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ASSESSING ALLELOPATHIC EFFECTS OF *ALEXANDRIUM FUNDYENSE* ON *THALASSIOSIRA* SP.

By

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B.A. Colby College, 2008

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Oceanography)

The Graduate School

The University of Maine

December, 2012

Advisory Committee:

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***ALEXANDRIUM FUNDYENSE* ON**

***THALASSIOSIRA* SP.**

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Thesis Advisor: Dr. Lee Karp-Boss

An Abstract of the Thesis Presented
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Production of allelopathic chemicals by the toxic dinoflagellate *Alexandrium fundyense* is one suggested mechanism by which this relatively slow grower outcompetes other phytoplankton, particularly diatoms. Despite well documented allelopathic potential of *Alexandrium* spp., the potency is variable. To further characterize allelopathic effects of *A. fundyense* on diatoms in the Gulf of Maine, I studied growth and nutrient acquisition by the chain-forming diatom *Thalassiosira* sp. in the presence and absence of allelochemicals. *Thalassiosira* cells, upon exposure to filtrate of *A. fundyense* cultures exhibited "bleaching" and both growth and nutrient utilization ceased for up to 4 days compared to controls. Results from this study support the existence of chemically mediated interactions, although the relatively high *A. fundyense* concentrations required to elicit a response suggest a greater role of such interactions in bloom maintenance than initiation. The magnitude of the effect was dependent on filtrate concentration and *Thalassiosira* cell size. *Thalassiosira* cultures that had undergone cell enlargement via sexual reproduction were less sensitive to *A. fundyense* filtrate, recovering earlier and showing less "bleaching." This difference in allelopathic effect did not appear to be related to either the total biovolume or total surface area of experimental cultures but cultures of cells with higher surface area/volume showed higher effects. These results demonstrate that competitor cell size,

independent from taxonomy, is likely to be important in shaping the outcome of allelopathic interactions. The findings presented here suggest a potential ecological impact of diatom cell size reduction and sexual reproduction that has not yet been described and that may be important in determining diatom survival and success.

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INTRODUCTION

The motivation for this study was the hypothesis that blooms of the harmful bloom-forming dinoflagellate *Alexandrium fundyense* in the Gulf of Maine are largely influenced by the relatively unstudied competitive interactions with diatoms (Townsend et al., 2005). The ecology of *A. fundyense*, a dinoflagellate that produces a suite of neurotoxins that cause paralytic shellfish poisoning, are of particular interest in the Gulf of Maine where proliferations of this species are of concern for human health and the shellfish industry. Evidence from several cruises in the Gulf of Maine shows alternating dominance between diatoms and *A. fundyense* in which patches with higher *A. fundyense* concentrations had low diatom concentrations and vice versa (Townsend et al., 2010). Attempts to model *A. fundyense* bloom dynamics (e.g. McGillicuddy et al., 2005) have rarely included biological interactions although it is likely that competition, either direct, allelopathic or both, between diatoms and *A. fundyense* is important in regulating bloom dynamics (Townsend et al., 2005). An initial experiment in which the Gulf of Maine diatom *Thalassiosira* was grown with *A. fundyense* revealed that *Thalassiosira* growth was inhibited in the presence of *A. fundyense* cells (see Figure 1.1). Many harmful bloom-forming dinoflagellates are known to release chemicals that influence success of competitors (i.e., allelopathic competition), but because the two species were grown together, I could not distinguish between direct competition for nutrients and possible chemically mediated interactions. I thus began my work using an experimental design commonly used in studies examining chemical interactions between phytoplankton. Namely, I grew *Thalassiosira* in cell-free filtrate from *A. fundyense* cultures to investigate the existence of chemicals released by *A. fundyense* that have the potential to limit *Thalassiosira* growth.

Following initial experiments to examine allelopathy, my cultures of *Thalassiosira* began to undergo sexual reproduction and showed variability in their response to allelochemicals. This unintended occurrence gave me the opportunity to examine allelopathic effects of *A. fundyense* on

cells of different sizes within a single species. Results from this study would not only add to current understanding of allelopathic interactions in the phytoplankton but also to understanding of the ecological implications of the unique life cycle of diatoms.

The first chapter of this thesis is a review of the literature on some aspects of the chemical ecology of both macro- and microalgae, a field that is quite broad and has been developing rapidly over the last few decades. This review will set the stage for understanding how my work on allelopathic interactions between two Gulf of Maine phytoplankton, the focus of the second part of the thesis, fits into the broader context of marine chemical ecology.

CHAPTER 1

ECOLOGY OF ALGAL PRODUCTION AND SENSING OF SECONDARY METABOLITES

Over the past few decades, recognition of the importance of biologically produced chemicals in life processes and communication in aquatic environments has resulted in the active and growing field of aquatic chemical ecology. Both planktonic and benthic macro- and microalgae release chemicals or make use of chemical signals in the environment in a variety of ecological contexts including reproduction, defense and predator avoidance, and competition (Hay, 1996; Cembella, 2003; Pohnert, 2010). These chemically mediated behaviors influence not only the individual responding to or producing a certain chemical, but also populations, communities, and ecosystem functioning (Hay and Kubanek, 2002; Hay, 2009). For example, toxins produced by the harmful bloom species *Karenia brevis* are known to cause massive mortalities in organisms from shellfish to manatees over thousands of kilometers (Hay and Kubanek, 2002).

The compounds of interest in algal chemical ecology are known as secondary metabolites. Unlike primary metabolites, chemicals that are required for normal functioning of algal cells and tissues, secondary metabolites are not essential for cellular maintenance and instead largely function as mediators in interactions between an alga and its environment (Maschek and Baker, 2008). Secondary metabolites are produced by both macro- and microalgae and these biologically active compounds can be classified into several broad categories according to their ecological roles. As will be emphasized in this review, these roles include intraspecific signaling, grazer deterrence, and competition (which includes anti-fouling). The well-known toxins (e.g., saxitoxin, domoic acid) produced by microalgae are also secondary metabolites whose ecological roles are less certain (Cembella, 2003). Although it is has been suggested in

some cases that they may serve in competitive interactions or grazer deterrence (Cembella, 2003) they will not be treated specifically here.

1.1 Pheromones

The first category of algal secondary metabolites comprises compounds released into the environment by a given organism with the purpose of inducing a response in another member of that species, i.e., pheromones. Pheromones are important as cues in initiation of reproduction and mate-finding. In order for sexual reproduction to occur in the aquatic environment, sexually mature male and female cells must locate each other in a continually diluting medium. Pheromones are essential to ensure that both types of gametes mature and are released at the same time and that they can reach each other.

Macroalgae rely on pheromones to coordinate initiation of sexual reproduction. For example, it has long been recognized that the pheromone lamoxirene induces release of male gametes in the orders Laminariales, Desmarestiales, and Sporochnales (Maier, 1993). In addition to stimulation of gamete maturation and release, pheromones are often necessary for sperm cells to locate a mature egg. In brown algae, these chemotactic pheromones, often the same chemicals that stimulate gamete release, are hydrocarbons with low solubility and are particularly well-studied (Pohnert and Boland, 2002). Because of the high activity of these chemicals, egg cells need only to release very small amounts – in the range of 10^{-15} mol per cell per hour (see Pohnert and Boland, 2002; Amsler and Fairhead, 2006 and references therein). Heterokont sperm use the pheromones produced by mature eggs and direct their movement towards them, either by swimming directly towards the source of the chemical, as in *Laminaria digitata* sperm (Maier and Müller, 1990), or by making U-turns as they sense decreasing pheromone gradients (Maier and Müller, 1986). Some of the most studied sexual pheromones that elicit such behavior include lamoxirene, fucoserratene, homosirene, and ectocarpene produced by *Laminaria digitata*, *Fucus spiralis, Hormosira banksii*, and *Ectocarpus siliculosus*, respectively (Amsler and Fairhead,

2006). Pheromones such as these impact success of the alga and thus have implications for community composition of algal communities.

In comparison with macroalgae, relatively little is known about sexual pheromones in phytoplankton. Although phytoplankton reproduce asexually most of the time, sexual reproduction is an important part of the life cycle of many groups and as with macroalgae it is likely that they rely on pheromones to coordinate sexual activity. Indeed, some microalgal pheromones are known that function as chemoattractants, as inducers of gamete production, or both. Initiation of sexual reproduction in the green alga *Volvox*, for example, is mediated by pheromones released by randomly developing male colonies that induce neighboring colonies to produce sexually mature males or females in the next generation. The responsible pheromone is effective at concentrations below 10^{-16} M (reviewed in Sekimoto, 2005). Several other green microalgae have been shown to release pheromones that serve in chemotaxis responses of sexual cell towards a mate (Sekimoto, 2005). Sato et al. (2011) demonstrated for the first time, that exudates from a sexually reproducing diatom culture stimulated gametogenesis in cells outside the size range typically necessary for sexual reproduction.

Intraspecific chemical signaling in planktonic microalgae is not limited to communication about sex. Poly-unsaturated aldehydes (PUAs) produced by diatoms have been suggested to be infochemicals within a population of diatoms by serving as signals of poor conditions for growth (Casotti et al., 2005). Since this role of PUAs was first recognized, several studies have investigated it further and have suggested that PUAs may control diatom populations by beginning the process of apoptosis, or programmed cell death, in neighboring cells (Vardi, 2008). Indeed, PUAs have recently been measured at active levels in seawater during diatom blooms (Vidoudez and Pohnert, 2008) and may be involved in termination of blooms (Leflaive and Ten-Hage, 2009).

1.2 Grazer Deterrence

Grazer-deterrent compounds make up the second category of secondary metabolites produced by algae. These metabolites have received significant attention over the past several decades particularly in tropical waters where the greatest diversity of bioactive compounds is thought to occur (Pereira and da Gama, 2008). A long-held hypothesis explaining the perceived higher diversity of secondary metabolites produced by tropical macroalgae is that these algae experience elevated grazing pressure compared to temperate species, leading to evolution of a wide variety of defenses including chemicals. In a recent meta-analysis, Pereira and da Gama (2008) revealed that the diversity of grazer-deterrent natural products produced by temperate seaweeds rivaled that of their tropical counterparts. They cited the fact that macroalgal species diversity is highest in temperate regions as the potential explanation. Another hypothesis, that tropical algae produce a greater diversity of metabolites at the species level than do temperature seaweeds, remains uninvestigated.

Examples of chemically mediated grazer-deterrence are known in species within all classes of macroalgae. The most common and diverse chemical defenses of macroalgae include phlorotannins and terpenes. These chemicals reduce grazing by consumers including herbivorous fishes and urchins as well as smaller grazers such as snails and amphipods (for reviews see Hay, 1996; Hay, 2009). The degree to which an alga is defended varies widely among and within species. As mentioned previously, there are geographical differences in macroalgal chemical defense. In addition, individuals of the same species often display variability in chemical content even within the same region as is demonstrated in individual algae from areas of high grazing pressure having higher deterrent loads (Hay, 1996). This is due to the fact that production of these secondary metabolites is often induced by grazer presence or grazer-inflicted damage as will be discussed later. An additional level of variability can occur within a single plant. The brown alga *Fucus vesiculosus* contains higher quantities of phlorotannin in basal tissues than in the actively

growing, and more expendable, apices (Tuomi et al., 1989). In *Halimeda*, a calcifying green alga, new uncalcified growth is protected from grazers by higher levels of potent secondary metabolites (Hay et al., 1988). In fact both chemical and structural defenses are utilized by *Halimeda*. Taken separately, its unique secondary metabolite and its $CaCO_3$ both fail to prevent grazing by urchins. Together, however, the two defenses act synergistically and greatly reduce grazing (Hay et al., 1994).

The chemical defenses of certain algae are not only important for their own defense, but can also be exploited by other algae that are not themselves chemically protected. There are numerous examples of such associational defenses in which palatable algae growing on or in close proximity to unpalatable ones are protected from grazing as well. A well-studied example involves the chemically defended *Dictyota* which produces diterpene alcohols. *Sargassum* specimens epiphytized by or experimentally attached to *Dictyota* were grazed at significantly lower levels than conspecifics growing without *Dictyota* (Pereira et al., 2010).

Planktonic microalgae also utilize secondary metabolites in grazer defense. Perhaps the most intensively studied example is that of the polyunsaturated aldehydes (PUAs) produced by diatoms. Diatom production of PUAs has been known for some time and has been suggested to play a role in defense against copepod grazers through their teratogenic effects on copepod offspring (Ban et al., 1997; Miralto et al., 1999). Because PUAs are effective against copepods only upon damage by a grazer, the evolutionary advantage of producing an expensive chemical that will only be used upon death of the individual cell has been questioned (Pohnert, 2010). Indeed, it is possible PUAs are produced for another use (e.g., as an infochemical as previously described) and damage to copepods is an unintended result.

Chemicals other than PUAs may also serve as grazing deterrents. For example, some copepods are known to avoid phytoplankton prey that contain paralytic shellfish toxins when given a choice between toxic and non-toxic food sources (Ianora et al., 2011). Two diatoms,

Phaeodactylum tricornutum and *Thalassiosira pseudonana* produce apo-fucoxanthin that seems to discourage grazing by copepods (Shaw et al., 1995). Although both the paralytic shellfish toxins and apo-fucoxanthin are held within cells, it appears that grazers are able to sense cells that contain them and preferentially select other food sources.

1.3 Allelopathy

Allelochemicals fall into a third class of algal-produced secondary metabolites – those used in competition. According to Rice (1984) allelopathy is any "direct or indirect" effect, either harmful or stimulatory, of one plant or microorganism on "another through production of chemical compounds that escape into the environment." Allelopathy, a long time subject of terrestrial plant and agricultural science, is most often thought of only as the negative effects – as a form of competition among organisms that differs from direct competition for resources (Willis, 1985). In the context of macro- and microalgal communities, allelopathic interactions fall into two ecological categories. The first type of allelopathic interaction in these aquatic systems involves benthic organisms competing for space and resources. These interactions are somewhat analogous to allelopathy in terrestrial systems in which an allelochemical acts against neighboring competitors, often by direct contact. Within this category researchers typically place anti-fouling chemicals in which benthic organisms use chemicals to prevent the establishment of epibiota such as other algae, invertebrates, or bacteria on their surfaces (Gross, 2003). The second category involves chemically mediated competition between pelagic microalgae, including cyanobacteria, an interaction that is complicated by the fact that the released chemicals are continuously diluted (Gross, 2003). This class of allelopathic interactions also encompasses the allelochemicals released by benthic macrophytes or macroalgae that act against planktonic algae.

Competition for space, nutrients, and light by macroalgae in the benthic environment is often mediated by chemicals that can either be released into the water or passed by direct contact with a competitor. Some crustose algae, which are more susceptible to shading, are known to be

allelopathic (e.g., Suzuki et al., 1998). Secondary metabolites produced by a number of macroalgae have been found to be active against fouling organisms including epiphytic diatoms, bacteria, fungi, and other algae (for examples of each see Hellio et al., 2000; Young Cho et al., 2001; Lam et al., 2008; Nylund et al., 2008). The compounds responsible for anti-fouling are of human interest in the development of nontoxic anti-fouling agents for use in marine engineering and ships (Hellio et al., 2000). Benthic microalgae have also been shown to use chemicals to control growth of nearby competitors. In one interesting example, the benthic diatom *Nitzschia cf pellucida* released cyanogen bromide (a compound never previously found to be produced naturally) each day around sunrise to prevent growth of competitors (VaneIslander et al., 2012).

In addition to competition for space and resources with neighboring species and epiphytes, submerged benthic macrophytes and algae compete with planktonic algae for resources, particularly for light. Eurasian water milfoil, *Myriophyllum spicatum*, is a particularly well-studied example (e.g., Hilt et al., 2006). This species produces phenolic compounds, (e.g., tellimagandin II, Gross et al., 1996), that inhibit growth of phytoplankton, including cyanobacteria. Recently, Bauer et al. (2009) demonstrated an increase in total phenolics produced by *M. spicatum* during the spring when competition with phytoplankton was highest. Interestingly, Bauer et al. (2010) found in another study that the bacterial community around or on phytoplankton targets may moderate the toxic effects of tannic acid, another common allelochemical, possibly by using it as a carbon source.

The compounds responsible for the inhibitory effects of macroalgae and macrophytes against phytoplankton must be active at very low concentrations due to the fact that they are diluted in the aqueous medium. This is also true in the case of allelochemicals that are produced by pelagic microalgae. The assumed expense of producing a compound that is constantly diluted raises questions regarding the evolutionary benefits of allelopathy in pelagic systems (e.g., Ianora et al., 2011), unless the chemical that acts allelopathically is a byproduct of another process. In spite of

these questions, however, it is clear that secondary metabolites released by phytoplankton (the donors) can influence the success of competitors (targets).

In one of the first comprehensive examinations of allelopathy in the plankton, Keating (1977) demonstrated the role of allelopathy in bloom succession in a lake. She demonstrated that, in general, secondary metabolites produced by a given algal species inhibited growth of the species preceding it in succession and stimulated later occurring species. Most of the species studied in the lake were cyanobacteria and indeed, since Keating's work, studies of allelopathy in freshwater systems revealed allelopathic activity of cyanobacterial secondary metabolites against eukaryotic algae, higher plants, and other cyanobacteria (for review see Gross, 2003). Although the identity and chemical nature of the responsible chemicals is often unknown, several of these allelochemicals have been identified. Cyanobacterin produced by *Scytonema hofmannii*, which damages target thylakoid membranes and inhibits PS-II electron transport and fischerellin A, produced by *Fisherella* strains, are two examples (as reviewed by Leão et al., 2012). Carbonic anhydrase activity was suppressed in the dinoflagellate *Peridinium gatunense* upon its exposure to exudates from *Microcystis* sp., an action that is likely to explain the negative correlation between observed abundances of these two species (Sukenik et al., 2002). The responsible metabolite, which is a compound other than the toxin microcystin, is unknown.

Although in freshwater systems allelochemicals produced by cyanobacteria have received the most attention, a few studies revealed allelopathic activity of compounds released by freshwater dinoflagellates (Rengefors and Legrand, 2001). Indeed, in the marine environment allelochemical production by eukaryotic phytoplankton is more often studied, particularly in those species that form harmful algal blooms. Often, the harmful species have relatively low growth rates (Smayda, 1997) and are thus unlikely to outcompete competitors via nutrient utilization alone. Allelopathy is one suggested mechanism by which these organisms can reach concentrations of concern in natural systems. In most cases, complete identification of the compounds responsible for the

allelopathic effects remains elusive as does understanding of the mechanism by which the chemicals act on target organisms. Allelochemicals vary widely and no one chemical has been found to be characteristic of on algal taxon (Leflaive and Ten-Hage, 2009). Table 1 summarizes the current state of knowledge of allelochemicals produced by harmful eukaryotic microalgae and their activity. Often, the responsible chemicals are distinct from the phycotoxins for which a given harmful bloom-former is known. For example, the active allelochemicals from both *Karenia brevis* and *Alexandrium* spp. are suites of compounds other than their well-known neurotoxic brevetoxins and saxitoxins, respectively (Tillmann and John, 2002; Prince et al., 2008a). In *K. brevis*, a suite of unstable polar compounds in the range of 500 – 1000 Da have been implicated in observed allelopathic effects (Prince et al., 2010). *A. tamarense* allelochemicals, on the other hand, seem to be large (7 – 15 kDa), stable, nonproteinaceous compounds (Ma et al., 2009).

Although the identities of allelochemicals in phytoplankton communities are largely unknown, their existence is clearly indicated by the effects that exudates have on competing phytoplankton. The strongest allelochemicals, such as those produced by *Prymnesium parvum*, quickly disrupt target cell membranes (Fistarol et al., 2003). Most allelochemicals, however, have more subtle modes of action such as inhibiting growth or photosynthesis without causing immediate death (see Legrand et al., 2003; Table 1, here). *Karenia brevis* and *Alexandrium* spp. have been particularly well-studied in terms of their production of metabolites known to inhibit growth of competing phytoplankton (Arzul et al., 1999; Kubanek et al., 2005; Hattenrath-Lehmann and Gobler, 2011) but dinoflagellates are not alone in their production of allelochemicals. The haptophytes *Prymnesium parvum* and *Phaeocystis pouchetii*, the diatom *Skeletonema costatum*, and the raphidophyte *Heterosigma akashiwo,* among many others, have also been shown to inhibit growth or cause death of other phytoplankton by their production of secondary metabolites (Granéli et al., 2008 and references therein).

The majority of studies have focused on laboratory experiments in which a target alga is cultured with donor species or in media containing filtrate from a culture of that donor (e.g. Arzul et al., 1999; Fistarol et al., 2005). Although these experiments are simplified and mostly unrepresentative of natural conditions in which organisms would be exposed to widely variable conditions, they are essential for determining the potential existence of allelopathic interactions in nature as well as the mode of action of the chemicals.

Recently, work has begun to move beyond the simplified one donor-one target system to further elucidate the nature of allelopathic interactions in the environment. Hattenrath-Lehmann and Gobler (2011), for example, examined *A. fundyense* allelopathy using laboratory and field experiments. The experimental results, namely allelopathic inhibition of autotrophic nanoflagellates and diatoms, were corroborated by similar changes in community structure during a natural bloom of *A. fundyense* and suggest a role of allelopathy in success of the bloom. In *K. brevis* on the other hand, clear allelopathic effects observed in the laboratory were not observed in mesocosm experiments using field assemblages of phytoplankton implying that competing interactions complicate and may alleviate allelopathy in the field (Poulson et al., 2010). Indeed, part of this reduced response of target species in a more natural setting may be due to the fact that some species have mechanisms that enable them to either avoid toxicity or even reduce allelochemical production by other species (Prince et al., 2008b).

One issue that emerges throughout studies of allelopathy in phytoplankton is that both the potency of the donor species and the 'sensitivity' of the target species to allelochemicals are greatly affected by different biotic and abiotic factors, leading to variability in the observed outcomes among and within different studies. Abiotic factors that may influence production by donor species, stability of the allelochemical and sensitivity of target species include light, temperature, pH, and nutrients (Granéli et al., 2008). Allelochemicals released by *P. parvum*, for example, are inactivated upon exposure to UV and visible light (Parnas et al., 1962 as cited in

Granéli et al., 2008). *P. pouchetii*, on the other hand, seems to increase production of its allelopathic metabolites in response to light (van Rijssel et al., 2007). Temperature effects are also complex, with some species exhibiting increased allelochemical production with higher temperatures (Granéli et al., 2008 and references therein). Under conditions of limited nutrients, namely nitrogen or phosphorus, allelochemical production increases in some allelochemical producers (Granéli and Johansson, 2003). Sensitivity of target species to a certain allelochemical may also increase when the cells are exposed to poor growth conditions such as nutrient-limitation (Fistarol et al., 2005). Although it has been suggested that differences in nutrient availability play a role in allelopathic interactions and is such a dynamic component of natural systems, the majority of studies were performed under nutrient replete conditions. Applications of results of such laboratory studies to field conditions should therefore be done cautiously.

Biotic factors also play a role in production of and response to allelochemicals. Allelochemicals produced by a given phytoplankton do not equally affect all target species and a given target species is unlikely to be sensitive to every allelochemical (Granéli et al., 2008). In some cases, allelochemical production varies with growth stage of the producing organism. *P. parvum* produces more allelochemicals in stationary stage, for example (Granéli et al., 2012). Experiments with *K. brevis* show more complicated interactions with inhibition of some target species observed only when *K. brevis* was in the stationary phase, while others were inhibited by extracts from *K. brevis* in exponential phase (Kubanek et al., 2005). This difference in effect on competitors suggests that *K. brevis* produces distinct chemicals or different amounts of chemicals at different growth stages. Sensitivity of the target can also vary with their growth stage (Poulson et al., 2010).

Also important in determining the degree of allelopathic effect is the concentration of both the producing species and the target. Generally, and fairly obviously, higher concentrations of the allelochemical producer result in stronger effects on a given target (Tillman et al., 2007). Higher

concentrations of the target species, however, tend to reduce the impact on the population of cells (e.g., Hattenrath-Lehmann and Gobler, 2011). Another source of variability is associated with differences in potency of strains of the same species as has been documented for *A. tamarense* and *A. fundyense* (Tillmann et al., 2009; Hattenrath-Lehmann and Gobler, 2011). It is important to emphasize that, as mentioned for both *A. fundyense* and *K. brevis*, often many allelopathic compounds are released by the same organism either at once or possibly at different times. Furthermore, more than one metabolite may be necessary for a response in a particular target (Leão et al., 2010).

While natural variability clearly plays a role in observed response differences, some variability may in part be explained by differences in experimental approach. Hilt et al. (2012) compared the responses of green algal targets to allelochemicals produced by the macrophyte *Myriophyllum verticillatum* in several different experimental systems both in the field and the laboratory and found that although the green algae were negatively affected in each of the experiments, both the parameter affected (c*hl a*, PSII activity, cell count, or biovolume) as well as the degree of the effect varied among experiment types. In particular, laboratory-based tests involving single additions of the allelopathic compound – the method most often used by researchers studying allelopathy- were most unrepresentative of field-based results. It is obvious therefore, that studies involving a combination of methods in both the natural setting and the laboratory are necessary to support the existence of allelochemical interactions and elucidate their ecological importance in the phytoplankton community.

Despite a growing body of research that supports the allelopathic potential of harmful algal species, the relevance of allelopathy as a mechanism for bloom formation and success in the field remains questionable. In a meta-analysis of published experimental work on allelopathy, Jonsson et al. (2009) revealed that the effects of allelochemicals have only been shown at high concentrations of the producing cells. This analysis suggests that while allelochemicals may aid

already established blooms to maintain dominance, there is little evidence supporting the role of allelopathy in bloom initiation (Jonsson et al., 2009). Similarly, Flynn (2008) used a model to investigate potential outcomes of chemically mediated competition in the phytoplankton and found that production of allelochemicals by a poor resource competitor did not provide an advantage against fast growing competitors. Flynn goes on to suggest that although allelochemicals may inhibit growth of other phytoplankton, their evolutionary role may instead be as a grazing deterrent.

1.4 Non-algal Secondary Metabolites That Influence Algal Ecology

Although the focus of this chapter has been on the production of secondary metabolites by algae, chemicals produced by non-algal members of the aquatic community can also be sensed by and greatly affect algae. In particular, chemicals produced by bacteria have been found to influence algal growth, morphology, and settlement (Goecke et al., 2010). Exudates of a naturally occurring epiphytic bacterium on *Oedogonium cardiacum*, for example, are necessary for formation of the alga's oogonial and antheridial reproductive structures (Machlis, 1973). The morphology of certain algae may also depend on the presence of bacteria (Provasoli and Pintner, 1980; Tatewaki et al., 1983). When grown axenically, green algae in the genera *Ulva* and *Monostroma* exhibit uncharacteristic morphology and normal growth can be restored only with addition of their natural bacterial assemblage (Provasoli and Pintner, 1980; Matsuo et al. 2005; Marshall et al., 2006). Bacterially produced cytokinins are likely the responsible metabolites (see Singh et al. 2011). Matsuo et al. (2005) isolated thallusin, a growth factor produced by some bacteria that appeared to be responsible for typical growth of *M. oxyspermum*. Interestingly, Singh et al. (2011) found that addition of bacterial isolates to axenic *U. fasciata* could contribute to normal morphogenesis of the algal thalli as well as induction of zoospores, but that different bacterial assemblages were responsible for each effect.

In addition to contributing to the morphology of some macroalgae, bacterial exudates are known to affect settlement of algal zoospores. In one of the first recognized instances of 'cross-kingdom signalling', *Ulva* zoospores were shown to exploit compounds produced by bacteria to locate suitable surfaces on which to settle (Joint et al., 2002). Destruction of N-acylhomoserine lactones (AHL), molecules that are produced by bacterial cells for quorum sensing, lead to reduced settling of zoospores on a surface (Tait et al., 2005). On the other hand, chemicals produced by some bacteria inhibit settlement and germination of algal spores (Egan et al., 2001).

Bacterial secondary metabolites can play a role in the success and ecology of planktonic algae as well. Growth of *Gambierdiscus toxicus* increased by more than 50% when grown with its natural epiphytic bacteria; an effect likely resulting from chemicals released by the bacteria (Sakami et al., 1999). Toxin production by phytoplankton is also likely to be influenced by their epiphytic bacteria. Domoic acid production by the diatom *Pseudo-nitzschia multiseries* is greatly enhanced by direct contact with bacteria (Kobayashi et al., 2009) and conversely domoic acid seems to control the population and diversity of the bacteria present (Guannel et al., 2011) although the mechanisms behind these phenomena are still unclear. Several researchers have investigated the role of bacteria in production of allelopathic metabolites, but a connection has yet to be identified (e.g., Tillmann and John, 2002).

Finally, secondary metabolites released by zooplankton and other grazers are, in some cases, sensed by algae and influence their behavior. Reports on morphological responses to the presence of exudates from grazers include transitions between colonial and motile, single-cell life forms in the haptophyte *Phaeocystis globosa* (Long et al., 2007), formation of coenobial colonies as well as spines in the chlorophycean algae *Scenedesmus* and *Desmodesmus* (Lürling, 2003), and increased silicification in the diatom *Thalassiosira weissflogii* (Pondaven et al., 2007). Chemical cues from select grazers such as those found in the saliva of grazing snails have been found to induce chemical production in a number of seaweeds (Coleman et al., 2007). In *Ascophyllum*

nodosum, a brown alga known for its production of deterrent phlorotannins, simulated grazing induced less of an increase in chemical content than did actual grazing by *Littorina obtusata* (Toth and Pavia, 2000). *A. minutum* increased its toxin 2.5 times in response to water-borne chemicals released by copepods and thus became more resistant to grazing (Selander et al., 2006). Many microalgae can form resistant cysts in response to environmental stressors such as temperature or nutrient depletion. Chemical cues from parasites and predators may also induce cyst formation (Toth et al., 2004) or delay excystment in some species of phytoplankton (Rengefors et al., 1998). Because the cysts are less susceptible to attack by parasites and zooplankton, this behavior represents another mechanism by which chemical cues from predators are sensed and used by algae as a survival strategy.

1.5 Conclusions and Future Prospects

The contribution of algal communities to what Pohnert (2010) describes as the "noise in the silent ocean" – the unceasing chemical signaling in aquatic systems – is unmistakable. Secondary metabolites produced by algae are involved in intraspecies communication, defense against predators, and competition with other algae. Algae can also sense chemicals produced by other organisms and respond in ways that influence their survival and ability to proliferate. Because of the potential significance of chemically-based interactions at levels beyond the individual, studies examining the processes controlling the chemical production as well as behavioral and ecological effects of chemical signals within algal communities are of great interest. These studies allow progress in understanding benthic and microbial communities and their control of carbon and nutrient cycling. Advancement has often been slowed, particularly in pelagic systems, by difficulties in characterizing and measuring the chemicals involved (Pohnert et al., 2007), but recent methodological advances have increased detection sensitivity (e.g., Vidoudez et al., 2011) and continuing bioassay development now allows measurement of previously unknown bioactive chemicals (Pohnert, 2005). These technological improvements will

enable future field studies to document and study novel chemically mediated interactions with the goal of discerning their ecological relevance. While designing experiments to investigate these interactions, it is essential to use study systems that are representative of natural situations, i.e., ecologically relevant concentrations of the metabolites and the organisms of interest. Furthermore, it is important, when possible, to investigate algal chemical ecology in both the field and the laboratory to gain a full understanding of a given interaction and its role in community dynamics.

Table 1.1 Summary of allelochemicals produced by some harmful eukaryotic algae and their modes of action. This table is an update of the tables provided by A) Granéli and Hansen (2006) and B) Granéli et al. (2008). Information added or updated here is in red. CP = cyst promotion, D = death, GI = growth inhibition, GR = grazing inhibition, HC = haemolytic/cytotoxic, U = unknown.

Species	**Alleochemical Identity or Description**	**Mode of Action/cellular target (if known)**	**Reference**
Bacillariophyceae			
Pseudo-nitzschia pugens	U	GI	A, B
Skeletonema costatum	U	GI	B, Yamasaki et al. (2007) A
Coscinodiscophyceae			
Rhizosolenia alata	U	GI	A,B
Dinophyceae			
Alexandrium catenella	U		B
A. fundyense	U	GI	Hattenrath-Lehmann and Gobler (2011); Lyczkowski (unpublished data)
A. minutum	U	GI, D, targets PSII - reduces number of active reaction centers	A,B, Lelong et al. (2011)
A. ostenfeldii	U	CP, loss of motility, target outer cell membrane	A,B, Tillmann et al. (2007)
A. tamarense	suite of high molec. weight, mostly lipophilic	GI,D, target sterols in cell membrane leading to lysis of cells	A,B, Ma et al. (2009), Ma et al. (2011)

Table 1.1 Continued.

Species	Alleochemical Identity or Description	Mode of Action/cellular target (if known)	Reference
Amphidinium klebsii	U	GI	A,B
Ceratium sp.	U	GI	A,B
Cochlodinium polykrikoides	U (but seem to be short-lived)	loss of motility, D	Tang and Gobler (2010)
Coolia monotis	U	GI,PI,D	A,B
Gambierdiscus toxicus	U	HC	A,B
Karenia brevis	suite of 500-1000 Da compounds, polar, aromatic functional groups	GI	A,B, Prince et al. (2010)
K. mikimotoi	H	HC,CP,GI	A,B
Ostreopsis lenticularis	U	GI	A,B
Peridinium aciculiferum	U	GI, D	A,B
Prorocentrum lima	U	GI	A,B
Prorocentrum minimum	polysaccharides	GI	Tameishi et al. (2009)
Prymnesiophyceae			
Chrysochromulina polylepis	fatty acids	D, GR, CP	A,B
Phaeocystis pouchetii	U, PUA		B
Prymnesium parvun	U, prymnesin	D	A,B
Raphidophyceae			
Chattonella antiqua	U		B
Heterosigma akashiwo	polysaccharide protein complexes	GI, bind specific target receptors on cell surface	B, Yamasaki et al. (2009)

CHAPTER 2

ASSESSING ALLELOPATHIC EFFECTS OF *ALEXANDRIUM FUNDYENSE* ON *THALASSIOSIRA* SP.

2.1 Introduction

In the phytoplankton community, production of allelopathic compounds has been suggested to confer a competitive advantage through the resulting inhibition or mortality of competitors. Increasing laboratory evidence suggests that release of allelopathic compounds is a widely occurring phenomenon among marine phytoplankton groups including dinoflagellates (Tillmann and John, 2002; Kubanek et al., 2005; Tang and Gobler, 2010), prymnesiophytes (e.g., Schmidt and Hansen, 2001), raphidophytes (e.g., Yamasaki et al., 2009) and one diatom (Yamasaki et al., 2011). Exudates from allelopathic donor species have been shown to cause a variety of negative effects on target species such as growth inhibition (Yamasaki et al., 2007), cyst promotion (Tillmann et al., 2007), cell lysis (Ma et al., 2009), loss of motility (Tang and Gobler, 2010), and death (Arzul et al., 1999). Potency and response to allelochemicals, however, vary between as well as within species (Granéli et al., 2008; Hattenrath-Lehmann and Gobler, 2011; Suikkanen et al., 2011), and different organisms may be positively or negatively affected or unaffected by a given allelopathic species or chemical (Tillmann and John, 2002). Poulson et al. (2010) revealed that *K. brevis* releases not one but a suite of allelochemicals and that competitor species differ in their sensitivity to each of these. Outcomes of allelopathic interactions also vary with growth phase of cultures (Kubanek et al., 2005; Prince et al., 2008a; Poulson et al., 2010; Yamasaki et al., 2011) and are influenced by abiotic factors such as light, temperature, pH, and nutrient availability (Granéli et al., 2008).

The relative abundance of 'donor' and 'target' cells can also influence experimental outcomes suggesting dose-dependent response. Perhaps obviously, increasing concentrations of donor cells or cell-free filtrate obtained from donor cultures typically leads to greater effects on

target algae (Granéli et al., 2008; Tillmann et al., 2008). Conversely, increasing concentrations of target cells leads to a decrease in the magnitude of negative effects as has been demonstrated in interactions between a number of dinoflagellate species and their competitors (Rengefors and Legrand, 2007; Tillmann et al., 2007; Poulson et al., 2010; Tang and Gobler, 2010). This trend of decreasing allelopathic effects with increasing target concentration is suggested to represent removal of an allelochemical from the system via binding to target cells or particles (Tillmann et al., 2007). Increasing exposure time to an allelochemical also leads to greater effects (Tang and Gobler, 2010), further supporting a dose-dependent nature of such interactions. An intriguing question in that context is whether vulnerability of cells to allelochemicals is dependent on their size. Only a few studies have examined the relationship between cell size and allelochemical effects by comparing responses of different species of varying cell sizes. Results from these experiments, however, are inconclusive (Schmidt and Hansen, 2001, Tillmann and Hansen 2009), largely because it is difficult to separate the effect of size from variations in physiological characteristics of the different species.

Allelopathy is of particular interest in the context of harmful algal blooms. Because many HAB-species, e.g., *Alexandrium* spp. and *Karenia brevis*, exhibit low growth rates, it has been hypothesized that one way by which they may compete with other phytoplankton is through allelopathy (Smayda 1997; Arzul et al., 1999; Kubanek et al., 2005, Townsend et al., 2005). Harmful blooms of *Alexandrium* spp., dinoflagellates that produce the suite of neurotoxins that cause Paralytic Shellfish Poisoning, occur worldwide. Although *A. fundyense* mostly occurs as a 'background' species in the Gulf of Maine (Anderson, 1998), summertime proliferations of *A. fundyense* reaching harmful levels are of significant concern in terms of the shellfish industry and human health. An unresolved question regarding the dynamics of *A. fundyense* in the Gulf of Maine is why blooms of this species are often restricted to off-shore waters. Townsend et al. (2005) proposed that biological interactions, chiefly competitive interactions with diatoms, are

important in determining the success of *A. fundyense* in in-shore regions. They hypothesized that diatoms prevent the initiation of *A. fundyense* blooms via direct competition or allelopathy but that once *A. fundyense* is established it prevents a second diatom bloom allelopathically (Townsend et al., 2005). A preliminary experiment in which the diatom *Thalassiosira* was grown with *A. fundyense* showed that growth of the diatom was inhibited in the presence of the dinoflagellate (Figure 2.1). Because the two cultures were grown together, however, I could not determine whether the effects were due to direct competition for nutrients or allelopathic interactions.

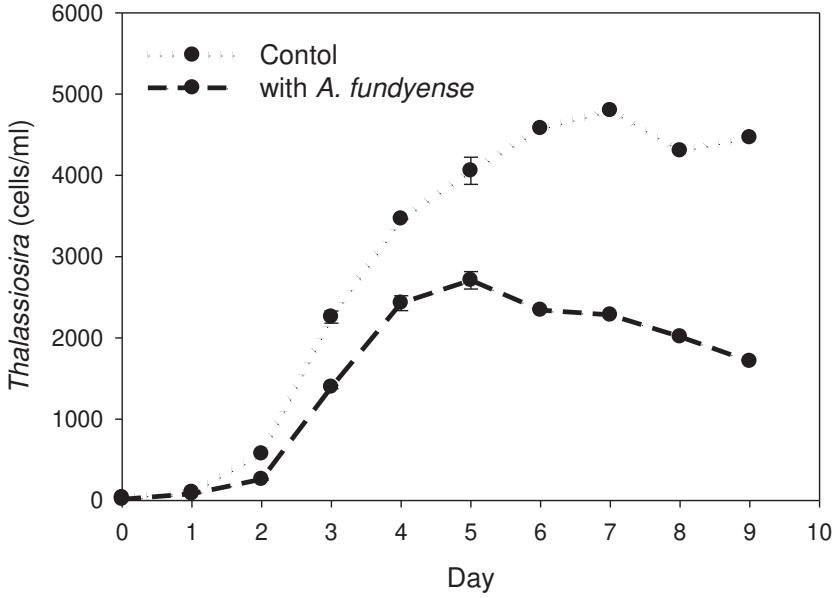

Figure 2.1. Cell concentrations of *Thalassiosira* grown with or without *A. fundyense* cells as a function of time.

Alexandrium spp. have been shown to be generally allelopathic against a wide range of target species, including diatoms (Tillmann et al., 2008), but individual strains of any given species vary in their allelopathic potential (e.g., Hattenrath-Lehmann et al., 2011). Although the allelochemicals produced by members of this genus remain to be fully characterized, they appear to be unrelated to the well-known PSP toxins (i.e., saxitoxin and its analogs; Tillmann and John,

2002) and spiruloids (Tillmann et al., 2007). Recently, Ma et al. (2009) characterized the allelochemicals released by *A. tamarense* as relatively stable and large (>5 kD), mostly lipophilic compounds. These allelochemicals were found to target specific sterols in the cell membranes of affected *Rhodomoas salina* leading to perforation of the membrane (Ma et al., 2011). The allelochemicals released by *A. minutum* have been demonstrated to result in decreased photosynthetic efficiency in target cells via a reduction in the number of photosynthetic reaction centers (Lelong et al., 2011). Recently, the allelopathic potential of North American East Coast strains of *A. fundyense* has been demonstrated, yet the magnitude of the effect was strain dependent and varied between target species (Hattenrath-Lehmann and Gobler, 2011). Although one strain from the Gulf of Maine was examined, its potential allelopathy was not tested against diatoms most relevant to competitive interactions within the Gulf of Maine. To further assess the role of allelopathy in the ecology of *Alexandrium* in the Gulf of Maine I examined allelopathic effects of a new *A. fundyense* strain isolated from the Gulf of Maine on the dominant spring-bloom former *Thalassiosira* sp. using environmentally realistic nutrient and target cell concentrations. A sexual reproduction event in my *Thalassiosira* culture resulted in the possession of cultures of varying cell sizes from a single initial isolate and provided the unique opportunity to examine size effects on the sensitivity of *Thalassiosira* to allelochemicals released by *A. fundyense* independently from taxonomic differences. This is of particular interest in terms of diatom ecology because although the diatom life cycle, basically diminution of cell size over successive vegetative divisions followed by size restoration via sexual reproduction, is well recognized, the implications of diatom cell size in terms of their ecology remain poorly understood.

2.2 Materials and Methods

2.2.1 Cultures and Growth Conditions

A. fundyense and the chain-forming *Thalassiosira* sp., both isolated from the Gulf of Maine in August 2010, were maintained as non-axenic batch cultures at 15.5°C in an incubator at the University of Maine in Orono, ME. Cultures were illuminated by cool fluorescent lights at an intensity of ~150 µmol quanta m^{-2} s^{-1} and a 14: 10 h light:dark cycle. Sterile media was prepared using nutrient-depleted GF/F filtered Gulf of Maine seawater with a salinity of 32-35. Macronutrients were augmented to reflect typical concentrations in the Gulf of Maine before the annual spring bloom (16 µmol/L NO_3^-, 16 µmol/L $Si(OH)_4$, 3 µmol/L PO_4^{3-}) and trace metals and vitamins were added at ¼ L1 concentrations (Guillard and Hargraves, 1993) using nutrient stocks obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota, East Boothbay, ME. Media prepared as such will hereafter be called 'GoM media.'

2.2.2 Initial Filtrate Experiments – General Effects of *A. fundyense* on *Thalassiosira*

As mentioned previously (Introduction), initial experiments in which *Thalassiosira* and *A. fundyense* were grown together revealed that *Thalassiosira* growth was inhibited in the presence of *Alexandrium* but I could not distinguish between allelopathy and direct competition for nutrients. To investigate existence of chemicals produced by *A. fundyense* that affect *Thalassiosira*, cultures of *A. fundyense* in late-exponential phase (~1000 cells/ml) were sterilely filtered through a 0.2 µm Millipore Steritop Vacuum bottle-top filter and the cell-free filtrate was diluted with sterile filtered Gulf of Maine seawater to final concentrations corresponding to several densities of *A. fundyense* cells. All cultures were made up to 2 L and supplemented with nutrients to achieve final concentrations matching GoM media. Control cultures consisted of *Thalassiosira* grown in GoM media, also filtered as described above, with no addition of *A. fundyense* filtrate. Filtrates and controls were immediately inoculated with exponentially growing *Thalassiosira* chains (cell size ~ 20 µm; mean +/- SD = 21 +/- 2.2, n = 92, raw data in Appendix

C) to a final density of ~20 cells/ml. In a first experiment, *Thalassiosira* was grown in control media or media containing *A. fundyense* filtrate diluted to correspond to 50, and 350 cells/ml. An analogous experiment was performed 2 months later. In addition to repeating the control and treatments from the previous experiment, this second experiment included two technical replicates of an intermediate filtrate dilution corresponding to 150 *A. fundyense* cells/ml.

To assess the effects on *Thalassiosira*, samples were taken daily from treatments and controls for nutrient analysis and cell counts until cultures reached stationary phase (7-9 days). Samples for nutrient analysis were filtered through 0.45 μm Millipore HA filters and frozen until analysis. Concentrations of NO_3^-+NO_2^-, NH_4^+, $Si(OH)_4$ and PO_4^{3-} were measured using a Bran Luebbe Autoanalyzer III following standard techniques. Samples for cell counts were preserved in formalin (0.2% final concentration) and kept in the dark until they were counted in triplicate using a compound microscope and a gridded Sedgwick-Rafter counting chamber. The entire area of the chamber was scanned and all encountered cells were counted. For cases in which *Thalassiosira* concentrations exceeded 2000 cells/ml, a 1:10 dilution was prepared and counted.

2.2.3 Evaluation of Allelopathic Effects as a Function of Cell Size

Following these initial experiments, *Thalassiosira* stock cultures became sexually reproductive, providing a unique opportunity to examine the role of cell size of an individual species in response to chemicals released by *Alexandrium.* Although all *Thalassiosira* originated from the same isolate, I obtained, in culture, cells ranging from 20 to 50 μm in size, the largest cells being the products of successful sexual reproduction and thus representing the maximal cell size for this species. Chains of three cell size classes were separated to start three new cultures of cells. The three cell size classes were 20 (“small”), 30 (“intermediate”) and 50 (“big”) μm. These size classes represent the mean and range of the cell diameters for each size class and the actual values are summarized in Table 2.1. In a series of 4 experiments spaced over 3 months, the effects of *Alexandrium* filtrate on these 3 target cell sizes were tested. The timing of these

experiments is outlined in Table 2.2. *A. fundyense* cultures were grown to late-exponential/early stationary phase, filtered, and augmented with nutrients as previously described. Filtrate was used at full strength (1000 cells/ml) as well as diluted to correspond to 350 cells/ml. *Thalassiosira* of each cell size was inoculated into 250 ml of treatment or control media at a concentration of 20 cells/ml. The cultures were monitored for 4 days - the period of time required for cells to be affected by and to begin to recover from allelopathic effects as determined in earlier experiments. Samples were taken daily for cell counts and nutrients as described above. While counting, cells were differentiated as either healthy-looking or abnormal ('bleached'). Specific growth rates were determined from the exponential phase of the growth curve (Equation 1) and based on calculations of daily growth rates (Equation 2)

$$r = \frac{Ln\left(\frac{N2}{N1}\right)}{t_2 - t_1} \qquad \textbf{(1)}$$

$$r = \left(\frac{1}{c_{mean}}\right) x \left(\frac{N2 - N1}{t_2 - t_1}\right) \qquad \textbf{(2)}$$

where N1 and N2 cells/ml are cell concentrations at time t_1 and t_2, respectively and c_{mean} represents the average number of cells at t_1 and t_2.

Table 2.1 *Thalassiosira* cell size classes. The average cell diameter (+/-SE) as well as the minimum and maximum values for each class are given. Raw data are given in Appendix C.

Thalassiosira cell diameters in the three size classes			
Cell Size Class	Average cell diameter +/- SE (µm)	Minimum (µm)	Maximum (µm)
Small (20 µm)	22.0 +/- 0.2	19.6	26.0
Intermediate (30 µm)	29.3 +/- 0.2	22.0	35.0
Big (50 µm)	48.0 +/- 0.2	40.1	55.5

Table 2.2 Summary of the timing of experiments to test the effects of *A. fundyense* filtrate on *Thalassiosira* cultures of 3 different cell sizes. The numbers in each column indicate the number of replicates of control (diatom in GoM media) and treatment (diatom in *A. fundyense* filtrate) included in each experiment.

Start Date	20-µm cells ("Small")	30-µm cells ("Interm-ediate")	50-µm cells ("Big")
8-Dec 2011	2		1
13-Dec 2011			1
1-Feb 2012		2	1
29-Feb 2012	1	1	

In the previous series of experiments, *Thalassiosira* was inoculated into treatments and controls at a density of 20 cells/ml regardless of cell size. A final set of experiments sought to examine if variations in the effects on cultures of different cell sizes were due to differences in the initial total biovolume or surface area of the target culture. For this set of experiments I only used 2 size classes (50- and 30-µm) because the smallest size class became sexually reproductive whenever it was inoculated into fresh media, making it an unreliable test subject. The 30-µm cells

in these experiments were the asexual descendents of the 50-µm cells used in previous experiments. One liter each of *A. fundyense* filtrate (1000 cells/ml) and control GoM media were prepared as described above and divided into 4 sterile bottles. Exponentially growing *Thalassiosira* chains from the two size classes were inoculated into 250 ml of filtrate or corresponding GoM media at varying initial cell concentrations. To determine total cell biovolumes, the valve diameter (d) and pervalvar length (h) of 40-50 cells from the 30- and 50-µm *Thalassiosira* cultures were measured and the average biovolumes and surface areas were calculated using the corresponding formulas for a cylinder (Eqs. 3, 4; Hillebrand et al., 1999).

$$V = \frac{\pi}{4} \times d^2 \times h \qquad \textbf{(3)}$$

$$SA = \pi d \times \left(\frac{d}{2} + h\right) \qquad \textbf{(4)}$$

Because previous experiments demonstrated that allelopathic effects were observed for both the intermediate and big size class at initial concentrations of around 30 cells/ml, we decided to test the corresponding total biovolumes (calculated by: 30 cells/ml x average cell biovolume). The biovolumes of interest were thus 3.3 x 10^5 and 10.5 x 10^5 µm^3 for intermediate and big cells, respectively. Each size class was inoculated into either *A. fundyense* filtrate or GoM media (controls) at cell densities matching both of those biovolumes (summarized in Table 2.3). A replicate of this experiment was performed on a later date. Because allelopathic effects were observed within 24 h (and recovery immediately followed) in earlier experiments, samples for cell counts and nutrients were only taken at the time of inoculation and after 1 day to determine the degree of allelopathic effect.

Table 2.3 Summary of target initial cell concentrations and biovolumes. The approximate targets for both the initial cell concentration and biovolume are presented for the four treatments in biovolume experiments in which the corresponding *Thalassiosira* concentrations were inoculated into *A. fundyense* filtrate (at 1000 cells/ml). Each treatment was paired with a control of GoM media.

Treatment/Control pair	Cell Size Class	Intended biovolume (x 10^5 μm^3)	Intended [*Thalassiosira*] (cells/ml)
A	Big	10.5	30
B	Intermediate	10.5	95
C	Intermediate	3.3	30
D	Big	3.3	10

2.2.4 Data Analysis

Means and standard error were calculated for triplicate cell counts on each day sampled. The uncertainty associated with all parameters derived from cell counts (e.g., growth rates, percent of cells bleached) were calculated by propagation of error. Student's t-tests were performed to determine the significance of differences in growth rates. Because of overall small sample sizes, non-parametric Mann-Whitney U-tests were used to rank and determine significance of differences in all other parameters. As a quantification of allelopathic effect, the percent of cells bleached after 24 hours as well as growth inhibition were used. Growth inhibition was defined as percent difference in growth rate over the first 24 h of the treatment relative to the control as shown in Equation 5 in which $r_{init\ control}$ and $r_{init\ treatment}$ represent the daily growth rate (Equation 2) for t_0 to t_1 for the control and treatment respectively.

$$Growth\ inhibition\ (\%) = \left[\frac{r_{init\ control} - r_{init\ treatment}}{r_{init\ control}}\right] x\ 100 \qquad \textbf{(5)}$$

2.3 Results

2.3.1 General Effects of *A. fundyense* Filtrate on *Thalassiosira*

Exposure of *Thalassiosira* (cell size ~ 20 μm) to cell-free filtrate from *A. fundyense* cultures resulted in bleaching and inhibition of *Thalassiosira* growth for a period of up to four days (Figure 2.2). The length of the period of inhibition was related to the concentration of cell-free filtrate added. Concentrations of filtrate corresponding to 50 *A. fundyense* cells/ml had no effect on *Thalassiosira* growth while filtrate concentrations corresponding to 350 *A. fundyense* cells/ml contributed to the longest period of growth inhibition as compared to control cultures. Macro-nutrient (NO_3^-+NO_2^-, $Si(OH)_4$ and PO_4^{3-}) utilization also ceased for roughly the entire period of growth inhibition (Figure 2.3). Addition of higher filtrate concentrations resulted in more variability between replications. Following the period of growth inhibition, filtrate-exposed cultures recovered, eventually attaining final cell densities similar to controls. While in one of the experiments the exponential growth rate of the recovering culture (exposed to filtrate corresponding to 350 *A. fundyense* cells/ml) exceeded that of the control (1.82 vs. 1.53 d^{-1}), in the second experiment this trend was reversed, with the exponential growth rate higher in the control culture (1.52 vs. 1.32 d^{-1}).

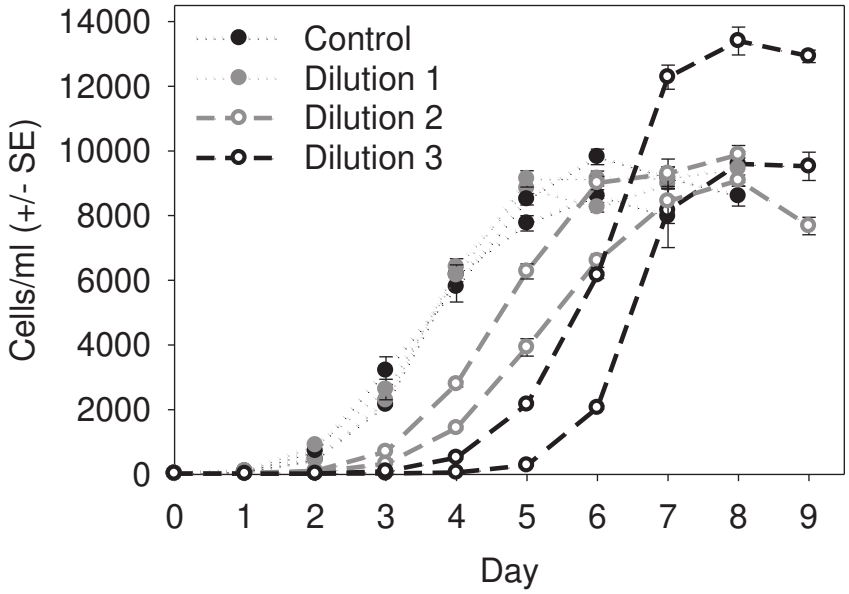

Figure 2.2 *Thalassiosira* cell concentrations as a function of time. Cells exposed to cell-free filtrate from *A. fundyense* cultures were inhibited relative to controls although the effect varied with concentration of filtrate added as shown in duplicate growth curves. Control cultures consisted of *Thalassiosira* grown in fresh GoM media, with no addition of *A. fundyense* filtrate. Data points are the mean and SE based of triplicate cell counts. Closed black circles, dotted line = control, closed gray circles, dotted line = *A. fundyense* filtrate diluted to correspond to 50 cells/ml, open gray circles = *A. fundyense* filtrate diluted to 150 cells/ml, open black circles = *A. fundyense* filtrate diluted to 350 cells/ml.

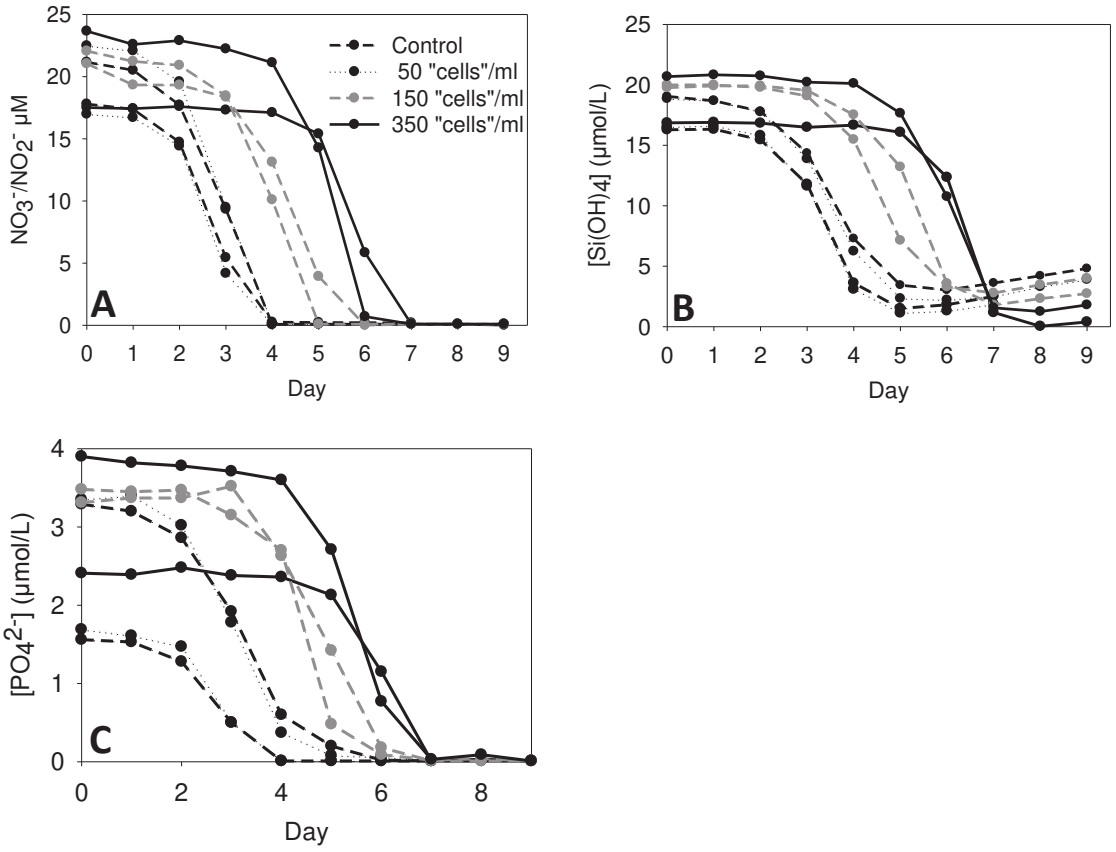

Figure 2.3 Nutrient concentrations in treatments and controls. Nitrate/nitrite (A), silicic acid (B), and phosphate (C) in treatments (*Thalassiosira* exposed to different concentrations of *A. fundyense)* and controls.

In addition to growth inhibition, *Thalassiosira* cells exposed to high concentrations of *Alexandrium* filtrate became discolored. Cells affected in this way bleached from golden brown to pale green or clear within 24 h (Figure 2.4). Total cell lysis was never observed and over time the percentage of bleached cells in the population decreased (data not shown).

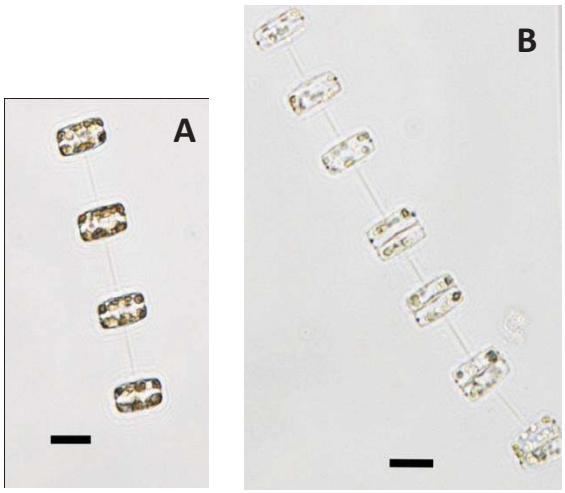

Figure 2.4 Microphotographs of *Thalassiosira*. A) A healthy chain in a control treatment (GoM media) and B) A chain exposed to high concentrations (350 "cells"/ml) of *A. fundyense* filtrate. *Scale bars* 20 µm.

2.3.2 Evaluation of Allelopathic Effects as a Function of Cell Size

Following sexual reproduction of *Thalassiosira* cultures, I obtained cultures of three different size classes based on valve diameter. The smallest cells were close in size to the size threshold at which sexual reproduction can occur. A culture of intermediate cell-size was acquired by isolating chains from populations that were further from the critical size required for sexual reproduction. Finally, the largest cell size cells (~50 µm) are the result of successful sexual reproduction and represent the initial (maximal) cell size for this strain (Figure 2.5). While 3 replicate experiments were attempted for each of the size classes (Table 2.2), during the final replication of the smallest cells, the culture initiated sexual reproduction and the control exhibited a very low growth rate compared to controls of previous experiments (Figure 2.6). Because of this difference, no data on the 20-µm cells from the 29-Feb experiment was used in the following analyses. The average maximum growth rate, calculated by Equation 2, attained by the 50-µm culture was lower than this growth rate for the other two classes. This difference was significant only between the 50- and 20-µm cultures (Student's t-test, $p<0.05$). The same trend is seen when

comparing exponential growth rates (Equation 1) among the size classes, with smaller cells having the faster growth rate (Data not shown, see Appendiex B for raw data). Initial growth rates (i.e. growth rate from t_0 to t_1) calculated using Equation 2 did not differ among the size classes (Student's t-tests, $p>0.2$).

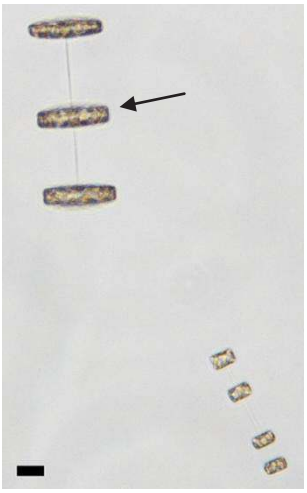

Figure 2.5 A microphotograph of two *Thalassiosira* chains of different cell sizes in the same culture. Larger cells (indicated by arrow) are the result of successful sexual reproduction. *Scale bar* 20 μm.

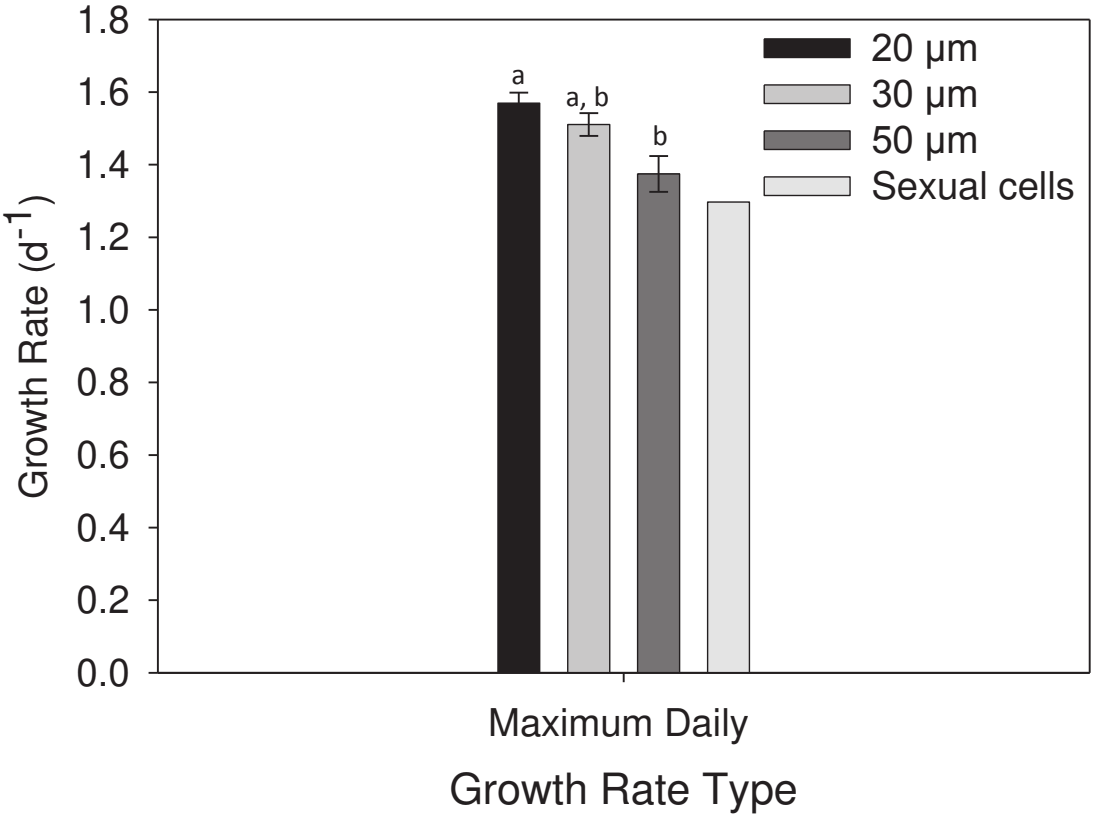

Figure 2.6 Daily maximum (Equation 2) growth rates of *Thalassiosira*. Growth rates are shown for cultures of the three size classes as well as a culture undergoing sexual reproduction. Bars represent the average growth rate (+/- propagated error) of each type from n = 3 experiments for the 30- and 50-µm cells and n = 2 experiments for the 20-µm cells. Because the growth rates for sexual cells come from a single experiment, no error bars are given. Same letter indicates no significant difference.

In these later experiments performed 6 months after the initial filtrate experiments, even small-celled *Thalassiosira* cultures were relatively unaffected by filtrate concentrations corresponding to 350 *A. fundyense* cells/ml. This is in stark contrast to earlier experiments in which a very strong affect was observed at these concentrations (Figure 2.2). Instead, filtrate concentrations corresponding to *A. fundyense* concentrations of 1000 cells/ml were required to elicit an effect for all cell sizes (Figure 2.7).

The effects of exposure to *A. fundyense* filtrate varied with cell size. The largest cells were least affected by the filtrate. This size class recovered faster, had the smallest reduction in initial growth rate relative to controls and exhibited the least amount of bleaching (Figures 2.7, 2.8, 2.9, 2.10). Culture recovery was determined by calculating the 95% confidence interval around the average maximum per day growth rate, as calculated for control cultures, for a given size class. When the daily growth rate of a treatment culture fell into this interval, the culture was considered to be recovered from its allelopathic inhibition. During the 4 days over which samples were taken, neither the small nor intermediate cells attained growth rates within the 95% confidence interval of the maximum growth in the controls. The largest cells, however, achieved their maximum growth rate between days 2 and 3 (Figure 2.8). Average daily growth rates of the treatments were significantly lower than in control cultures during the first two days of the experiments for the 50-µm cells and for the 30-µm cells (Mann-Whitney U-tests $n_{1,2}$=3,3, U=6, $p<0.05$). Growth rates then increased during the latter half of the experiment (Figure 2.8). As described for earlier experiments, utilization of nutrients (data not shown, but raw data is available in Appendix B) displayed a pattern similar to that of growth, with cultures not taking nutrients during periods of growth inhibition. The average proportion of cells bleached was close to 100% for the 20- and 30-µm cultures exposed to the highest concentrations of *A. fundyense* filtrate but was only 30% in the 50-µm size class. The intermediately sized cells bleached somewhat (17% of the total number of cells) when exposed to the lower concentration of filtrate (350 "cells"/ml) while no bleaching was observed at this filtrate concentration for the other two size classes. The 30-µm cells were slightly more affected than 20-µm cells at both filtrate concentrations showing slightly slower growth and recovery than the 20-µm cells (Figures 2.7).

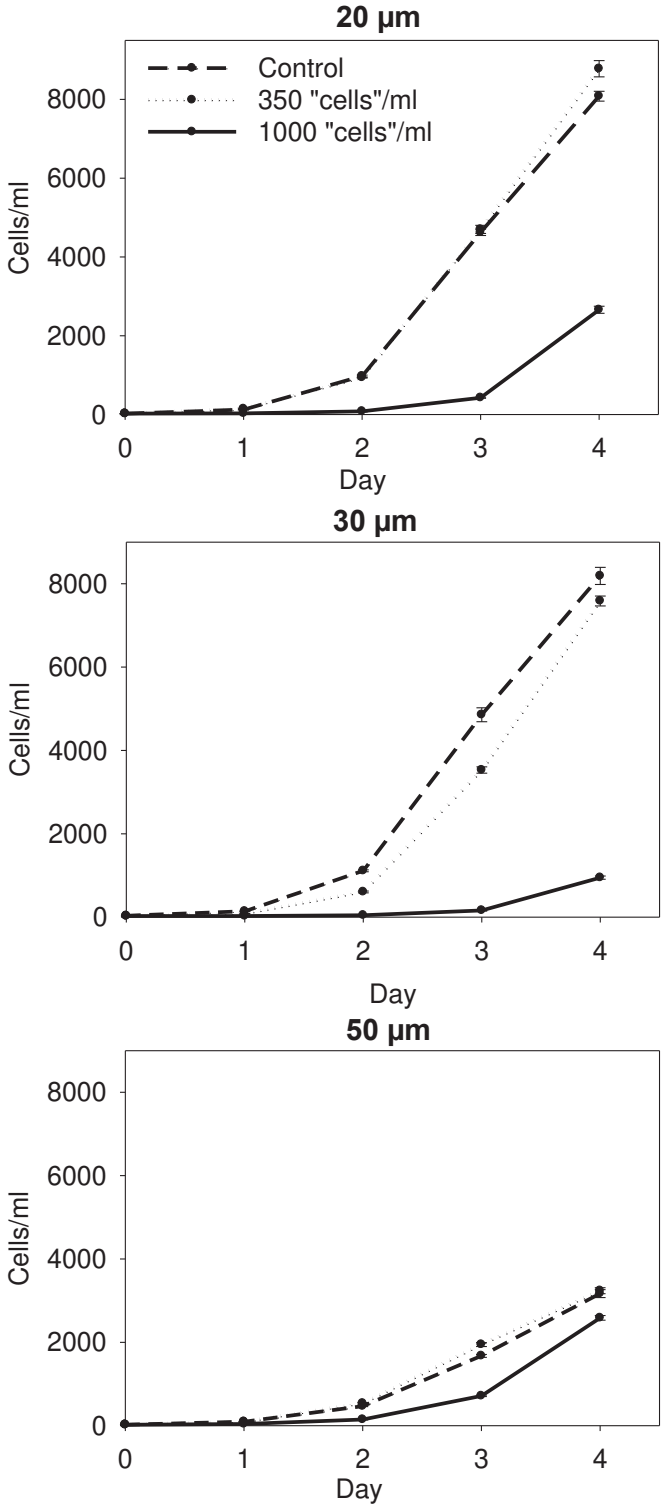

Figure 2.7 Cell concentrations as a function of time for *Thalassiosira* cultures of different cell sizes in two concentrations of *A. fundyense* filtrate. Data points are the average of number of cells/ml on each day in replicate experiments. Error bars represent the propagated SE of triplicate cell counts for each daily sample.

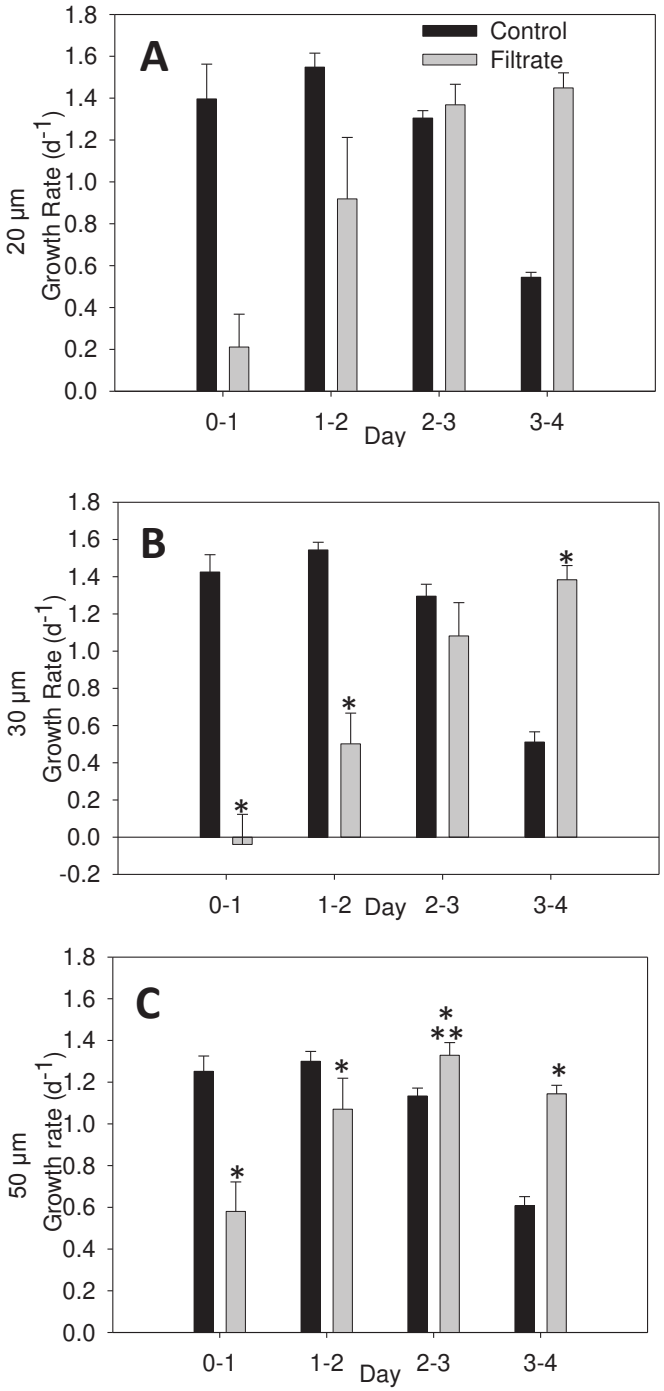

Figure 2.8 Daily specific *Thalassiosira* growth rates. For A) 20 µm cells; B) 30 µm cells; C) 50 µm cells. Filtrate treatment media corresponds to 1000 *A. fundyense* cells/ml. Data are the means (+/- SE) of growth rates for each day for n = 3 replicates for the 30 and 50 µm cells and n = 2 replicates for the 20 µm cells. * indicates significant difference from the control by a Mann-Whitney U-test. Significance was only calculated for the 30 and 50 µm size class. ** indicates that the culture had recovered (i.e., reached the maximum growth rate as determined by control cultures).

2.3.3 Testing the Biovolume or Surface Area Dependence of Allelopathic Interactions

In order to determine whether the difference in allelopathic effect between size classes was due to the total available biovolume or surface area in the inoculated culture, data from all experiments that included different cell sizes were combined. Growth of larger cells, with the lowest surface area to volume ratio, was less inhibited than the growth of intermediate or small cells. Small cells (20 µm) displayed the greatest inhibition of growth (Figure 2.9). A Mann-Whitney U-Test indicated that growth of intermediate size cells (30 µm) was significantly more inhibited compared to the large cells (50 µm) ($U = 35$, $p<0.05$). Decreasing cell size (increasing surface area:volume) was also related to a larger percent bleaching upon exposure to *A. fundyense* filtrate (Figure 2.10). However, a Mann-Whitney U-Test showed that differences between bleaching in the 50- and 30-cells was not significant ($U = 49$, $p>0.05$). In one experiment bleaching was abnormally low compared to previous and later experiments. If the results of this experiment are removed, the higher bleaching of the intermediately sized cells compared to the largest cells significant at $p < 0.05$ ($n_{1,2}=5,5$, $U = 15$). Mann-Whitney U-tests also showed significant differences in bleaching, but not in growth inhibition, between the 20- and 50-µm cultures ($n_{1,2}=7,2$, $U = 28$) but no difference in either effect between the 20- and 30-µm size classes.

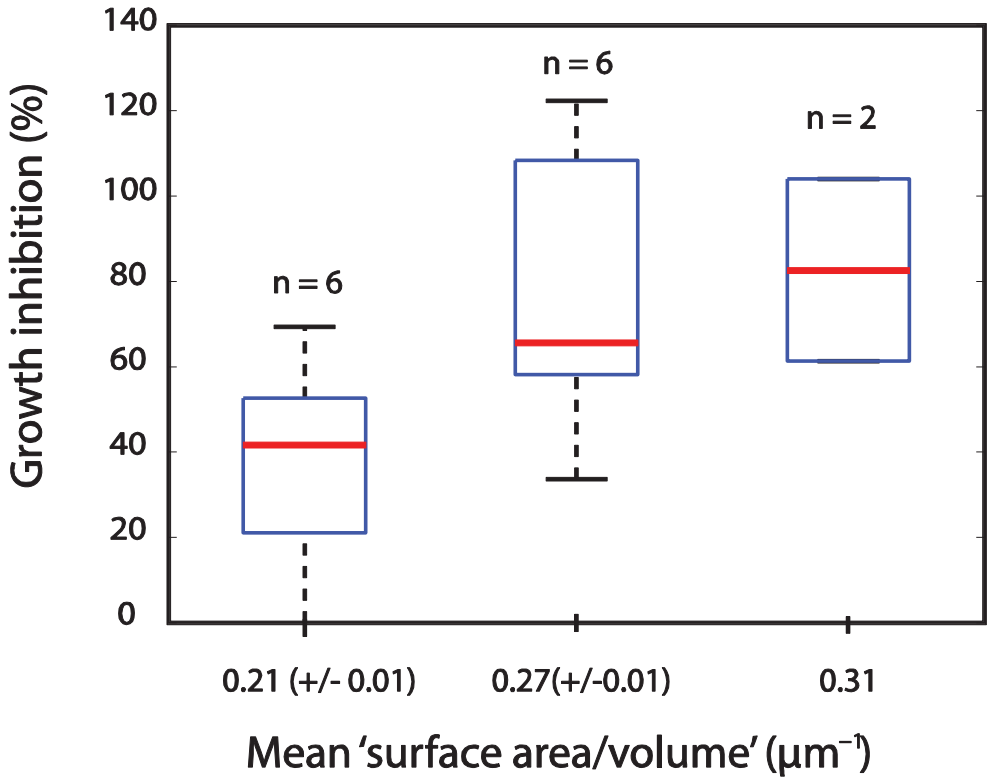

Figure 2.9 Box and whisker plots of growth inhibition of three *Thalassiosira* size classes. The three given 'surface area/volume' correspond to the 50-, 30-, and 20-µm size classes from left to right. Percent growth inhibition is percent difference in initial growth rate (t_0-t_1) of the treatment relative to the control upon exposure to *A. fundyense* filtrate (1000 "cells"/ml). The horizontal line within the box indicates the median, boundaries of the box indicate the upper and lower quantile, and the whiskers indicate maximal and minimal values of growth inhibition calculated for each size class.

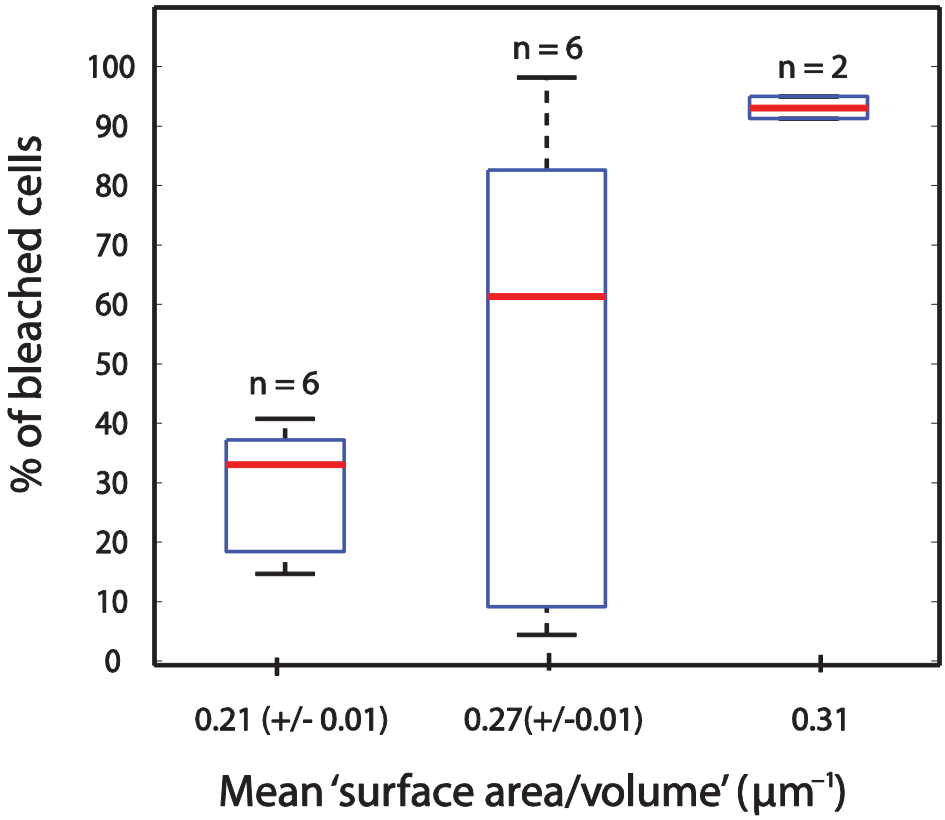

Figure 2.10 Box and whisker plots of the percentage of bleached cells in each of three *Thalassiosira* size classes. The three given 'surface area/volume' correspond to the 50-, 30-, and 20-µm size classes from left to right. Percent of cells bleached in *Thalassiosira* upon exposure to *A. fundyense* filtrate (1000 "cells"/ml). The horizontal line within the box indicates the median, boundaries of the box indicate the upper and lower quantiles, and the whiskers indicate the highest and lowest values of the results.

Despite the variability, within the 30-µm size class, as biovolume or surface area increased, the degree of growth inhibition decreased (Figures 2.11). This trend existed, but was less clear for the bleaching effect (Figure 2.12). Larger cells however, did not show a difference in the degree of growth inhibition or bleaching for two biovolume ranges tested. For a given biovolume, smaller cells exhibited higher bleaching and greater growth inhibition than the larger cells. A similar trend is observed when comparing bleaching and growth inhibition with surface area. Namely, for a given surface area, small cells were generally more affected by the chemicals in the media than the larger cells (Figure 2.12). For one experiment, a culture of intermediately

sized cells exhibited relatively low bleaching (Figures 2.11b and 2.12b). These cells did not, however, show any abnormality in the degree of growth inhibition when compared to earlier or later experiments.

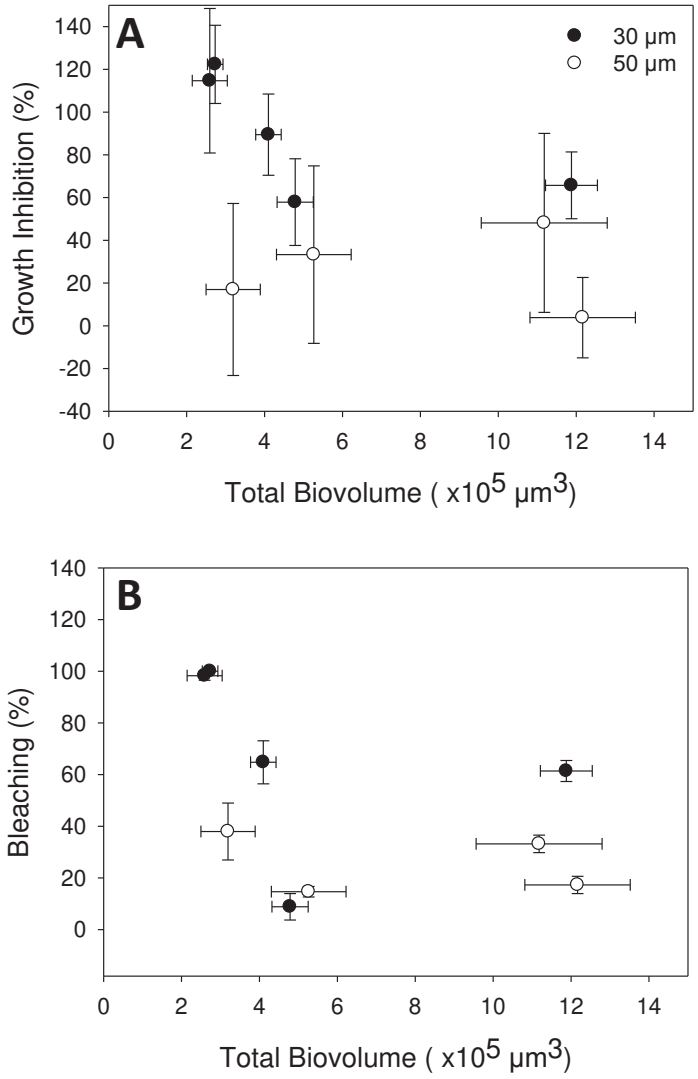

Figure 2.11 Responses of *Thalassiosira* to chemicals released by *A. fundyense* as a function of total cell biovolume. (A) Growth inhibition as percent change in initial growth rate of the treatment relative to the control. (B) Percent of bleached cells. Intermediate size cells (30 µm) are marked by full circles and large cells (50 µm) are marked by open circles. Error bars indicate the propagated standard error of both the specific effect and the estimated biovolume.

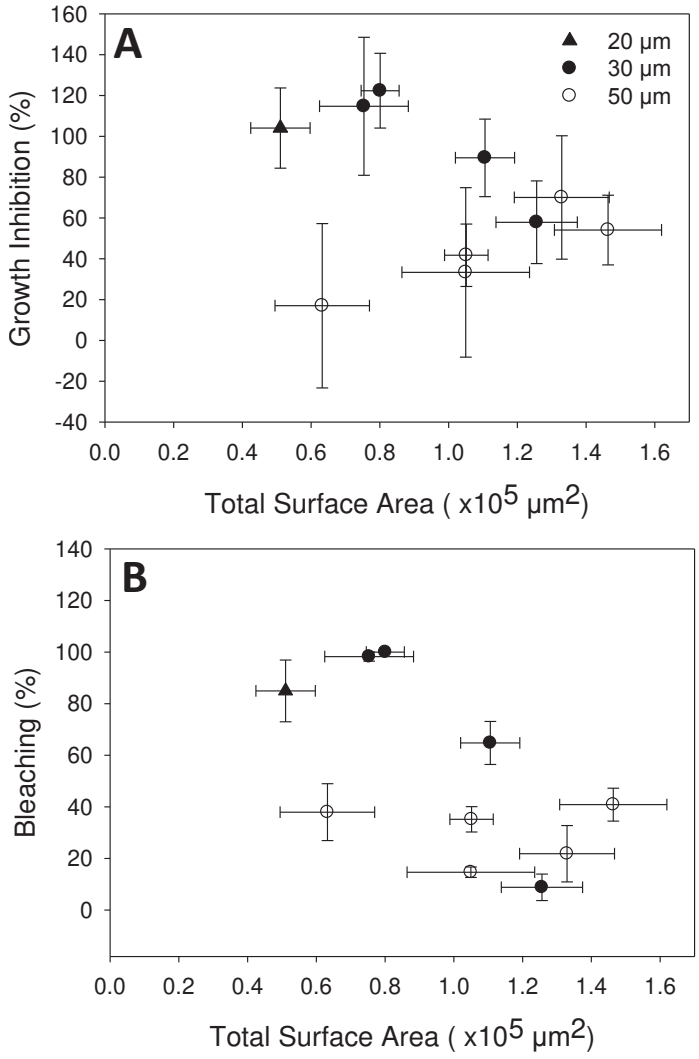

Figure 2.12 Responses of *Thalassiosira* to chemicals released by *A. fundyense* as a function of total cell surface area. (A) Growth inhibition as percent change in initial growth rate of the treatment relative to the control. (B) Percent of bleached cells. Intermediate size cells (30 µm) are marked by full circles and large cells (50 µm) are marked by open circles. Error bars indicate the propagated standard error of both the specific effect and the estimated biovolume.

As mentioned previously, *Thalassiosira* was affected by a filtrate concentration corresponding to 350 "cells"/ml of *A. fundyense* in the initial experiments. In experiments performed 5 months later, *Thalassiosira* was negatively affected at a concentration equivalent to 1000 "cells"/ml but not at the lower concentration (350 "cells"/ml). Over the next year during which 6 experiments were carried out, there was no obvious change in response of *Thalassiosira* in terms of growth inhibition (Figure 2.13) or bleaching (Figure 2.14).

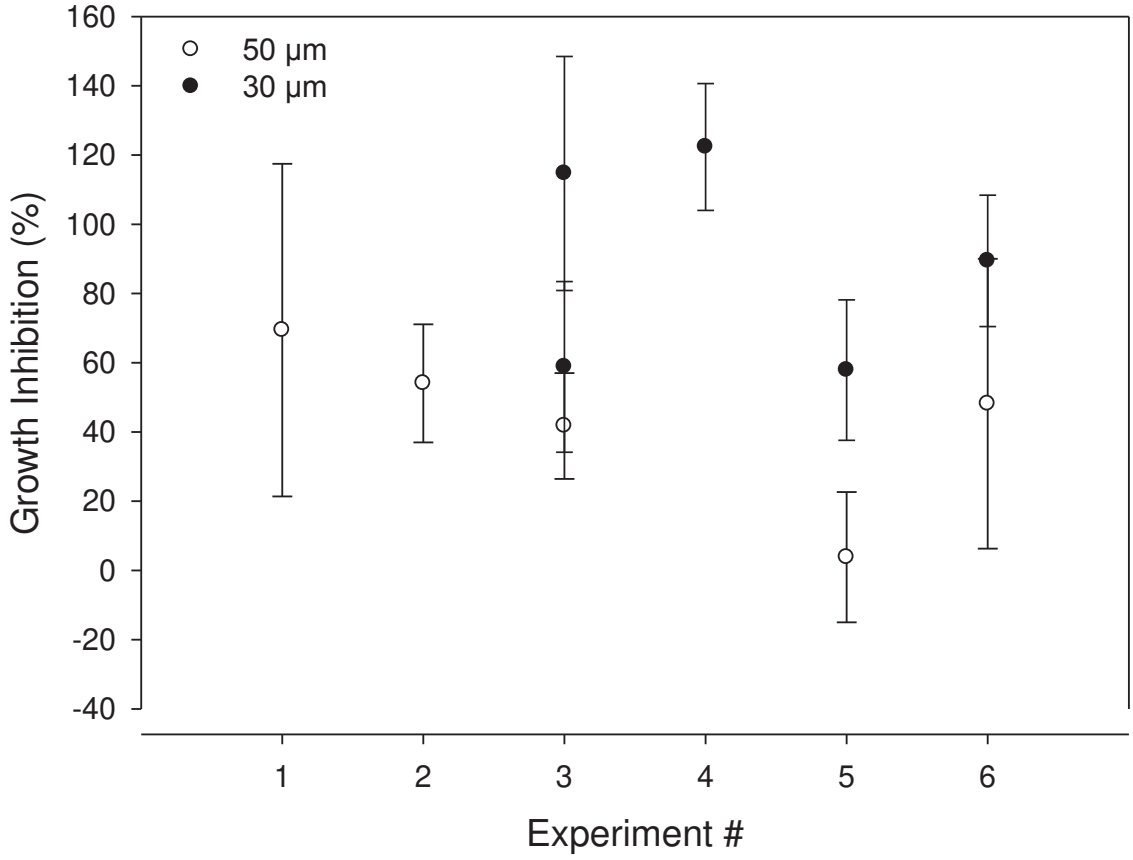

Figure 2.13 *Thalassiosira* growth inhibition over the course of the study period. Growth inhibition of two size classes of *Thalassiosira*, as percent change in initial growth rate of the treatment relative to the control, is plotted against the date on which each experiment was started ranging from December 2011 to September 2012. Experiment #: 1 = 8 December, 2011; 2 = 13 December, 2011; 3 = 1 February, 2012; 4 = 29 February, 2012; 5 = 12 September, 2012; 6 = 27 September 2012.

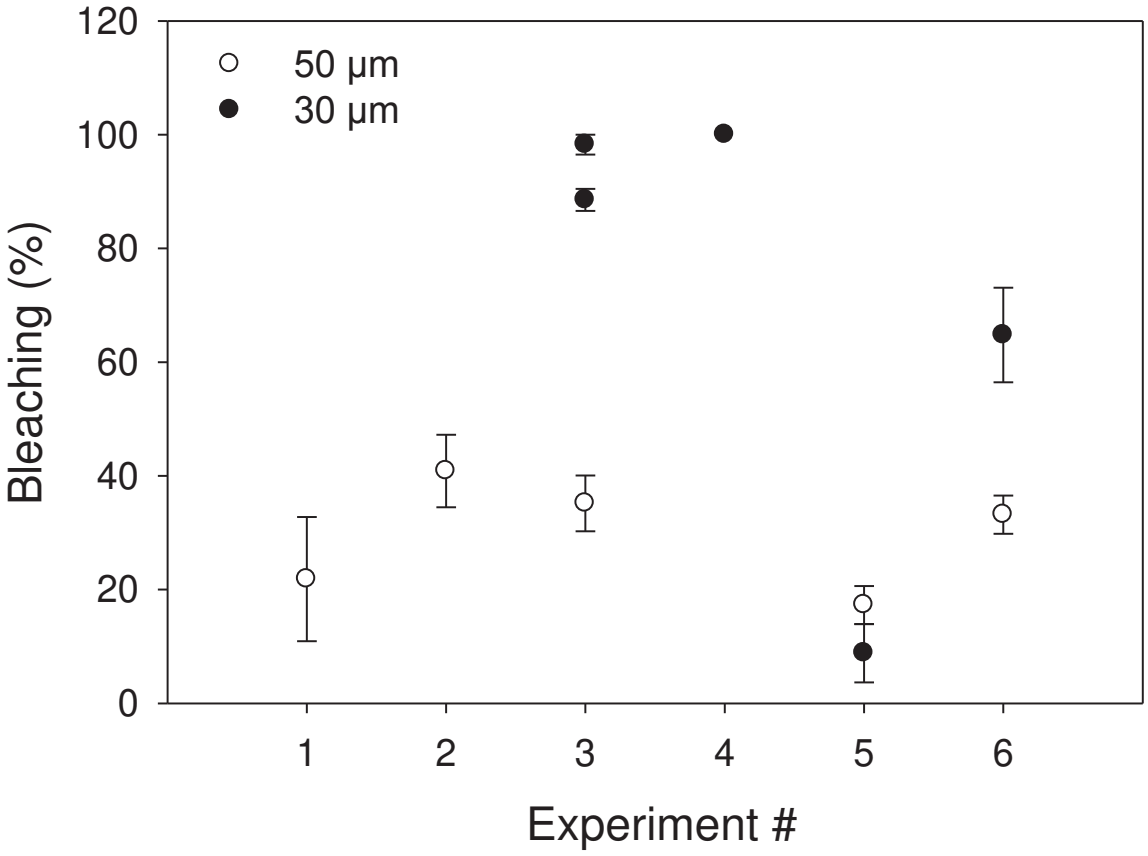

Figure 2.14 *Thalassiosira* bleaching over the course of the study period. Percent of cells bleached upon exposure to *A. fundyense* filtrate corresponding to 1000 cells/ml is plotted against the date on which each experiment was started ranging from December 2011 to September 2012. 1 = 8 December, 2011; 2 = 13 December, 2011; 3 = 1 February, 2012; 4 = 29 February, 2012; 5 = 12 September, 2012; 6 = 27 September 2012.

2.4 Discussion

2.4.1 General Effects of *A. fundyense* on *Thalassiosira*

Cell-free filtrate of *A. fundyense* cultures negatively affected the common Gulf of Maine diatom *Thalassiosira* sp. *Thalassiosira* exposed to high concentrations of *A. fundyense* exhibited marked reductions in growth and nutrient utilization relative to control cultures. The observed growth inhibition effect is similar to that seen in protistan targets exposed to filtrate from a

variety of donor dinoflagellates including *Prymnesium parvum* (Fistarol et al., 2003), *Karenia brevis* (Kubanek et al., 2005), and several *Alexandrium* species such as *A. ostenfeldii* (Tillmann et al., 2007), *A. tamarense*, and *A. catenella* (Arzul et al., 1999). More recently, *A. fundyense* isolates, including one from the Gulf of Maine, were confirmed to inhibit growth of *Rhodomonas salina* as well as diatoms from the Long Island Sound (Hattenrath-Lehmann and Gobler, 2011). No other studies to my knowledge have measured nutrient utilization in experiments examining allelopathic interactions. The fact that nutrient utilization ceased throughout the period of growth inhibition confirms that *A. fundyense* would benefit immediately from reduced competition for nutrients.

In addition to inhibition of growth and nutrient utilization, *A. fundyense* filtrate caused bleaching of *Thalassiosira* cells. Filtrate from an *A. ostenfeldii* culture was also reported to cause bleaching of *T. weissflogii* cells in which the target's "cell content was conspicuously granular" (Tillmann et al.,2007). In their study, cell discoloration was usually an initial step towards complete lysis of target cells, something that was never observed in my experiments even at the highest concentrations of *A. fundyense* filtrate tested. Rather, in my experiments, *Thalassiosira* cultures recovered fully after a few days and eventually reached cell concentrations similar to those in the control. In one of the two initial experiments, final *Thalassiosira* concentrations were higher in the 350 cells/ml treatment than in the control (Figure 2.2). This was probably due to the fact that this treatment started with higher nutrient concentrations than the others (Figure 2.3). Additionally, in one of the two experiments, the exponential growth rate of the recovering treated culture was higher than that of the control. Taken alone, this may have suggested an enhancing affect of *A. fundyense* filtrate on *Thalassiosira*. However, in the second experiment the opposite effect was seen and thus the possibility that *Thalassiosira* growth is enhanced following inhibition cannot be determined without further study. What is clear, however, is the immediate negative effect on *Thalassiosira* in terms of growth and success in competing for nutrients. The

observed recovery suggests that the putative allelochemicals may become inactive (e.g., break down; Arzul et al., 1999) and thus that a single addition may underestimate allelopathic effects. Alternatively, *Thalassiosira* may be able to escape toxic effects by virtue of a high growth rate, as has been suggested for *Chaetoceros gracile* (Arzul et al., 1999) or some other protective mechanism. Finally, it cannot be ruled out that chemicals present in *A. fundyense* filtrate are not capable of completely lysing *Thalassiosira* cells and act only to inhibit growth on some segment of the population, at least at the concentrations tested here. Had live cells continuously releasing chemicals been tested, a stronger effect on *Thalassiosira* may have been observed (e.g., Rengefors and Legrand, 2001; Kubanek et al., 2005).

Growth inhibition or cell death resulting from an abnormally high pH often associated with algal cultures can sometimes be mistaken for allelopathic effects on a culture (Schmidt and Hansen, 2001) and it is important to recognize this possibility in interpreting results of experiments examining allelopathic potency. It is unlikely that increases in pH can be implicated in the results presented here because the effects were seen within the first 24 h of exposure before photosynthesis of the cultures could significantly increase the pH. As measured in one experiment (data not shown), the initial pH of treatment cultures with high *A. fundyense* filtrate concentrations was not different from the pH of the controls. Additionally, the fact that *Thalassiosira* did eventually recover and was able to successfully grow suggests that any pH differences were not a concern. Limitation of nutrients may also cause retardation of cell growth or discoloration of cells (personal observation). Despite the low nutrient concentrations used in these experiments, it is not likely that the measured negative effects are due to nutrient limitation because the cultures were harvested in mid-exponential phase and the negative effects occurred within the first 24 h before nutrients in the experimental cultures were appreciably drawn down. This was confirmed by nutrient analyses. In fact, the environmentally relevant nutrient

concentrations used here are a strength of this study and are intended to maintain culture conditions as close to natural as possible.

The concentration of *A. fundyense* filtrate required to inhibit growth or cause bleaching in *Thalassiosira* was high (equivalent to 350 or 1000 cells/ml) relative to typical field concentrations of this species in the Gulf of Maine (Anderson et al., 2005). In the Bay of Fundy however, *A. fundyense* concentrations can be close to the 350 cells/ml range (Page et al., 2006), and in these localized areas of higher donor cell abundance allelopathy may be important. Additionally, as discussed above, it is possible that because of degradation of the specific allelochemicals my study underestimates the allelopathic potential of *A. fundyense* against *Thalassiosira* although experiments using a continuous dose of low filtrate concentrations will be necessary to elucidate this further. If allelopathy is indeed used, it would most likely play a role in maintaining already established blooms (e.g., Kubanek et al., 2005) or in localized regions of higher cell abundance of the producing species (Tang and Gobler, 2010). According to a meta-analysis of published experimental work on allelopathy (Jonsson et al., 2009) the majority of studies revealed allelochemical effects only at high concentrations of donor cells indicating that while allelochemicals may be important in already established blooms there is little evidence supporting their role in bloom initiation (Jonsson et al., 2009). Jonsson et al. (2009) suggest that allelopathy may be a side effect of chemicals designed for another purpose such as grazer deterrence. Thus other possible explanations for the apparent lack of sensitivity of *Thalassiosira* to *A. fundyense* filtrate diluted to closer to Gulf of Maine concentrations of *Alexandrium* are that the responsible chemicals are intended for a different competitor species, another purpose altogether, or are a byproduct of some other cellular process.

Hattenrath-Lehmann and Gobler (2011) described *Alexandrium* blooms that they suggest were mediated by allelopathic competition with other phytoplankton. Although the results of their laboratory studies provide convincing evidence of the ability of *Alexandrium* to inhibit

competitors via the release of chemicals (whatever their intended purpose), it is difficult to determine whether allelopathy can be implicated in their field observations. In particular, full understanding of allelopathy in the field, and in the laboratory, is limited by the fact that most allelochemicals remain to be identified and are not easily measurable. Further complicating the study of allelopathy are the widely variable conditions in the field. Cembella (2003) referred to definitive confirmation of the existence of allelopathy in marine systems as "one of the great challenges of marine chemical ecology."

2.4.2 Variability of Allelopathic Effects

The observed effects of *A. fundyense* filtrate were highly variable within and among experiments. A distinct change occurred between early experiments intended to examine allelopathy in general and later experiments that focused on *Thalassiosira* cell size. Namely, the concentration of *A. fundyense* filtrate necessary to elicit a response increased from the equivalent of 350 to 1000 cells/ml. This is an interesting occurrence and could indicate either a change in sensitivity of our *Thalassiosira* cultures or an alteration in the production or identity of the chemicals by *Alexandrium* (Martins et al., 2004; Hattenrath-Lehmann and Gobler, 2011). Because *Thalassiosira* underwent sexual reproduction at the same time, it is difficult to rule whether the potency of *A. fundyense* changed or whether the change had to do with an alteration in *Thalassiosira* physiology. This change in the observed allelopathic effect was only a one-time occurrence over the time period of the experiments described here.

The degree of the negative effects on *Thalassiosira* cells in a size class for a given filtrate concentration also varied between experiments. For example, the percent of cells in 50-µm cultures that bleached upon exposure to *A. fundyense* filtrate varied from about 15 to 40% in different experiments. Growth inhibition was also variable. The effects were variable not only between experiments, but also within experiments. In two replicates of *Thalassiosira* exposed to filtrate corresponding to 150 *A. fundyense* cells/ml one culture was clearly more affected than the

other (Figure 2.2) despite the fact that the two replicates were started at the same time with the same stocks of *Thalassiosira* and *A. fundyense* filtrate. The experiments carried out here include both biological replicates in which a treatment was repeated within a single experiment as well as replicates of the entire experimental set-up that were performed at different times. The use of the latter type of replication ensures that the effects observed were not due to a one-time experimental error or a difference in either the donor or target at a given time. The patterns observed in the experiments here are strengthened by the fact that the effects were seen over multiple distinct experiments, carried out over the course of a year.

The variability observed here is certainly not unique to my experimental system. The allelopathic potential of a given donor species is known to vary among strains (Tillmann and Hansen, 2009; Hattenrath-Lehmann and Gobler, 2011) and even among different batch cultures of a single strain (Tillmann et al., 2007). Production of allelochemicals also varies with growth stage of the donor and *Alexandrium* spp. have been shown to be most potent during stationary phase (Wang et al., 2006; Ma et al., 2009).Target species sensitivity seems to vary with growth phase as well (Poulson et al., 2010). Abiotic factors such as light, temperature, pH, and nutrient availability also influence both the production of allelochemicals by donors and a target's sensitivity (Granéli et al., 2008). Nutrient limitation was not shown, however, to increase the allelopathic potential of *A. tamarense* on a *Rhodomonas* bioassay (Zhu and Tillmann, 2012). To minimize these sources of variability in my study, I maintained consistent culture conditions in terms of nutrients, light, and temperature as well as harvested donor and target cells consistently in late exponential and exponential phase, respectively. Nevertheless, variability exists in my results and is probably due to factors beyond my control such as subtle differences in culture status. In spite of the variability of the allelopathic effects, it is clear that *Thalassiosira* is inhibited by *A. fundyense* filtrate. Support for the findings described is strengthened by the fact that the trends hold despite the variability.

2.4.3 Target Size Dependence of Allelopathy

Another source of variability inherent in studies comparing allelopathic effects on a variety of targets is that of target cell size. It has long been accepted in the terrestrial plant literature that germination of smaller seeds is more inhibited than that of larger seeds for a given allelochemical concentration (Williams and Bartholomew, 2011). Some studies of allelopathy in the marine phytoplankton community have taken this into account by correcting for total cell volume of the target cells (e.g. Tang and Gobler, 2010). Others have looked for relationships between size and the magnitude of the observed effect. Schmidt and Hansen (2001) found a negative relationship between dinoflagellate cell volume and the percentage of dinoflagellate cells with reduced motility upon exposure to *Chrysochromulina polylepis*. In order to obtain this relationship however, they had to exclude from their analysis one dinoflagellate species that was not affected. In a second experiment, the effect of *C. polyepis* on growth of a number of algal isolates from different classes, no relationship was found between cell volume and the degree of growth reduction in the targets. However, when several species for which changes in pH may have caused changes in growth were removed, a slight negative correlation was observed (Schmidt and Hansen, 2001). This negative trend could mean that small cells are more sensitive to allelochemicals or may indicate size-dependent response time (Schmidt and Hansen, 2001). On the other hand, Tillmann and Hansen (2009) observed that while their smallest target, *Chrysochromulina ericina,* was among the most resistant to *A. tamarense* allelochemicals, *Ceratium lineatum*, the largest, was most susceptible. Together, those two studies suggest that size as well as physiological and phylogenetic differences should be considered.

Sexual reproduction of different batch cultures of *Thalassiosira* allowed me, for the first time, to examine target sensitivity as it relates to cell size within a single species. All of the *Thalassiosira* cultures used in my experiments came from a single initial isolate obtained from the Gulf of Maine. Following sexual reproduction, I obtained several cultures of different cell

sizes. Diatoms have a unique life history in which the mean cell size of a population decreases with successive asexual divisions. Restoration of a species' maximum size seems to be required once cells reach a minimum size threshold and this is often achieved through sexual reproduction (see Chapter 3). Cell size plays a role in a number of aspects of phytoplankton ecology such as absorption of light, metabolism, nutrient uptake, and susceptibility to grazing (reviewed by Finkel et al., 2010) and has also been shown to be correlated with DNA content (von Dassow et al., 2008). Despite the important role of diatoms in marine microbial systems – indeed, they are responsible for up to 40% of oceanic carbon fixation (Falkowski and Raven, 1997) – the ecological implications of diminishing cell size associated with cell division are relatively unstudied.

Thalassiosira cultures of different sizes exhibited different growth rates. Cells within the largest size class (50 μm) had low growth rates and in general growth rate increased as size decreased. The slowest growth rate, however, was observed for small cells in the experiment started on 29 February 2012 that initiated sexual reproduction shortly after inoculation into fresh media. The observed increase in growth rate with decreasing size until the population reaches a critical cell size at which growth rate decreases has been observed for other diatoms including *Pseudo-nitzschia delicatissima* (Amato et al., 2005) and *Thalassiosira weissflogii* (von Dassow et al., 2006). The critical cell size after which growth rate begins to decrease may be the size threshold for sexualization (von Dassow et al., 2006). This is supported by the fact that my cultures of *Thalassiosira* in that size range were able to become sexual, but it should be noted that not every culture that reached the critical size did initiate sexual reproduction. Increasing growth rate with decreased size is generally ascribed to lower metabolism of larger cells (Amato et al., 2005) or superior nutrient uptake in cells with larger surface area-to-volume ratios (von Dassow et al., 2006). Based on these differences in growth rate, it is clear that the physiology of *Thalassiosira* varies with cell size.

Thalassiosira cells of different sizes responded differently to *A. fundyense* filtrate. The 50-µm cells recovered faster, bleached less, and had a lower degree of growth inhibition than cells in the other two size classes. The effects are clearly dose-dependent since higher concentrations of filtrate elicited greater negative effects. Without knowing the nature of the chemical or details of the response and assuming equal diffusion of the allelochemical to all cells in a culture, there are several possible explanations for the negative relationship between cell size and chemically mediated effects of *A. fundyense* filtrate on *Thalassiosira*. First, the response may be biovolume dose-dependent and thus a larger biovolume of cells would need to take up a greater amount of the allelochemical to be inhibited. The allelochemical concentration in the filtrate may not be high enough for the population of big cells to get the dose required for a negative effect. Second, particularly if the chemical acts upon the cell membrane, the effects may be related solely to the amount of cell surface area available for binding. Additionally effects could be related to surface-to-volume ratio, and associated higher uptake rates of smaller cells may mean that they get more of the toxin before it degrades. Finally, differences in response could be because of physiological differences, including growth rate, between size classes that are independent of physical size characteristics. If either of the first two possible explanations are the case, the results of the experiments in which *Thalassiosira* of different sizes were inoculated into *A. fundyense* filtrate at the same concentration, may have been biased towards showing that smaller cells are more sensitive to the putative allelochemicals when in fact the cultures of smaller cells simply had a lower starting biovolume or surface area available.

To distinguish between the possibilities given above and to determine whether the previous results were biased towards the smaller cells, I ran an experiment that corrected for the total inoculated biovolume for two cell sizes. The observed allelopathic effects were somewhat related to overall biovolume or surface area, at least within the 30-µm size class, suggesting a dose-dependent response. Because the higher biovolume or surface area cultures started with a

higher cell concentration, this trend is similar to that shown in other studies in which increasing target concentrations results in a decreased allelopathic effect (Tillmann et al., 2007; Hattenrath-Lehmann and Gobler, 2011) and has been suggested to be due to adsorption or absorption of chemicals to cell surfaces reducing their effect on other cells (Tillmann et al., 2007). The results, however, also suggest that total culture biovolume or surface area alone are not sufficient to explain differences in response between cells of different sizes because small cells exhibited a greater response to allelochemicals a given total biovolume or surface area. The surface area to volume ratio, on the other hand, did appear to be positively related to increasing allelopathic effect. This suggests that effects of the allelopathic chemical depend not only on the quota per volume but also on the flux of the chemical into a cell. Without knowing the mechanisms by which the allelochemical acts upon *Thalassiosira* cells, I cannot determine definitively if the difference in effect are due to differences in flux related to SA:volume or because of some other physiological difference between big and small cells. Although big and small cells had different maximum and exponential growth rates, it is unclear whether such differences account for size-related variations in allelopathic effects because there was no difference in the initial (t_0-t_1) growth rate between the size classes.

Other than changes in growth rate with cell size diminution and probable alteration of nutrient uptake and gas exchange associated with greater surface-to-volume ratios, I am not aware of any reports of other ecological impacts that may result from cell size changes linked to the diatom life cycle. Large cells resulting from sexual reproduction are genetically distinct from the parent strain because of genetic recombination. While it is possible that a newly established culture of large cells is genetically more resistant to *A. fundyense* allelochemicals than the small strains, the fact that decreased susceptibility was seen in a number of experiments in which the 50-µm cells originated from distinct sexual reproduction events suggests that resistance is linked to a more common change associated with sexual reproduction or the establishment of a

population of maximally sized cells. Resistance to harmful chemicals in the environment would certainly be beneficial to large cells since they are likely to make up a small percentage of the population especially at the beginning of a sexual reproductive event. With such a resistance, growth of larger cells could outpace growth of small cells that are fated to either reproduce or die, ensuring that the products of expensive sexual reproduction survive.

2.4.4 Conclusions and Future Work

I have observed, for the first time, differences in susceptibility of a diatom to chemicals in the environment linked to cell size differences associated with the unique diatom life cycle. Growth of *Thalassiosira* was inhibited and cells became bleached in the presence of a filtrate from a dense *Alexandrium fundyense* culture. *Thalassiosira* cells in the largest cell size class (50 μm) were less affected by chemicals in *A. fundyense* filtrate than were smaller cell sizes and it appears that either physiological differences between size classes or more efficient transport of the chemical into smaller cells leads to the observed differences. Without knowing the identity or mechanism of action of the allelochemical(s) involved it is difficult to fully elucidate the observed effects and their cause. A focus of future work should be on deciphering the cellular components that are the targets of the chemicals as well as identifying the chemicals acting. Additionally, examination of allelopathic effects on other diatom targets in association with cell size changes will be essential. For future studies, a broader suite of biovolumes should be tested and the effects on individual cultures should be tested frequently over long periods of time as the culture goes through cycles of size diminution and restoration. Unfortunately, a lack of understanding about the mechanisms that control diatom sexual reproduction and the fact that many attempts at reproduction fail will make these studies quite challenging.

Laboratory-based culture work is essential in the study of marine microbial ecology in that it enables researchers to isolate specific components of a very complicated system in order to piece together functioning in nature. Culture work does, however, come with challenges

(Lakeman et al., 2009 provides a good review), particularly when it comes to applying laboratory results to more dynamic field conditions. As observed in the experiments presented here, changes can occur in cultures that influence the outcome of various manipulations. With this in mind, experiments attempting to elucidate the ecology of phytoplanktonic organisms should be carried out as soon as possible after isolation from the field of the experimental strains and cultures should be maintained in conditions as similar as possible to those of the natural environment. I have attempted to do this by using media with nutrient concentrations relevant to the Gulf of Maine, an experimental facet that is lacking in other studies of allelopathy in marine phytoplankton. Much work on allelopathy among phytoplankton has been carried out on strains that have long been in culture and with extremely high nutrient concentrations and thus their relevance to field conditions should be interpreted carefully. All this being said, because of the usefulness of comparing results of experiments on a given donor or target species, there is value to using phytoplankton cultures that have been widely used as experimental systems for years. Another caveat in terms of studies investigating allelopathic interactions is that the results of one experiment at one point in time may not give the full picture. As seen here, one diatom responded very differently to chemicals in *A. fundyense* filtrate depending on the size of the cells which in turn relates to the time within their life cycle at which they are harvested to test. Future work in the field of phytoplankton allelopathic interactions must therefore make specific attempts to design experiments that are most relevant to conditions in the ecosystems in which those interactions take place. Namely, experiments should use relevant donor and target species and concentrations, environmentally realistic nutrient complications and recognize the variability inherent both in culture and in nature.

CHAPTER 3

OBSERVATIONS ON *THALASSIOSIRA* SEXUAL REPRODUCTION

Diatoms, single-celled eukaryotic algae characterized by complex silica walls or frustules, dominate the microbial community in many marine systems and are often the basis for some of the most efficient marine food webs (Allen et al. 2006). Indeed, diatoms are known to contribute to up to 40% of the annual oceanic carbon fixation (Falkowski and Raven, 1997). This fact, along with their negatively buoyant, silica frustules, renders them ecologically important in terms of carbon sequestration and nutrient cycling (Smetacek, 1985). There are two main groups of diatoms based on cell morphology: centric diatoms, which exhibit radial symmetry and pennate diatoms with bilateral symmetry. The diatom used for this thesis work, *Thalassiosira,* is a centric diatom and the focus of this overview will be on this group.

The silica frustule of a diatom consists of two parts; the epitheca and the hypotheca. These two pieces fit together like a petri dish with the smaller hypotheca fitting into the epitheca (Figure 3.1). During vegetative (asexual) reproduction the hypovalves of the two new cells are formed inside the frustule of the parent cell, with each one of the parent valves serving as epitheca for a new cell. Thus, one daughter cell is smaller than the parent cell and over successive cycles of mitotic division, the mean cell size of the population decreases. This size reduction can continue until some critical size threshold, or cardinal point,- usually around 1/3 of its maximal size (Drebes, 1977) - at which sexual reproduction is necessary to "reset" the population to the maximal cell size. At this time, cells differentiate into egg cells and spermatogonia via meiosis. Diatoms have a gametic life history in which the gametes are the only haploid stage (Figure 3.2). Once the critical cell size is reached, a variety of cues including temperature, day length, and nutrient changes may induce sexual reproduction. The timing of sexual reproduction varies by species and can either be synchronous, in which the majority of the population undergoes sexual reproduction at once, or asynchronuous in which a subset of the population becomes sexualized

over a longer time period. Fusion of the gametes results in the production of an auxospore, a specialized cell in which the larger frustule of the initial cell forms (Figure 3.2). Centric diatoms exhibit oogamous sexual reproduction in which a larger non-motile egg is fertilized by motile sperm. Pennate diatoms, on the other hand, are typically isogamous and sexual reproduction often involves parent cell pairing prior to fertilization.

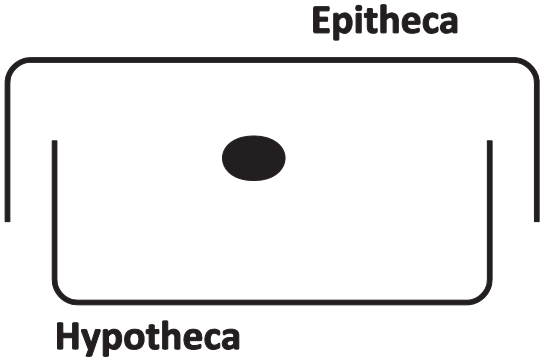

Figure 3.1 A basic centric diatom. The valve, or frustule, consists of two parts – the epitheca and hypotheca.

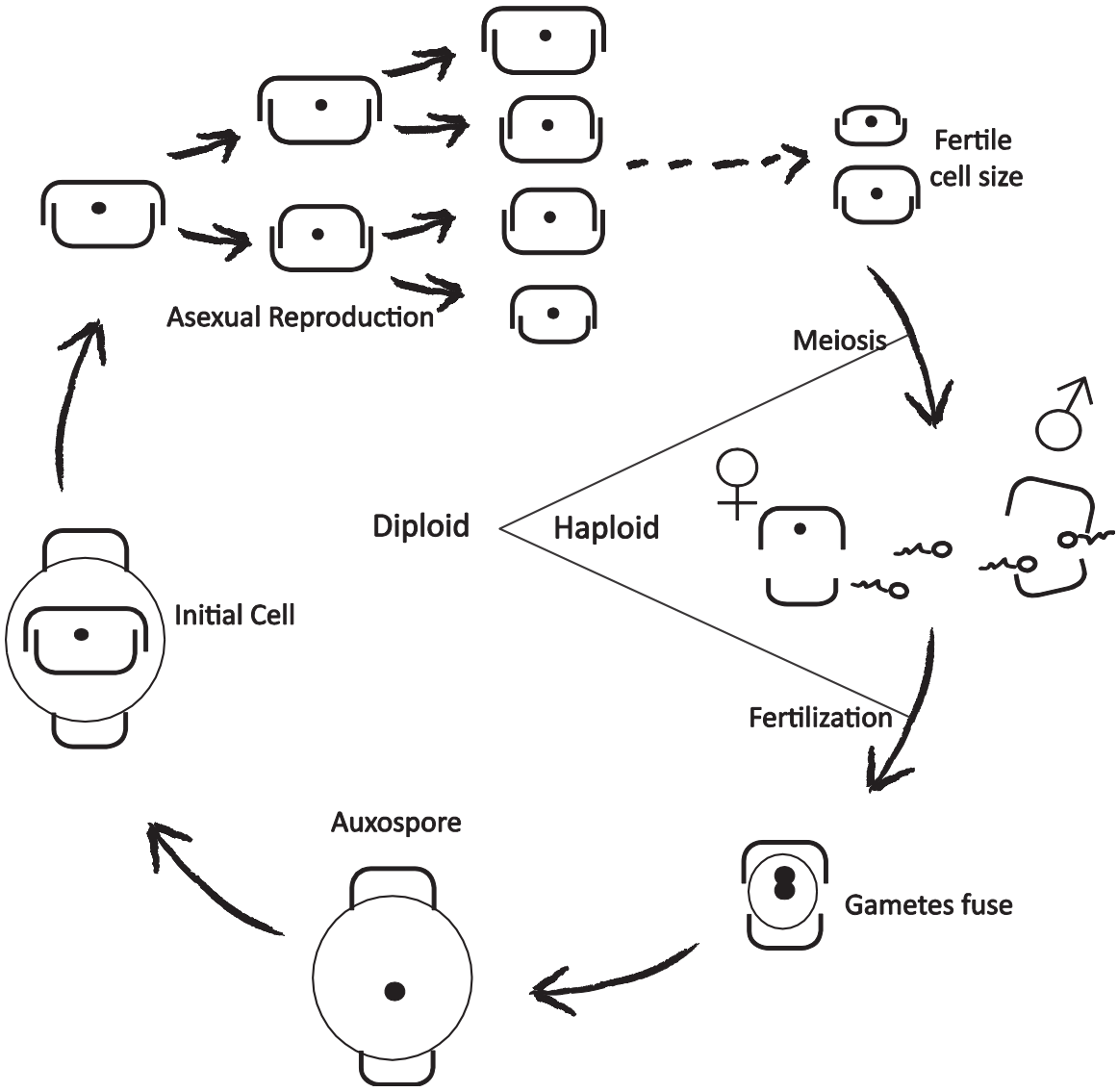

Figure 3.2 Life cycle of a typical centric diatom. (after Hasle and Syvertsen, 1996)

Although examination of *Thalassiosira* sexual reproduction was not the focus of my thesis, I feel its occurrence in my cultures is an important phenomenon to document, particularly because the cultures resulting from these reproductive events make up an important part of my research.

In general, sexual reproduction in my *Thalassiosira* isolate followed patterns previously described for other *Thalassiosira* spp. including *T. punctigera* (Chepurnov et al., 2004) and *T. weissflogii* (von Dassow et al., 2006). Besides reaching a certain critical size, the trigger that induced sexualization of our cultures remains unknown although it most often occurred within a day or so of transfer of an aliquot to fresh media. Reaching a cardinal size alone might be sufficient even without another external trigger as has been shown in *Ditylum brightwellii* (Koester et al., 2007). Other *Thalassiosira* spp. are known to require a dark period for induction of spermatogenesis (Armbrust et al., 1990; von Dassow et al., 2006) but it is unclear if this is the case for my isolate as well. The first evidence of sexual reproduction in my cultures of *Thalassiosira* sp. was observed in cultures with cell sizes of 20-24 μm (Figure 3.3). Cells that had differentiated into spermatogonangia produced flagellated sperm cells that were seen swimming erratically across the slide. Rarely, cells bent at the girdle were observed (Figure 3.3C). These cells may be oogonia, based on eggs described for other centrics including *T.weissflogii* (Chepurnov et al., 2004, von Dassow et al., 2006). It is also possible that they represent early auxospore formation. While Chepurnov et al. (2006) observed sperm only rarely in sexually reproductive cultures of *T. punctigera*, in our cultures, sperm were the most obvious sexual cells prior to auxospore development and were often observed without subsequent observation of auxospores or initial cells (discussed below). Developing auxospores, although compressed initially (Figure 3D), expanded roughly isometrically, and eventually formed globular auxospores as is characteristic of other radially centric diatoms (Medlin and Kaczmarska, 2004; Mills and Kaczmarska, 2006). Additionally, fertilized cells within a chain remained attached to sibling vegetative cells during early auxospore development as described for *T. angulata* (Mills and

Kaczmarska, 2006). Newly formed vegetative cells were 50-55 µm in size and had a slower growth rate as was discussed in Chapter 2.

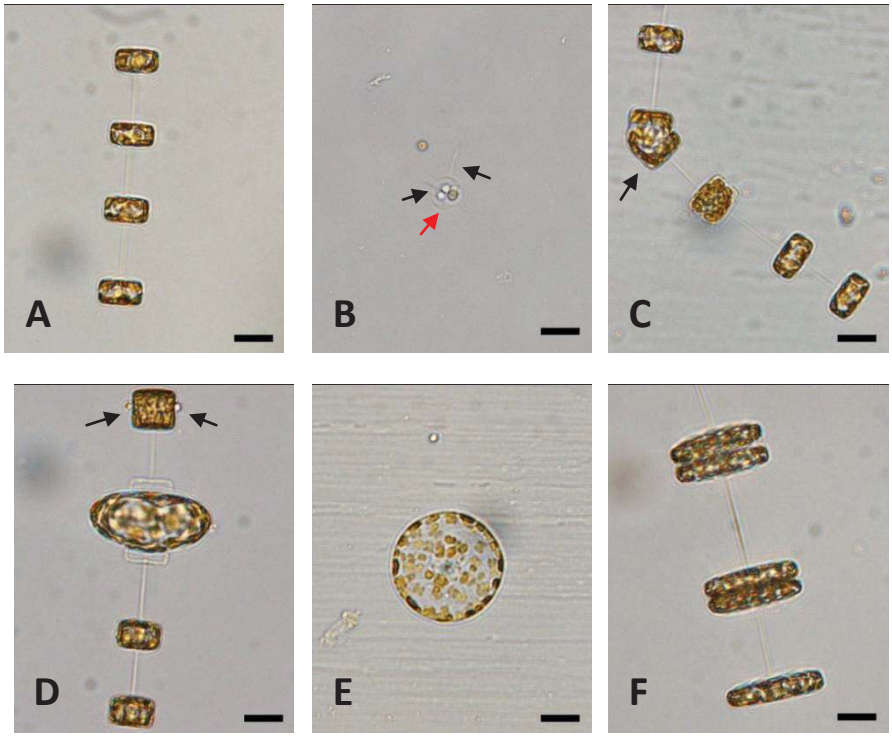

Figure 3.3 Micrographs of various stages of *Thalassiosira* sexual reproduction. **(A)** A chain of asexually reproducing cells near the cardinal size of ~20 µm. **(B)** Spermatocytes, with visible flagella (black arrows), within a frustule of a spermatogonial cell (red arrow). **(C)** An oogonium or developing auxospore (arrow). **(D)** A more advanced auxospore as well as sperm cells attaching to a possible (arrows). **(E)** Valve view of a newly formed and released initial cell. **(G)** Vegetatively dividing cells of the initial size. *Scale bars*, 20 µm.

Initiation of sexual reproduction in our *Thalassiosira* cultures did not always result in successful establishment of a population of maximally sized cells. Instead, sexual reproduction apparently failed in many cases, with auxospores aborting instead of developing into vegetative cells. As mentioned previously, actively swimming sperm cells were often observed in our cultures but no further sexual activity was observed. This has been previously observed in *T.*

weissflogii (Armbrust et al., 1990) and may be due to a lack of the correct cue for egg development if it differs from that used for induction of spermatogenesis. When this occurred, small cells continued to dominate the culture and reproduce vegetatively, further diminishing in size. This diminution seemed to continue until the cultures were no longer viable. Some diatoms, including other *Thalassiosira* spp. (e.g. *T. weissflogii*; von Dassow, 2006), are known to undergo asexual enlargement to increase cell size Although cells that enlarge this way do not typically reach the maximal cell size (Chepurnov et al., 2004; von Dassow et al., 2006). This process did not frequently occur in our cultures, although occasionally an intermediately sized cell or chain of cells was observed in cultures dominated by smaller cells.

Other than lack of a cue for induction of egg formation, failure of sexual reproduction in our cultures may have been promoted by frequent transfers necessitated by low nutrient concentrations in our growth media. It is likely that reproductive success is density dependent (Sarno et al., 2010). Dilution with each transfer would decrease the likelihood of a spermatocyte locating an egg because of lower cell densities, or perhaps even due to dilution of pheromones responsible for initiation of sex or egg location. Indeed, it is likely that diatoms utilize pheromones to communicate with conspecifics about sex as their brown algal relatives do (Pohnert and Boland, 2002), but evidence for such pheromones remains rudimentary. Recently, Sato et al. (2011) provided evidence for sex pheromones in the pennate diatom *Pseudostaurosira trainorii*, but such experimental evidence is lacking for centric diatoms.

As discussed in Chapter 2 of this thesis, although sexual reproduction is clearly an important component of the diatom life cycle, it remains understudied. Indeed, sexual reproduction has been examined in relatively few species of diatoms (Chepurnov, 2004) and we are only starting to delve into its molecular and genetic basis since the sequencing of the first diatom genomes (Armbrust et al., 2004; Bowler et al., 2008). Continued exploration of the

ecological consequences of diatom sexual reproduction including cell susceptibility to predation or parasitism and competitive interactions among species is also necessary.

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APPENDICES

APPENDIX A

Stephanopyxis turris as a Target of *Alexandrium fundyense* Allelochemicals

S. turris is another common Gulf of Maine diatom. Unlike *Thalassiosira,* which tends to proliferate during the early spring and later in the fall, *S. turris* is found at higher densities during the late summer. It is thus a possible target of allelochemicals released by *A. fundyense* blooms that are reaching their end later in the season.

Methods

To examine the existence of such an interaction, I performed an allelopathic test analogous to those performed using *Thalassiosira*. *S. turris*, also isolated from the Gulf of Maine in August 2010, and *A. fundyense* cultures were maintained as previously described (Chapter 2). Filtrate of a late-exponential phase *A. fundyense* culture and sterile GoM media controls were prepared as discussed in the Methods section of Chapter 2. The effects of the filtrate on *S. turris* were tested at full-strength filtrate (~1000 "cells"/ml) and with filtrate diluted with GoM media to a concentration corresponding to 350 *A. fundyense* "cells"/ml. *S. turris* was inoculated to a density of ~20 cells/ml into 1 L duplicate controls, full-strength filtrate, and diluted filtrate. The cultures were monitored for 5 days and samples for nutrient analysis and cell counts were collected daily.

Results

As with *Thalassiosira*, chemically mediated effects of *A. fundyense* on *S. turris* were dependent on filtrate concentration. Only in the full-strength filtrate treatments was *S. turris* growth inhibited and even then, it was only slightly inhibited. Unlike in experiments with *Thalassiosira*, there was no obvious period of time over which growth was entirely inhibited (Figure A.1). Growth inhibition, measured as in Chapter 2 as percent change in t_0-t_1 growth rate in the treatment relative to the control, was different in the two replicates. In one of the two, growth inhibition was 70% while in the other, growth rate was actually higher for the first day in

the treatment than in the associated control. Additionally, cells did not exhibit the bleaching response that was characteristic of *Thalassiosira* cultures exposed to high concentrations of *A. fundyense* filtrate.

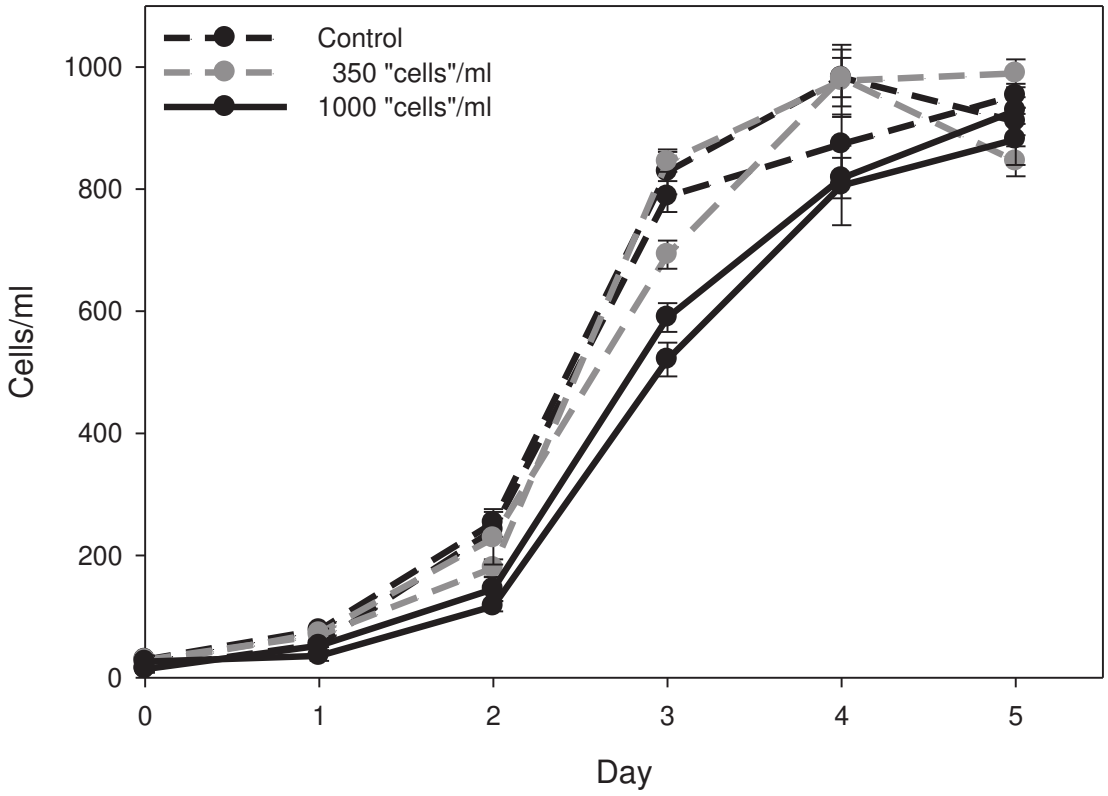

Figure A.1 *Stephanopyxis turris* cell concentrations as a function of time. Growth of *S. turris* cells exposed to higher concentrations of *A. fundyense* filtrate was slightly inhibited relative to both the control and a lower concentration of filtrate corresponding to 350 *A. fundyense* "cells"/ml.

Discussion

Stephanopyxis turris growth appeared to be relatively little affected by *A. fundyense* filtrate. Although the results are based on one experiment with duplicates of each treatment and control, it appears that the effect of the filtrate on *S. turris* is variable with one replicate showing stimulation of growth and the other showing inhibition. Cells of *S. turris* are quite large with an

average biovolume of 70252 μm^3 and an average surface area of 8204 μm^2. It is possible that the sheer size of this organism may protect it from toxic chemicals in the environment, at least at the concentrations tested here, as discussed in Chapter 2, but it cannot be ruled out that *S. turris* is not as susceptible as *Thalassiosira* due to some physiological difference.

Because these results are based on a single experiment with high variability, it is difficult to draw any firm conclusions about the role allelopathy may play in interactions between *A. fundyense* and *S. turris*. Given the relatively small impact of the filtrate and the high concentration of *A. fundyense* required (1000 cells/ml) to illicit this minimal response, it seems unlikely that *A. fundyense* can influence *S. turris* population growth to any major extent in the field through allelopathy. These results reveal the need for further studies examining *A. fundyense* allelopathy against other members of the Gulf of Maine phytoplankton community to fully understand its role, if any, in *Alexandrium* bloom dynamics.

APPENDIX B

Raw Data from Allelopathy Experiments

Table B.1 Raw data from initial filtrate experiments. *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented.

Treatment	[*A. fundyense* filtrate]	time (day)	count 1	count 2	count 3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
Control 17 –May-12	0	0	16	32	18	17.79	16.29	0.01	1.56
		1	111	72	103	17.38	16.33	0.01	1.53
		2	485	449	487	14.72	15.46	0.01	1.28
		3	2052	2180	2230	5.44	11.78	0.10	0.50
		4	5820	6130	6580	0.25	3.61	0.00	0.01
		5	8223	7600	7460	0.22	1.50	0.08	0.01
		6	8620	8590	8620	0.21	1.81	0.02	0.01
		7	8260	8110	7550	0.19	2.50	0.20	0.01
Filtrate	10	0	21	32	21	17.73	16.66	0.13	1.50
		1	110	85	93	17.13	16.47	0.01	1.54
		2	684	629	635	13.38	15.23	0.06	1.19
		3	3414	4450	3710	1.51	8.95	0.08	0.09
		4	6470	6850	6420	0.03	2.52	0.20	0.01
		5	9608	10630	8800	0.06	1.19	0.32	0.01
		6	9820	8290	8350	0.13	1.58	0.01	0.01
		7	9600	7750	8410	0.14	2.35	0.14	0.01
Filtrate	50	0	12	25	41	16.99	16.51	0.03	1.69
		1	50	43	82	16.71	16.62	0.01	1.61
		2	374	476	465	14.42	15.81	0.01	1.47
		3	2294	2324	2249	4.18	11.63	0.14	0.50
		4	6880	6360	5990	0.05	3.10	0.01	0.01
		5	8920	8250	9360	0.09	1.13	0.22	0.01
		6	8090	8130	8550	0.09	1.31	0.09	0.01
		7	8790	9770	8390	0.12	1.86	0.01	0.01
Filtrate	350	0	19	29	17	17.51	16.86	0.08	2.41
		1	13	37	18	17.43	16.90	0.01	2.39
		2	27	0	52	17.60	16.83	0.01	2.48
		3	30	18	30	17.31	16.49	0.12	2.38
		4	48	66	48	17.11	16.67	0.01	2.36

Table B.1 continued.

Treatment	[A. fundyense filtrate]	time (day)	count 1	count 2	count 3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
Filtrate	350	5	247	279	312	15.39	16.08	0.01	2.13
		6	2052	2140	1986	5.84	12.35	0.02	1.15
		7	10430	7240	6800	0.09	1.17	0.01	0.01
		8	8680	9460	10650	0.09	0.03	0.13	0.01
		9	9790	8670	10110	0.01	0.39	0.01	0.01
Control 22-July-12	0	0	18	21	26	21.16	19.05	0.47	3.29
		1	72	83	102	20.51	18.68	0.59	3.20
		2	749	711	712	17.71	17.81	0.25	2.86
		3	4003	2530	3090	9.34	14.35	0.45	1.92
		4	6660	5700	5040	0.08	7.29	0.23	0.60
		5	8810	8510	8200	0.09	3.43	0.33	0.20
		6	9380	9860	10210	0.09	3.02	0.29	0.02
		7	8500	9530	9480	0.09	3.61	0.62	0.01
		8	8120	8520	9170	0.09	4.20	0.84	0.03
Filtrate	50	0	16	33	21	22.48	18.86	0.66	3.35
		1	106	97	101	22.08	18.69	0.54	3.40
		2	890	935	896	19.59	17.76	0.42	3.02
		3	2658	3150	2060	9.48	13.88	0.22	1.78
		4	6800	5850	5810	0.10	6.24	0.47	0.37
		5	9410	8630	9370	0.09	2.33	0.41	0.08
		6	9460	8700	9280	0.09	2.21	0.46	0.06
		7	8660	9810	9030	0.09	2.51	0.64	0.01
		8	9290	10110	8960	0.09	3.30	0.40	0.03
Filtrate	150	0	11	21	24	22.06	20.00	0.42	3.48
		1	38	35	24	21.22	19.99	0.61	3.45
		2	114	94	127	20.91	19.78	0.41	3.47
		3	668	695	734	18.45	19.08	0.58	3.15
		4	2650	2950	2770	10.10	15.48	0.40	2.70
		5	6380	5830	6610	0.09	7.13	0.45	0.48
		6	9350	9030	8640	0.09	3.29	0.26	0.09
		7	9730	9770	8390	0.09	2.78	0.46	0.02
		8	9680	10030	9940	0.09	3.46	0.30	0.04

Table B.1 continued.

Treatment	[A. fundyense filtrate]	time (day)	count 1	count 2	count 3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
Filtrate	150	0	32	20	31	21.04	19.76	0.60	3.31
		1	38	40	23	19.33	19.91	0.84	3.37
		2	67	101	48	19.32	19.92	0.78	3.37
		3	308	341	327	18.36	19.54	0.27	3.52
		4	1400	1521	1372	13.12	17.53	0.43	2.63
		5	3390	4220	4170	3.93	13.21	0.28	1.42
		6	6320	6490	7000	0.00	3.57	0.63	0.18
		7	7620	8520	9200	0.09	1.79	0.52	0.01
		8	8790	9090	9380	0.09	2.32	0.48	0.01
		9	7240	7630	8170	0.09	2.73	0.22	0.01
Filtrate	350	0	31	16	26	23.66	20.68	0.63	3.90
		1	12	21	21	22.59	20.83	0.36	3.82
		2	23	21	20	22.90	20.75	0.43	3.78
		3	63	115	102	22.23	20.22	0.35	3.71
		4	488	449	616	21.12	20.12	0.13	3.60
		5	2186	2120	2170	14.27	17.65	0.43	2.71
		6	6020	6350	6090	0.70	10.75	0.42	0.77
		7	12980	11710	12150	0.09	1.56	0.25	0.03
		8	14190	12700	13310	0.09	1.25	0.29	0.09
		9	13120	13120	12540	0.09	1.80	0.84	0.01

Table B.2 Raw data from cell size experiment 1 (8-December-2011). *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts

Treatment	Thalas cell Size (µm)	[A. fundyense filtrate]	time (day)	1	2	3	NO3+NO2 (uM)	Si(OH)4 (uM)	NH4 (uM)	PO4 (uM)
C	50	0	0	15	15	23	16.77	16.62	0.22	2.92
			1	117	112	53	15.39	16.55	0.03	3.64
			2	383	461	428	10.15	14.11	0.01	2.02
			3	1790	1550	1730	2.49	10.31	0.01	1.34
			4	2610	2910	3450	0.09	1.96	0.07	0.20
F	50	350	0	29	35	27	16.77	16.01	0.20	3.60
			1	96	54	67	15.28	15.77	0.07	3.43
			2	546	568	462	10.36	13.71	0.01	2.24
			3	1835	1744	1905	2.15	8.64	0.12	1.19
			4	3310	3160	2790	0.01	1.72	0.00	0.04
F	50	1000	0	28	22	20	17.22	16.46	0.18	4.55
			1	24	27	56	17.09	16.61	0.01	4.32
			2	112	106	165	15.09	15.87	0.01	3.77
			3	646	751	674	9.11	14.23	0.18	4.35
			4	2410	2190	2490	0.84	5.31	0.01	0.71
C	20	0	0	16	15	21	16.81	16.38	0.62	3.40
			1	122	147	132	16.20	16.73	0.22	3.16
			2	930	883	979	11.70	14.92	0.01	2.42
			3	4450	4890	4930	2.53	14.25	0.01	1.43
			4	7740	8240	8580	0.01	2.59	0.02	0.08
C	20	0	0	26	28	24	16.88	16.97	0.70	3.55
			1	149	99	91	16.00	16.88	0.01	3.13
			2	950	982	1107	11.22	16.72	0.12	2.41
			3	4570	4480	4430	1.94	8.68	0.01	1.17
			4	7870	8040	8020	0.09	2.33	0.00	0.01
F	20	350	0	27	27	24	16.82	15.97	0.62	3.89
			1	136	96	162	16.20	16.06	0.01	3.37
			2	903	921	927	12.25	14.62	0.01	2.76
			3	4930	4390	4360	2.49	8.70	0.04	1.23

Table B.2 continued.

Treatment	Thalas cell Size (μm)	[A. fundyense filtrate]	time (day)	1	2	3	NO3+NO2 (uM)	Si(OH)4 (uM)	NH4 (uM)	PO4 (uM)
F	20	350	4	8060	9360	8440	0.09	2.50	0.00	0.01
F	20	350	0	23	15	18	16.70	15.97	0.60	3.86
			1	139	177	143	15.89	16.07	0.12	3.50
			2	1006	951	918	12.26	14.79	0.01	3.07
			3	5010	4680	4830	2.12	8.60	0.01	1.21
			4	9230	8850	8740	0.09	2.52	0.01	0.00
F	20	1000	0	36	24	23	17.30	16.72	0.16	4.65
			1	16	31	31	17.31	16.63	0.01	4.48
			2	105	58	71	17.11	16.52	0.01	4.19
			3	472	401	390	15.13	15.68	0.01	3.61
			4	2550	2480	2860	7.17	12.23	0.01	1.99
F	20	1000	0	22	15	24	17.26	16.61	0.16	4.71
			1	30	28	42	17.32	19.27	0.07	4.32
			2	125	83	36	17.11	16.41	0.01	4.20
			3	413	418	453	15.30	15.71	0.01	3.83
			4	2590	2520	2950	6.89	12.33	0.02	2.35

Table B.3 Raw data from cell size experiment 2 (13-December-2011). *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts.

Treatment	Thalas cell size (µm)	[*A. fundyense* filtrate]	time (day)	1	2	3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
C	50	0	0	21	34	30	22.21	17.02	0.44	3.12
			1	98	91	99	21.21	20.61	0.18	3.16
			2	476	414	451	12.05	14.53	0.21	2.40
			3	1539	1515	1541	3.14	8.53	0.25	1.15
			4	2910	2710	2680	0.09	2.91	0.25	0.34
F	50	350	0	29	28	17	17.28	17.52	0.36	3.26
			1	100	116	110	16.48	16.98	0.22	3.00
			2	493	582	543	8.52	14.58	0.33	2.41
			3	1814	1859	1755	1.47	8.65	0.27	1.14
			4	3120	3460	3150	0.09	2.88	0.31	0.23
F	50	1000	0	29	20	26	17.23	18.30	0.26	3.52
			1	40	50	35	16.10	18.17	0.15	3.36
			2	68	110	135	13.42	17.68	0.29	3.22
			3	461	529	549	8.90	15.16	0.25	2.42
			4	2160	2440	2180	1.34	7.72	0.33	0.99

Table B.4 Raw data from cell size experiment 3 (1-February-2012). *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts.

Treatment	Thalas cell Size (µm)	[*A. fundyense* filtrate]	time (day)	1	2	3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
C	50	0	0	22	24	21	18.54	17.28	0.80	3.13
			1	85	101	99	17.22	16.97	0.69	2.73
			2	549	509	526	12.09	14.97	0.90	2.11
			3	1829	1920	1660	3.54	8.65	0.87	0.99
			4	3950	3770	3530	0.40	2.66	1.03	0.18
F	50	350	0	14	10	25	17.30	17.14	0.88	3.17
			1	94	69	119	16.04	18.66	0.63	2.74
			2	599	469	572	10.85	14.63	0.71	2.13
			3	2080	2460	2070	3.10	8.34	0.93	1.04
			4	3390	3560	3200	0.30	2.90	0.51	0.08
F	50	1000	0	23	19	20	17.20	17.63	0.59	3.44
			1	49	35	48	17.21	17.62	0.45	3.14
			2	198	200	242	14.78	16.92	0.83	2.83
			3	995	924	901	8.00	13.27	0.87	1.84
			4	3080	3340	2990	1.13	5.73	0.44	0.56
C	30	0	0	36	34	28	18.55	16.97	0.75	3.08
			1	159	184	134	17.42	17.04	0.83	2.79
			2	1325	1299	1229	10.35	14.20	0.75	1.73
			3	5420	5620	5580	0.31	5.56	0.72	0.41
			4	7930	7790	8140	0.04	1.69	0.88	0.06
C	30	0	0	34	46	29	18.30	16.99	0.84	3.10
			1	197	188	140	17.31	17.00	0.76	2.85
			2	1284	1166	1223	10.81	14.30	0.74	1.86
			3	5110	5690	5330	0.54	6.82	0.84	1.23
			4	9400	7440	7750	0.25	1.63	0.71	0.01
F	30	350	0	14	17	7	17.46	17.03	0.76	3.20
			1	38	68	48	16.94	17.02	0.73	2.93
			2	461	405	337	14.40	16.36	0.93	2.48
			3	3020	2760	2600	3.95	10.98	0.68	1.14

Table B.4 continued.

Treatment	Thalas cell size (µm)	[*A. fundyense* filtrate]	time (day)	1	2	3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
F	30	350	4	7270	6490	6880	0.09	2.12	0.86	0.01
			0	43	25	42	17.20	17.00	0.90	3.14
			1	56	41	58	16.91	16.98	0.55	2.85
			2	532	551	441	13.79	16.19	0.97	2.24
			3	3130	2950	2570	3.50	10.08	0.72	1.09
			4	7450	6920	7140	0.16	2.48	0.93	0.01
F	30	1000	0	29	10	16	17.21	17.65	0.70	3.46
			1	33	28	35	17.24	17.70	0.73	3.19
			2	65	29	60	17.28	17.69	0.72	3.03
			3	197	138	258	16.10	17.25	0.74	2.83
			4	1330	1158	1520	9.64	14.84	0.93	2.01
F	30	1000	0	20	36	35	17.25	17.69	0.82	3.41
			1	38	22	15	17.37	17.75	0.85	3.32
			2	49	31	29	17.69	17.93	0.92	3.11
			3	131	80	87	17.06	17.65	0.78	3.00
			4	380	368	355	15.01	16.91	0.92	2.66

Table B.5 Raw data from cell size experiment 4 (29-February-2012). *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts.

Treatment	Thalascell size (µm)	[*A. fundyense* filtrate]	time (day)	1	2	3	NO3+NO2 (µM)	Si(OH)4 (µM)	NH4 (µM)	PO4 (µM)
C	30	0	0	33	33	40	20.53	18.75	0.44	2.72
			1	227	233	213	19.50	17.29	0.03	2.24
			2	1510	1354	1516	12.49	15.32	0.03	1.46
			3	5560	6550	4940	1.62	7.25	0.00	0.38
			4	8810	8710	8630	0.19	2.00	0.00	0.01
F	30	350	0	48	19	27	22.01	17.99	0.25	2.99
			1	152	118	163	20.80	22.39	0.03	2.35
			2	979	909	826	16.06	16.43	0.03	2.10
			3	4770	4870	5100	4.27	9.17	0.03	0.73
			4	8690	8320	9120	0.35	2.15	0.17	0.01
F	30	1000	0	33	40	33	18.71	22.37	0.13	3.49
			1	18	40	16	19.05	19.10	0.00	3.13
			2	46	45	44	19.10	19.38	0.13	3.16
			3	244	151	171	18.48	18.63	0.05	3.03
			4	1239	1095	1079	12.78	16.60	0.02	2.19

Table B.6 Raw data from biovolume-normalized experiment 1 (12-September-2012**).** *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts.

Treatment	**Thalass. cell Size (µm)**	**[*A. fundyense* filtrate]**	**time (day)**	**1**	**2**	**3**	**NO3+NO2 (µM)**	**Si(OH)4 (µM)**	**NH4 (µM)**	**PO4 (µM)**
C	50	0	0	48	54	28	17.64	16.18	0.49	2.3
			1	112	97	114	14.41	15.99	0.09	2.46
F	50	1000	0	31	31	42	17.58	16.31	0.44	2.63
			1	94	84	91	15.49	18.60	0.37	2.41
C	30	0	0	48	32	47	16.86	18.09	0.03	3.28
			1	120	120	146	15.99	17.15	0.34	3.11
F	30	1000	0	34	43	47	16.93	17.22	0.01	3.33
			1	69	70	52	16.40	17.18	0.23	3.19
C	50	0	0	19	12	15	17.53	16.46	1.19	2.87
			1	34	31	34	16.73	16.43	0.09	2.59
F	50	1000	0	14	20	11	16.87	17.22	1.30	3.48
			1	18	22	34	16.59	17.26	0.29	3.11
Cl	30	0	0	109	119	123	17.56	16.66	0.15	2.96
			1	389	412	378	13.06	16.02	0.39	2.21
F	30	1000	0	168	115	118	16.94	17.58	0.29	3.61
			1	261	275	313	14.87	17.11	0.00	3.04

Table B.7 Raw data from biovolume-normalized experiment 2 (27-September-2012). *A. fundyense* filtrate concentration (as corresponding concentration of cells/ml), raw counts, and nutrient data are presented. C= control, F=filtrate. 1,2,3 indicate triplicate cell counts

Treatment	**Thalass. cell Size (µm)**	**[*A. fundyense* filtrate]**	**time (day)**	**1**	**2**	**3**	**NO3+NO2 (µM)**	**Si(OH)4 (µM)**	**NH4 (µM)**	**PO4 (µM)**
C	50	0	0	31	38	43	17.29	16.80	0.84	3.27
			1	66	129	143	15.07	18.72	0.66	2.85
F	50	1000	0	42	27	29	17.38	17.61	0.66	3.91
			1	56	62	49	16.64	17.53	0.37	3.78
C	30	0	0	29	37	39	17.35	16.73	0.83	3.36
			1	100	111	106	16.50	16.75	0.45	2.93
F	30	1000	0	40	43	33	17.41	17.70	0.57	3.97
			1	53	44	32	17.20	17.65	0.44	3.64
C	50	0	0	11	13	14	17.28	16.95	0.87	3.38
			1	24	27	38	16.87	16.88	0.10	2.98
F	50	1000	0	9	13	6	17.36	17.66	0.65	3.95
			1	23	15	18	17.38	17.81	0.60	3.60
C	30	0	0	108	129	111	17.44	17.06	1.04	3.37
			1	364	322	302	14.60	16.67	0.19	2.77
F	30	1000	0	112	102	122	17.40	17.90	0.40	4.10
			1	152	177	139	17.11	18.18	0.12	3.56

APPENDIX C

Raw Cell Size Measurements

Table C.1 Cell measurements of 20-µm *Thalassiosira* in initial experiments. Cell dimensions measured using the measuring function on a Nikon DS Camera Control Unit DS-L2. Biovolume and surface area are calculated by $V = \pi/4 * d^2*h$ and $SA = \pi * d * (^d/_2 + h)$.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Initial Filtrate May and July 2011	19.1	12.31	3527.0748	1311.6966
	18.37	10.89	2886.2641	1158.5495
	19.1	9.7	2779.2547	1155.0851
	19.31	10.43	3054.4902	1218.4395
	19.13	15.07	4331.4477	1480.5307
	18.73	13.4	3692.0726	1339.5388
	19.46	8.82	2623.2769	1134.0615
	18.48	12.06	3234.753	1236.6063
	19.02	5.06	1437.6773	870.60255
	19.59	10.13	3053.291	1226.2602
	19.42	10.8	3198.9841	1251.3095
	18.78	12.87	3565.0006	1313.3202
	19.17	15.11	4361.1254	1487.2399
	19.05	10.33	2944.2871	1188.2689
	19.32	13.05	3825.7328	1378.3964
	18.54	9.26	2499.8867	1079.2822
	18.98	9.92	2806.6869	1157.3684
	19.06	14.61	4168.5584	1445.4732
	18.08	13.15	3376.0781	1260.3919
	18.06	11.07	2835.783	1140.417
	18.53	11.62	3133.6237	1215.7934
	18.73	12.53	3452.3634	1288.3463
	19.1	9.82	2813.6372	1162.2856
	18.31	14.72	3875.9162	1373.3512
	19.32	9.49	2782.0846	1162.3202
	18.79	12.26	3399.6477	1278.306
	19.99	11.76	3690.8194	1366.2235
	18.48	11.48	3079.1844	1202.9334
	19.18	11.78	3403.5519	1287.6653

Table C.1 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
	20.89	9.42	3228.6254	1303.6976
	19.95	7.84	2450.709	1116.5511
	20.83	12.37	4215.3935	1491.0362
	19.63	8.8	2663.2575	1147.9771
	19.51	7.74	2313.9042	1072.3118
	19.46	10.88	3235.9697	1260.0005
	18.79	8.32	2307.1019	1045.7258
	19.16	11.9	3431.0564	1292.9438
	18.9	9.48	2659.6337	1123.9896
	20.14	13.47	4291.1768	1489.4153
	18.88	9.04	2530.826	1096.1092
	18.98	11.86	3355.5753	1273.0456
	22.16	12.91	4979.1549	1670.1285
	24.22	13.5	6219.7357	1948.6489
	23.75	11.76	5209.8405	1763.4741
	25.55	14.11	7234.3365	2157.9969
	24.33	12.14	5644.0751	1857.7514
	24.21	13.27	6108.7223	1929.9707
	24.19	13.2	6066.4629	1922.2967
	24.17	13.1	6010.5536	1912.3548
	23.68	9.93	4373.2321	1619.5337
	23.33	10.75	4595.4477	1642.8706
	23.53	14.58	6340.0292	1947.4665
	23.74	14.72	6515.6706	1983.1197
	23.25	14.18	6020.2153	1884.8496
	23.01	11.85	4927.6683	1688.2873
	23.27	13.89	5907.2438	1866.0016
	22.49	14.62	5807.8603	1827.4765
	21.64	15.12	5561.0421	1763.5066
	23.22	13.02	5513.4736	1796.7039
	22.98	13.65	5661.3836	1814.9521
	23.21	15.19	6426.8466	1953.794
	23.93	15.7	7061.1418	2079.808
	21.94	15.51	5863.743	1825.1749
	21.82	15.51	5799.7753	1811.0792

Table C.1 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
	23.32	15.06	6432.3837	1957.5591
	22.71	14.64	5930.1438	1854.6281
	22.43	14.52	5737.3989	1813.4405
	22.86	17.39	7137.4309	2069.7604
	23.15	9.04	3805.0494	1499.285
	22.91	10.33	4258.3405	1567.9511
	22.79	11.46	4674.8012	1636.3471
	23.86	10.61	4744.016	1689.5624
	22.27	18.19	7085.3778	2051.6728
	21.74	8.98	3333.3836	1355.7198
	24.08	13.54	6166.2554	1935.1155
	23.6	15.3	6692.761	2009.237
	22.59	15.7	6292.4822	1915.7968
	23.32	11.55	4933.2027	1700.4096
	23.35	11.89	5091.4972	1728.6386
	23.47	14.61	6320.716	1942.5006
	23.31	8.51	3631.65	1476.6935
	23.07	13.82	5776.8769	1837.6427
	24.82	11.72	5670.496	1881.5206
	23.78	11.46	5089.7705	1744.4102
	23.79	7.93	3524.9418	1481.6905
	22.68	7.6	3070.3619	1349.5
	22.7	10.75	4350.6091	1576.0428
	22.4	12.03	4740.799	1634.734
	22.78	9.36	3814.8123	1484.9837
	22.94	11.96	4943.1967	1688.5564

Table C.2 Cell measurements of 20-µm *Thalassiosira*. Cell dimensions measured using the measuring function on a Nikon DS Camera Control Unit DS-L2. Biovolume and surface area are calculated by $V = \pi/4 * d^2*h$ and $SA = \pi * d * (^d/_2 + h)$.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 1	21.76	11.73	4362.2	1545.6
8-Dec-11	19.73	10.8	3301.9	1280.9
	22.57	15.01	6005.3	1864.5
	22.58	12	4805.3	1652.1
	22.27	11.83	4608.0	1606.7
	22.51	9.97	3967.7	1501.0
	21.86	15	5629.7	1780.7
	19.61	12.24	3696.8	1358.1
	19.55	15.03	4511.7	1523.5
	22.84	10.95	4486.4	1605.1
	23.46	13.25	5727.5	1841.1
	20.73	10.61	3581.0	1366.0
	20.91	17.32	5947.7	1824.6
	22.78	13.01	5302.4	1746.2
	21.75	10.38	3856.7	1452.3
	20.85	13.09	4469.3	1540.3
	22.57	11.9	4761.0	1643.9
	21.83	15.51	5805.1	1812.3
	23	10.36	4304.3	1579.5
	22.2	11.93	4617.8	1606.2
	24	11	4976.3	1734.2
	20.18	9.93	3176.0	1269.2
	23.2	10.01	4231.5	1575.0
	20.73	13.82	4664.4	1575.1
	23.79	16.42	7298.8	2116.2
	23.83	15.48	6904.1	2050.9
	22.31	14.8	5785.6	1819.2
	22.46	16.54	6553.1	1959.3
	21.02	10.73	3723.5	1402.6
	22.38	15.76	6199.6	1894.8
	21.78	13.11	4884.4	1642.2

Table C.2 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 1	20.67	15.27	5124.0	1662.7
8-Dec-11	21.86	12.8	4804.0	1629.7
	23.16	15.24	6420.2	1951.4
	22.8	18.63	7606.3	2151.0
	20.13	10.98	3494.5	1330.9
	20.02	15.43	4857.2	1600.0
	20.61	12.48	4163.5	1475.3
	23.99	13.88	6273.9	1950.1
	21.78	11.18	4165.3	1510.1
	20.94	16.48	5675.5	1772.9
	21.19	11.39	4016.8	1463.5
	25.94	10.45	5522.6	1908.6
	21.37	13.01	4666.3	1590.8
	25.55	11.1	5691.1	1916.4
	20.45	9.36	3074.3	1258.2
	22.21	12	4649.1	1612.1
	20.37	12.87	4194.2	1475.4
	21.22	17.11	6051.0	1847.9
	20.91	11.85	4069.3	1465.2
	22.2	8.65	3348.2	1377.4

Table C.3 Cell measurements of 30-µm *Thalassiosira*. Cell dimensions measured using the measuring function on a Nikon DS Camera Control Unit DS-L2. Biovolume and surface area are calculated by $V = \pi/4 * d^2 * h$ and $SA = \pi * d * (^d/_2 + h)$.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 3	28.18	12.86	8020.7	2385.9
1-Feb-12	25.73	13.91	7232.6	2164.3
	26.03	13.01	6923.3	2128.2
	28.26	15.43	9678.3	2624.4
	30.55	9.83	7205.5	2409.5
	27.47	8.74	5179.9	1939.6
	27.68	9.73	5855.1	2049.6
	24.91	17.28	8421.3	2327.0
	23.86	14.27	6380.5	1963.9
	30.22	11.51	8255.7	2527.3
	28.55	13.31	8520.8	2474.2
	30.98	14.26	10749.1	2895.5
	26.38	17.11	9351.7	2511.1
	27.72	10.45	6306.6	2117.0
	30.32	11.57	8353.8	2546.1
	24.26	11.24	5195.6	1781.1
	30.17	11.4	8149.8	2510.3
	26.47	15.24	8386.5	2367.9
	28.18	15.91	9923.0	2655.9
	26.38	14.09	7701.1	2260.8
	28.76	11.14	7236.9	2305.8
	29.54	11.29	7737.6	2418.4
	29.82	11.24	7850.0	2449.8
	30.27	18.72	13471.6	3219.5
	30.33	11.5	8308.7	2540.8
	28.78	18.55	12067.4	2978.3
	28.19	11.85	7396.0	2297.7
	29.71	17.75	12305.3	3043.2
	25.46	16.71	8507.1	2354.8
	26.95	12.13	6919.4	2167.9
	28.65	11.9	7671.6	2360.4
	27.55	16.01	9543.9	2577.9
	30.17	14.55	10401.7	2808.9
	30.03	15.83	11212.0	2910.0

Figure C.3 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 3	31.02	11.64	8796.8	2645.8
1-Feb-12	25.97	16.37	8671.3	2395.0
	30	12.93	9139.7	2632.3
	30.09	10.21	7260.4	2387.4
	28.66	11.18	7212.5	2296.9
	29.57	11.36	7801.4	2428.8
	30.88	12.23	9159.5	2684.3
	30.91	17.7	13281.9	3219.6
	30.91	8.38	6288.3	2314.5
	30.56	13.21	9689.5	2735.2
	28.22	16.91	10576.6	2750.1
	31.31	14.04	10809.9	2920.9
	29.82	11.47	8010.7	2471.3
	28.87	10.73	7024.0	2282.4
	29	13.91	9187.8	2588.3
	30.81	12.08	9006.2	2660.3
	25.35	15.11	7626.2	2212.8
Cell size 4	22.53	12.23	4875.7	1663.0
29-Feb-12	22.84	11.73	4806.0	1661.1
	28.11	10.46	6491.5	2164.9
	28.26	18.41	11547.5	2888.9
	28.95	16.18	10650.4	2788.0
	28.14	14.75	9173.4	2547.8
	27.02	13.91	7976.0	2327.6
	26.91	15.65	8900.9	2460.5
	24.36	10.54	4912.3	1738.7
	26	16.18	8590.4	2383.5
	26.46	15.48	8512.2	2386.6
	26.24	12.24	6619.1	2090.6
	28.46	12.66	8053.7	2404.2
	27.75	12.24	7402.8	2276.7
	28.14	15.24	9478.1	2591.1
	29.18	9.49	6346.4	2207.5
	29.64	12.64	8721.5	2557.0
	28.32	9.72	6122.7	2124.6
	29.51	15.51	10608.2	2805.8

Figure C.3 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 4	22.07	17.4	6656.5	1971.5
29-Feb-12	27.12	11.61	6706.6	2144.5
	24.96	17.02	8328.0	2313.2
	28.65	11.4	7349.3	2315.4
	26.27	16.42	8899.9	2439.2
	28.26	13.43	8423.8	2446.8
	27.36	13.51	7942.9	2337.1
	26.88	10.92	6196.9	2057.1
	27.76	12.86	7783.4	2332.0
	26.68	14.66	8195.9	2346.9
	28.31	10.91	6867.4	2229.2
	27.82	11.83	7191.0	2249.7
	24.56	10.09	4780.1	1726.0
	26.46	14.61	8033.8	2314.2
	26.47	15.33	8436.1	2375.4
	27.73	15.01	9065.1	2515.5
	28.18	13.85	8638.2	2473.5
	24.28	12.92	5982.0	1911.5
	29.82	14.16	9889.4	2723.3
	26.82	15.93	8999.6	2472.1
	26.69	14.27	7983.8	2315.5
	27.13	14.37	8307.0	2380.9
	27.06	11.36	6533.2	2115.9
	29.38	13.56	9192.9	2607.5
	28.24	13.31	8336.8	2433.6
	28	10.95	6742.5	2194.7
	26.91	14.66	8337.8	2376.8
	23.91	9.93	4458.6	1643.9
	23.29	15.05	6411.6	1953.2
	26.05	14.91	7946.6	2286.2
	28.31	16.91	10644.2	2762.9
	24.83	11.18	5413.6	1840.5
Biovolume 1	32.36	17.7	14557.3	3444.3
12-Sep-12	30.91	18.01	13514.6	3249.7
	31.5	12.23	9531.0	2768.9
	31.3	19.12	14711.8	3419.0

Figure C.3 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Biovolume 1	31.21	14.42	11031.7	2943.9
12-Sep-12	30.91	18.01	13514.6	3249.7
	32.14	16.39	13297.2	3277.5
	32.54	17.82	14819.5	3484.9
	33.18	12.63	10920.6	3045.8
	33.25	16.07	13953.7	3415.3
	34.79	17.68	16806.6	3833.6
	30.06	11.79	8367.2	2532.8
	31.83	13.64	10853.7	2955.4
	31.36	14.61	11284.8	2984.2
	31.41	13.76	10662.1	2907.5
	30.22	13.5	9683.1	2716.2
	35.04	11.29	10887.1	3171.4
	31.69	12.13	9567.4	2785.1
	31.18	15.48	11819.9	3043.5
	30.04	15.33	10865.1	2864.2
	30.53	12.87	9421.5	2698.5
	31.33	17.68	13629.9	3282.0
	30.95	13.82	10397.3	2848.4
	31.5	14.47	11276.6	2990.6
	30.91	15.27	11458.5	2983.6
	29.89	18.01	12637.3	3094.5
	33.54	10.99	9709.9	2925.0
	31.6	12.28	9630.8	2787.6
	29.82	12.93	9030.3	2608.1
	32.72	14.12	11872.7	3133.1
	29.63	16.36	11280.7	2901.9
	31.63	18.72	14709.4	3431.7
	31.6	18.32	14367.8	3387.2
	32.37	11.25	9258.2	2790.0
	30.88	12.54	9391.7	2714.4
	31.69	14.54	11468.3	3025.0
	33.25	12.03	10445.7	2993.2
	31.82	10.84	8620.2	2674.1
	31.52	17.7	13811.3	3313.3
	32.27	12.41	10149.8	2893.9

Figure C.3 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Biovolume 2	32.41	13.67	11277.6	3041.8
27-Sep-12	30.94	12.93	9721.4	2760.5
	30.46	14.16	10318.4	2812.4
	31.44	14.55	11295.8	2989.8
	30.79	17.28	12866.3	3160.6
	31.33	17.49	13483.4	3263.3
	31.1	11.82	8979.0	2674.1
	29.79	12.66	8824.0	2578.8
	31.19	17.37	13271.5	3230.1
	31.55	13.56	10601.0	2907.6
	30.09	16.06	11420.4	2940.4
	33.73	14.52	12974.5	3325.7
	31.52	14.27	11134.9	2973.7
	32	14.26	11468.6	3042.1
	31.18	14.37	10972.3	2934.7
	31.36	14.12	10906.3	2935.9
	31.83	15.03	11959.8	3094.4
	30.13	14	9982.0	2751.2
	29.61	13.12	9034.4	2597.7
	32.82	14.12	11945.4	3147.9
	30.48	13.21	9638.8	2724.3
	29.78	11.8	8219.0	2497.0
	30.46	16.42	11965.3	3028.7
	30.94	13.7	10300.3	2835.3
	30.55	15.55	11398.4	2958.4
	28.91	12.41	8146.3	2440.0
	33.18	16.22	14024.7	3420.1
	29.49	13.64	9316.5	2629.7
	29.91	14.67	10307.5	2783.7
	29.54	15.9	10897.0	2846.3
	31.34	13.62	10506.7	2883.8
	28.69	13	8404.2	2464.7
	29.14	13.19	8796.6	2541.3
	28.72	14.35	9296.3	2590.4
	30.32	15.28	11032.4	2899.5
	29.14	13.17	8783.2	2539.5

Figure C.3 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
	30.48	14.24	10390.3	2822.9
	30.64	14.26	10514.5	2847.3
	30.04	13.56	9610.6	2697.2
	30.12	14.35	10224.7	2782.9

Table C.4 Cell measurements of 50-µm *Thalassiosira*. Cell dimensions measured using the measuring function on a Nikon DS Camera Control Unit DS-L2. Biovolume and surface area are calculated by $V = \pi/4 * d^2 * h$ and $SA = \pi * d * (d/2 + h)$.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 1	48.82	10.64	19917.1	5375.7
8-Dec-11	46.09	9.59	16000.1	4725.4
	45.93	18.74	31049.3	6017.8
	46.15	13.91	23268.1	5362.3
	48.43	14.04	25863.4	5820.4
	48.2	15.03	27424.8	5925.3
	46.07	12.1	20170.3	5085.2
	45.54	16.54	26940.9	5624.0
	46.05	15.88	26448.4	5628.4
	47.35	12.41	21852.5	5367.8
	46.13	17.3	28913.6	5849.8
	45.83	18.23	30073.0	5924.0
	46.35	16.54	27907.8	5783.0
	46.05	16.72	27847.4	5749.9
	47.27	17.35	30448.1	6086.4
	48.77	19.09	35661.6	6661.0
	47.94	20.89	37707.2	6756.3
	45.85	15.86	26186.2	5586.7
	46.3	16.46	27712.9	5761.5
	45.46	8.36	13569.2	4440.2
	47.94	13.25	23916.7	5605.6
	47.72	13.21	23626.2	5557.4
	45.37	21.84	35308.6	6346.3
	47.24	12.8	22434.7	5405.0
	48.17	14.16	25805.2	5787.6
	47	21.67	37596.2	6669.6
	45.49	11.83	19226.8	4941.1
	47.03	13.88	24111.8	5525.1
	46.28	13.01	21885.4	5256.0
	46.14	14.03	23458.6	5377.8
	45.93	15.46	25614.9	5544.5
	45.96	13.21	21915.6	5225.4
	47.88	18.74	33741.8	6419.9

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 1	45.99	14.47	24037.3	5413.0
8-Dec-11	49.8	14.37	27990.2	6143.8
	46.61	12.92	22045.0	5304.4
	48.79	16.91	31615.1	6331.2
	49.82	11.57	22554.4	5709.6
	44.76	17.4	27379.1	5593.8
	48.91	15.91	29892.0	6202.3
	45	18.7	29741.1	5824.5
	46.95	20.73	35888.9	6520.1
	47.55	11.64	20670.2	5290.4
	45.51	16.54	26905.4	5618.2
	45.89	13.21	21848.9	5212.4
	50.42	15.46	30867.8	6442.1
	47.64	18.1	32263.5	6274.0
	44.9	15.55	24621.4	5360.2
	48.91	15.91	29892.0	6202.3
	44.54	11.83	18432.1	4771.5
	40.14	20.58	26042.9	5126.1
Cell size 2	49.16	13.64	25889.8	5902.7
13-Dec-12	48.48	17.35	32026.9	6334.3
	46.42	17.11	28956.8	5880.0
	47.63	17.68	31501.7	6209.1
	48.58	17.68	32770.8	6405.4
	48.09	14.57	26464.2	5833.9
	46.32	18.55	31258.7	6069.6
	49.02	19.26	36349.0	6740.6
	47.82	14.37	25808.7	5750.8
	49.19	21.82	41466.6	7172.7
	46.56	20.18	34358.7	6357.0
	45.97	11.93	19800.6	5042.4
	47.83	17.87	32108.1	6278.7
	47.56	11.25	19986.0	5234.0
	46.63	18.32	31285.7	6099.2
	47.55	12.28	21806.7	5386.0
	48.42	12.92	23790.4	5648.1

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 2	46.02	15.43	25665.5	5557.5
13-Dec-12	46.01	16.39	27250.4	5694.3
	45.36	17.82	28796.8	5771.4
	45.18	11.9	19077.8	4895.4
	46.74	16.11	27641.6	5797.2
	44.99	15.86	25213.0	5421.1
	47	18.46	32027.1	6195.6
	49.49	14.2	27315.8	6055.1
	45.77	13.82	22738.4	5277.8
	46.26	17.15	28824.7	5853.9
	48.63	16.67	30962.4	6261.5
	46.6	17.11	29181.8	5916.0
	46.15	21.71	36315.6	6493.1
	44.54	14.12	22000.1	5091.9
	45.82	12.93	21320.6	5159.1
	48.7	15.13	28183.0	6040.3
	47.31	12.76	22430.9	5412.3
	45.83	12.66	20884.5	5122.1
	47.63	17.31	30842.4	6153.7
	44.82	14.35	22640.5	5176.0
	47.64	19.94	35543.4	6549.4
	47.29	13.25	23272.6	5481.3
	47.97	16.14	29169.8	6046.9
	46.07	13	21670.5	5215.5
	45.29	16.01	25792.0	5499.9
	47.18	15.51	27115.5	5795.4
	46.19	16.24	27212.7	5707.9
	47.77	15.28	27385.7	5877.6
	47.27	15	26324.0	5737.4
	47.24	17.82	31233.3	6150.1
	48.35	16.07	29505.2	6113.1
	49.03	15.03	28377.4	6091.2
	47.87	19.68	35419.5	6559.2
	48.25	16.07	29383.3	6092.8
Cell size 3				
1-Feb-12	42.25	13.51	18940.8	4597.2

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 3	44.77	17.04	26824.6	5545.1
1-Feb-12	43.12	18.92	27629.2	5483.6
	47.03	17.73	30799.8	6093.9
	43.84	18.55	28001.1	5573.8
	43.42	11.69	17309.5	4556.0
	41.02	10.54	13929.1	4001.4
	43.87	12.57	19000.3	4755.5
	41.28	10.46	13999.1	4033.2
	41.85	12.36	17002.0	4376.2
	41.72	14.12	19302.5	4584.7
	41.07	12.83	16996.8	4304.9
	43.78	14.75	22204.1	5039.4
	44.12	16.91	25852.6	5401.5
	44.65	14.64	22923.1	5185.2
	42.99	16.77	24342.1	5168.0
	43.34	14.89	21966.6	4977.9
	41.46	12.87	17375.1	4376.4
	43.29	16.82	24756.6	5231.2
	43.5	12.08	17952.9	4623.2
	42.64	14.37	20520.2	4780.9
	45.37	13.51	21841.5	5159.0
	42.6	18	25655.6	5259.6
	42.74	21.45	30774.2	5749.5
	43.73	18.01	27049.7	5478.1
	45.05	15.01	23925.5	5312.3
	44.28	16.18	24916.3	5330.7
	43.53	15.56	23156.7	5104.3
	41.55	19.73	26752.2	5287.2
	43.5	18.01	26765.9	5433.6
	44.01	14.24	21662.2	5011.3
	43.05	16.22	23609.5	5104.8
	43.01	17.42	25309.1	5259.5
	48.65	19.72	36657.5	6731.8
	43.77	11.44	17213.5	4582.4
	43.07	12.64	18415.6	4624.2

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Cell size 3	44.52	17.4	27086.3	5547.0
1-Feb-12	42.83	10.7	15415.9	4321.2
	42.08	20.86	29010.5	5539.1
	49.01	17.73	33447.8	6502.9
	42.51	15.86	22510.0	4956.7
	43.1	16.87	24612.7	5202.2
	45.46	12.28	19931.8	5000.0
	43.23	13.01	19095.8	4702.5
	45	17.3	27514.5	5626.6
	43.41	14.95	22126.4	4998.9
	41.25	17.56	23467.3	4948.4
	43.41	14.95	22126.4	4998.9
	44.85	10.98	17346.7	4706.8
	42.35	19.11	26918.9	5359.8
	44.42	13.21	20471.5	4942.8
Biovolume 1	52.18	16.91	36161.1	7048.9
12-Sep-12	51.92	18.54	39252.6	7258.5
	52.39	20	43113.8	7603.1
	51.46	19.28	40099.3	7276.6
	50.7	14.24	28748.5	6305.8
	51.75	17.82	37481.6	7103.8
	53.07	19.12	42293.7	7611.8
	52.71	16.01	34935.5	7015.4
	50.69	13.5	27243.8	6186.0
	53.74	20.73	47020.2	8036.3
	50.79	12.28	24879.7	6011.5
	50.92	20.27	41278.2	7315.4
	50.68	16.53	33345.4	6666.4
	51.37	15.7	32539.3	6678.9
	50.91	12.35	25139.9	6046.5
	51.44	12.54	26060.9	6183.0
	55.52	17.73	42923.7	7934.4
	52.27	13.64	29269.1	6531.5
	50.91	16.82	34239.1	6761.4

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Biovolume 1	52.67	19.29	42029.0	7549.5
12-Sep-12	53.93	14.89	34013.0	7091.3
	54.5	15.24	35552.3	7275.0
	52.58	17.82	38693.6	7286.3
	50.51	15.38	30817.8	6448.0
	50.55	18.66	37449.3	6977.2
	53.29	17.93	39990.9	7462.5
	50.46	15.62	31236.7	6475.7
	50.39	10.54	21019.3	5657.0
	53.56	18.03	40622.5	7539.9
	50.06	14.12	27791.1	6157.0
	53.35	15.53	34716.0	7073.7
	52.08	20.55	43776.8	7622.8
	53.49	22.93	51527.5	8347.6
	53.04	16.76	37031.5	7211.7
	53.11	19.34	42844.9	7657.6
	47.78	19.56	35071.2	6522.1
	52.03	19.29	41013.8	7405.4
	53.18	18.32	40692.3	7503.1
	53.68	16.67	37726.8	7337.6
	50.81	11.65	23621.9	5914.9
	50.19	18.55	36700.2	6881.8
	52.5	12.8	27708.8	6440.7
	52.36	17.37	37401.5	7163.7
	53.25	13.27	29552.9	6674.0
	53.66	20.46	46269.7	7972.0
	52.47	17.08	36931.7	7140.0
	51.7	17.02	35729.8	6963.0
	50.06	12.1	23815.3	5839.4
	53.79	19.82	45039.8	7894.2
	50.35	17.91	35660.3	6815.2
Biovolume 2	49.82	16.72	32593.7	6515.7
27-Sep-12	51.6	15.19	31764.9	6644.7
	49	13.56	25570.6	5858.9

Table C.4 continued.

Experiment	Valve Diameter, d (µm)	Pervalvar length, h (µm)	Biovolume (μm^3)	Surface Area (μm^2)
Biovolume 2	51.11	15.78	32374.9	6637.0
27-Sep-12	51.36	15.93	33003.2	6713.9
	52.15	15.62	33364.1	6831.1
	49.54	21.37	41191.4	7181.0
	51.91	17.82	37713.7	7138.8
	50.24	17.37	34434.1	6706.4
	52.2	16.24	34755.0	6943.4
	53.56	15.67	35305.3	7142.8
	52.47	16.71	36131.7	7079.0
	52.77	15.62	34162.1	6963.7
	50.48	17.27	34563.8	6741.6
	50.51	15.9	31859.7	6530.6
	49.51	21.85	42065.6	7249.0
	52.38	15.01	32344.6	6779.7
	52.17	15.24	32577.4	6773.0
	50.05	17.28	33997.1	6651.9
	52.76	16.06	35111.1	7034.4
	48.66	18.09	33641.3	6484.7
	51.14	16.27	33419.4	6722.1
	51.11	19.14	39268.4	7176.5
	51.23	16.77	34567.8	6821.6
	51.81	17.08	36008.5	6996.5
	52.23	16.12	34537.8	6930.1
	47.69	17.31	30920.2	6165.9
	51.04	19.03	38935.9	7143.5
	51.89	15.01	31742.3	6676.4
	50.19	17.68	34978.9	6744.6
	50.63	16.14	32494.5	6593.8
	51.17	15.78	32451.0	6649.6
	50.78	17.4	35239.1	6826.3
	49.96	17.13	33580.9	6609.3
	48.95	15.33	28849.4	6121.3
	49.5	16.76	32253.3	6455.2
	52.32	15.46	33238.0	6841.0
	51.23	16.06	33104.3	6707.3
	51.8	16.91	35636.3	6966.7
	51.27	18.96	39143.1	7182.9

BIOGRAPHY OF THE AUTHOR

Emily Lyczkowski was born in Poughkeepsie, NY on May 30, 1986. She was raised in Millbrook 2004. She attended Colby College and received a Bachelor's degree in Biology with a minor in Chemistry in 2008. Following graduation she worked as a sea-going research technician at Bigelow Laboratory for Ocean Sciences in Boothbay Harbor, ME. She entered the School of Marine Sciences graduate program in Oceanography at The University of Maine in the fall of 2010. She is a student member of the American Association for the Advancement of Science and the Association for the Sciences of Limnology and Oceanography. She is a candidate for the Master of Science degree in Oceanography from The University of Maine in December, 2012.