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The Effect of Nutrient Release from Fish Farms on the Lower Trophic Levels of the Marine Food Web in North Patagonia, Chile

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Abstract

A mesocosm experiment was performed over 16 days during austral summer in 2010, in Northern Patagonia, Chile. Nutrients were added in 8 different concentrations along a gradient, simulating different levels of nutrients released by salmon farms. Nitrogen loading rate ranged from L_N = 0 µg l^{-1} d^{-1} to L_N = 42 µg l^{-1} d^{-1}, and Silicate and Phosphorus were added in Redfield ratios. The goal of the study was to look at the effect of nutrient addition on the lower trophic levels of the marine pelagic food web represented by three groups of organisms: Phytoplankton, Ciliates and Copepods.

Nutrient addition was found to have a very clear effect on chlorophyll *a*, POC and ciliate biomass, which showed a very clear linear increase with increasing loading rate. POC and chl *a* values showed that phytoplankton growth started immediately after nutrient addition. At the maximum level POC values had become 13 times larger than at the original level. Phytoplankton community composition, in the mesocosms with the highest loading rates, was found to shift from larger cells in the beginning of the experiment to smaller cells towards the end. Ciliate biomass was found to increase linearly with food concentration, indicating that ciliate population growth was closely tied to food availability. Ciliate biomass peaked on day 8 with 117 µg C l^{-1} found in the treatments with highest nutrient additions. A maximum of 91 ciliates ml^{-1} was found on day 16 during the experiment. Both these values are much higher than what has previously been reported in the area, indicating that ciliates might play a more important role in the Patagonian marine food web than previously thought. Copepod concentration and biomass were found to be uncorrelated to both nutrient addition and food concentration. This study found that nutrients released from fish farms have the potential to cause drastic increases in chl *a* and phytoplankton and ciliate biomass, as well as affect the community structure of phytoplankton.

Sammendrag

Det ble utført et 16 dager langt mesocosmeforsøk i Nord Patagonia, Chile under den australske sommeren i 2010. Næringsstoffer ble tilsatt i 8 ulike konsentrasjoner som fulgte en gradient, for å simulere næringsstoffutslipp fra lakseoppdrett. Målet var å studere hvordan næringsstoffene påvirket de lavere trofiske nivåene i næringsnettet, representert med 3 grupper organismer: phytoplankton, ciliater og copepoder. Nitrogentilsetningene varierte fra L_N = 0 µg l^{-1} d^{-1} til L_N = 42 µg l^{-1} d^{-1}, og fosfor og silisium ble tilsatt i Redfield-ratioer.

Chl *a*- og POC-verdier viste at phytoplanktonen reagerte raskt og kraftig på næringsstofftilsetningene. POC- verdiene viste at phytoplanktonbiomassen ble 13 ganger så stor ved maks. nivå som ved det originale nivået. Chl *a* og POC viste en nær lineær økning i takt med økt næringsstofftilsetning. I mesocosmene med de høyeste næringsstofftilsetningene oppstod det en endring i phytoplanktonens samfunnsstruktur fra store alger til mindre alger over tid. Ciliater reagerte raskt med tilsatte næringsstoffer, og nådde en topp på dag 8 med 117 µg C l^{-1}. Maks antall ciliater ble funnet på dag 16 med 91 individer ml^{-1}. Dette er et desidert høyere antall ciliater enn det som har blitt funnet tidligere i området, og viser at ciliater kan spille en viktig rolle i det patagoniske næringssnettet under riktige omstendigheter. Biomassen til ciliater hadde en klar lineær korrelasjon både tilsatt næringsstoffer og matkonsentrasjon. Det ble ikke funnet noen korrelasjon mellom verken copepoder og mengden tilsatte næringsstoffer eller mellom copepoder og matkonsentrasjon. Disse resultatene indikerer at næringsstoffutslipp fra lakseoppdrett kan føre både til en kraftig økning i biomassen til organismer i lavere trofiske nivåer, i tillegg til endringer i phytoplanktonens samfunnsstruktur.

Table of Contents

1. Introduction

Production of Atlantic salmon in the fjords of Norway and Chile is a very large and profitable industry (Mente et al. 2006, FAO 2010). This growing industry presents a number of environmental challenges. In particular the fate of nutrients and waste to the nearby environment has been of great concern (Islam 2005, Iriarte et al. 2010). While the effect on the seafloor in proximity of salmon farming sites has been widely studied, the effect on the pelagic marine food web is little documented and understood (Soto and Norambuena 2004; Olsen and Olsen 2008). It is likely that the nutrients released from salmon farms have an impact on the pelagic planktonic food web (Olsen and Olsen 2008).

Phytoplankton has the capacity to absorb nutrients such as ammonia and phosphate directly, thus contributing to phytoplankton growth (Cloern 2001; Olsen and Olsen 2008). Since phytoplankton is a food source for a number of organisms, in particular for both ciliates and copepods, an increase in phytoplankton biomass can lead to a subsequent increase in ciliate and copepod biomass (Cloern 2001; Olsen and Olsen 2008). Copepods may also prey on ciliates (Pierce and Turner 1992), thus possibly benefiting from population growth from both groups. Changes on one level of the pelagic food web has the potential of changing the dynamics of the entire food web, through a process called trophic cascade, in which predators indirectly influence the plant basis of the food web (Stibor et al. 2004). An increase in nutrient availability can also cause changes in phytoplankton community structure, which subsequently can affect higher trophic levels of the food web (Cloern 2001). By looking at the changes in phytoplankton, ciliate and copepod biomass exposed to different levels of nutrient addition, we wish to understand how they are affected by the nutrient release.

The WAFOW, 'Can Waste Emission From Fish Farms Change The Structure Of Marine Food Webs?', -project was funded by the Norwegian Research Council and undertaken over a 3 years period, from 2009 to 2011. The goal of the project was to study the effect of nutrient release from salmon aquaculture on the marine food web, in the tempered coastal fjords of Norway and Chile. To do this, a series of mesocosm-experiments were undertaken in Chile and Norway. The first of these took place in the period of time from the 18th of January 2010 to the 3rd of February 2010 at the Huinay Scientific Field Station, in the Comau fjord of Northern Patagonia, Chile (42°22´ S, 72°24´ W) (Sanchez et al. 2011). This thesis is based on this first experiment and focuses on the lower trophic levels of the pelagic marine food web, with special attention on the role of ciliates.

1.1 List of abbreviations

CAS – Cage Aquaculture Systems

Chl a – Chlorophyll a

CNLR – Critical Nutrient Loading Rate

DIN – Dissolved Inorganic Nitrogen

DIP – Dissolved Inorganic Phosphorous

DON – Dissolved Organic Nitrogen

DOP – Dissolved Organic Phosphorous

DW – Dry Weight

HAB – Harmful Algae Blooms

HNF – Heterotrophic NanoFlagellates

ISA – Infectious Salmon Anemia

L_N – Loading rate of Nitrogen

POC – Particulate Organic Carbon

PON – Particulate Organic Nitrogen

POP – Particulate Organic Phosphorous

WAFOW – Research project 'Can Waste Emission From Fish Farms Change The Structure Of Marine Food Web'

WW – Wet Weight

2. Theory

2.1 Aquaculture of Atlantic salmon

Atlantic salmon, *Salmo salar*, is a species of finfish that is extensively used in aquaculture both in Norway and Chile (Buschmann et al. 2006; Maroni 2000). In 2010 these two countries stood for 35.4% and 28% of the world's salmonid production respectively, placing them as the largest producers of Atlantic salmon in the world (Buschmann et al. 2009; FAO 2010). In Chile the production exceeded 370 000 tons in 2005 (Vielka et al. 2005), but the production was heavily reduced after an outbreak of Infectious Salmon Anemia virus (ISA) in 2007-2008 (FAO 2010; Iriarte et al. 2010).

Production of Atlantic salmon in Chile starts in land-based hatcheries, before the fish larvae are moved to fresh water cages until the fish reaches about 100g, when they are transferred to a marine environment (Vielka et al. 2005). The production in marine waters takes place in Cage Aquaculture Systems (CAS), which are intensive, open, floating cage systems that allow for a high degree of interaction with the environment (Islam 2005; Olsen et al. 2007; Soto and Norambuena 2004). The cage systems vary in shape and size, but are either square-metal cages, or round-plastic cages, and can contain a maximal density-biomass of 20 kg/m^3 (Vielka et al. 2005).

One of the reasons that both Norway and Chile have been able to dominate the production of salmon is the presence of very long (over 1800 and 1500 km respectively), fjord-dominated coastlines (FAO 2010; Iriarte et al. 2010). These coastlines offer near ideal environmental conditions for salmon farming (Soto and Norambuena 2004), with an abundance of sheltered areas, such as fjords, bays and channels (Buschmann et al. 2009; Tironi et al. 2010). Atlantic salmon production takes place in 3 regions in the south of Chile from 41°S to 46 °S, namely region X, XI and XII (Soto and Norambuena 2004; Vielka et al. 2005). Region X is today the largest producer of salmon, and so further growth in the industry is expected to take place in region XI and XII (Soto and Norambuena 2004; Vielka et al. 2005). Region X contains over 375 salmon farms (Vielka et al. 2005).

2.2 Ecological impact of salmon production

The growing production of Atlantic salmon has led to growing concerns in regard to effects of CAS on the environment (Mente et al. 2006; Reid et al. 2009). CAS affect the environment in a variety of ways, including the release of large amount of nutrients from feed and feces (Islam 2005; Iriarte et al. 2010), the use of potentially harmful chemicals such as copper paints used in net treatments (Buschmann et al. 2006; Buschmann et al. 2009), the escapes of farmed fish and their effect on local wild fauna (Buschmann et al. 2006; Tironi et al. 2010), and the introduction of pathogens (Olsen and Olsen 2008; Buschmann et al. 2009; Tironi et al. 2010).

Nutrient emission from fish farms occurs mainly in different forms of nitrogen and phosphorous, which are summarized in Figure 2.1. Uneaten fish feed and fecal matter form Particular Organic Nitrogen and Phosphorous (PON/POP) (Olsen and Olsen 2008). The larger of these particles sink to the seafloor (Islam 2005; Olsen and Olsen 2008), while the smaller remain in suspension, and are thus available for filter feeders (Cloern 2001; Olsen and Olsen 2008). Dissolved Organic Nitrogen and Phosphorous (DON/DOP) are complex chemical compounds from fish feed and feces, that are not directly available for consumption and have a long residential time in the marine ecosystem (Olsen and Olsen 2008). These compounds can aggregate and form marine snow, while the smaller of these compounds can to some extent be available for bacteria. Dissolved Inorganic Nitrogen and Phosphorus (DIN/DIP) are excreted through the fish's gills and are dispersed in the photic zone, mostly in the form of ammonium (NH_4) and phosphate (PO_4) (Olsen and Olsen 2008). About 50% of the nitrogen and 28% of the phosphorus from the feed is lost in this form (Mente et al. 2006). The dissolved inorganic nutrients can be assimilated directly by phytoplankton (Cloern 2001; Olsen and Olsen 2008; Buschmann et al. 2009).

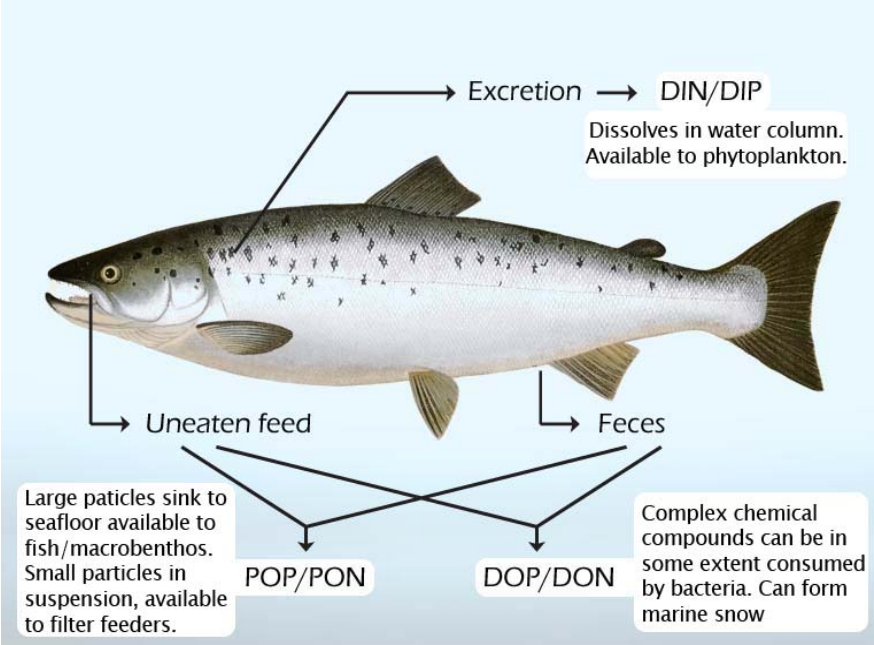

Figure 2.1: Main forms of nutrient emission from Atlantic salmon farms

One of the most documented effects of nutrient emission from fish farms is the impact on sediments directly below or in close proximity to the fish farms (Olsen and Olsen 2008). PON and POP, as well as aggregations of DON/DOP, sink to the seafloor and accumulate (Cloern 2001; Islam 2005; Olsen and Olsen 2008; Tironi et al. 2010). This can result in changes in the physical-chemical properties of the sediments as well as a loss of biodiversity (Cloern 2001; Buschmann et al. 2006; Mente et al. 2006; Tironi et al. 2010).

CAS production has the potential to cause eutrophication in coastal waters in the same way as human and agricultural waste (Merceron et al. 2002; Islam 2005; Olsen and Olsen 2008; Iriarte et al. 2010). Buschmann, Lopez et al. 1996 showed that the

production of 100 t salmon created the same amount of nutrient waste as a population of 2800-3200 people. This means that the Chilean salmon production of 2007 of 370 000 t is the equivalent of approximately 11 million people. However, unlike the environmental impacts on the benthos, the pelagic eutrophication process is little documented and understood (Soto and Norambuena 2004; Olsen and Olsen 2008). The eutrophication process has been hypothesized to have a number of direct and indirect effects on the marine environment.

The direct effects include changes in chlorophyll, primary production, sedimentation rates, nutrient ratios, phytoplankton community, and Harmfull Algae Blooms (HAB) (Cloern 2001; Olsen and Olsen 2008). Since DIN and DIP are directly available to phytoplankton, increases in primary production and chlorophyll can occur quite rapidly following nutrient addition (Cloern 2001; Merceron et al. 2002; Islam 2005; Olsen and Olsen 2008). This can result in phytoplankton blooms as well as changes in phytoplankton community (Cloern 2001, Mente et al. 2006). Changes in nutrient ratios can occur with regards to N:Si and P:Si ratios, as silicate is not released from fish farms and thus Si can become depleted in natural waters (Cloern 2001; Iriarte et al. 2010). This can lead to growth of dinoflagellates over diatoms (Cloern 2001; Buschmann et al. 2006; Mente et al. 2006; Iriarte et al. 2010). The occurrence of HABs has been linked to emission from CAS (Buschmann et al. 2006; Mente et al. 2006; Iriarte et al. 2010). Buschmann et al. (2006) reported an increase of HABs in southern Chile, possibly in connection to salmon production. An initial increase in phytoplankton biomass is followed by a stepwise increase in grazer and predator biomass, as carbon is transported through the food chain (Soto and Norambuena 2004; Olsen and Olsen 2008). Biomass that is not assimilated into the food web will sink to the seafloor, affecting sedimentation rates (Cloern 2001; Olsen and Olsen 2008).

Indirect effects of eutrophication include changes in water transparency, seasonal cyles, nutrient cycling and food web structure. Increases in phytoplankton biomass can reduce water transparency (Cloern 2001). Changes in seasonal cycles can occur, as a result of nutrient emission since nutrient availability may change. Normally temperate coastal areas experience a nutrient rich environment during the winter, which is then depleted through the summer, but nutrient emission from CAS occur mainly during the summer, thus possibly changing seasonal cycles (Cloern 2001; Mente et al. 2006).

The effects of nutrient emission on the coastal environment depend on several physical and biological factors (Cloern 2001; Olsen and Olsen 2008). The main physical factor being local hydrodynamics (Cloern 2001; Soto and Norambuena 2004; Olsen and Olsen 2008), such as tidal currents, water transport and residence times, which have a big effect on nutrient dispersal and thus on the local eutrophication process (Aure and Stigebrandt 1990; Cloern 2001; Soto and Norambuena 2004). Of the biological factors, the integrity and elasticity of the food web is of much importance (Cloern 2001; Soto and Norambuena 2004; Olsen and Olsen 2008). The assimilation capacity of the food web is crucial for how carbon is transported through the food chain (Olsen and Olsen 2008). Olsen and Olsen (2008) stipulated a

Critical Nutrient Loading Rate (CNLR) above which the food web loses its buffer capacity and its ecosystem integrity, and placing this limit between 3 and 20 mg N m^{-3} day^{-1} for coastal ecosystems.

2.3 The marine food web

The marine pelagic food web in temperate coastal waters is characterized by alternating seasons of high and low productivity due to climatic changes in temperature, solar radiation and precipitation. The high productivity season in Northern Patagonia starts in late winter/early spring (September) and lasts until late summer (April) (Buschmann et al. 1996; Sanchez et al. 2011).

The marine food web has traditionally been divided into the classical food chain and the microbial loop (Calbet and Saiz 2005). The classical food chain is based on carbon fixation through photosynthesis performed by phytoplankton (Calbet and Saiz 2005). Increases in solar radiation trigger phytoplankton growth, which is then eaten by larger planktonic organisms such as copepods and euphausiids, who in turn are food for larger organisms such as fish larvae or jellyfish (Pierce and Turner 1992). The microbial loop has dead organic material as its primary carbon source. Organic waste material, so called “marine snow”, in form of waste material and dead organisms sinks sink through the water and taken up by bacteria. The bacteria are in turn prey for Heterotroph Nanoflagellates (HNF) and ciliates (Azam et al. 1983). Ciliates and nanoflagellates can both be preyed upon by other planktonic organisms such as copepods (Calbet and Saiz 2005). The microbial loop is called a loop because all marine organisms contribute to the production of organic waste material, thus enabling a cyclic carbon transport (Azam et al. 1983). The classical food chain and microbial loop merge with ciliates and to a certain amount with HNF since these organisms can have dead organic material as their original food source but can be prey for organisms belonging to the classical food chain (Pierce and Turner 1992).

Phytoplankton is a general term used to describe autotrophic marine planktonic algae. Phytoplankton varies greatly in size and can be classified as pico- (0.2 to 2 µm), nano- (2 to 20 µm) and microphytoplankton (20 to 200 µm). Nanophytoplankton usually consists of dinoflagellates, while diatoms usually dominate microphytoplankton (Buschmann et al. 1996). Diatoms require silicate to grow, and often have the ability to form long chains or other patterns (Takeda 1998).

Ciliates are a group of marine protists that are found in all kind of marine environments, but are usually more abundant in coastal waters (Pierce and Turner 1992). The group is characterized by their cilia, which are arranged both in simple manner or more complex structure around the organism’s mouth and over the cell, dependent of the species. The cilia’s function includes both feeding and locomotion processes (Laybourn-Parry 1982). Ciliates reproduce asexually through binary fission, which allows them to exhibit very fast growth (Laybourn-Parry 1992, Pierce and Turner 1992).

Ciliates have been found be both autotrophic, heterotrophic and mixotrophic, though heterotrophy is most common. Ciliates are classified as microplankton, as their size usually is between 20 – 200 µm. However, smaller ciliates of about 10 µm are also common. These smaller ciliates are usually bacteriovore, while the bigger heterotrophic ciliates often graze on phytoplankton as well as HNF. Strictly carnivorous ciliate species have also been described. As a general rule the size of the food consumed by ciliates is proportional to the ciliate's cell size (Pierce and Turner 1992). Ciliates are commonly classified on whether or not they have lorica. Both belong in the subclass Choreotricha, with loricated ciliates belonging to the class Spirotrichea. These ciliates are commonly called tintinnids. Marine aloricate ciliates are mostly a part of the Oligotrichida order. Important families in this order are *Strombidium, Halteria*, *Laboea* and *Tontonia* (Laybourn-Parry 1992). Tintinnids are generally less abundant than aloricated ciliates (Ohman and Snyder 1991; Pierce and Turner 1992). The most common marine species of both tintinnid and aloricate marine species are shown in Figure 4.2. In this thesis the terms tintinnids and aloricate ciliates will be used to describe the different types of ciliates independent of further classification.

Copepods are marine crustaceans that are abundant in most temperate coastal waters. They are a very important food resource for a number of fish larvae, and thus important for a number of commercial fisheries (Mauchline 1998). Copepods are usually in the size class of 500 – 5000 µm, and are considered to be a part of mesoplankton (Tokle 1999). Pelagic marine copepods usually belong to 1 of 3 main groups: Calanoida, Cyclopoida or Harpacticoida (Tokle 1999), as illustrated in Figure 2.3. Calanoid copepods are considered the dominating species in terms of biomass, while cyclopoid copepods can dominate in terms of numbers (Gismervik et al. 2002).

Copepods commonly have a lifespan that varies from several months up to several years (Peterson 1998; Arnkværn et al. 2005). Their life starts as eggs that hatch as nauplii, become copepodites, before finally evolving into adult copepods. Calanoid copepods have 6 nauplii stages and 5 copepodites stages, before evolving to its final adult stage (Tokle 1999).

Copepods are usually classified either as filter/suspension feeders, ambush feeders or cruising/filter feeders (Tiselius and Jonsson 1990; Gismervik 2006). *Calanus* and *Pseudocalanus*, among others are considered filter feeders, and are typically labeled as algivores. However both filter feeding and cruising copepods have been found to have tremendous impact on ciliate community, thus deviating from their algivore behavior (Gismervik 2006). It has been observed that copepods will adapt their behavior to prey availability, meaning that certain copepods may switch from one category to another according to food resources available (Tokle 2006).

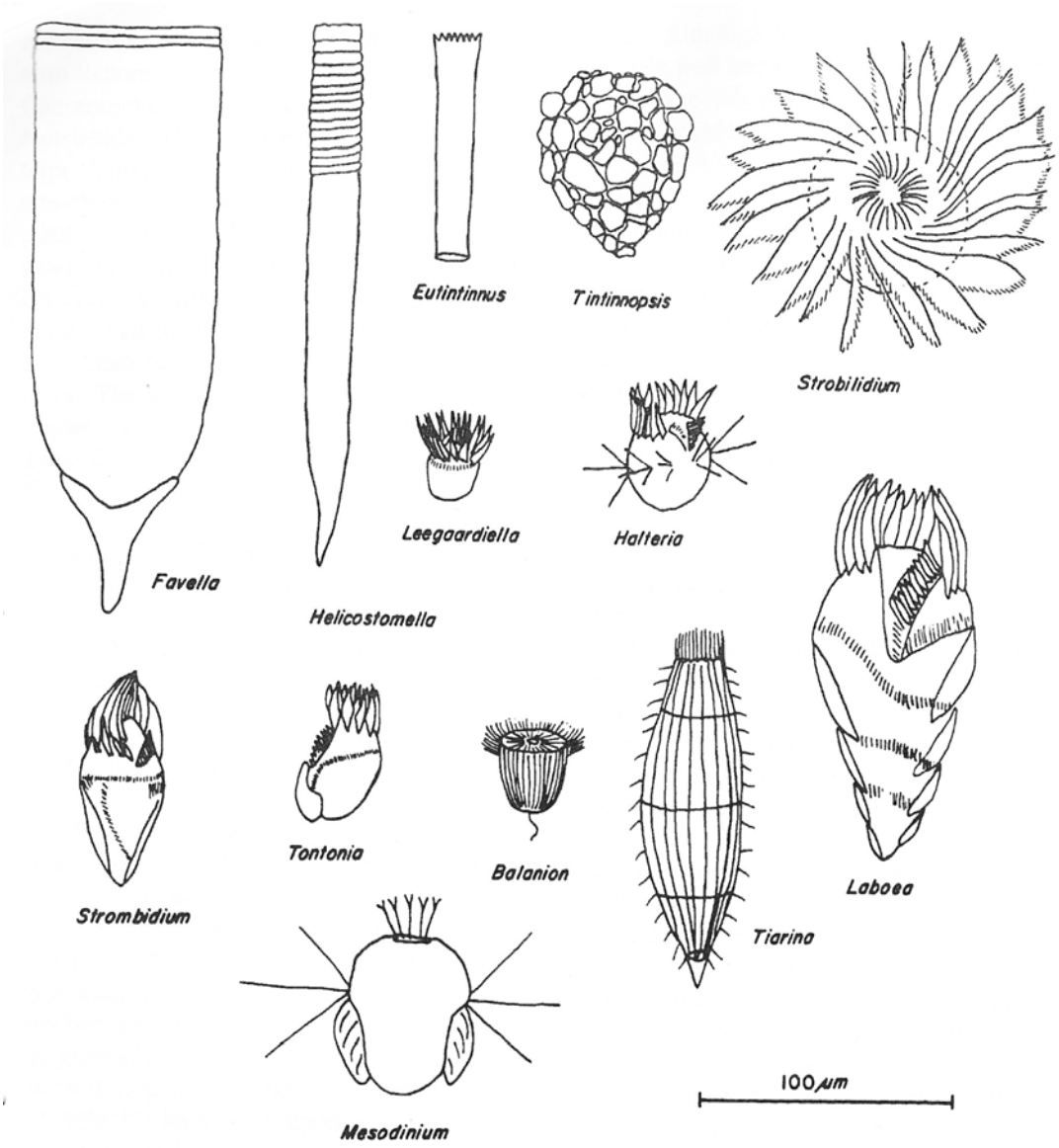

Figure 2.2: Common species of marine ciliates (Pierce and Turner 1992).

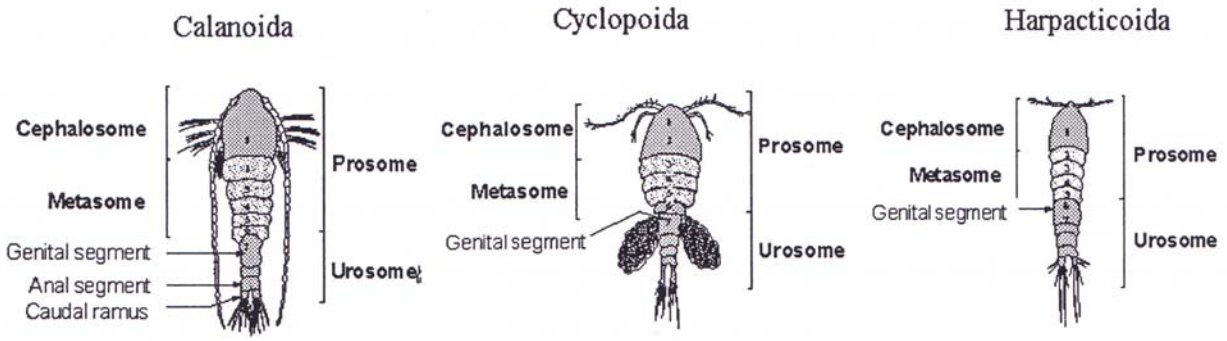

Figure 2.3: Physiology of the 3 most common marine pelagic copepod groups (Tokle 1999).

The prey size of copepods varies with the copepods size and predatorial behavior (Hansen et al. 1994). Calanoid copepods can feed on food particles in the size specter of 5-10 µm to 50-200 µm (Berggreen et al. 1988). The ubiquitous cyclopoid copepod, *Oithona similis* (Ward and Hirst 2007), can feed upon particles from 8-10 µm to 35-40 µm (Eaton 1971).

Phytoplankton, ciliates and copepods interact in a number of ways. Phytoplankton can be grazed upon by both ciliates and copepods, and is the base of the classical food chain (Calbet and Saiz 2005). Ciliates have been reported to graze between 10 and 80 % of primary production (PP) in marine environments (Vargas and Martinez 2009), and can in some cases control phytoplankton blooms (Montagnes and Lessard 1999). Ciliates, in turn, can be preyed upon by copepods, and form on average 30% of copepods daily carbon intake. The state of the food web has been known to heavily influence what part ciliates and phytoplankton make up of copepods diet (Calbet and Saiz 2005). Calbet and Saiz (2005) reported that ciliate consumption by copepods declined in environments with high phytoplankton concentrations, while in less rich environments (<50 µg C l^{-1}) ciliate and phytoplankton formed equal parts of copepods diet. Since copepods may feed on both ciliates and phytoplankton, they have the potential to indirectly affect ciliate grazing on phytoplankton through predation, a process called trophic cascades (Stibor et al. 2004).

2.4 Local conditions

The Comau fjord, our study site, is situated in region X, and is a sill-free fjord that runs north-south parallel to the Pacific Ocean, causing it to be relatively protected from ocean circulation and winds. The Comau fjord is narrow with a max width of 10 km at the mouth and a length of approximately 34 km. Its depth varies from 600m at the mouth to only 50m at the head (Haussermann and Fosterra 2009). The fjord is heavily influenced by freshwater input both from rain and river influx (Huinay and Vodudahue rivers), creating a two layers system within the fjord, with an upper freshwater layer and a lower saline water layer (Haussermann and Forsterra 2009, Iriarte et al. 2010; Sanchez et al. 2011).

Productivity in the region is highly affected by seasonal changes such as solar radiation and freshwater discharge (Gonzalez et al. 2010; Sanchez et al. 2011). Productivity peaks in springtime with high primary production values of up to 1-28 mg C m^{-3} h^{-1} and chlorophyll a values at 1-14 mg m^{-3}, while phytoplankton biomass has been found to reach 164.6 ± 2.2 µg C l^{-1} (Sanchez et al. 2011). Nanophytoplankton have been found to dominate both the freshwater and saline water layer. Nanoflagellates made up the biggest part of the nanophytoplankton while the diatom species *Thalassiosira minima* made up 71% of the microphytoplankton at 15m (Sanchez et al. 2011). Other common diatom genera in the microphytoplankton were *Skeletonema*, *Chaetoceros* and *Rhizosolenia* (Gonzalez et al. 2010). Ciliates have been found in the fjord region, but have not been found in large numbers (Vargas and Martinez 2009). Marine crustaceans such as copepods and euphausiids are the most important part of the mesozooplankton (Palma and

Silva 2004). The most common copepod species in the area are calanoids, and have been reported to be *Calanus chilensis* (Marin and Antezana 1985), *Calanoides patagoniensis* (Antezana 1999), *Calanus australis* (Sanchez et al. 2011), *Drepanopus forcipatus* (Gonzalez et al. 2010), *Paracalanus* sp. and *Acartia* spp. (Gonzalez et al. 2011). Other important organisms in the regions' marine food web are gelatinous carnivores and chaeognaths (Palma and Silva 2004). Cladoecerans can make up a large component of the zooplankton in the freshwater layer (Sanchez et al. 2011).

2.5 Focus of the thesis

In this thesis the effect of nutrient addition, simulating nutrients released from fish farms on the lower levels of the marine pelagic food web in Chilean North Patagonia, will be examined. 3 groups, namely phytoplankton, ciliates and copepods, will in this thesis represent the lower levels of the food web. Changes in numbers and biomass in relation to nutrient addition will be examined, as well as changes in community structure.

The interactions between these 3 groups will also be focused on. In particular copepods role both as grazers and as predators will be investigated, to see if and how they might influence ciliate populations.

3. Material and Methods

3.1 Introduction

A mesocosm experiment was undertaken from the 18th of January 2010 to the 3rd of February 2010 at the Huinay Scientific field station, in the Comau fjord, Northern Patagonia, Chile (42°22´ S, 72°24´ W, Figure 3.1). A gradient of nutrients were added to the mesocosms to study the effect of nutrient release from fish farms on the pelagic food web.

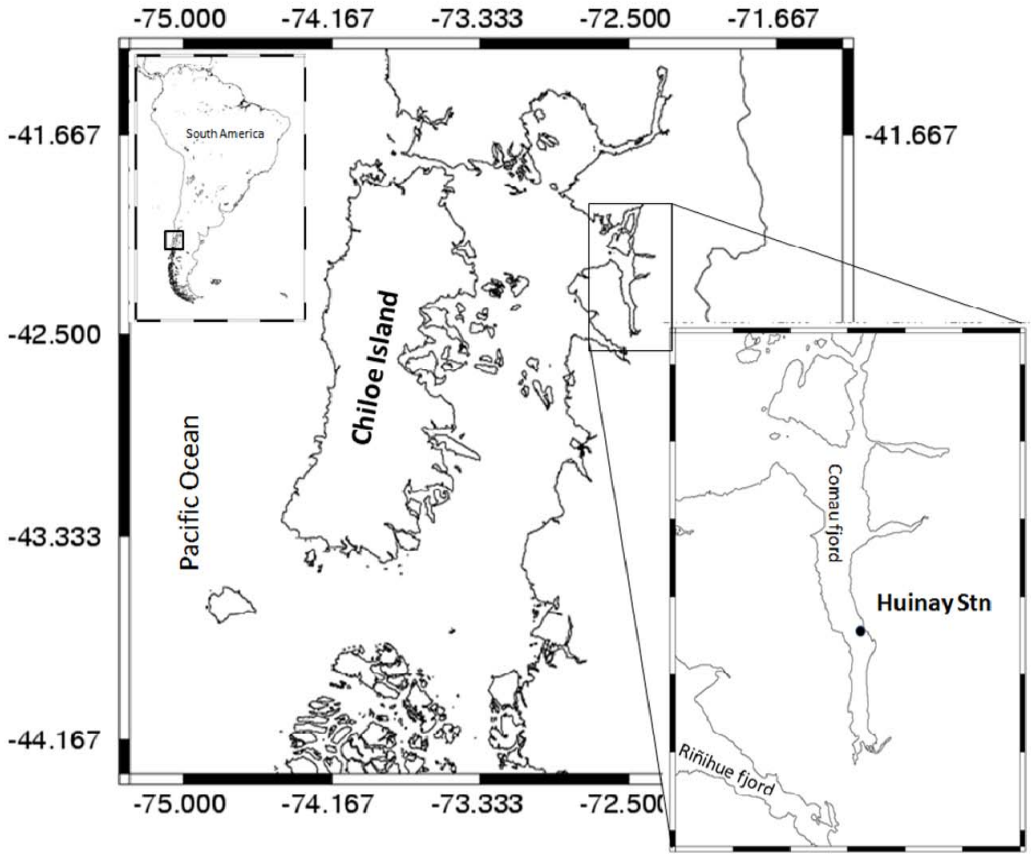

Figure 3.1: Location of Huinay Field Station and Comau fjord, where the mesocosm experiment was undertaken (used with permission from Sanchez et al. 2011).

3.2 Local conditions

Several CTD-casts were done upon arrival, in the middle of the fjord from 0-30 and 0-65 m, measuring salinity, temperature, density and fluorescence.

3.3 Mesocosms

Plastic cans with a volume of 35 L were used as mesocosms. These were filled with seawater several times over a period of two days, before being filled with 200 µm mesh filtrated seawater pumped up from approximately 20 m depth, using a Watson

Marlow industrial peristaltic pump. A day zero sample was taken directly from the pumped water before starting the experiment.

Zooplankton was collected from below the brackish water layer in the middle of the fjord using a 200 µm –plankton net with a 0.25 m^2 opening. A 20 l gatherer was attached to the tail of the net for collecting zooplankton. The net was towed horizontally at approximately 20 m depth for approximately 10 minutes. This was repeated 3 times until approximately 60 l of seawater was collected. The samples were then transported to a cold room, and larger zooplankton such as krill, mysis and gelatinous plankton was removed. The samples were then filtered through a 190 µm filter as to create two samples of 3.6 l each. The samples were then mixed in a slow eight-shaped motion, before a small subsample of known volume was counted in a stereomicroscope. Based on the number of copepods in this subsample the main sample was diluted to a volume of 12 l, obtaining a sample with a concentration of approximately 0.5 copepods l^{-1}. After unification, 500 ml of sample was added to each mesocosm, accounting for approximately 0.5 copepods l^{-1} in each mesocosm.

The mesocosms were then incubated at 2 m depth for a period of 16 days. They were suspended in the water. Natural mixing was assumed to occur through natural wave action. In addition the mesocosms were mixed once per day before nutrient addition or sampling.

3.3 Nutrient addition and sampling

The mesocosms were organized into 8 treatments, with 3 replicates in each treatment, including a zero-addition treatment. The 8 treatments had an increasing nutrient loading rate as shown in Table 3.1 in a ratio of 16:16:1 for N:P:Si as determined from Olsen et al. (2007). N was as added as NH_4Cl, P as $NaH_2PO_4*H_2O$ and Si as SiO_3.

Table 3.1: Loading rate for nitrogen, phosphorus and silicon (L_N, L_P, L_{Si} respectively), as daily added nutrients in µg L^{-1} d^{-1}. Nutrient ratio for all treatments (Tr.) were 16:16:1 (N:Si:P). N was added as NH_4Cl, P as $NaH_2PO_4*H_2O$ and Si as SiO_3.

	L_N	L_P	L_{Si}
Zero	0.0	0.0	0.0
Control	3.68	0.51	7.40
Tr. 1	7.00	0.97	14.1
Tr. 2	9.80	1.35	19.7
Tr. 3	14.0	1.93	28.1
Tr. 4	17.2	2.37	34.5
Tr. 5	28.0	3.87	56.3
Tr. 6	42.0	5.80	84.4

Nutrients were added manually at the same time every day, directly after the sampling. Sampling volume and frequency can be seen in Table 3.2.

Table 3.2: Volume and frequency of sampling

Samples	Volume (ml)	Frequency
Nutrients	10	Day 0, 2, 4, 6, 8, 10, 12, 14, 16
Chlorophyll	500	Day 2, 4, 6, 8, 10, 12, 14, 16
Ciliate	250	Day 0, 2,4, 6, 8, 10, 12, 14, 16
Phytoplankton	250	Day 4, 10, 16
Zooplankton	Total volume	Day 16

Ciliate and phytoplankton samples were fixed with 2 % Lugol's acid solution, which was added to the 250 ml bottles prior to sampling.
At the end of the experiment the remaining water in the mesocosms was filtered through a 200 µm filter. The volume of water filtered was measured. The mesozooplankton and nauplii was fixated with 5% Lugol's acid solution.

3.4 Analyses

3.4.1 Nutrients, Chlorophyll *a* and POC

Phosphate (PO_4), silicon (Si), nitrate (NO_3) and nitrite (NO_2) were analyzed from the nutrient samplings. This was done with an autoanalyser (Technicon model) according to Atlas et al. (1971).
Chlorophyll a analyses were done on site using methanol extraction according to Pepe et al. (2001).
Fractionated Particulate Organic Carbon (POC) measurements were done at 2 – 10 µm and 10 - 40 µm size fractions using a Casy Counter (Casy 1 Model TTC). The lugol fixated phytoplankton samples were used. When necessary the samples were distilled with 0.2 µm filtered seawater. Capillary tubes of 600 µm were used, measuring 3 times 200 µl for every sample.

3.4.2 Phytoplankton analyses

Phytoplankton samples were preserved with 2 % Lugol's acid solution and counted on day 4, day 10 and day 16. The samples were gently turned about 200 times, then a subsample of 10 ml was transferred to a sedimentation chamber for 12 hours, before being counted in a light microscope, as described in (Edler and Elbrachter 2010).

Cells were counted and measured, and the biovolume for the different genera was determined using shape formulas from Hillebrand et al. (1999). An overview of the shapes used for the different genera can be seen in Table 3.3. For the unidentified silicoflagellates, shape was determined upon viewing the organism.

Table 3.3: An overview over the different shapes used for determining biovolume of phytoplankton and ciliates. All shapes are taken from Hillebrand et al. (1999), except for ciliates and silicoflagellates where shapes were determined upon direct observation.

Group	Genus	Shape
Diatoms	*Asterionellopsis*	Prism on triangle
	Ceratulina	Cylinder
	Chaetoceros	Elliptic prism
	Coccinodiscus	Cylinder
	Cylindrotheca	Prolate spheroid + 2 cylinders
	Eucampia	Elliptic prism
	Leptocylindrus	Cylinder
	Melosira	Cylinder
	Pseudinitzschia	Prism of parallelogram
	Skeletonema	Cylinder + 2 half spheres
	Rhizosolenia	Cylinder
	Thalassionema	Box
	Thalassiosira	Cylinder
Dinoflagellates	*Alexandrium*	Ellipsoid
	Dinophysis	Ellipsoid
	Diplopsalys	Cone + half sphere
	Gyrodinium	Ellipsoid
	Prorocentrum	Ellipsoid
	Protoperidinium	Two cones
	Silicoflagellate un.	Sphere
Ciliates	Aloricate ciliates	Sphere or cone
	Tinntinids	Cone

3.4.3 Ciliate analyses

Ciliate samples were counted following the same procedure as the phytoplankton samples. The counts and identification were then undertaken with a Leica DMIRB reverse microscope. The entire sample was usually counted, unless a large number of ciliates were present (200 or more), in which case only half the sample was counted by skipping every other row in the ocular.

Ciliates were grouped into two distinct categories either as tinntinids or aloricate ciliates (Putt and Stoecker 1989). The aloricate ciliates included all types of strombidium and strombilidium. Pictures of the 30 first ciliates, independent of category, were taken with a SONY DFWX700 camera. Using ImageJ software, diameter, length and width of the ciliates were measured. Cell volume was calculated using two different shapes, either a sphere or a cone, for oligotriches (Table 3). For tintinnids the entire length of the lorica was measured as well as the width. The formulas used for calculating biovolume were taken from Hillebrand et al. (1999).

The specific growth rate of the ciliate community was determined using equation 3.1 (Gismervik et al. 2002):

$N_t = N_0 * e^{\mu t}$ (Equation 3.1)

Where N_0 is the number of ciliates on day 0, N_t is the number of ciliates on the day at the end of the growth, t is the number days in the growth period and μ is the growth rate.

3.4.4 Zooplankton analyses

3.4.4.1 Mesozooplankton samples

Zooplankton filtered through 200 µm mesh was refiltered through 90 µm mesh size and concentrated to a known volume. A subsample with a known volume was then analyzed under a Leica M205C Stereolupe microscope with a magnification of 20. Copepods were identified as Calanoid, Cycloid or Harpacticoid copepods, and the prosome for each individual was measured. A minimum of 100 Calanoid copepods was counted for each sample, except in samples with a very low density where the entire sample was counted. The samples were also analyzed in their entirety with regards to Cycloid or Harpacticoid copepods, as well as other type of zooplankton. Other types of zooplankton such as polychaete- and decapode larvae were not measured.

3.4.4.2 Microzooplankton samples

A minimum of 100 copepod nauplii were counted per sample, unless the sample contained less in which case the entire sample was counted. The sample was also analyzed in its entirety in regards to copepods and other zooplankton, where the prosome of copepods were measured while other zooplankton groups were only counted. The length of 30 individual nauplii was measured and the average length was then used as an average length for all the samples.

3.4.5 Biomass calculations

The biomass of the different zooplankton groups was determined by conversion rates or length-weight relationship from literature, by using the general Equation 3.2. The same formula was used for converting cell counts to biomass.

$W = a * L^b$ (Equation 3.2)

W is dryweight, carbon or wetweight, a and b are species-specific coefficients and L is either the animals length or in case of phytoplankton cells, cell volume in μm^3 (Tokle 1999, Menden-Deuer and Lessard 2000). An overview of how this equation

was used for both phytoplankton and zooplankton groups can be seen in Table 3.4. Literature derived conversion factors can also be seen in Table 3.4.

Table 3.4: Length-weight relationship and species-specific coefficients used for phytoplankton and zooplankton in biomass conversion. The general formula, pg C = a * volume^b, was used for cell counts, where a and b are group specific coefficients and volume is the cell volume in µm^3. For calanoid copepods the same formula indicates µg C, and uses L, length of the individual, instead of volume. For cyclopoid copepods and nauplii the formula indicated wet weight (WW) and dry weight (DW) respectively. For ciliates and harpacticoids a general conversion rate from literature was used.

Taxa	a	b	Length/Vol.	Formula	Conv. rate	Reference
Phytoplankton						
Diatoms	0.288	0.811	μm^3	$pgC = a*vol^b$		Menden-Deuer 2000
Ciliates						
Aloricate			pg μm^{-3}		0.19	Putt 1989
Tinntinids			pg μm^{-3}		0.053	Verity 1984
Copepods						
Calanoid >800 µm	$3.39 * 10^{-6}$	2.18	µm	$\mu gC = aL^b$		Tokle 1999
Calanoid < 800 µm	$4.47 * 10^{-7}$	2.42	µm	$\mu gC = aL^b$		Tokle 1999
Cyclopoid	1.91	3	mm	$WW = aL^b$		Krylov 1968
Harpacticoid			µm		0.7	Tokle 1999
Nauplii	5.29	2.37	mm	$DW = aL^b$		Botrell et al. 1987

Phytoplankton biomass was calculated by using group-specific coefficients as presented by Menden-Deuer and Lessard (2000). Biomass was then converted from pg C to µg C. Casy count results were also converted to carbon using the diatom coefficients. Casy count counts all cells, diatoms coefficients were chosen because they contain less carbon than other groups of phytoplankton, and therefore the POC values can be assumed not to indicate restrictive biomass values.

Ciliate biomass was calculated by multiplying cell volume with the following conversion rates: 0.19 pg μm^{-3} for oligotriches (Putt and Stoecker 1989) and 0.053 pg μm^{-3} for tinntinids (Verity and Langdon 1984).

Calanoid copepods were divided by size into two groups: >800 µm and <800 µm. The biomass of the bigger Calanoid copepods was determined by using coefficients for *Calanus* spp., *Paraeuchaeta* sp., *Temora longicornis*, *Metridia* sp. and unknown calanoid copepods, as presented by Tokle (1999). The biomass of the smaller calanoid copepods was determined by using coefficients for *Pseudocalanus* sp., *Paracalanus* sp., *Microcalanus* sp. and copepodites, also from Tokle (1999).

The biomass of cycloid copepods was determined by using the regression line of Kryvlov (1968), based on *Oithona* sp. For conversion from wet weight to carbon, the dry weight was assumed to be 28 % of wet weight (Tande et al. 1992), and the dry weight was again assumed to contain 45 % carbon (Tokle 1999). The length of the cycloid copepods was also converted from µm to mm.

The biomass of Harpacticoid copepods was found by using Tokle 1999s conversion rate of 0.7 µg C.

For calculating the biomass of nauplii the regression line established by (Botrell et al. 1976) was used. Conversion from dry weight to carbon was done in a similar manner as for cycloids. Rectification from µm to mm was also made.

The low numbers of other types of zooplankton, as well as a lack of measurements of these groups, resulted in the omission of their biomass.

4. Results

4.1 Local Conditions

Data from the CTD showed typical summer conditions for the Comau fjord with a stratified water column. Surface temperature was registered to be close to 16°C., while surface salinity was of 5 psu. The underlying brackish water layer was approximately 8 m deep with salinity measurements going from 10 to 15 psu. The marine water underneath had a salinity of 30 psu.

4.2 Nutrients

Nutrient measurements are summarized in Table 4.1. All nutrient values can be seen in Appendix A. All nutrients showed both little variation and low concentrations throughout the experiment. NO_3 concentrations were close to zero in all treatments. Some NO_3 was found in the treatments with the lowest nutrient concentrations at the end of the experiment (0.46 to 0.83 µg l^{-1} for L_N = 0 and L_N = 3.68 µg l^{-1} d^{-1}). The concentration of NO_2 was found to be zero throughout the experiment in all treatments with the exception of L_N = 28 and L_N = 42 µg l^{-1} d^{-1} on day 4, where values of 0.03 µg l^{-1} were registered for both treatments. Silicate measurements showed some variations throughout the experiment. On day 0, the silicate concentration was measured to be 8 µg l^{-1}, dropping to 0.30 – 1.22 µg l^{-1} on day 4 and day 8 throughout the treatments. On day 10, the silicate concentration was registered to be 0 µg l^{-1} in all treatments, increasing on day 12 and 16 to values up to 1.17 µg l^{-1}. On day 16 a particularly high silicate concentration of 3.36 µg l^{-1} was found in the mesocosm with L_N = 28 µg l^{-1} d^{-1}. The phosphate concentration was found to be 0.42 µg l^{-1} on day 0 of the experiment, and varied little from this original concentration throughout the experiment, changing to 0.13 µg l^{-1} for L_N = 0 µg l^{-1} d^{-1} and to 0.69 µg l^{-1} for L_N = 28 µg l^{-1} d^{-1}. These were the lowest and highest concentrations of phosphate measured during the course of the experiment.

Table 4.1: Nutrient concentrations (µg l^{-1})

Concentration (µg l^{-1})	NO_3	NO_2	Si	PO_4
Start	0.47	0	8	0.42
Lowest	0	0	0	0.13
Highest	0.83	0.03	3.36	0.69
Average	0	0	2	0.42

4.3 Algae response

Figure 4.1 (A, B and C) shows phytoplankton response over time as chlorophyll *a* in µg l^{-1} (A), 2-10 µm fractionated Particulate Organic Carbon (POC) in mg C l^{-1} (B) and 10-40 µm fractionated POC in mg C l^{-1} (C) for different loading rates (µg l^{-1} d^{-1}). The chlorophyll *a* response starts on day 6 - 8 with stabilization on day 10 until day 16. The highest amount of chlorophyll *a* was found to be 19.3 µg l^{-1} on day 16 for L_N = 42 µg l^{-1} d^{-1}, which along with L_N = 28 µg l^{-1} d^{-1} showed a generally higher response. The treatments with a lower loading rate (L_N = 0, L_N = 3.68 and L_N = 7.00 µg l^{-1} d^{-1}) exhibited a modest increase in chlorophyll *a* during the experiment. A large standard error was found for L_N = 28 µg l^{-1} d^{-1} on day 10, 12 and 14. The complete chlorophyll *a* values can be seen in Appendix B.

For 2-10 µm POC, an initial growth response was recorded on day 2 followed by a plateau until day 8, where the treatments with a higher loading rate (L_N = 14, L_N = 17.2, L_N = 28 and L_N = 42 µg l^{-1} d^{-1}) showed a second growth period. A peak was registered on day 14, with a maximum biomass of 1.06 mg C l^{-1} for L_N = 42 µg l^{-1} d^{-1}. POC in the size range of 10-40 µm displayed an initial growth response on day 2 independent of loading rate. A succeeding peak was reach on day 4 for all treatments. The treatments with a high nutrient addition (L_N = 14, L_N = 17.2, L_N = 28 and L_N = 42 µg l^{-1} d^{-1}) maintained a high biomass throughout the experiment whereas the treatments with the lowest loading rate (L_N = 0, L_N = 3.68, L_N = 7 and L_N = 9.8 µg l^{-1} d^{-1}) decreased over time, remaining however above the initial levels. Maximum biomass was found to be 0.603 mg C l^{-1} on day 10 for L_N = 42 µg l^{-1} d^{-1} at 0.603 mg C l^{-1}. The total maximum biomass for all cells was recorded on day 14 with 1.584 mg C l^{-1} for L_N = 42 µg l^{-1} d^{-1}. The full 2-10 µm and 10-40 µm POC values are shown in Appendix B.

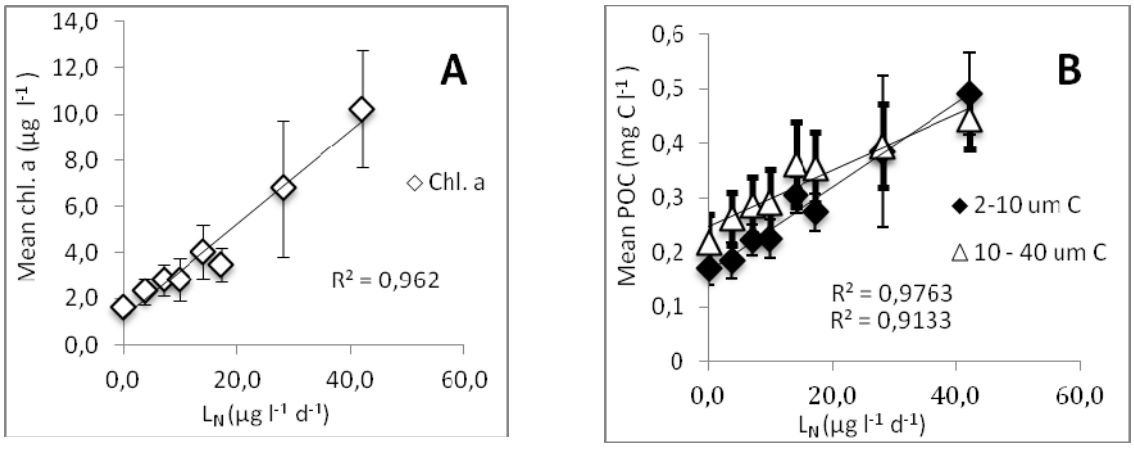

Figure 4.2: Mean chlorophyll *a* in µg l^{-1} (A) and mean POC in mg C l^{-1} (B) as a function of the loading rate (L_N µg l^{-1} d^{-1}). SE ± 2 and R^2 included.

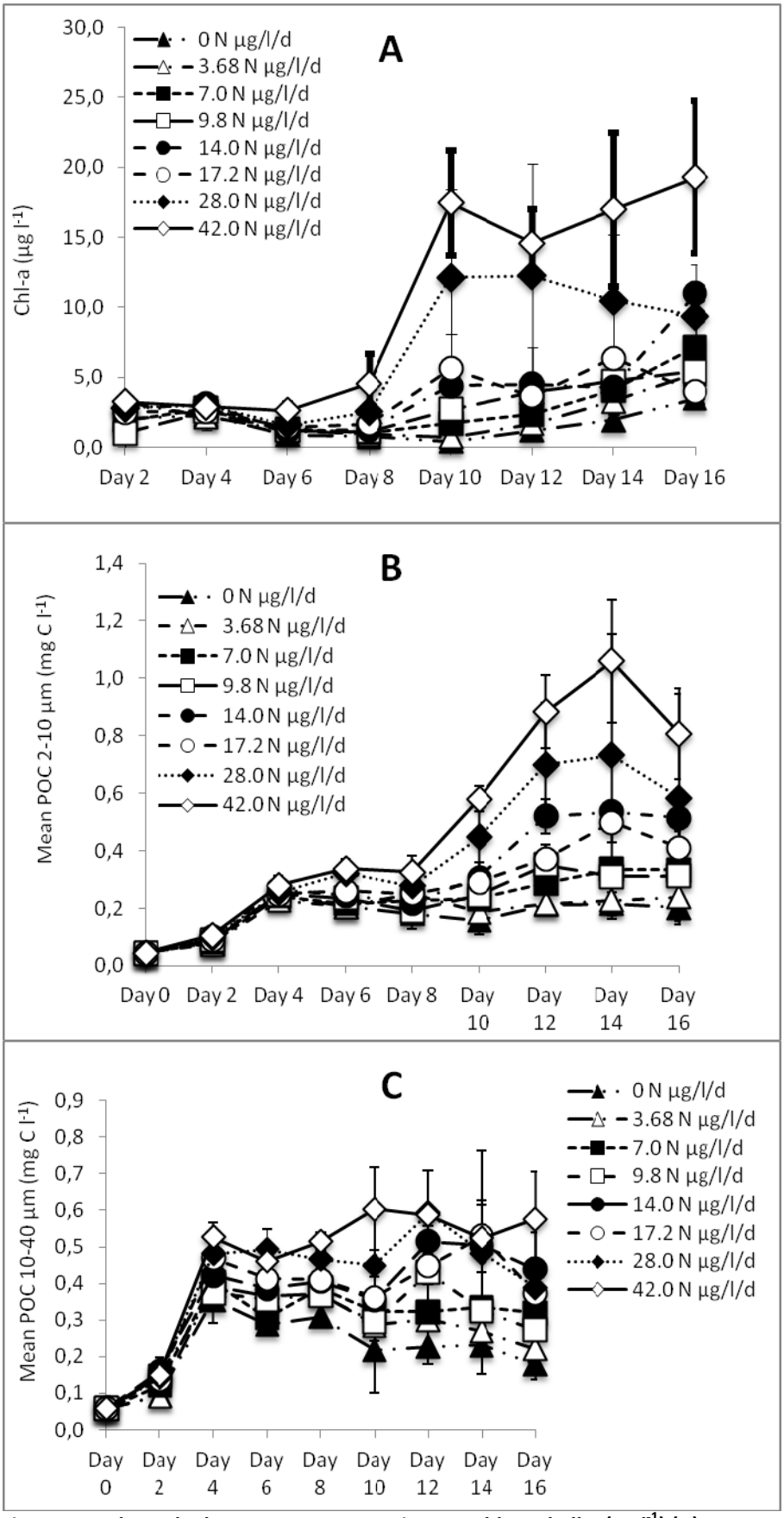

Figure 4.1: Phytoplankton response over time as: chlorophyll *a* (µg l^{-1}) (A), mean POC of size fraction 2 – 10 µm (mg C l^{-1}) (B), and mean POC of size fraction 10 – 40 µm (mg C l^{-1}) (C), according to loading rate (L_N µg l^{-1} d^{-1}). SE ± 2 included.

Figure 4.2 (A – B) shows mean chlorophyll *a* (A) and fractionated POC (B) as a function of the loading rate. There was a close to linear trend of increased response with increased loading rate. Both chlorophyll *a* and small cell (2-10 µm) response was especially visible in the treatments with higher loading rates (L_N = 28 and L_N = 42 µg l^{-1} d^{-1}).

Table 4.2 summarizes the changes in the phytoplankton community recorded during the experiment. Diatoms dominated throughout the experiment in all treatments. *Thalassionema* sp. was present in high numbers during the whole experiment, and *Thalassiosira* sp. was also present during the entirety of the experiment independent of loading rate but in smaller numbers. *Skeletonema* sp. dominated in all treatments on day 4 and day 10 but decreased considerably in numbers on day 16. *Cylindrotheca* sp., *Leptocylindrus* sp. and *Pseudonitzschia* sp. appeared in large numbers on day 16 after being present only in low numbers on day 4 and 10. Dinoflagellates and flagellates were present in larger numbers on day 10 and day 16 than on day 4, but were in general a minor component of the community. A general increase both in diversity and numbers was recorded from day 4 to day 10, which was maintained to day 16. Highest diversity was in general recorded in treatments with an intermediary loading rate (L_N = 7, L_N = 9.8 and L_N = 14 µg l^{-1} d^{-1}). Numbers were however found to be highest for L_N = 28 and L_N = 42 µg l^{-1} d^{-1}.

4.3 Zooplankton

4.3.1 Microzooplankton

The ciliate community was in general dominated by aloricate ciliates throughout the experiment. Tintinnids were found in low numbers on day 12 and 16 for L_N = 0 and L_N = 14 µg l^{-1} d^{-1}. The response in ciliate over time is shown in Figure 4.3, in terms of numbers, A (individuals ml^{-1}); individual size, B (pg C $cell^{-1}$) and biomass, C (µg C l^{-1}). Ciliate numbers increased from day 4 to day 8 after which they seemed to stabilize, except for L_N = 42 µg l^{-1} d^{-1}, which oscillated during the rest of the experiment. The lowest ciliate counts were throughout the experiment found for L_N = 3.68 µg l^{-1} d^{-1}. The maximum amount of ciliates were found on day 16 at 91 individuals ml^{-1} for L_N = 42 µg l^{-1} d^{-1}. Ciliate biomass values over time for the different treatments showed a growth response from day 4, with a peak at day 8, followed by a decrease in biomass in most treatments. The maximum biomass was recorded on day 8 at 117 µg C l^{-1} for L_N = 42 µg l^{-1} d^{-1}. The treatment with L_N = 3.68 µg l^{-1} d^{-1} had the lowest recorded biomass over time which corresponds with the low counts in this treatment. Maximum specific growth rate was found to be 0,77 day^{-1}, in the growth period between day 4 and 8, for L_N = 42 µg l^{-1} d^{-1}. Ciliate size (Figure 4.3 B) had a similar response independent of loading rate, with an increase of carbon content from day 0 to day 8, followed by a general decrease. Maximum cell size was registered on day 8 for L_N = 9.8 µg l^{-1} d^{-1} at 1584.3 pg C $cell^{-1}$. The complete ciliate counts and biomass values are shown in Appendix C.

Table 4.2: Presence of phytoplankton genera for day 4, 8 and 16 for the different treatments according to loading rate (L_N). Phytoplankton was divided into 3 groups: diatoms (1), dinoflagellates (2) and flagellates (3). The amount of cells present is indicated as: not present (-), barely present (+), present (++), abundant (+++), very abundant (++++) and dominating (+++++).

		Day 4								Day 10								Day 16							
	Genus	L_N 0	L_N 3.7	L_N 7	L_N 9.8	L_N 14	L_N 17	L_N 28	L_N 42	L_N 0	L_N 3.7	L_N 7	L_N 9.8	L_N 14	L_N 17	L_N 28	L_N 42	L_N 0	L_N 3.7	L_N 7	L_N 9.8	L_N 14	L_N 17	L_N 28	L_N 42
1	*Asterionellopsis*	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	Ceratulina	++	+	+	+	++	++	+	++	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	+
	Chaetoceros	++	+++	+++	++	++	+++	+++	+	+	+	+	+	+	+	+	++	+	+	+	++	+	+	+	++
	Coccinodiscus	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-
	Cylindrotheca	+	-	+	+	-	-	-	+	+	-	+	-	-	+	+	+	++	++	+++	+++	+++	+++	+++++	+++++
	Eucampia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	++	+	-
	Leptocylindrus	-	-	-	-	-	-	-	-	-	-	+	+	+	++	+	++++	+++	++	-	++	+++++	+	+++++	+++++
	Melosira	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	Pseudinitzschia	+	+	+	+	+	+	+	+	-	+	++	+	+	++	+	++	++	++	+++	+++	+++	+++++	+++++	+
	Skeletonema	+++++	+++++	+++++	+++++	+++++	++++	++++	+++++	++++	+++	+++++	++++	++++	+++++	+++	++++	-	+	-	++	+++	++	+	-
	Rhizosolenia	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	+	+	+	+	++	+	+	-
	Thalassionema	++	++	+++	++	++	+++	++	++	++	+++	++++	++++	++++	+++	+++	+++	++++	++++	++++	+++	++	+++	++	++
	Thalassiosira	++	+	++	++	++	++	++	++	++	++	++	++	+++	++	++	+++	+	+	++	++	+	+	+	+
2	*Alexandrium*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+
	Dinophysis	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	++	-	-	+	-	+	-	+	+
	Gyrodinium	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-
	Prorocentrum	-	-	-	-	-	-	-	-	-	-	+	+	+	++	+	++++	+++	++	-	-	-	-	-	-
3	*Diplopsalys*	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-
	Protoperidinium	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+	++	+	-	-	+	+	+	+	+

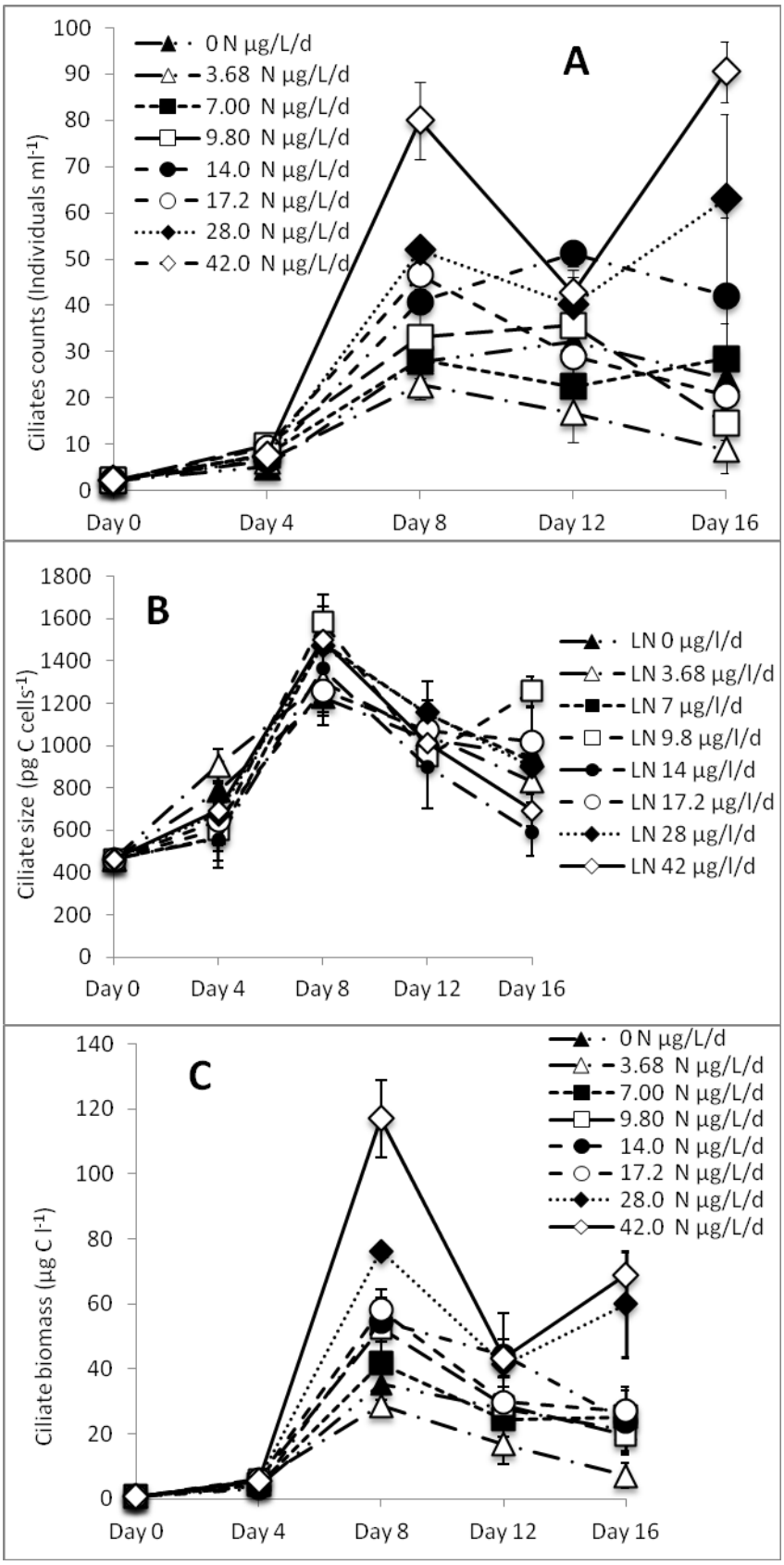

Figure 4.3: Ciliate response over time as: individuals ml^{-1} (A), size, pg C cell^{-1} (B) and biomass, µg C l^{-1} (C), according to loading rate (L_N µg l^{-1} d^{-1}). SE ± 2 included.

4.3.2 Mesozooplankton

Calanoid copepods were found to dominate the mesozooplankton in terms of biomass, while their offspring were dominating in terms of numbers. Cyclopoid and harpactipoid copepods were present in all samples, but were only present in very small numbers and varied in size between 100 - 400 µm. Other types of mesozooplankton that were found in some numbers were ostracods and appendicularia. In general 1-3 ostracods were found in all treatments. In the treatment with L_N = 3.68 µg l^{-1} d^{-1} over 7 appendicularia per liter were found, although this was not included in biomass calculations. Figure 4.4 shows nauplii and copepod counts (A) and biomass as well as nauplii ratio (B) on day 16. Pronounced variation in both the number and biomass of nauplii and copepods present, accounts for a high SE for the different treatments. A small increase in biomass can be seen with an increasing loading rate. However the treatment with the highest loading rate (L_N = 42 µg l^{-1} d^{-1}) showed a lower amount of individuals present as well as biomass. The nauplii ratio was found to increase somewhat with nutrient addition, but not for the two treatments with the highest loading rate (L_N = 28 and L_N = 42 µg l^{-1} d^{-1}). Complete mesozooplankton values can be seen in Appendix D.

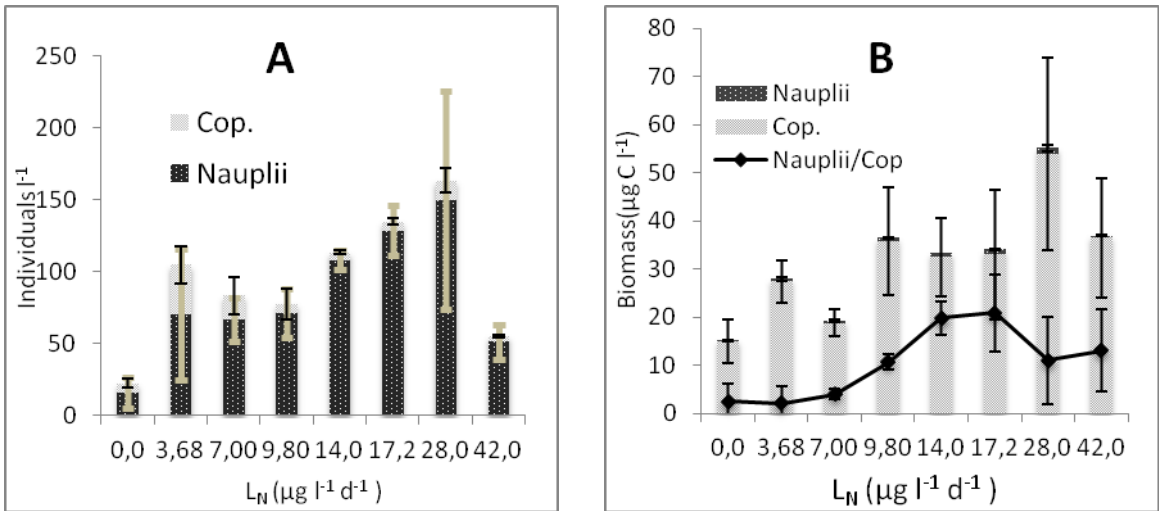

Figure 4.4: Copepod and nauplii response on day 16 as counts (individuals l^{-1}), A, and biomass (µg C l^{-1}), B; according to loading rate (L_N µg l^{-1} d^{-1}). Nauplii ratio is indicated in B as nauplii biomass/copepod biomass. SE ± 2 included.

4.4 Grazing and predation

Figure 4.5 (A-F) summarizes the general grazing and predation responses of ciliates and copepods. Figure A shows ciliate and copepod mean biomass in (µg C l^{-1}), as a function of loading rate (L_N µg l^{-1} d^{-1}). Figure B shows ciliate biomass (µg C l^{-1}) as a function of food biomass, both large (10-40 µm) and small (2-10 µm) POC cells. C to E show the relationship between copepods and their food groups on day 16, as ciliate biomass (C), ciliate size (D) and POC (E) as a function of copepod biomass. Nauplii production (individuals l^{-1}) as a function of POC in the size range of 10-40 µm (mg C l^{-1}) on day 16 is shown in Figure F.

Ciliate mean biomass showed a clear positive correlation with an increase in loading rate (A), with R^2 = 0.95, while copepod mean biomass (A) showed no such trend, R^2 = 0.48. There was also a clear relationship between food biomass and ciliate biomass, both for small and large cells, whit R^2 = 0.95 and 0.76 respectively (B). There was no clear trend between copepod biomass and their food groups, neither for ciliate biomass (C), ciliate size (D) nor POC biomass (E). Nauplii numbers did not show any clear correlation with POC in the size range of 10- 40 µm either (F).

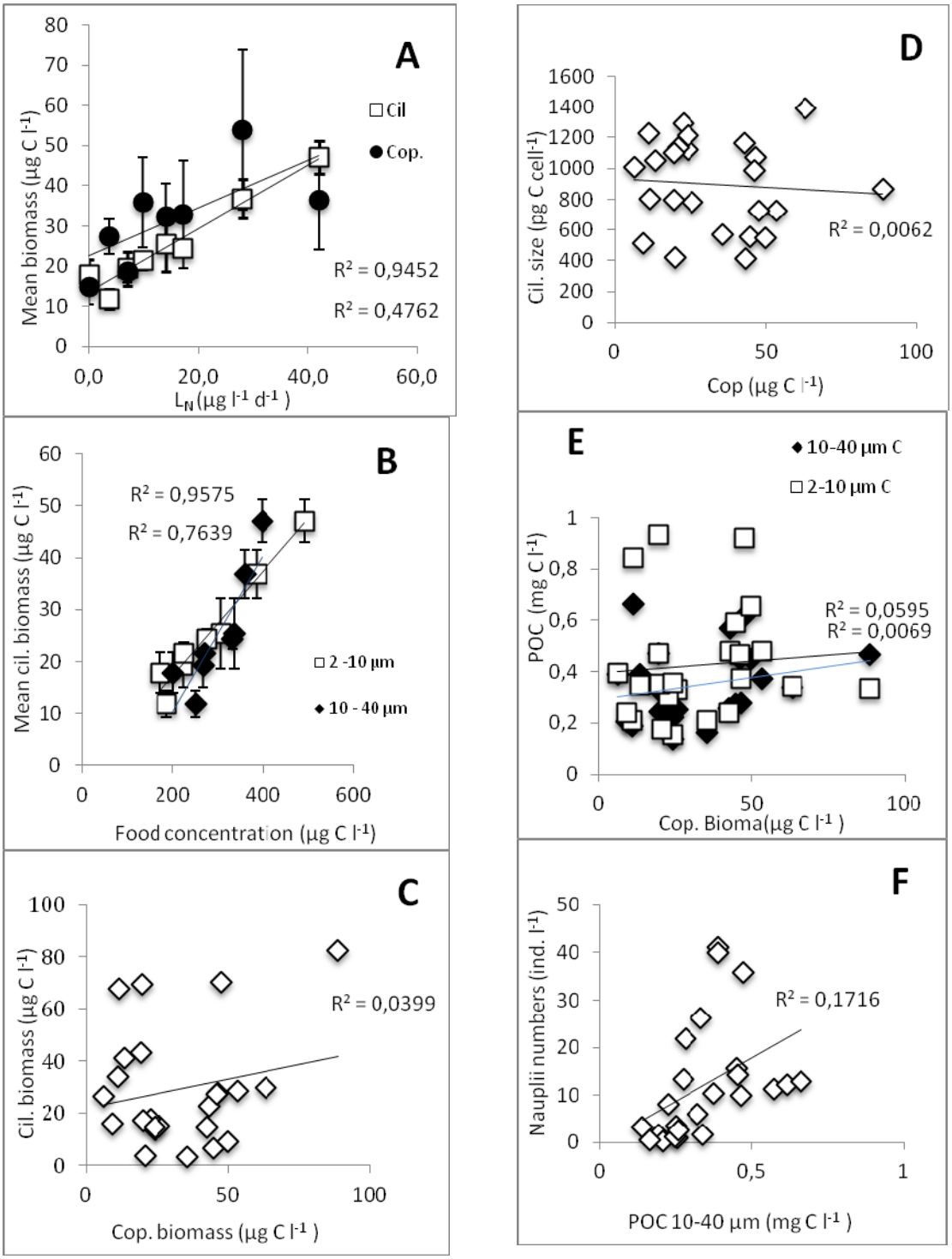

Figure 4.5 (A-F): Grazing and predation responses. A: Mean biomass of micro- and mesozooplankton (µg C l^{-1}), as a function of loading rate, L_N, (µg l^{-1} d^{-1}). SE±2 and R^2 included. B: Mean ciliate biomass (µg C l^{-1}) as a function of food biomass (µg C l^{-1}) with linear trends. SE±2 included and R^2 included. C: Ciliate biomass (µg C l^{-1}) as a function of copepod biomass (µg C l^{-1}) on day 16. Linear trend and R^2 included. D: Ciliate size (pg C $cell^{-1}$) as a function of copepod biomass (µg C l^{-1} l) on day 16. Linear trend and R^2 included. E: POC biomass (mg C l^{-1}) as a function of copepod biomass (µg C l^{-1}) on day 16. Linear trend and R^2 included. F: Nauplii numbers (individuals l^{-1}) as a function of POC biomass in the size range 10-40 µm (mg C l^{-1}) for day 16. Linear trend and R^2 included.

5. Discussion

5.1 Response to nutrient addition

Nutrient addition affected chlorophyll *a*, POC and ciliates almost immediately, but reaching peaks in growth at different times (Figure 4.1 and 4.3). Chl *a* peaked on day 10, while large and small POC particles reach their highest levels on day 4 and 14, respectively, and ciliates peaked on day 8. A near linear correlation was found for all these three groups in relation to loading rate (Figure 4.2 and 4.5A). The phytoplankton showed potential for not only rapid, but also extensive growth, with total maximum POC values being 13x higher than the original level on day 0. Ciliate biomass growth was found to be directly tied to food availability (Figure 4.5B), thus confirming that phytoplankton growth triggered rapid ciliate growth. Copepod biomass did however not show any correlation with nutrient addition (Figure 4.4 and 4.5A), nor with food availability (Figure 4.5C, D and E).

5.1.1 Phytoplankton response

The extensive growth of phytoplankton as shown by the chlorophyll *a* and POC values (Figure 4.1) was expected as a consequence of nutrient addition. Earlier mesocosm experiments have shown similar results, with both immediate growth and later severe phytoplankton blooms registered (Olsen et al. 2001; Olsen et al. 2006; Olsen et al. 2007). However the same nutrient loading rates seem to have less impact in natural waters, as shown by Olsen et al. (2001). The same results were found by Soto and Norambuena (2004) and Merceron et al. (2002) when studying chlorophyll and phytoplankton in proximity of fish farms. This can largely be explained by hydrological processes, which prevent the phytoplankton from fully assimilating nutrients from fish farms (Cloern 2001; Merceron et al. 2002; Soto and Norambuena 2004; Olsen and Olsen 2008).

The POC particles of both size fractions exhibited a period of growth from day 2, but the larger particles (10 - 40 µm) grew more rapidly than the smaller particles (2 – 10 µm), reaching levels between 0.35 - 0.55 mg C l^{-1} on day 4, while the smaller particles only reached levels of 0.2 mg C l^{-1}. This changed on day 8 when a new growth period was registered for the smaller POC particles, reaching levels of 1.2 mg C l^{-1} on day 14. This growth took place on a much larger scale in the mesocosms with high loading rate, and was almost unnoticeable in the mesocosms with low loading rate. This indicates that nutrient additions affected the phytoplankton community. Phytoplankton community structure changes have been mentioned as a possible result of nutrient addition earlier, with a similar shift as seen here, from large diatoms towards smaller algae such as dinoflagellates (Cloern 2001; Buschmann et al. 2006; Mente et al. 2006; Iriarte et al. 2010). Although dinoflagellates became more abundant in the later phase of this experiment (Table 4.2), there was no clear trend linking them to increased nutrient loading rates.

Nutrient addition has also been linked to HABs (Buschmann et al. 2006; Mente et al. 2006; Iriarte et al. 2010,). Some genera with the potential to cause HABs, such as *Protoperidinium* sp., *Dinophysis* sp., and *Alexandrium* sp. (Buschmann et al. 2006), were found in our experiment, and were more present towards the end of the experiment than at the beginning (Table 4.2).

A reason as to why the smaller phytoplankton organisms dominated in the latter part of the experiment is the possibility that nutrient resources became too scarce for the nutrient requirements of the larger algae, thus allowing smaller organisms with other nutritional needs to outcompete them. Table 4.1 shows that almost all nutrients except phosphate became depleted during the course of the experiment, supporting this hypothesis. Unfortunately identification to the species level was not possible in this experiment, thus making it difficult to know the nutritional requirements of the different dominating genera.

Grazing can also be an explanation as to why the larger POC fraction stopped its growth. Copepods are particularly known to graze on larger phytoplankton species. However Figure 4.5E shows that the copepods exerted no grazing pressure on the phytoplankton.

It is also important to note that the fractionated POC values probably encompasses other material than just fixated phytoplankton cells such as dead cells, organic debris and ciliates. Ciliate biomass values were therefore subtracted from the 10 – 40 µm POC fraction to avoid that they were counted twice. Comparing the chl. *a* values with the total POC values, it is possible to assume that for the most part, the measured POC was composed of phytoplankton because of the very similar values. It is also important to note that ciliates have the potential to feed on dead organic material as long as it is in within the right size range (Pierce and Turner 1992), making POC independently of its composition an ideal measurement of food available for ciliates.

5.1.2 Ciliate response

As seen in Figure 4.3, the ciliates in this experiment exhibited a growth response typical for ciliates, with a peak followed by either a decline or stabilization in numbers and biomass (Gismervik et al. 2002; Gismervik 2005). This typical response grew in magnitude with an increasing loading rate, which fits with the linear correlation between nutrient addition and ciliate growth shown in Figure 4.5A. In the mesocosms with the highest loading rates L_N = 28 and 42 µg l^{-1} d^{-1}, ciliates bloomed on day 8 (Figure 4.3). Earlier experiments of similar nature, such as Olsen et al. (2007), reported the same type of fast growth as seen here, with ciliate blooms occurring within 5 -7 days of the experiment. This typical response is assumed to be due to ciliates' ability for fast growth, and thus being capable of rapidly utilizing new food resources (Laybourn-Parry 1992; Pierce and Turner 1992; Gismervik et al. 2002; Calbet and Landry 2004).

Figure 4.3A shows a maximum of 91 ciliates ml $^{-1}$. This value is high above the average values of 1-10 individuals ml $^{-1}$ commonly found in coastal temperate marine environments (Montagnes and Lessard 1999), but is not unheard of during ciliate blooms where values of up to 1.2 * 10^3 ciliates ml^{-1} have been found (Pierce and Turner 1992). However previous studies from northern Chilean Patagonia have not registered ciliate abundances of this magnitude. Vargas and Martinez (2009) found aloricate ciliates to be present at 0-18 individuals ml^{-1} in the Chilean fjord region.

Ciliates, especially aloricate ciliates can be hard to sample accurately because of their fragility. Formaldehyde is particularly unsuited for conserving ciliate (Modigh and Castaldo 2005). Although Lugol's acid solution is a better-suited conservation agent for ciliates (Ohman and Snyder 1991), it has been known to cause up to 30 % cell shrinkage (Ohman and Snyder 1991; Calbet and Saiz 2005). Lugol's acid solution is also a much more effective conservation chemical if added prior to sampling instead of after (Personal observation). Another reason why ciliates may not have been registered in such numbers earlier, might be because of ciliates' tendency to form short term blooms, thus making timing essential in getting an accurate estimation of ciliate population size and structure (Montagnes and Lessard 1999; Olsen et al. 2002; Gismervik 2006,). It must also be taken into account that since our study was conducted in an experimental setting; ciliate population dynamics might have been affected in such a way that allowed for the registered growth. This is supported by Olsen et al. (2001) study where responses to nutrient addition were much higher in mesocosms than in a natural occurring lagoon, although loading rates were the same.

Despite this, it seems ciliates may play a larger role in the Northern Patagonian food web than previously assumed. The extent of the bloom recorded during this experiment indicates that ciliates in the Chilean fjords have the potential for very rapid growth under favorable circumstances, and thus can affect the rest of the food web both as grazers and as a potential food source for larger predators. Ciliates' role in containing phytoplankton blooms, both harmful and otherwise, would be especially useful to further study to assess the food web's capacity to assimilate nutrients.

Ciliate size (Figure 4.3B) changed in a similar manner in all treatments independent of nutrient loading, signifying that the increase in biomass registered in the treatments with a higher loading rate was in the most part due to numbers. The ciliate size changes observed in this experiment fit with previous studies on ciliate dynamics, where an initial growth was recorded followed by stabilization or a decline in size (Montagnes and Lessard 1999, Ohman and Snyder 1991).

Figure 4.6 B shows a linear correlation between food biomass (POC size spectrum 2-10 µm) and ciliate biomass, strongly suggesting that an initial increase in food biomass led to a consecutive increase in ciliate biomass. Ciliate biomass also showed a correlation to larger POC (10-40 µm), although this correlation was weaker than

with the smaller POC. This can be explained by ciliates' preference for food particles in the size range of 3 -7 µm (Stibor et al. 2004; Gismervik 2006). Food concentration has previously been found to directly affect ciliate growth (Pierce and Turner 1992; Montagnes and Lessard 1999; Calbet and Saiz 2005). Olsen et al. (2007) found that ciliate biomass increased in a linear mode over the entire food range, further supporting that initial ciliate growth is dependent on food availability.

5.1.3 Copepod and nauplii response

Copepod biomass showed no clear trend of increasing with nutrient loading (Figure 4.5A). Copepod numbers and biomass increased slightly with increasing nutrient loading but high standard errors showed a large variation between mesocosms (Figure 4.4). On day 16 of the experiment a maximum of 34.6 copepods l^{-1} with a body mass of 0.7 µg C ind^{-1} were found in the treatment with L_N = 3.68 µg l^{-1} d^{-1}. The biggest copepods were found in the treatment with L_N = 28 µg l^{-1} d^{-1}, where 13.7 copepods l^{-1} with a body mass of 3.4 µg C ind^{-1} were counted. This shows that the copepod population in the mesocosms was dominated by smaller species.

The highest copepod biomass of 53.9 µg C l^{-1}, was found in the mesocosm with L_N = 28 µg l^{-1} d^{-1}, while the mesocosm with the highest loading rate had noticeably lower biomass value. Similar results were found in Gismervik et al. (2002) and Olsen et al. (2007), studies where copepod fitness seemed to peak in a mesocosm with medium nutrient addition. A possible explanation for this might be that higher nutrient loadings favored the growth of large diatoms, which might have been too unhandy for optimal grazing by copepods (Gismervik et al. 2002). This might be especially relevant in this experiment since the copepods on average consisted of small species. It must also be taken into account that no direct measurement of size and biomass was made at the beginning of the experiments and thus direct biomass growth is hard to assess from day 0 to the end of the experiment. The large variations in copepod numbers and biomass found between mesocoms with similar loading rate seem to indicate that there was no clear trend in how the copepod populations reacted to the increases in food availability.

High standard errors can also be explained by the copepod addition process, which might have created initial differences in the copepod communities present in each mesocosm, since only a specific volume was added to each mesocosm. It is also possible that small species of copepods and nauplii entered the mesocosms through the pump during the filling of the mesocosms, increasing the risk of different concentrations of copepods in the different mesocosms.

It is also necessary to note that copepods have a longer life cycle than both phytoplankton and ciliates (Peterson 1998, Arnkværn, Daase et al. 2005), and thus require a longer experimental period before effects of increased nutrient loadings can be observed (Gismervik et al. 2002; Gismervik 2006). Olsen et al. (2007) found in their mesocosm experiment that copepods reacted after 11 days of nutrient addition, while other studies assess 2 – 3 weeks to be necessary before seeing any response from mesozooplankton (Gismervik et al. 2002; Olsen et al. 2006). This

would be very close to the end of the experiment, and thus direct copepod growth would be difficult to observe during the course of this study. However, increased egg and nauplii production have been registered as a result of food availability due to nutrient addition (Gismervik et al. 2002). Neither egg production nor nauplii production was measured during this experiment, but an assessment of nauplii production can be made indirectly through nauplii to copepod ratio (Figure 4.4B).

Both the highest concentration and biomass of nauplii was found in treatments with medium nutrient addition. The nauplii to copepod ratio was also highest in these medium treatments with the exception of the treatment with L_N = 28 µg l^{-1} d^{-1} which despite of a high number of ciliates had low nauplii to copepod ratio. This indicates that nauplii also have higher fitness in treatments with medium nutrient addition.

5.2 Ciliate population dynamics

All mesocosms showed a peak on day 8 followed by a decline in biomass (Figure 4.3C). Despite this registered decline, the ciliate populations in the mesocosms with a high nutrient addition level seemed to stabilize at level above the original one. Ciliate populations' decline after their original peak is still not well understood despite several studies on the matter (Gismervik et al. 2002). It is possible that the ciliate population decimates its own food source (Montagnes and Lessard 1999; Gismervik et al. 2002). As shown in Figures 4.1 and 4.6 B, food availability does not seem to be a restricting factor in ciliate population growth in our experiment. However since phytoplankton growth was measured in terms of chlorophyll *a* and POC, there is the possibility that the ciliates actively grazed some phytoplankton species rather than others, which would result in a reduction of food availability. Indeed Gismervik (2006) found that the impact of phytoplankton grazing by ciliates was species dependent. This highlights the need for more research on ciliate species distribution, behavior and food preference. Unfortunately ciliate species identification remains difficult and costly to undergo in large scale or in the field (Pierce and Turner 1992).

Copepod predation has also been linked to ciliate populations' decline (Laybourn-Parry 1992; Montagnes and Lessard 1999; Gismervik et al. 2002; Gismervik 2006), and ciliates have been noted to make up an important part of copepod diet independent of copepod body weight (Calbet and Saiz 2005). An explanation for copepods' predation on ciliates has been the nutritional value of ciliates (Pierce and Turner 1992; Sanchez et al. 2011). In Norwegian fjords copepods have been found to be P-limited and thus might actively exploit a food source with higher P value than phytoplankton, such as ciliates (Gismervik 1997). However, P-limitation does not seem to occur in Northern Patagonia during the summer period (Iriarte et al. 2007; Silva et al. 2009). In concordance to this, P-levels were stable throughout our experiment, and P was not found to be a limiting nutrient, as seen in Table 4.1. Ciliate behavior such as active swimming, with a following higher chance of encounter with copepods, might also be a possible explanation for enhanced copepod predation (Calbet and Saiz 2005; Gismervik 2006; Sanchez et al. 2011).

Several species of copepods have been found to actively select for ciliates under certain circumstances (Stibor et al. 2004; Calbet and Saiz 2005). In particular *Calanus australis*, a common copepod in the Comau fjord, has been shown to positively select for ciliates, being capable of removing up to 60% of ciliate stock (Sanchez et al. 2011). *C. finmarchicus*, *Pseudocalanus* sp., *Centropages hamatus*, *Acartia clausi* and *Oithona similis* have also been shown to impact ciliate populations (Gismervik 2006; Castellani et al. 2008). Both *Pseudocalanus* sp. and *Centropages hamatus* were shown to decimate ciliate populations (Gismervik 2006). It is natural to assume that at least some of these copepods were present in our study, notably *Calanus australis* and *Acartia* sp., which are common in the study area (Gonzalez et al. 2011; Sanchez et al. 2011). However since identification to species level was not possible during this experiment, it is hard to assess what kind of impact these species might have had. This lack of identification is unfortunate since different copepods exhibit different predation and grazing behavior (Gismervik 2006, Castellani et al. 2008). More knowledge about the identity of the copepods present would have given a clearer idea of the state of the food web.

Calbet and Saiz (2005) noted that 7 copepods l^{-1} with a standard body mass of 10 µg C ind^{-1} would be necessary to consume 50% of a ciliate population. Smaller copepods, such as the ones found in our experiment, have also been known to feed on ciliates (Turner 2004; Castellani et al. 2008). However, no correlation was found between neither ciliate and copepod biomass nor ciliate size and copepod biomass (Figure 4.5C and D). This means that the copepods did not exert any predation pressure on the ciliates, and likely did not cause the decline in ciliate populations.

One reason why the copepods did not feed on ciliates might be due to the trophic state of the system. Ciliate consumption by copepods has been found to increase when phytoplankton is scarce (Stibor et al. 2004; Calbet and Saiz 2005). Based on this, it might be possible that copepods had a larger impact on ciliate populations in the mesocosms with lower phytoplankton biomass. Copepod biomass was not found to correlate with POC (Figure 4.5D and E), indicating that copepods were not capable of exerting grazing pressure on the phytoplankton. This increases the uncertainty of whether the copepods were primarily grazers or predators.

Nauplii also have the potential to heavily feed on ciliates (Gismervik et al. 2002); however, no correlation was found between ciliate and nauplii biomass (not shown), indicating that nauplii did not regulate ciliate population.

Another factor to take into account when looking into the food web dynamics concerning ciliates is the possibility of other predators that were not included in this study. In particular euphausiids have been found to play a large role in Chilean Northern Patagonia (Palma and Silva 2004), and might be a factor in ciliate population regulation (Calbet and Saiz 2005; Sanchez et al. 2011). Other possible predators are cladocera and chaetognatha, both of which are common in the area (Pierce and Turner 1992; Palma and Silva 2004; Sanchez et al. 2011). These predators were not the primary focus of this study, and would have required a larger of volume of water than our mesocosms allowed. However some cladocera and chaetognatha

were found in some of the mesocosms, and might have contributed to grazing and predation in these mesocosms.

6. Conclusion

Increases in nutrient addition were found to be directly tied to increases in chl *a* and POC, indicating that the phytoplankton took full advantage of the added nutrients. Based on POC values, phytoplankton showed a severe increase in biomass from day 0 to its maximum values on day 14 (13x the original level). This supports the hypothesis that nutrient addition has the potential to cause severe algae blooms. A change in phytoplankton community structure was also registered as smaller algae became more dominant towards the end of the experiments in treatments with high nutrient loadings.

Changes in ciliate biomass was linearly correlated to both nutrient addition increases and increases in food concentration. This indicates that food availability is inherently important for ciliate growth, which is supported by previous studies (Pierce and Turner 1992; Montagnes and Lessard 1999; Calbet and Saiz 2005). Ciliates bloomed on day 8 of the experiment, and both ciliate biomass and ciliate numbers were found to be much higher than previously recorded in the study area (Vargas and Martinez 2009; Gonzalez, Castro et al. 2011). This suggests that ciliates may in fact be more important to the Chilean Patagonian marine food web than previously thought. In particular, it is possible that ciliates can play a crucial role as buffer in the food web by being capable to exploit phytoplankton blooms faster than other organisms.

Copepods showed very little response to increases in nutrient addition, which is not unsurprising, due to their longer life cycle (Peterson 1998; Arnkværn, Daase et al. 2005). An experimental period of up to 3 weeks might be necessary to register growth in copepod biomass due to the lag in their response (Gismervik, Olsen et al. 2002; Olsen, Agusti et al. 2006). Unexpectedly, neither copepod biomass nor nauplii biomass showed any correlation to food concentration. No evidence of either grazing pressure on phytoplankton, nor predation pressure on ciliates was found for either of these groups. It is possible that the copepod concentrations in the mesocosms were too low to affect its food source. There were also several weaknesses in the mesozooplankton addition and sampling, making it difficult to assess the mechanisms behind the copepods' lack of response.

Nutrient release from fish farms was thus found to affect the lower trophic levels of the food web by causing drastic increases in phytoplankton and ciliate biomass and numbers. High levels of nutrient addition were also found to change the phytoplankton community structure, which can have consequences further up in the food chain (Cloern 2001). Copepods' ability to assimilate this increase in biomass is uncertain, and thus the fate of these changes remains uncertain.

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Appendix A - Nutrients

Table A1: Nutrient concentrations, silicate (Si), phosphate (PO_4), nitrate (NO_3) and nitrite (NO_2), in µg l^{-1} according to nutrient additions (zero, control and 1-6 increasing gradient) during the experiment.

Si	Day 0	Day 4	Day 8	Day 10	Day 12	Day 16
Zero	8	1.08	1.22	0	0.98	0
Cont.	8	0.34	0	0	1.15	0
1	8	1.05	0.42	0	0	0
2	8	0.72	0.31	0	0.7	0.11
3	8	0.3	0.75	0	1.06	0
4	8	0	0.38	0	1.17	0.82
5	8	0.56	0.92	0	0.82	3.36
6	8	0	0	0	2	
PO_4						
Zero	0.42	0.37	0.47	0.52	0.53	0.13
Cont.	0.42	0.41	0.37	0.48	0.4	0.23
1	0.42	0.42	0.39	0.53	0.37	0.23
2	0.42	0.46	0.36	0.6	0.32	0.31
3	0.42	0.4	0.42	0.6	0.24	0.54
4	0.42	0.34	0.4	0.4	0.26	0.28
5	0.42	0.27	0.69	0.53	0.42	0.48
6	0.42	0.34	0.59	0.59	0.55	
NO_3						
Zero	0.46	0	0	0	0	0.83
Cont.	0.46	0	0	0	0	0.13
1	0.46	0	0	0	0	0
2	0.46	0	0	0	0	0
3	0.46	0	0	0	0	0
4	0.46	0	0	0	0	0
5	0.46	0	0	0	0	0
6	0.46	0	0	0	0	
NO_2						
Zero	0	0	0	0	0	0
Cont.	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0.01	0	0	0	0
6	0	0.03	0	0	0	

Appendix B – Chlorophyll *a* and POC

Appendix B contains chlorophyll *a* (Table B1) and POC results (Table B2 and B3).

Table B1: Chlorophyll *a* measurements from the different treatments (zero, control and 1-6 increasing gradient) from day 2 to day 16 in µg l^{-1}. Standard deviation (Sd) included.

Date	Zero	sd	Cont	sd	1	sd	2	sd	3	sd	4	sd	5	sd	6
Day 2	**2.0**	**0.1**	**3.3**	**0.2**	**1.9**	**0.0**	**1.0**	**1.2**	**2.8**	**0.3**	**2.5**	**0.6**	**2.8**	**0.5**	**3.3**
Day 4	**2.3**	**0.0**	**2.2**	**1.0**	**2.8**	**0.7**	**2.6**	**0.8**	**3.1**	**0.3**	**2.6**	**0.1**	**3.0**	**0.8**	**2.9**
Day 6	**0.9**	**0.0**	**1.2**	**0.3**	**1.3**	**0.1**	**1.2**	**0.2**	**1.2**	**0.3**	**1.4**	**0.1**	**1.6**	**0.2**	**2.6**
Day 8	**0.8**	**0.3**	**0.9**	**0.2**	**1.1**	**0.2**	**1.1**	**0.5**	**1.2**	**0.6**	**1.7**	**0.6**	**2.6**	**0.6**	**4.6**
Day 10	**0.4**	**0.3**	**0.7**	**0.0**	**1.7**	**0.2**	**2.7**	**0.8**	**4.4**	**0.8**	**5.6**	**2.5**	**12.2**	**6.3**	**17.5**
Day 12	**1.2**	**0.3**	**1.7**	**0.4**	**2.4**	**0.5**	**3.9**	**0.6**	**4.5**	**2.6**	**3.7**	**0.8**	**12.3**	**7.9**	**14.6**
Day 14	**2.0**	**0.5**	**3.3**	**0.5**	**4.1**	**1.8**	**4.7**	**1.6**	**4.3**	**2.3**	**6.4**	**0.6**	**10.5**	**4.8**	**17.0**
Day 16	**3.5**	**0.7**	**5.3**	**2.0**	**7.2**	**1.8**	**5.4**	**1.9**	**11.0**	**2.1**	**4.0**	**0.6**	**9.4**	**2.5**	**19.3**

Table B2: Fractionated POC in the 2 – 10 µm range in mg C l^{-1} according to nutrient additions (zero, control and 1-6 increasing gradient) during the experiment. Standard Error (SE) included.

POC	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16
Zero	0.04	0.08	0.24	0.21	0.18	0.16	0.21	0.22	0.20
Cont.	0.04	0.08	0.23	0.21	0.25	0.19	0.22	0.23	0.24
1	0.04	0.08	0.24	0.22	0.23	0.23	0.29	0.33	0.33
2	0.04	0.09	0.25	0.23	0.19	0.24	0.35	0.31	0.31
3	0.04	0.10	0.27	0.24	0.22	0.31	0.52	0.54	0.51
4	0.04	0.09	0.25	0.26	0.25	0.29	0.37	0.50	0.41
5	0.04	0.10	0.25	0.32	0.28	0.45	0.70	0.73	0.58
6	0.04	0.11	0.28	0.34	0.33	0.58	0.88	1.06	0.81
SE									
Zero	0.02	0.01	0.01	0.01	0.05	0.05	0.04	0.05	0.05
Cont.	0.02	0.01	0.01	0.02	0.03	0.02	0.04	0.05	0.10
1	0.02	0.01	0.03	0.03	0.02	0.03	0.07	0.03	0.03
2	0.02	0.01	0.01	0.01	0.03	0.05	0.07	0.05	0.07
3	0.02	0.01	0.00	0.03	0.02	0.05	0.06	0.03	0.08
4	0.02	0.01	0.02	0.04	0.01	0.05	0.05	0.07	0.06
5	0.02	0.01	0.02	0.05	0.03	0.13	0.21	0.42	0.36
6	0.02	0.01	0.03	0.02	0.06	0.04	0.13	0.21	0.16

Table B3: Fractionated POC in the 10 – 40 µm range in mg C l^{-1} according to nutrient additions (zero, control and 1-6 increasing gradient) during the experiment. Standard Error (SE) included.

POC	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16
Zero	0.06	0.09	0.36	0.29	0.31	0.22	0.23	0.23	0.18
Cont.	0.06	0.09	0.39	0.36	0.37	0.29	0.30	0.27	0.22
1	0.06	0.12	0.40	0.31	0.39	0.32	0.32	0.33	0.32
2	0.06	0.15	0.37	0.36	0.37	0.29	0.43	0.33	0.27
3	0.06	0.16	0.42	0.39	0.41	0.36	0.51	0.50	0.44
4	0.06	0.14	0.47	0.41	0.41	0.36	0.45	0.53	0.37
5	0.06	0.14	0.48	0.49	0.47	0.45	0.59	0.48	0.39
6	0.06	0.15	0.53	0.46	0.51	0.60	0.59	0.52	0.58
SE									
Zero	0.03	0.02	0.06	0.03	0.03	0.12	0.05	0.08	0.04
Cont.	0.03	0.00	0.05	0.06	0.06	0.04	0.10	0.03	0.06
1	0.03	0.01	0.04	0.03	0.09	0.08	0.08	0.03	0.08
2	0.03	0.01	0.02	0.02	0.07	0.12	0.13	0.07	0.07
3	0.03	0.03	0.06	0.09	0.07	0.03	0.03	0.19	0.18
4	0.03	0.01	0.01	0.02	0.04	0.06	0.04	0.26	0.10
5	0.03	0.05	0.04	0.05	0.02	0.15	0.12	0.15	0.08
6	0.03	0.03	0.04	0.03	0.02	0.11	0.00	0.09	0.13

Appendix C – Ciliates

Table C1: Ciliate counts (#) in individuals ml^{-1} and biomass in µg C l^{-1} according to nutrient addition (zero, control and 1 -6 increasing gradient) during the course of the experiment. Standard deviation included.

		Day 0		Day 4		Day 8		Day 12		Day 16	
		#	Bio.	#	Bio.	#	Bio.	#	Bio.	#	Bio.
Zero	Aloricate	2.13		4.9		27.5		23.1		16.4	
	Tintinnids	0		0.1		0.5		9.1		7.6	
	Total	2.13	0.68	5	4.04	28	35.2	32	27.9	24	21.2
	St. Dev	0.72	0	0.48	0.37	3.64	7.93	8.03	4.8	5.13	6.48
Cont.	Aloricate	2.13		5.9		21.8		16.4		8.3	
	Tintinnids	0		0.1		1.2		0.6		0.7	
	Total	2.13	0.68	6	5.82	23	28.7	17	16.9	9	7.2
	St. Dev	0.72	0	0.32	0.79	3.06	1.66	6.41	6.27	5.06	3.8
1	Aloricate	2.13		7.5		27.8		19.9		25.9	
	Tintinnids	0		0.5		0.4		2.1		2.1	
	Total	2.13	0.68	8	4.49	28.2	41.7	22	24.5	28	25.4
	St. Dev	0.72	0	0.77	1.34	3.98	6.8	1.8	5.26	7.65	7.87
2	Aloricate	2.13		9.5		32.9		31.2		13.3	
	Tintinnids	0		0.1		0.1		4.8		1.7	
	Total	2.13	0.68	10	5.82	33	52.5	36	28.8	15	19.6
	St. Dev	0.72	0	0.84	0.88	0.78	1.11	3.23	3.88	3.51	5.1
3	Aloricate	2.13		6.3		39.4		39.4		27.9	
	Tintinnids	0		0.7		1.6		11.6		14.1	
	Total	2.13	0.68	7	3.34	41	54.8	51	44	42	24
	St. Dev	0.72	0	1.5	0.19	6.48	9.73	2.64	13.1	16.9	10.5
4	Aloricate	2.13		8.2		44.9		27.9		19.4	
	Tintinnids	0		0.8		1.6		1		1	
	Total	2.13	0.68	9	5.99	47	58.1	29	30	20.4	27.1
	St. Dev	0.72	0	0.41	0.95	2.85	3.93	5.31	4.6	2.73	0.35
5	Aloricate	2.13		7.1		50		33.6		59.7	
	Tintinnids	0		0		2.2		6.4		3.2	
	Total	2.13	0.68	7.1	5.66	52.2	76.3	40	41.9	63	59.9
	St. Dev	0.72	0	2.18	2.63	0.67	1.1	6.13	3.46	18.6	16
6	Aloricate	2.13		7.5		79.4		42.6		90.5	
	Tintinnids	0		0.5		0.6		0.3		0.5	
	Total	2.13	0.68	8	5.45	80	116.8	43	43.3	91	68.9
	St. Dev	0.72	0	1.61	1.31	8.42	11.8	4.8	5.99	6.5	1.35

Appendix D - Mesozooplankton

Table D1: Mesozooplankton counts (#) in individuals l^{-1} and biomass in µg C l^{-1} according to nutrient addition (zero, control and 1 -6 increasing gradient) on the last day of the experiment.

	Zero		Cont.		1		2		3		4		5		6	
	#	Bio.	#	Bio.	#	Bio.	#	Bio.	#	Bio.	#	Bio.	#	Bio.	#	Bio.
Crustacea																
Calanoid cop	6.04	14.7	34.3	27.3	16.53	18.60	6.37	35.70	5.18	32.40	6.01	32.90	13.40	53.80	3.33	36.20
Cyclopoid cop	0.15		0.17		0.02		0.14		0.47		0.13		0.13		0.27	
Harpacticoid cop	0.32		0.14		0.20		0.19		0.03		0.04		0.13		0.38	
Total cop	6.51	14.9	34.55	27.4	16.76	18.70	6.70	35.80	5.68	32.40	6.18	32.90	13.66	53.90	3.98	36.50
Cop Nauplii	16.2	0.14	70.49	0.60	67.13	0.57	71.20	0.60	108.26	0.91	128.90	1.10	150.01	1.27	51.49	0.43
Amphipoda	0.00		0.00		0.00		0.00		0.01		0.00		0.00		0.05	
Ostracoda	0.05		0.00		0.09		0.17		0.11		0.17		0.16		0.05	
Cladocera	0.01		0.02		0.00		0.00		0.00		0.00		0.00		0.00	
Decapoda larvae	0.08		0.00		0.00		0.01		0.01		0.00		0.02		0.03	
Appendicularia	0.00		2.72		0.01		0.05		0.03		0.03		0.00		0.00	
Gastropoda	0.07		0.03		0.00		0.00		0.00		0.00		0.00		0.00	
Bivalvia	0.00		0.00		0.00		0.00		0.00		0.01		0.00		0.00	
Polychaete	0.01		0.02		0.02		0.01		0.05		0.06		0.01		0.03	