630	0,0620	0,0625
		38	0,0560	0,0540	0,0550
21	Vest Jamtøya/ Hemnefjorden	39	0,0630	0,0610	0,0620
		40	0,0660	0,0660	0,0660
22	Midt. Snillfjorden/ Hemnefjorden	41	0,0380	0,0400	0,0390
		42	0,0430	0,0420	0,0425
23	Snillfjorden	43	0,0520	0,0520	0,0520
		44	0,0400	0,0380	0,0390
18	Reference station 2	33	0,0410	0,0410	0,0410
		34	0,0450	0,0460	0,0455

B.3 Silicate – SiO_3

Table VII. Silicate concentration (µg (SiO3)SI / L

	Date	Station	Station	Coordinates	Depth (m)	Sampling (m)	Parallel 1 (µg/L)	Parallel 2 (µg/L)	Mean (µg/L)	SD (µg/L)
1 (February)	12.02.	2	Vest Frøyfjorden	N 63°37.427` E 8°15.977	255	7	101	100	101	0,3
	12.02.	3	Vest Torsøya	N 63°37.541` E 8°25.468	161	7	96	79	88	11,8
	12.02.	4	Vest Langøya	N 63°37.888` E 8°34.131	131	7	95	100	97	3,9
	12.02.	5	Øst Langøya	N 63°37.912` E 8°34.815	145	7	99	99	99	0,2
	12.02.	6	Storhallaren	N 63°40.544` E 8°39.562	89	7	94	97	95	2,5
	13.02.	7	Øst Frøyfjorden	N 63°40.979` E 8°52.059	102	7	78	99	88	14,6
	13.02.	8	Inntian Nord Frøya	N 63°44.771` E 8°52.412	44	7	106	99	102	4,8
	12.02.	1	Reference station 1	N 63°36.634` E 8°08.379	316	7	96	98	97	1,3
2 (April)	16.04.	9	Fillfjorden	N 63°36.605` E 9°00.709	179	4+6+8	22	19	20	2,1
	16.04.	10	Øst Frøyfjorden	N 63°40.979` E 8°52.059	103	4+6+8	25	24	25	1,2
	16.04.	11	Midt. Frøyfjorden	N 63°40.148` E 8°40.509	108	4+6+8	31	30	30	0,7
	16,04.	12	Øst Torsøya	N 63°36.524` E 8°30.403	82	4+6+8	26	28	27	1,5
	16.04.	13	Inntian Frøya	N 63°43.790` E 8°51.018	30	4+6+8	21	25	23	2,8
	16.04.	14	Inntian Nord Frøya	N 63°44.771` E 8°52.412	45	4+6+8	27	34	31	4,8
	17.04.	15	Øst Mausen	N 63°51.334` E 8°43.462	132	4+6+8	35	32	33	2,0
	17.04.	16	Sørvest Mausen	N 63°49.548` E 8°34.149	91	4+6+8	33	35	34	1,3
	18.04.	19	Nordøst Hemnskjel	N 63°31.856` E 9°12.735	168	4+6+8	38	32	35	4,3
	18.04.	20	Nord Røstøya	N 63°27.441` E 8°54.001	170	4+6+8	29	26	27	2,5
	18.04.	21	Vest Jamtøya/ Hemnefjorden	N 63°26.595` E 9°06.699	207	4+6+8	29	31	30	1,3
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	N 63°21.473` E 9°13.332	400	4+6+8	18	20	19	1,2
	18.04.	23	Snillfjorden	N 63°22.674` E 9°25.325	199	4+6+8	24	18	21	4,3
	17.04.	18	Reference station 2	N 63°38.141` E 8°24.425	407	4+6+8	19	21	20	1,5

Appendix C – Macronutrient concentration in µM

Table VIII. Macronutrient concentration in µM

	Date	Station	Station	NH4 µM	NO3 µM	NH4+NO3 µM	PO4 µM	Si µM
1 (February)	12.02.	2	Vest Frøyfjorden	0,37	6,45	6,82	0,47	3,12
	12.02.	3	Vest Torsøya	0,51	6,33	6,84	0,47	3,47
	12.02.	4	Vest Langøya	0,73	6,38	7,11	0,50	3,54
	12.02.	5	Øst Langøya	0,74	6,13	6,87	0,50	3,40
	12.02.	6	Storhallaren	0,49	6,22	6,71	0,44	3,14
	13.02.	7	Øst Frøyfjorden	0,58	7,07	7,65	0,52	3,64
	13.02.	8	Inntian Nord Frøya	0,48	6,35	6,83	0,50	3,58
	12.02.	1	Reference station 1	0,62	6,39	7,01	0,49	3,44
2 (April)	16.04.	9	Fillfjorden	1,22	3,30	4,52	0,35	0,72
	16.04.	10	Øst Frøyfjorden	1,26	2,23	3,48	0,30	0,87
	16.04.	11	Midt. Frøyfjorden	1,07	1,39	2,46	0,24	1,08
	16,04.	12	Øst Torsøya	1,00	0,94	1,94	0,22	0,97
	16.04.	13	Inntian Frøya	1,23	1,75	2,98	0,25	0,81
	16.04.	14	Inntian Nord Frøya	0,95	1,76	2,71	0,27	1,10
	17.04.	15	Øst Mausen	1,12	1,33	2,45	0,24	1,19
	17.04.	16	Sørvest Mausen	1,09	1,56	2,65	0,25	1,22
	18.04.	19	Nordøst Hemnskjel	0,77	1,37	2,14	0,20	1,25
	18.04.	20	Nord Røstøya	0,66	0,63	1,29	0,16	0,97
	18.04.	21	Vest Jamtøya/ Hemnefjorden	0,62	0,67	1,29	0,16	1,06
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	0,29	0,22	0,52	0,09	0,67
	18.04.	23	Snillfjorden	0,10	0,51	0,61	0,09	0,75
	17.04.	18	Reference station 2	0,91	0,45	1,36	0,17	0,72

Appendix D Chlorophyll a

Table IX. Chlorophyll a concentration measured during cruise 1 and cruise 2 (>200µm)

	Date	Station	Station	A1 (µg chl a/L)	A2 (µg chl a/L)	B1 (µg chl a/L)	B2 (µg chl a/L)	Mean (µg chl a/L)
1 (February)	12.02.	2	Vest Frøyfjorden	0,298	0,326	0,310	0,343	0,319
	12.02.	3	Vest Torsøya	0,254	0,242	0,278	0,290	0,266
	12.02.	4	Vest Langøya	0,166	0,156	0,170	0,178	0,167
	12.02.	5	Øst Langøya	0,154	0,168	0,161	0,149	0,158
	12.02.	6	Storhallaren	0,168	0,190	0,156	0,158	0,168
	13.02.	7	Øst Frøyfjorden	0,192	0,209	0,185	0,190	0,194
	13.02.	8	Inntian Nord Frøya	0,204	0,209	0,192		0,202
	12.02.	1	Reference station 1	0,310	0,322	0,310	0,312	0,313
2 (April)	16.04.	9	Fillfjorden	1,094	1,097	1,025	0,912	1,032
	16.04.	10	Øst Frøyfjorden	1,702	1,610	1,265	1,358	1,484
	16.04.	11	Midt. Frøyfjorden	1,594	1,670	1,685	1,651	1,650
	16,04.	12	Øst Torsøya	1,073	0,979	1,313	1,378	1,186
	16.04.	13	Inntian Frøya	0,744	0,775	0,677	0,629	0,706
	16.04.	14	Inntian Nord Frøya	1,668	1,637	1,111	1,147	1,391
	17.04.	15	Øst Mausen	1,058	1,066	1,217	1,126	1,117
	17.04.	16	Sørvest Mausen	1,222	1,195	1,147	1,106	1,168
	18.04.	19	Nordøst Hemnskjel	1,159	1,178	1,135	1,145	1,154
	18.04.	20	Nord Røstøya	2,734	2,717	3,019	3,005	2,869
	18.04.	21	Vest Jamtøya/ Hemnefjorden	2,126	2,299	1,951	1,848	2,056
	18.04.	22	Midt. Snillfjord/ Hemnefjorden	2,225	2,016	2,126	2,112	2,120
	18.04.	23	Snillfjorden	3,780	4,248	4,056	4,073	4,039
	17.04.	18	Reference station 2	1,649	1,673	1,584	1,654	1,640