

# GRAZER-INDUCED RESPONSES IN MARINE PHYTOPLANKTON

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## ABSTRACT

Phytoplankton are the most important primary producers in the world's oceans, yet little is known of how they avoid the constant threats from the pelagic grazers. From land it is well known that plants defend themselves by having thorns or producing compounds unpalatable to grazers. This thesis shows how different species of phytoplankton cope with different threats from pelagic grazers.

In paper I the focus was on the dinoflagellate *Alexandrium minutum*, a producer of the highly potent neurotoxins paralytic shellfish toxins (PST). These toxins can cause mass mortalities in marine mammals and can be deadly to humans, who ingest the toxins via filter feeders that accumulate the toxins from algae in their diet. The suggested purpose of these toxins is to act as a defence against zooplankton grazers, like copepods. It has been shown that *A. minutum* is able to sense water borne cues from the copepods and respond by an increase in PST production. The results of paper I show that not only is *A. minutum* able to sense copepods, it can also recognize different species and respond by either increasing PST production or not. Cues from one of the copepod species tested, *Centropages typicus*, resulted in a more than 20 fold increase in PST, whereas another copepod, *Pseudocalanus sp.*, did not have any effect on the PST content. It seems likely that *A. minutum* can recognize copepods that have the same distribution area as itself, these species would be the most significant grazers on *A. minutum* and a defence against them would benefit the alga. This strengthens the suggested role of PST as a grazer deterrent.

Another group of substances that have been suggested to act as defence against grazers are the polyunsaturated aldehydes (PUA) produced by some phytoplankton, among others, diatoms. The role of PUA has been debated and various effects have been shown for a number of organisms. PUA has been shown to have negative effect on the reproduction of copepods, but results are contradictory. In paper II we investigate another possible defensive effect of PUA, as a structuring agent on the microbial community of bacteria and viruses. Bacteria can infect diatoms or compete for nutrients, and viral infections can terminate phytoplankton blooms. Compounds that affect these organisms can be of great benefit for the producing organism. The results in paper II show that PUA have no effect on either bacteria or viruses, and further questions the role of these compounds.

Perhaps the most interesting findings in this thesis are the ones presented in paper III and IV, where diatoms are shown to use chain length plasticity as a defence against copepods. Previously, different factors such as nutrient uptake and flotation has been suggested to be the driving force behind chain formation, but chain formation as a defence has been suggested before. Here I present further support for this. The diatoms responded to cues from copepods by reducing their chain length, and thereby size, with reduced grazing as a result. Reducing chains to single cells would make the diatom *Skeletonema marinoi* too small for copepods to handle, while larger species like *Thalassiosira rotula* would still be large enough to be caught. But by reducing chain length the diatoms also reduce the encounter rate with grazers, and thus larger species like *T. rotula* can escape grazing. Lower grazing rates were also observed on single cells than longer chains. All diatoms did not respond to grazer cues in the same way, *Chaetoceros affinis* did not reduce chain length when subjected to copepods. *C. affinis* has long spines that may act as a defence which could be the reason why it does not reduce its size. I suggest that chain length plasticity may be an evolutionarily adopted trait in chain forming diatoms and that size-selective predation may have played a key role in the evolution of chain formation and chain length plasticity in diatoms.

## POPULÄRVETENSKAPLIG SAMMANFATTNING

Havets skogar står inte med 100 meter höga träd, istället finns där miljoner små, små växtplankton som följer med vattnets rörelser. Trots att de är så små så producerar de lika mycket syre som jordens gröna växter gör på land. De förökar sig genom att dela på sig, men ibland har de sex också. (Varför är växtplankton så många? För att de har så roligt! Varför har de så roligt? För att de är så många!) Har du badat mycket i sommar? Vet du vad du simmat runt i? Vad som kan se ut som ett ganska fridfullt blågrönt hav är egentligen ett slagfält, där växtplanktonen gör allt de kan för att försvara sig mot en mängd olika djur som vill äta upp dem. Vem är det då som äter alla plankton? Betarna är många och varierar enormt i storlek, från några tusendels millimeter långa ciliater till den 20 meter långa valhajen. En av alla betare är hoppkräftorna, små kräftdjur. Hoppkräftorna är även de många, antagligen de talrikaste flercelliga organismerna i världen, och kanske den största källan till protein i havet. Vem vet, snart kanske vi äter hoppkräftor till middag?

Hur gör växtplanktonen då för att försvara sig? Ett sätt att försvara sig mot betare är att producera gifter. I den här avhandlingen visar jag att växtplankton (dinoflagellaten *Alexandrium minutum*) inte bara känner av att det finns hoppkräftor i närheten och producerar gift som svar på det, utan även kan känna igen olika sorters hoppkräftor och producera mer eller mindre gift som svar. De känner igen hoppkräftorna på lukten och lukten av en av dem, *Centropages typicus*, gör att *A. minutum* producerar mer än 20 gånger så mycket gift än *A. minutum* som inte känt lukten av hoppkräftor. Gifterna som *Alexandrium* producerar, paralytiska skaldjurstoxiner, är mycket starka nervgifter som kan ge dödliga förgiftningar även hos människor. Vi får i oss det när vi äter t.ex. musslor som i sin tur ätit giftiga alger.

Ett annat sätt att motverka att man blir uppäten är att ändra storlek och på så sätt göra att betarna antingen inte hittar en, eller att de inte kan fånga och hantera en. Det här gör en del växtplankton, som kiselalger. Man brukar säga att kiselalger lever i glashus, eftersom deras skal är gjorda av kisel (dvs. glas). Skalen ser ut som små askar, där locket är lite större än botten. De kiselalger jag tittat på kan antingen förekomma som ensamma celler eller sitta ihop i långa kedjor. Kedjorna bildas när algerna delar på sig och sedan sitter ihop efter delningen. När kiselalgerna känner av betare kan de ändra sättet de växer på, genom att splittra upp kedjorna i mindre delar kan de undkomma betarna, de blir för små för hoppkräftorna att hantera, eller för svåra att hitta.

Det är inte bara mot djur som vill äta upp dem som växtplankton måste skydda sig, i havet finns det även mängder av bakterier och virus, och de kan infektera växtplankton på samma sätt som vi kan bli infekterade och bli förkylda. Men ofta är det värre för ett encelligt plankton att bli infekterat, om infektionen leder till att cellen spricker är det slutet för den algen. En del av alla de ämnen som produceras av kiselalger är fleromättade aldehyder, och dessa ämnen skulle kunna vara ett försvar mot mikroorganismer. Då många bakterier och framförallt virus bara infekterar en viss art av växtplankton skulle det vara fördelaktigt att kunna försvara sig mot dessa angripare. Det vi har sett här är att aldehyderna inte har någon effekt mot varken bakterier eller virus. Kanske har just de här ämnena andra funktioner?

Vi vet mycket om hur växter på land försvarar sig, en del har taggar och andra är giftiga. Växtplankton verkar också ha en rad sätt att försvara sig på, och det finns mycket mer att ta reda på när det gäller de här försvaren.

*”Överarbета och vara för ambitiös är farligt,  
då är det bättre att gå och dricka ett glas  
champagne och lugna ner sig lite.”*

- Gudmar Olofsson

## LIST OF PAPERS

This thesis is based on the following papers, which in the text will be referred to by their roman numerals.

**I.** Bergkvist, J., Selander, E., Pavia H. 2008. Induction of toxin production in dinoflagellates: the grazer makes a difference. *Oecologia* 156(1): 147-154.

**II.** Paul, C., Reunamo, A., Lindehoff, E., Bergkvist, J., Mausz, M. A., Larsson, H., Richter, H., Wängberg, S-Å., Leskinen, P., Båmstedt U., Pohnert G., 2012. Diatom Derived Polyunsaturated Aldehydes Do Not Structure the Planktonic Microbial Community in a Mesocosm Study. *Mar. Drugs* 10(4), 775-792; doi:10.3390/md10040775

**III.** Bergkvist, J, Thor, P., Jakobsen, H. H., Wängberg, S-Å., Selander E., 2012. Grazer-induced chain length plasticity reduces grazing risk in a marine diatom. *Limnology & Oceanography*, 57(1), 318-324 | DOI: 10.4319/lo.2012.57.1.0318

**IV.** Bergkvist J., Eggertsen M., Hansen G. Grazer-induced colony size plasticity – a general response in colony forming phytoplankton? *Manuscript*

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## *Abbreviations*

DMS	Dimethylsulfide
DMSP	Dimethylsulfoniopropionate
DOC	Dissolved Organic Carbon
HPLC	High Performance Liquid Chromatography
PST	Paralytic Shellfish Toxin
PUA	Polyunsaturated Aldehydes



# 1. INTRODUCTION

## 1.1. PELAGIC INTERACTIONS

The marine food web is complex, and interactions between different levels in the food web can have large effects on how energy and matter is transferred within it (fig. 1). Most of the world's oceans are nutrient poor habitats, and since small size facilitates nutrient uptake, much of the marine primary production is dominated by pico- and nanoplankton less than 20  $\mu\text{m}$  in size (Wolfe 2000). Phytoplankton are the most important primary producers in the oceans, and even though they constitute less than 0.5% of the global biomass they account for nearly half of the world's primary production (Field et al. 1998) and form the base for the marine food web. Phytoplankton have high turnover rates compared to vascular plants and seaweeds (Shurin et al. 2006). In most terrestrial systems, the primary producers have the highest biomass, but aquatic systems often have ratios of heterotroph-to-autotroph biomass greater than 1 (Del Giorgio et al. 1999).

The daily turnover in pelagic systems is in the order of 1000 times that of forests (Cebrian 1999) and grazer induced mortality is much higher in aquatic compared to terrestrial ecosystems (Cyr and Pace 1993), therefore it is important to be able to avoid predation. Avoid being eaten is as important as the ability to photosynthesize and take up nutrients. Likewise, for the grazers (the term grazer is used for consumers of phytoplankton throughout the thesis) it is important to find and exploit the primary producers.

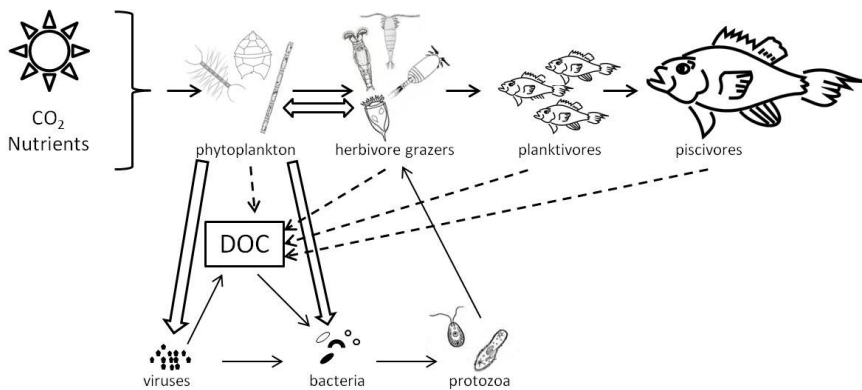
Grazing pressure in the pelagic is high, an average of 23% of the phytoplankton primary production is consumed by mesozooplankton grazers (like copepods) per day (Calbet 2001) and the lion's share of what is left is consumed by microzooplankton (Calbet and Landry 2004). But there are other loss factors for phytoplankton than grazing by zooplankton and sinking, bacteria and virus infecting phytoplankton is an important mortality cause (Brussaard 2004). It is now known that viruses can infect a wide range of phytoplankton species, including bloom forming species (Bettarel et al. 2005) and diatoms (Nagasaki et al. 2005). Viruses infecting phytoplankton are mostly host specific, infecting only one species and in some cases only one host strain (Brussaard 2004). Since phytoplanktons are unicellular, a lytic infection results in cell death and can have significant effects on the population. However, the fact that phytoplankton blooms still occur suggests that some algal species have means to protect themselves and reduce the impact of grazing and virus infections (Brussaard 2004).

Pelagic food webs are strongly size-structured, more so than terrestrial systems. In the pelagic system there is a strong positive correlation between body size and place in the trophic hierarchy (Shurin et al. 2006). Terrestrial consumers vary in size from much larger (e.g. grazing mammals) to much smaller (e.g. insects) than the plants they consume, while pelagic consumers typically are larger than their prey (Hansen et al. 1994). Primary producers in both systems fix carbon at similar rates per square meter despite lower

biomass in the aquatic system, but there are less storage in phytoplankton communities than forests or grassland (Cebrian 1999). The majority of primary producers in pelagic systems are unicellular, while terrestrial plants are predominantly multicellular and structurally complex. Phytoplankton lack the transport and structural tissue that terrestrial plants have, and are therefore almost entirely made up of nutrient rich (high nitrogen and phosphate) metabolically active material (Shurin et al. 2006).

Many aquatic consumers, as filter-feeding mollusks, whales and planktivorous fish, are mainly limited by the size of their prey (Shurin et al. 2006), while other consumers, like copepods, have selective feeding based on size, shape and quality of the food particles (Price et al. 1983). Selective feeding in copepods allows them to structure the phytoplankton community and can have large implications for the pelagic food web.

Copepods are not important grazers during the spring bloom in temperate waters, since only small overwintering populations are present and the low temperature results in low grazing rates, but heterotrophic dinoflagellates are. They are different from other pelagic grazers in having an optimum prey size similar to their own size, shorter generation time than mesozooplankton and have been shown to be important grazers on diatoms (Tiselius and Kuylenstierna 1996).



**Figure 1.** Illustration of the pelagic food web, showing where phytoplankton and herbivore grazers fit in. Block arrows indicating which interactions the papers in this thesis have looked at. Modified after (Azam et al. 1983).

## 1.2. DEFENCES IN PLANTS, TERRESTRIAL AND AQUATIC

Plants are under constant threat of being attacked, large grazers can remove the whole plant, while smaller grazers feed on sections of it, and parasitic microorganisms invades the smallest parts. There are many ways by which a plant can defend itself, among the most common is morphological defences such as thorns, spines and hairs. Having only one form of defence can be effective against some of the grazers, but not all, as for example thorns can be effective against large grazers, but not against bacteria infecting the plant (Karban and Baldwin 1997). Thus, many plants also have chemical defences against smaller grazers.

While defences in terrestrial plants and even seaweeds are quite well studied, defence in phytoplankton is a relatively new idea, traditionally other factors such as nutrient limitation, abiotic factors, competition and predation was assumed to shape the structure of phytoplankton communities. But phytoplankton are not defenceless morsels floating around for the consumers to wolf down. It is well known that not all phytoplankton species are grazed to the same extent. This is primarily due to properties as size, shape and cell-wall structure, but evidence is mounting that macroalgae and phytoplankton are also defending themselves against predation. Life in the pelagic has been called “a watery arms race” (Smetacek 2001) and that is exactly what it is. Of all the phytoplankton species in the ocean, about 200 are known to be, suspected to be or have the potential to produce compounds harmful for other organisms (Landsberg 2002). Looking at phytoplankton, there are some differences compared to terrestrial plants and also large multicellular aquatic plants. For example, single celled phytoplanktons are not subjected to partial predation, except maybe chain forming or colonial forms. Therefore, the defence should aim to deter the grazer and shift the grazing towards other phytoplankton species. As well as terrestrial plants, phytoplankton defend themselves by changing their morphology, growth form and biochemical composition (Van Donk et al. 2011). Results from paper III and IV in this thesis suggests that diatoms are one of the groups of phytoplankton that changes growth form as a defence against grazers.

## 1.3. INDUCIBLE DEFENCES IN PHYTOPLANKTON

Inducible defences are, in contrast to constitutive defences that are always expressed, only expressed following damage or stress (Karban and Baldwin 1997). Inducible defences also gives the producer the possibility to cope with resistance in herbivores with new or modified toxins (Tollrian and Harvell 1999). For unicellular organisms, with no tissue to sacrifice, it is better to repel grazers before ingestion than cause post-ingestion toxic effects (Wolfe 2000). The first evidence of inducible morphological defense in phytoplankton was discovered using the green algae *Scenedesmus subspicatus*, *Scenedesmus* responded to the water flea *Daphnia magna* by increasing both the proportion of cells in colonies and the proportion of armoured cells (Hessen and Van

Donk 1993). Since then the number of cases of phytoplankton with inducible defences has increased. Colony formation in *Phaeocystis globosa* increased when grazed by heterotrophic dinoflagellates (Jakobsen and Tang 2002) and suppressed colony formation in response to cues from grazing copepods (Long et al. 2007). It was also shown that grazer-induced morphotypes benefited from lower grazing rates (Long et al. 2007). Another example is the dinoflagellate *Alexandrium tamarense*, which responded to grazer cues by simultaneously splitting up chains and reducing swimming speed. As single cells swim slower than chains, and single cells has a reduced hydrodynamical signal compared to chains this reduces encounter rate with copepod grazers several fold (Selander et al. 2011). Reduced encounter rates with grazers could also be an important factor for chain length plasticity in diatoms (paper III and IV).

#### **1.4. CHEMICALLY MEDIATED INTERACTIONS IN PHYTOPLANKTON**

Considering that all multicellular organisms evolved from unicellular microbes, the chemical signalling systems common in plants most likely originated in unicellular microbes (Wolfe 2000). In the pelagic system, chemical cues are important for a number of interactions, both between members of the same species and between different species. Chemical cues determine if an organism feed on, mate with, flee from or fight with another organism (Hay 2009). Understanding the role of chemical cues is of great importance in order to explain the structure and function of marine systems (Hay 2009). An example of how phytoplankton metabolites can have effect over hundreds or thousands of kilometres is dimethylsulfide (DMS) and its effect on zooplankton, fishes, seabirds and possibly even global weather patterns (Hay and Kubanek 2002). Some phytoplankton strains have the potential to cleave dimethylsulfoniopropionate (DMSP) to DMS and acrylate when injured by grazers (Wolfe 2000). Many grazers avoid feeding on DMS producing phytoplankton and in areas with high productivity DMS is released into surface waters and the atmosphere. Seabirds use DMS as a cue for finding food and oxidation products of DMS are important in the formation of aerosols, affecting cloud formation (Hay and Kubanek 2002) and ultimately the heat reflection capacity of the atmosphere.

Omnivorous copepods are able to discriminate between *Alexandrium* species with different toxin content, and choose the nontoxic species (Teegarden 1999). The selection occurs before ingestion, and suggests that the copepods can recognize toxic cells by chemosensory means. As a result, it seems like selective grazing is a more important factor for the duration of a harmful algal bloom than physical incapacitation of the grazers (Teegarden 1999). The effect on copepod grazer vary with the species of algae, and is also dependant on if the alga is provided singly or as part of an assemblage. When the effect of several "harmful" species of phytoplankton were tested on copepod grazers, only one (*Alexandrium* sp.) showed to be toxic to the copepod *Acartia tonsa* (Colin and Dam 2002), although this could be due to other factors that correlate with toxin content.

## 1.5. AIM OF THESIS

The general aim of this thesis was to investigate defences in marine phytoplankton, in particular inducible defences. Phytoplankton have various ways to defend themselves, with different consequences for the potential predator. Defences include changes in biochemistry, behaviour, life history and morphology of the cell.

### *Paper I.*

The aim of this paper was to investigate if paralytic shellfish toxin (PST) production in *Alexandrium minutum* was dependant on the species of copepod present. Three different species of copepods and two different strains of *A. minutum* were used. Previous findings showed that *A. minutum* respond to cues from copepods with an increase in PST content, here the question was if the various copepods all had the same effect on *A. minutum*.

### *Paper II.*

The mesocosm study in this paper was designed to investigate the effect of polyunsaturated aldehydes (PUA) and the PUA producing diatom *Skeletonema marinoi* on the microbial community. PUA have been shown to have a negative effect on microorganisms and has been suggested to structure the bacterioplankton community. The aim of this paper was to see if ecologically relevant levels of PUA had an effect on the microbial community of bacteria and viruses.

### *Paper III.*

Some diatoms form long chains of connected cells, but the reason for chain formation is still unclear. In paper III, I wanted to look at the correlation of chain length in *Skeletonema marinoi* and presence of grazers. Other species of phytoplankton can sense grazers and respond by changing size, I wanted to see if *S. marinoi* could sense grazers and respond by altering the chain length. I subjected *S. marinoi* to direct and indirect grazing (water borne cues from caged grazers) from three species of copepods and one species of heterotrophic dinoflagellate.

### *Paper IV.*

Following the results in paper III, I wanted to continue looking at chain formation and chain length plasticity in diatoms, this time investigating more species. I looked at *Thalassiosira rotula*, *Chaetoceros affinis* and several strains of *Skeletonema marinoi*. Here as well in paper III the diatoms were exposed to direct grazing and water borne cues from caged grazers.

## 1.6. RESEARCH AREAS

### 1.6.1. Toxin production in dinoflagellates

Some species of dinoflagellates produce bioactive compounds that render copepod grazers incapable of feeding (Huntley et al. 1986). The genus *Alexandrium* produces paralytic shellfish toxins (PST's), a group of toxins that have been suggested to deter copepod grazers and thereby move grazing pressure towards competing species (Colin and Dam 2002). PST's are sodium-ion channel blockers that inhibit nerve conduction (Turner and Tester 1997). PST's are highly lethal, LD<sub>50</sub> (Lethal Dose 50%) in mice is 10 µg kg<sup>-1</sup>, compared to a LD<sub>50</sub> for sodium cyanide at 10 mg kg<sup>-1</sup> (Landsberg 2002).

Selander and co-workers (Selander et al. 2006) found that waterborne cues from the copepod *Acartia tonsa* induced paralytic shellfish toxin production in *Alexandrium minutum*, and that the increase in toxicity correlated to a decrease in grazing by the copepod. Several species of copepods have been shown to be able to discriminate between toxic and non-toxic *Alexandrium* cells, and rejecting the toxic cells without causing mortal damage to the cells (Teegarden 1999). In paper I we look closer at the inducing capabilities of three species of copepods, to evaluate if all copepods have the same impact on PST production.

### 1.6.2. Polyunsaturated aldehydes

Studies have shown that a diatom diet resulted in high egg production rates, but reduced hatching success in copepods (Ianora and Poulet 1993). Later it was suggested that some diatoms produce terratogenic compounds that induce egg mortality and production of abnormal larvae in copepods (Poulet et al. 2007). Negative effects have been shown for a wide range of organisms, including reduced growth rate in bacteria, inhibition of embryonic cell cleavage in echinoderms and disturbance of cytoskeleton in molluscs (Adolph et al. 2004). The compounds responsible for the terratogenic effects in copepods are identified as reactive volatile polyunsaturated aldehydes (PUA), derived from the oxidative transformation of free fatty acids (Pohnert et al. 2002). The production of PUA is initiated when the cell is wounded, as would occur e.g. during zooplankton grazing (Pohnert 2000). The effect of PUA on copepods ranges from reduced egg hatching success, malformation of nauplii, and larval mortality (Ianora et al. 2003; Paffenhofer et al. 2005) to no effect at all (Dutz et al. 2008). Evidence suggests that the PUA alone is not enough to explain the negative effect on copepods, since species of diatoms that do not produce PUA have a negative effect on copepod reproduction success as well (Fontana et al. 2007). The effects shown on egg hatching success and nauplii development in copepods (Ianora et al. 2004) does not fit well in the suggestion that a defense should be direct in order to benefit the producing organism. When the slackening of grazing pressure due to reduced reproductive success of copepods appears, the phytoplankton community might not be the same as the one triggering the effect. Considering the effect of PUA shown on bacteria, another possible function of PUA is structuring of diatom

associated bacterial communities (Ribalet et al. 2008). Regarding the effect of PUA on various organisms (Adolph et al. 2004) it can be assumed that PUA also affect viruses. A negative effect on bacterial growth or viral distribution would be a more direct defence, particularly considering viruses, since many of them are species or even strain specific (Brussaard 2004). Chain forming diatoms would benefit if a cell infected by bacteria or viruses has a defence that kills pathogens, even if the cell itself dies. This would protect the other cells in the chain from being infected. The effect of PUA on bacteria and viruses is investigated in paper II.

### 1.6.3. Chain formation in diatoms

Chain length may have great importance on the grazing impact, as size selective feeding is common in the pelagic. Changes in chain length not only affect the size of the organism, but also modify the encounter rate with grazers, thereby modifying the risk of being detected by a grazer. In paper III and IV we look into the reason why diatoms form chains. It has been suggested that chain formation could aid the uptake of nutrients, especially chains like those of *Skeletonema* and *Thalassiosira*, which have large gaps between the cells that reduces intra-chain competition for nutrients. Longer chains would allow the diatom to stay in the euphotic zone longer, and thereby acquire more nutrients and light (Takabayashi et al. 2006). However, even though the cells are separated by gaps, which in theory allows water to flow between the cells, the gaps contain linking spines that suppress such flows (Pahlow et al. 1997). The evolution of chains in diatoms appear to have started with compact, fused cells, as in *Melosira* sp. (Pahlow et al. 1997). Cells in chains like these have a lower ability to obtain nutrients than cells in chains with gaps between the cells, which contradicts the theory that chain formation should be correlated to the nutrient supply in the pelagic (Pahlow et al. 1997). Pahlow et al. suggests that chain formation is either a primitive property of diatoms or that other factors than nutrient have led to the evolution of chains. One of these factors could be the impact of grazers on chain length, where size-selective predation may have played a role in the evolution of chain formation and chain length plasticity (paper III and IV).

Improved flotation has been another suggested reason for chain formation, where the structure of the chains, with long silica rods between the cells, could decrease sinking rate of chains by causing an increased surface area ratio of the chains, and thereby increase the frictional resistance (Smayda and Boleyn 1966). Smayda and Boleyn also suggest that the diatoms have increased buoyancy during active growth, independent of the morphological properties, and that the flotation at that time is physiological in nature. Morphological characteristics such as chain formation as an adaptation to flotation should then become more important as the cells age (Smayda and Boleyn 1966). This is further supported by the result that diatom chains follow Stokes' Law when the cells are dead (Waite et al. 1997).

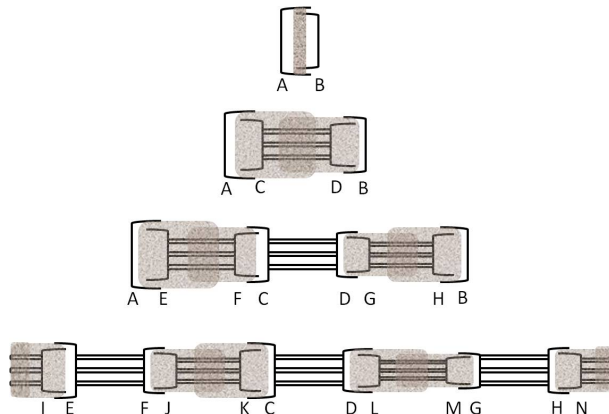
## 1.7. STUDY ORGANISMS

### 1.7.1. Diatoms

Diatoms are the largest group of microalgae and carries out about 20% of the photosynthesis on earth, each year they generate as much organic carbon as the entire world's rainforests put together. This carbon serves as the base for the marine food web, close to the coast it supports the important fisheries and out at sea they sink and provide food for deep-water organisms. Diatoms are the most diverse group of phytoplankton, comprising an estimated 200 000 different species (Armbrust 2009). The earliest fossils of diatoms are 190 million years old (Sims et al. 2006), but molecular-clock-based estimates suggests that the diatoms originated about 250 million years ago (Sorhannus 2007).

Diatoms have a cell wall, called frustule, made of silica. The frustule is in two parts (valves) which has given the diatoms their name, from the Greek *diatmos* meaning "cut in half". Size ranges from a couple of micrometers to a few millimetres, and diatoms occur either as single cells or as chains of connected cells. There is a limit to how long the chains can be, either because separation valves which lack interlocking structures are formed, or because cells within the chain breaks or die (Round et al. 1990).

Diatoms reproduce asexually by cell division, where each daughter cell inherit one of the valves from the parent cell, and thus only form one new valve. The new daughter cells are formed within the parent cell, which results in the new cell wall is smaller than the surrounding parental cell wall, which leads to a reduction in cell size in one of the daughter cells (Round et al. 1990). With time, this leads to a reduction in cell size in the whole diatom population, original cell size is restored with sexual reproduction, some species also restore cell size with vegetative enlargement (Round et al. 1990).



**Figure 2.** Cell division in *Skeletonema costatum* showing how the cells stay attached after division and how a chain of cells is formed, adapted from (Housley et al. 1975).

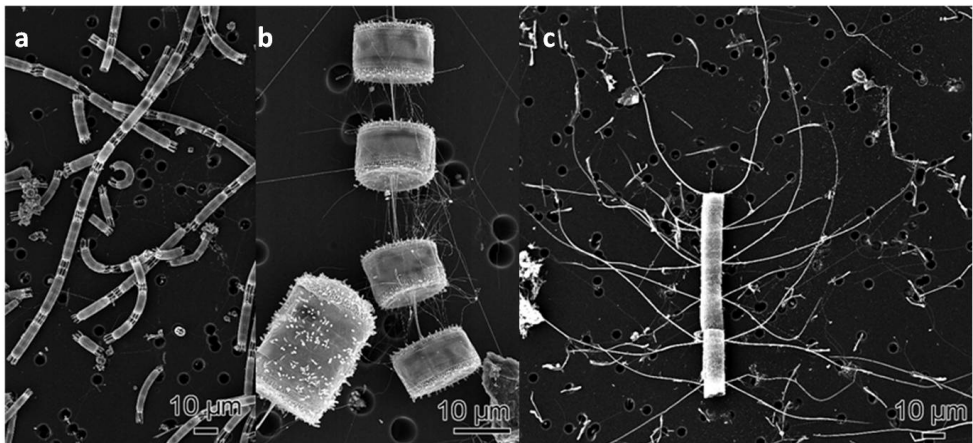


All diatom species used in this thesis are centric, chain forming diatoms. *Skeletonema marinoi* and *Thalassiosira rotula* belong to the same order (Thalassiosirales) while *Chaetoceros affinis* belongs to another order (Chaetocerotales) (Medlin et al. 1996), which separates them evolutionary. Another common feature in *Skeletonema marinoi* and *Thalassiosira rotula* is that they produce polyunsaturated aldehydes, something that *Chaetoceros affinis* does not (Wichard et al. 2005a). All three species form long chains of connected cells, however, the way they stay attached in chains vary.

Many *Skeletonema* species have a worldwide distribution and are among the most important contributors of phytoplankton blooms in coastal waters. *Skeletonema* can form long chains of >20 cells, connected with long processes on the edge of the valve (fig. 2). The cells are cylindrical, 2-12  $\mu\text{m}$  in diameter, and in most species each cell contains one or two chloroplasts (Sarno et al. 2005). *Skeletonema marinoi* (fig. 3 a) is a bloom forming species that often dominate the spring bloom on the Swedish west coast (Tiselius and Kuylenstierna 1996). Growth rate in *Skeletonema marinoi* is normally one cell division per day (Balzano et al. 2011).

*Thalassiosira* consists of a large number of species, mainly marine but freshwater species have been recorded. It is widely distributed around the world and form long chains connected with a single silica thread (Round et al. 1990). Growth rate varies with salinity, nutrients, and light conditions, but *Thalassiosira* can divide more than two times per day (Krawiec 1982). *Thalassiosira rotula* (fig. 3 b) has a diameter of 8-55  $\mu\text{m}$ .

*Chaetoceros* consists of a number of species that, as in *Skeletonema* and *Thalassiosira*, has a worldwide distribution. *Chaetoceros* form chains of cells connected by fusion of cells or interlocking setae. *Chaetoceros affinis* (fig. 3 c) has a diameter of 7-30  $\mu\text{m}$ .

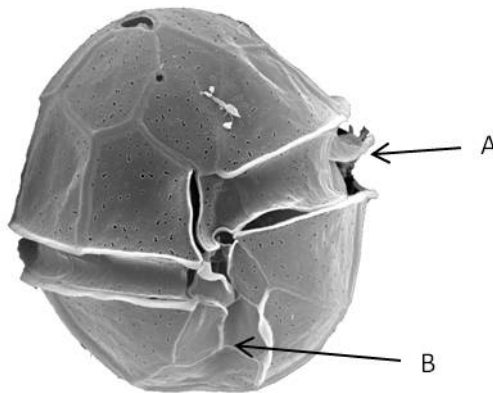


**Figure 3.** Scanning electron micrograph of diatom species used a) *Skeletonema marinoi* b) *Thalassiosira rotula* c) *Chaetoceros affinis*. Pictures show chains of connected cells. (Photo: G. Hansen).

### 1.7.2. Dinoflagellates

Dinoflagellates are important primary producers, and form a significant part of the plankton production in both oceans and lakes. They are usually unicellular and both photosynthetic and heterotrophic species are known, some are mixotrophic. Dinoflagellates normally have two flagella, one longitudinal and one transverse, one propelling them forward and one allowing them to change direction (fig. 4). Dinoflagellates are capable of producing a number of toxins, and account for 75% of all harmful algal bloom species (Smayda 1997), the blooms they form are called red tides, since the cells can be abundant enough to change the colour of the water and can persist for several weeks (Turner and Tester 1997). Contradictory to their being a dominating group of phytoplankton, dinoflagellates are poor competitors for nutrients and often have low growth rates (Smayda 1997).

Dinoflagellates of the genus *Alexandrium* produce paralytic shellfish toxins (PST), a group of highly potent neurotoxic alkaloids (Schantz et al. 1975). The PST's accumulate in shellfish feeding on the algae without any apparent affect on the shellfish (Asp et al. 2004). PST can accumulate in the food web and lead to mass mortalities in marine mammals and seabirds (Landsberg 2002). When humans are affected it is mainly through the consumption of filter feeders (mussels), and if the dose is high enough it is lethal.



**Figure 4.** Scanning electron micrograph of *Alexandrium tamarense*. The picture shows the two furrows where the flagella dinoflagellates use for propulsion are situated A: the furrow in which the transverse flagellum is situated, B: the furrow in which the longitudinal flagellum is situated. (Photo: T. Alpermann).

### 1.7.3. Copepods

Copepods (fig. 5) are probably the most abundant metazoan on earth (Turner 2004), outnumbering even the insects in number of individuals (Humes 1994). In the aquatic food web they are an important link between primary producers and higher trophic levels, like fish.

Copepods have different feeding strategies; some are ambush feeders, where the non-mobile grazer detects the prey by hydromechanical, chemical or possibly visual cues (Kiørboe 2011). The ambush feeders detect the prey before they make physical contact, and then attack it. Copepods are discriminating feeders, capturing, handling and ingesting or rejecting particles based on the quality of the particles (Huntley et al. 1986), even if they eat a wide variety of phytoplankton and other protozoa (Turner 2004). Selective feeding allows copepods to reject particles after capturing, and enables them to discriminate between food particles and non-nutritious particles (Donaghay and Small 1979). Copepods are able to feed selectively on particles of different size, larger cells are detected at a greater distance and, as follows, are cleared at a higher rate than smaller cells (Price et al. 1983). Higher clearance rate on specific food items, e.g. ciliates, are probably mainly the consequence of high encounter rate due to the high swimming velocity of many ciliates (Kiørboe 2008).

Most of the experiments in this thesis (paper III and IV) were carried out with the copepod species *Acartia tonsa*, this species can be kept in culture to ensure a continuous supply of copepods. Other species used were *Acartia clausi* (paper I and III), *Centropages typicus* (paper I), *Pseudocalanus* sp. (paper I), *Centropages hamatus* (paper III), and *Temora longicornis* (paper III). The experiments in paper I was carried out using copepods caught in the wild.



**Figure 5.** Picture of copepod (*Calanus* sp.) as representative of copepods used in this thesis. (Photo: E. Selander).

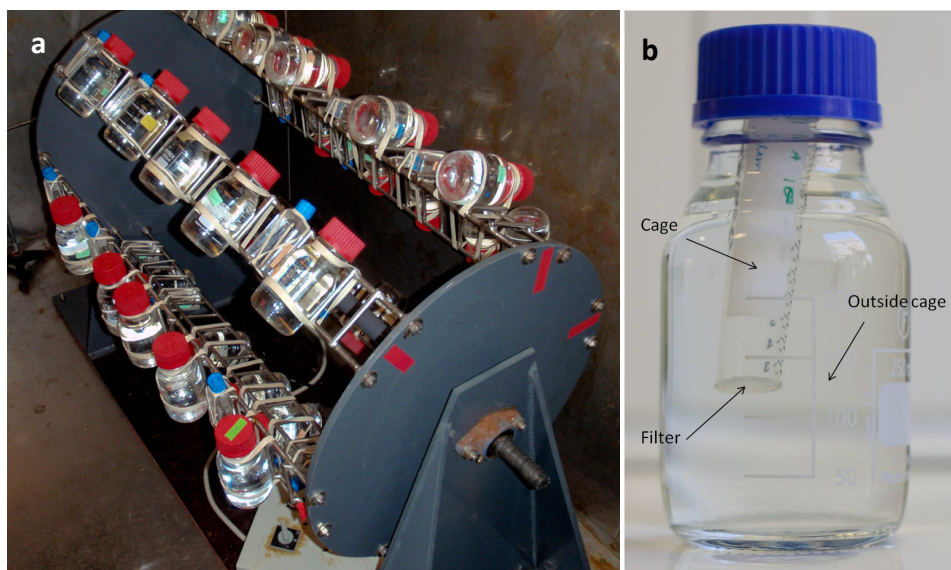
## 2. METHODOLOGY

### 2.1. EXPERIMENTAL DESIGN

Filtered natural seawater was used in all experiments, with added nutrients to ensure the growth of the algae.

#### 2.1.1. Plankton wheel

The experiments in paper III and IV were carried out on a plankton wheel revolving at 0.2-0.5 rounds per minute. Plankton wheels can be constructed in various ways, but the general idea is a revolving structure with the possibility to attach bottles or other containers. The revolving of the wheel is to ensure that the algae stays suspended and does not sink to the bottom, as would happen if the bottles are left standing. The wheels used in paper III and IV looked like the one in figure 6 a. In paper I, no wheel was used, instead the contents of the bottles were stirred by gently lifting and sinking the cage, *A. minutum* also stay suspended by swimming.



**Figure 6.** a) Plankton wheel with experimental set-up. b) Blue-cap bottle with cage made of centrifuge tube with polycarbonate filters in both ends (top end not visible in this picture).

### 2.1.2. On the use of cages

Both in the induction experiment with *A. minutum* and in the chain length experiments with diatoms (paper I, III and IV), cages were used to separate the grazers and grazed cells from the other cells. The use of cages is convenient when looking at chemically mediated traits. It assures that the effect seen is not due to selective grazing (e.g. selective removal of the least toxic cells in a population) or mechanical damage (e.g. fragmentation of diatom chains) by grazers. The cages were made from polypropylene centrifuge tubes, with a nylon or polycarbonate filter in either one or both ends (fig. 6 b). In all cases where cages were used, the permeability of the cages was tested prior to the experiment, to ensure that there was an exchange of water and thereby also chemical cues between the compartments.

### 2.1.3. Mesocosms

Phytoplankton are difficult to study in their natural environment, therefore mesocosms are useful in the study of these organisms and arguably more relevant for natural systems than smaller sized bottle incubations. Mesocosms are enclosures of varying size designed to mimic close to nature conditions. In the mesocosms it is possible to manipulate different factors and look at the results. The mesocosms used in the study in paper II are located at the Umeå Marine Science Centre (UMSC) at Umeå University. This facility comprises 12 insulated cylindrical tanks, five meters in height, made of inert polyethylene (fig. 7). The tanks are computer controlled, with possibilities to control temperature, light, and hydrographical properties. By heating the lowest part of the tanks, thermal advection resulted in a slow mixing of the water column. Sampling can be performed from fixed outlets, or by a Ruttner water sampler.



**Figure 7.** The mesocosm facility in Umeå, top view of six of the tanks. Picture from [www.umf.umu.se](http://www.umf.umu.se).

## 2.2. ANALYSIS

### 2.2.1. Cell counting

Where available, chain length and cell number were analysed with particle counter or flow cytometer. The advantage of particle counter and flow cytometer are the short processing time of each sample, and the large number of cells counted. The particle counter used in paper I, III and IV was a Multisizer 3 Coulter Counter, Beckman Coulter, equipped with a 100 µm aperture tube. Two different types of flow cytometers were used, one FACSCalibur (Becton Dickinson, San Jose, CA, USA) for counting bacteria and viruses (paper II), and the other CytoBouy (CytoBouy, <http://cytobouy.com/>) for counting and measuring chain length on diatoms (paper III). In diatoms, the auto fluorescence of the chloroplasts of each cell generates peaks in red fluorescence and the frustules generate peaks in forward scatter, enabling the detection of the cells and also the detection of number of cells per chain. There is no need for staining of the cells, and samples can be analysed straight from the experimental container if needed. The approach for counting virus and bacteria differs from that of counting diatoms. To detect and count bacteria and virus, samples need to be fixed in glutaraldehyde and frozen in liquid nitrogen prior to analysis. Before detection in the flow cytometer, samples are diluted and stained with the fluorescent dye SYBR Green I (Molecular probes Inc. Eugene, OR, USA).

Where particle counter and flow cytometer were unavailable, chain length and cell number were analysed by counting in an inverted microscope. Samples were transferred to multiwell plates and preserved in Lugol's solution, a total of 100 units per sample were then counted, and the number of cells per chain was noted.

### 2.2.2. Chemical analysis

Paralytic shellfish toxins (paper I) were extracted by vacuum filtration of a known number of cells on glass fibre filters that were subsequently freeze dried and extracted through consecutive freeze-thaw cycles in 0.05 M Acetic acid (aq.). Samples were stored frozen until analysis with High Performance Liquid Chromatography with fluorescent detection (Asp et al. 2004).

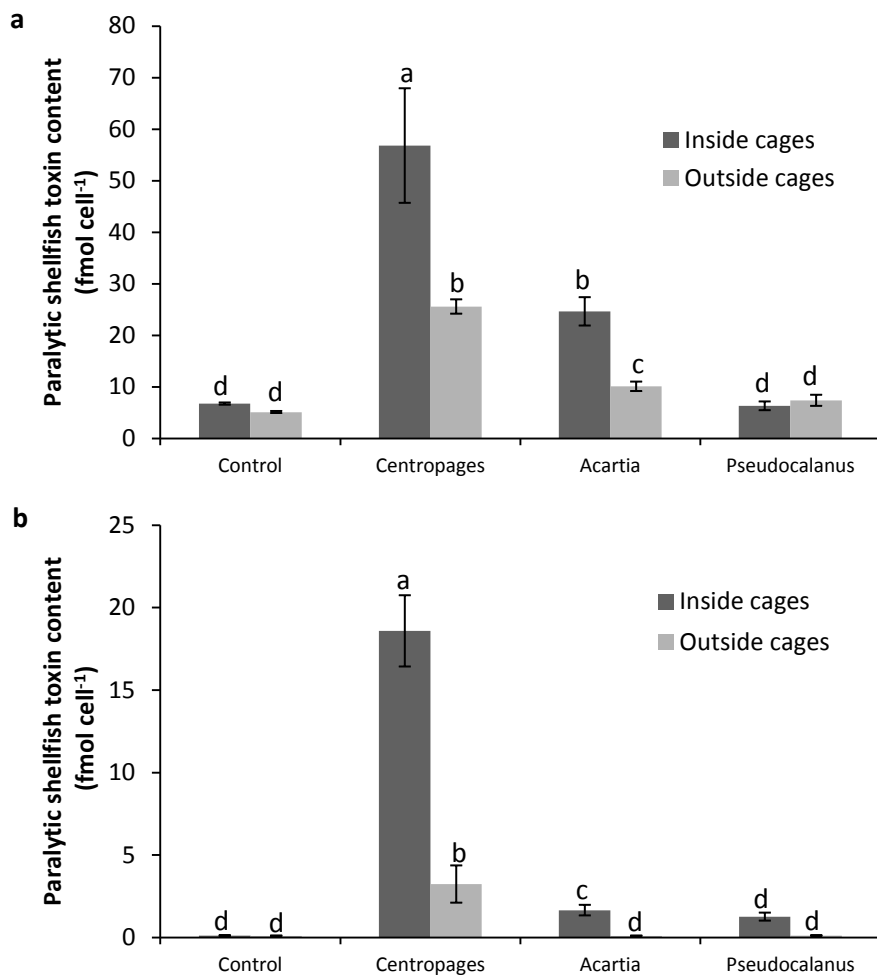
### 3. RESULTS & DISCUSSION

#### 3.1. GRAZER SPECIFIC RESPONSE IN PST PRODUCTION

*Alexandrium minutum* seem to be able to discriminate between copepod grazers, and respond by increased toxin production to “known” species, i.e. species with the same geographical distribution in evolutionary time as *A. minutum*. In the experiment in paper I, the copepod *Centropages typicus* induced the strongest response, followed by *Acartia clausi*, while *Pseudocalanus sp.* did not have an effect on toxicity (fig. 8). This suggests that toxin production in *Alexandrium* is not simply a general defence against all copepods, but rather a species specific response against grazers commonly co-occurring with *A. minutum*. Toxin content after exposure to *A. clausi* was similar to levels previously described (Selander et al. 2006), while the effect of *C. typicus* was considerably stronger, more than 20 times higher than controls for one of the strains (fig. 8 a). The species specific response in PST also contradicts the suggestion that cues from injured *A. minutum* cells elicit the effect. If the cues would originate from injured *A. minutum* cells, all treatments should show an increase in PST, since all species of copepods were grazing on the cells. Even though *Pseudocalanus* were feeding on *A. minutum*, there was no response in PST production. It has also been shown that both grazing and starving copepods induce increased toxicity (Selander et al. 2006).

Being able to discriminate between copepod species is an advantage for *A. minutum*, if there is a cost coupled to the toxin production *A. minutum* does not have to spend energy or precious compounds on producing toxins against non-threatening copepods. Here, the production of PST could not be coupled with any apparent cost in terms of reduction in growth rate in *A. minutum*, but it is possible that other costs than reduced growth rate or costs that only appear in more complex environments was present. The theory of inducible defenses assumes that inducible defenses have to be costly to be favored in front of constitutional (constantly up-regulated) defenses (Tollrian and Harvell 1999).

As well as the inducing effect of copepods seem to be species specific, the effect of PST's on copepods is species specific and even population specific. The copepod *Eurytemora herdmani* has been shown to be able to ingest large quantities of toxic *Alexandrium spp.* without any apparent physiological effects (Teegarden and Cembella 1996), while *Acartia clausi* suffered reduced hatching success after ingestion of toxic *A. minutum* cells (Frangoulos et al. 2000). Copepods have also been shown to discriminate between cells with different toxin content and prefer the less toxic cells (Teegarden 1999). The role of PST's as grazer deterrents is under debate, and grazers seem to be able to develop a resistance against the toxins over time (Colin and Dam 2004; Bricelj et al. 2005).



**Figure 8.** Paralytic shellfish toxin (PST) content in fmol cell<sup>-1</sup> in *Alexandrium minutum* cultures exposed to different copepod grazers (*Centropages typicus*, *Acartia clausi* and *Pseudocalanus sp.*). a) *A. minutum* no. 83 and b) *A. minutum* A5. Letters above bars (a-d) indicate significant differences in PST content. Bars show mean values of five replicates  $\pm$ SE. Note the different scales on the y-axes. (Paper I).

All copepod species in the experiment in paper I was grazing on the *A. minutum* cells. Toxicity is not a sure way to escape predators since the toxin containing species effect on herbivores feeding on the phytoplankton seems to be very species dependant. For a single celled organism there is no real use in being toxic if you still get eaten, since there is no benefit from the reduced grazing. Chain or colony forming species may form an exception, they could get away with partial predation, where the grazer consumes a part or a cell, and then rejects the rest. If grazers are unable to distinguish between toxic and non-toxic cells it might seem useless to produce toxin since this means death for the cell,

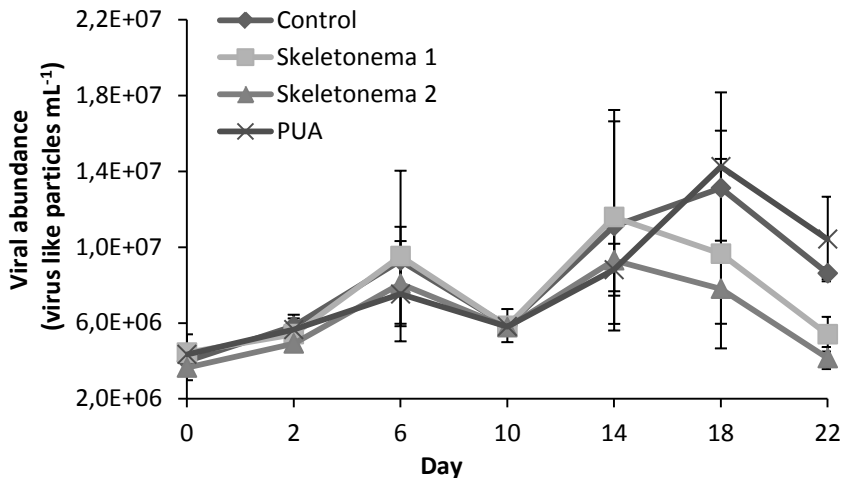


which eliminates the possibility of evolution through natural selection. However, *A. minutum* and most other species of phytoplankton reproduce through division, theoretically an entire bloom may consist of only a few clones, by sacrificing itself an individual can help genetically identical cells and thereby secure its gene representation in future generations (Wolfe 2000). This might not be an optimal strategy because reducing grazing pressure by killing the grazer may allow competitive species to avoid grazing too, without paying the prize of producing the defence, and thereby decrease the success of toxic prey through competition (Wolfe 2000). On the other hand, if the toxins deter the grazer without harming the *A. minutum* cell, it can escape predation and the grazer might choose a prey from a competitive species, thereby giving the *A. minutum* cell increased fitness (Guisande et al. 2002) (paper I).

### 3.2. NO EFFECT OF PUA ON BACTERIA OR VIRUSES

Neither the release of PUA from *S. marinoi*, nor the presence of the PUA producing algae itself did affect the abundance of bacteria or viruses. This suggests that PUA does not play a significant role in diatom-bacteria or diatom-virus interactions. Viral and bacterial abundance was the same in all mesocosms at the beginning of the experiment, consisting of the natural microbial community in the Bothnian Sea. No significant differences in virus abundance between the treatments were observed until day 14. After day 14, the PUA treatment and the control contained significantly more virus-like particles than the two treatments with added *S. marinoi* (*Skeletonema* 1 and *Skeletonema* 2). During the entire duration of the experiment, control and PUA as well as *Skeletonema* 1 and *Skeletonema* 2 did not differ in their virus abundance (fig. 9). Since there was no increase in viral abundance at the decline of the *S. marinoi* bloom, the termination of the bloom was not likely due to lysis by viruses. However, viral termination of phytoplankton blooms has been reported for the coccolithophorid *Emiliana huxleyi* (Bratbak et al. 1993) and lysis of diatoms from virus infections has been observed (Nagasaki et al. 2005).

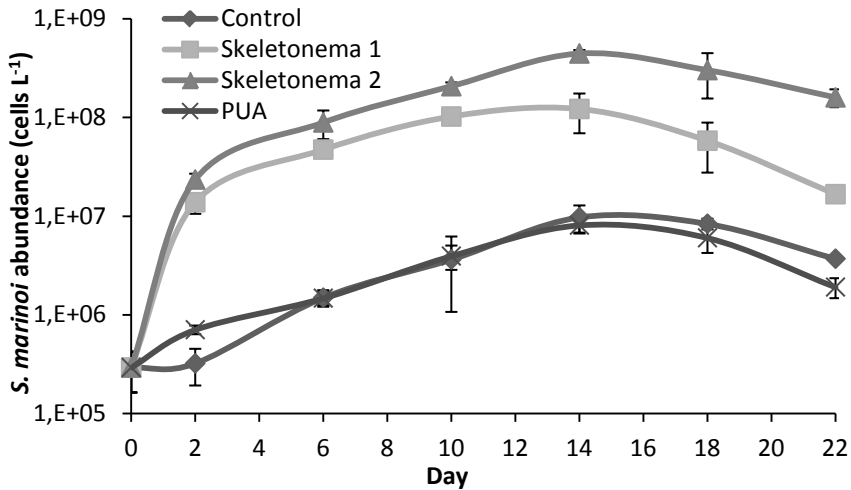
Similar to the virus abundance, there was no difference in bacterial cell number during the first ten days of the experiment. After ten days, there was an enhanced bacterial growth in control and PUA, compared to *Skeletonema* 1 and *Skeletonema* 2. *S. marinoi* could suppress bacterial growth either by competition for nutrients or from antibacterial activity of the algae. Such activity was observed in *S. costatum*, where extracellular metabolites had a negative effect on growth rate of the bacteria *Listeria monocytogenes* (Terekhova et al. 2009). PUA, on the other hand, had no effect on the bacterial abundance, even when concentrations way above those reached in nature were applied.



**Figure 9.** Viral abundance during the experiment. Values are mean  $\pm$ STDEV. (Paper II).

A problem in dealing with bioactive compounds is that the natural, and ecologically relevant, concentration of the compound is not known. The concentrations used in some experiments with PUA are higher than the concentration in the sea (Adolph et al. 2004; Ribalet et al. 2008), and this could explain the effect on bacteria seen in these experiments, and the absence of effect on the bacteria in paper II. In the experiment in this thesis, the concentration of PUA added were considered to be on the same level as what can be expected to occur during a bloom (Vidoudez et al. 2011). However, local concentrations of PUA around a damaged cell can be higher, and Ribalet et al. (Ribalet et al. 2007) estimated concentrations to be 47, 4.7 and 0.5  $\mu\text{mol PUA L}^{-1}$  at a distance of 1, 10 and 100  $\mu\text{m}$  from the cell surface. This was considered and a higher concentration of PUA was also added, without effect (paper II).

Another suggested function for PUA is allelopathy, the production of compounds that either harm or benefit competing species. The PUAs decadienal, octadienal and heptadienal, all released by *S.marinoi*, have been shown to have negative effect on growth rate, chlorophyll degradation and cell size in phytoplankton (Ribalet et al. 2007). Here, as well as no significant effect on bacteria or viruses, there was no effect on the phytoplankton community in the mesocosms. The two *Skeletonema* treatments showed a higher *S. marinoi* cell abundance during the whole experiment, with *Skeletonema 2* having the highest concentration (fig. 10). This was to be expected since *Skeletonema* was added to these two treatments. It is possible that PUA has another role, as cell-cell signalling where the PUA decadienal has been shown to activate the production of nitric oxide and thereby triggering cell death and initiate the termination of a bloom (Vardi et al. 2006). Many different possible effects of PUA have been investigated during more than a decade of research, with uncertain support for only a few. The ecological role of polyunsaturated aldehydes is still under debate, and it is not clear to what extent it has an influence on the plankton.



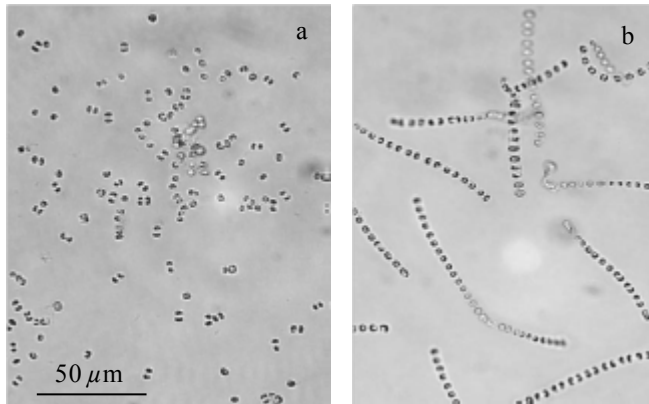
**Figure 10.** Number of *Skeletonema marinoi* cells L<sup>-1</sup> during the experiment. Values are mean  $\pm$ STDEV. (Paper II).

### 3.3. CHAIN FORMATION IN DIATOMS AS DEFENCE AGAINST GRAZING

One of the major findings in this thesis is that diatoms can adjust their chain length in the presence of grazers to reduce grazing loss (paper III and IV). Chain length plasticity may be an evolutionarily adopted trait in chain forming diatoms and suggests that size-selective predation may have played a key role in the evolution of chain formation and chain length plasticity. Significantly lower copepod grazing rates were observed on single or two-celled chains than on longer chains. Splitting up long chains into single or double units is an advantageous adaptation for diatom populations growing in environments with abundant copepod grazers.

All *S. marinoi* clones (fig. 11) and *T. rotula* were able to adjust chain length when subjected to cues from copepods. Single cell *S. marinoi* would fall into the lower prey size spectra of *A. tonsa*, which would lower the grazing pressure. However, single cell *T. rotula* would still be large enough to be fully retained by *A. tonsa* (Berggreen et al. 1988). This contradicts the theory in paper III, that diatoms change size to become smaller than what can be efficiently handled by the inducing grazer, and instead suggest that the effect of size on encounter rate may be an important factor in the evolution of grazer-induced chain size plasticity. A chain of cells is detected at a larger distance which results in higher encounter rates for chains and colonies than for single cells (Selander et al. 2011). Shorter chains or single cells would then be more difficult for the grazers to find. Neither *S. marinoi* nor *T. rotula* showed any difference between high and intermediate grazer

concentrations, grazer-induced chain length plasticity seems to be a very sensitive system where the diatoms are able to sense low natural densities of copepods. That the diatoms are able to sense cues from copepods in these concentrations suggests that the response in this study is likely to occur in nature. Further evidence of that these process occurs in the pelagic system is the previous findings where the presence of grazers have shown an effect on chain length of diatoms (Martin 1970; O'Connors et al. 1976; Deason 1980). They all considered the reduction in chain length as a result of direct mechanical damage from the grazing copepods, but the results presented in this thesis show that chemical cues are more likely responsible for the reduction in chain length.

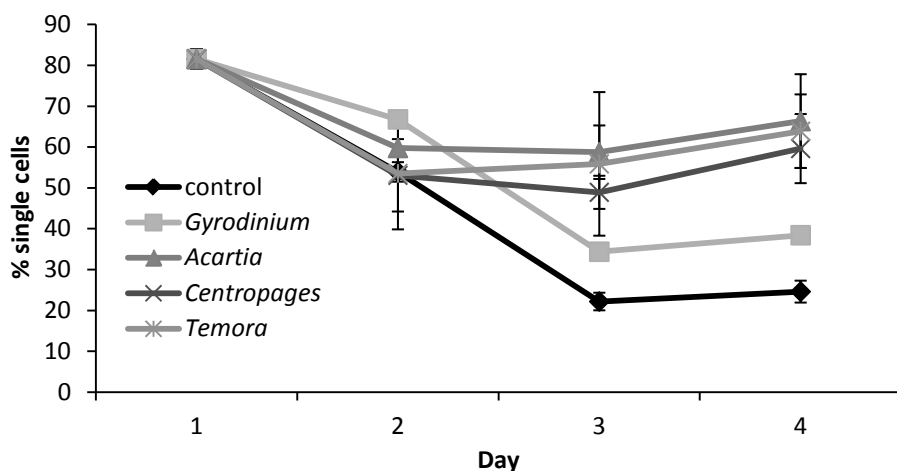


**Figure 11.** Picture of *Skeletonema marinoi* cells showing effect of grazers on chain length. a) With copepods *Acartia clausi* and *Centropages hamatus*. b) Control without grazers. (Paper III).

The cue responsible for the reduction in chain length seems to originate from the copepod grazers, not from damaged diatom cells, since grazing on *Rhodomonas* also resulted in reduced chain length (paper III). Grazer specific cues are more reliable than cues from injured algal cells, as cells also lyse because of other factors, such as viral or bacterial infections or at the end of algal blooms. The compounds released by viral-lysed cells and grazer-damaged cells are probably similar (Pohnert et al. 2007), and it would be difficult for an intact algal cell to discriminate between the cues (Van Donk et al. 2011). Grazer-specific cues, on the other hand, would be more reliable. Algae could use cues that are not directly linked to feeding, which would allow the alga to respond to grazers before they start to feed on the alga. These cues could be sex pheromones or aggregation pheromones, and as these pheromones are grazer-specific it would also enable the alga to only respond to cues from grazers that actually pose a threat to them (Van Donk et al. 2011).

Reduction in chain length appears to be correlated to cell division, there was a significant difference in the number of single cells in controls and copepod treatments after three days (fig. 12). Diatom chains are formed by cell division, where the cells stays attached after division. This could be explained by that the effect is coupled to cell divisions and

that the reduction in chain length occurs when cells divide and daughter cells do not stick together after division. This would be a fast enough process to track changes in grazing pressure. Timing of the response could also reflect the necessary build up of copepod cues, it is possible that it takes a couple of days to reach threshold concentrations. There were no apparent differences in morphology between grazed treatments and control, beside that of reduction in chain length. Chain forming diatoms end a chain by producing a terminal valve during cell division, these valves lack interlocking structures and cells do not stay attached after division (Round et al. 1990). We were not able to identify any higher proportion of terminal valves in scanning electron microscope (SEM) micrographs of our samples (paper IV), and perhaps the grazer cues induce a different type of chain splitting, not involving the formation of terminal valves.



**Figure 12.** Percentage of single *Skeletonema marinoi* cells per day in cultures exposed to different copepod grazers (*Acartia tonsa*, *Centropages hamatus* and *Temora longicornis*) or protozoan grazer (*Gyrodinium dominans*). Values are mean of three replicates  $\pm$ SE.

*Chaetoceros affinis* did not show any changes in chain length. *Chaetoceros* belongs to a different phylogenetic branch of diatoms than *S. marinoi* and *T. rotula*, and have a very different morphology. The long spines in *C. affinis* could act as grazing deterrents (Pahlow et al. 1997), and have been shown to damage the gills of rainbow trout (Yang and Albright 1992). The spines could be another type of morphological defense present in *C. affinis* and explain the absence of effect of copepod grazers on the chain length of *C. affinis*.

## 4. CONCLUSIONS & FUTURE DIRECTIONS

The overall aim of this thesis was to investigate some of the various chemically induced defences occurring in phytoplankton. The results in this thesis suggest that grazer-induced responses in phytoplankton are more common than previously known, and responses are both chemical and morphological and that these responses are present in phytoplankton has large effects on pelagic food webs and the results in this thesis adds to our understanding of chemical interactions in phytoplankton.

The perhaps most important finding of this thesis is how some diatom species uses chain length plasticity as a defence against copepod grazers. By reducing the chain length and thereby reducing the size, not only do the diatoms fall outside the handling size of some copepods but they also benefit from reduced encounter rates with grazers. This may explain the effect seen in *Thalassiosira rotula*, which would not be small enough to avoid *Acartia tonsa*, even as a single cell. That not all species of diatoms investigated responded to grazers by shortening of their chains also suggests that there are different strategies to handle grazing pressure in this very important group of marine primary producers, the long spines in *Chaetoceros* could e.g. be an example of such defence. Looking for grazer-induced chain length plasticity in other diatom species, and in more complex natural conditions would give new insights in this field.

That *A. minutum* is able to sense waterborne cues from copepods and also recognize different species of copepods strengthens the suggested role of PST as grazer deterrents, but this has yet to be confirmed. To find the cue(s) responsible for the changes in chain length and increase in PST production is the next challenge. The results from paper I, III and IV hint that the cue(s) most likely originate from the copepods.

Considering the result that PUA did not affect virus, bacteria or the phytoplankton community, this further questions the role of PUA in the plankton community. Maybe PUA are simply pure secondary metabolites, with no apparent function in the plankton or have other functions than the suggested grazer defence?

All things considered, there is no question that the future will have much to reveal about defences in phytoplankton and their role in the population dynamics of the pelagic ecosystem.

## 5. ACKNOWLEDGEMENTS

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*“The great tragedy of science is the slaying of a beautiful hypothesis by an ugly fact.”*

- Thomas Henry Huxley