THE ROLE OF ALLELOPATHY FOR HARMFUL ALGAE BLOOM FORMATION

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Abstract: Strong evidence has accumulated on the last years that some algal species are able to kill not only their grazers but also other algal species, a process called allelopathy. Killing the nutrient-competing phytoplankton species enable these species to freely utilize limiting resources such as nitrogen and phosphorus. While for some algal species, like e.g. the flagellate *Prymnesium sp.,* the allelochemicals seem to be the same substances as their toxins, for some other algal species they are not. *Alexandrium* spp. are among the latter case: their internal toxins (such as saxitoxins) are not able to inhibit the growth of other algal species. However, these species by producing other substances than their internal toxins also cause allelopathic effects. Emphasis is placed here on the flagellate species *Prymnesium parvum*; which is not only able of allelopathy but mixotrophy as well. Mixotrophy, i.e. the capability to ingest bacteria, other algae and even potential grazers, also contributes to the bloom-forming ability of *Prymnesium* spp. Allelopathy, mixotrophy and grazer deterrence increase dramatically when *Prymnesium* spp. cells are grown under N or P deficiency, and so does toxicity, but decrease in intensity or cease completely if cells are grown with high amounts of N and P in balanced proportions. *Prymnesium* filtrates from nutrient deficient cultures have almost the same strong effect on grazers and other plankton cells as *Prymnesium* cells grown together with their target. It seems that toxin production in *Prymnesium* spp. works not only as a defense mechanism, but also, by killing competitors, improve the algae competitive ability under conditions of severe nutrient depletion. We

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can assume thus that a consequence of the increased input of N and P to aquatic ecosystems is provoking an unbalanced nutrient situation for *Prymnesium* spp., as well as many of the other HAB species producing toxins, to growth but ideal to produce toxins instead.

Keywords: HABs, allelopathy, phytoplankton, nutrients, toxins

1. Defining Allelopathy

Harmful effects of plants on other plants or crops dates back at least 2 millennnia, while experimental work, testing allelopathy, started in the late eighteenth century (Willis, 1985). Phytoplankton allelopathy was probably first observed in 1917 by Harder (Inderjit and Dakshini, 1994) when he reported auto-inhibition in the freshwater cyanobacteria *Nostoc punctiforme*. In 1937 the term allelopathy was coined by Molisch (Rizvi et al., 1992) to describe both detrimental and beneficial biochemical interaction between all classes of plants (including microorganisms). The term has evolved since then, including and excluding organisms. We propose to use a definition of allelopathy that is based on Rice's concept (Rice, 1984), but updating the definition along with the taxonomic classification to: "any direct or indirect, harmful or beneficial effect by plants, protists (e.g. microalga, ciliates), bacteria, or viruses on another trough the production of chemical compounds that leak into the environment". Keating (1999) has also suggested using the term allelochemistry, which we believe is more appropriate, since allelopathy evokes only negative effects (pathos = to suffer). In this paper we use the term allelopathy in the context of the negative/positive effect of allelochemicals produced by certain algae on other algal groups/species; i.e. grazer deterrence and or predator avoidance are not discussed. We consider allelopathy as competition strategy, while grazer deterrence and or predator avoidance are toxic effects.

2. Allelopathy in Aquatic Environments

Although phytoplankton allelopathy was observed nearly a 100 years ago by Harder (Inderjit and Dakshini, 1994), only in recent years research on the subject has gained momento (Fistarol et al., 2003; Granéli and Johansson, 2003; Gross, 2003; Legrand et al., 2003; Skovgaard et al., 2003; Fistarol et al., 2004a, 2004b; Suikkanen et al., 2004; Kubanek et al., 2005; Granéli and Pavia, 2006; Granéli and Hansen, 2006; Tillmann et al., 2007; Granéli and Weberg, 2008). It seems that the main reason for the increase in the

interest on phytoplankton allelopathy isbecause most of the phytoplankton species producing allelochemicals are harmful to the marine ecosystems where they occur.

Harmful algal blooms (HABs) have increased worldwide in waters ranging from fresh to coastal estuarine and marine waters (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993; Van Dolah, 2000), causing enormous impacts in the aquatic ecosystems they occur (Maestrini and Granéli, 1981; Granéli and Hansen, 2006). Fish kills are usually the first direct effect seen from the impact of such blooms. The most deleterious impacts occur when they affect entire ecosystems, causing death of phytoplankton, zooplankton seaweeds, and shellfish. The toxins produced by such blooms are secondary metabolites released in the water with capability to punctuate holes in the cells membranes of the target. Blooms caused by high discharges of nitrogen and phosphorus to the coastal areas, which produces high algal biomass that when decomposing causes oxygen depletion in the water column and mostly in the bottom waters, also cause devastating consequences to the benthic community (Allen et al., 2006). However, the difference between these two types of bloom affecting the aquatic ecosystems is that, in the first case, the cause of the deleterious effect on the flora and fauna is the result of a direct action of the chemicals liberated by the HA-species into the water on the other organisms, and not oxygen deficiency provoked by the decomposing bloom. It seems logical to assume that the effect on the entire ecosystem from the action of released chemicals is a secondary effect, while the main purpose of these chemicals would be to kill/inhibit the growth of the other phytoplankton species competing with the HA-species (allelopathy) and also to decrease losses by killing their grazers (grazer deterrence).

Both laboratory and filed work have demonstrated allelopathic effects of several phytoplankton groups. Here we are going to discuss some of the most important findings of the latest works, emphasizing the results of our group, and also the allelopathic effect of the flagellate species *Prymnesium parvum* which is not only able of allelopathy but mixotrophy as well.

3. Testing Allelopathy in Aquatic Systems

To mimic natural conditions when testing phytoplankton allelopathy, an important detail that should be considered is the amount of allelochemicals to which the target species will be exposed. This can be done by having the allelopathic species at natural cell concentrations, and by exposing the target organisms to a continuous addition allelochemicals. Experiments involving just a single addition of allelopathic filtrate to a target species have demonstrated that the allelopathic effect may be lost some time after the exposure, either due to the degradation of the chemicals by light or bacteria,

or the recovery of the target species (Gleason and Paulson, 1984; Windust et al., 1997; Suikkanen et al., 2004; Fistarol et al., 2005). Several studies demonstrated that the toxins produced by some microalgae (e.g. prymnesins produced by *Prymnesium parvum* and nodularin produced by *Nodularia*) can be inactivated, for example by light through photodynamic and/or photooxidative processes, and are sensitive to temperature (Reich and Parnas, 1962; Twist and Codd, 1997; Kvernstuen, 1993 *cited in* Larsen and Bryant, 1998; Fistarol et al., 2003). Some toxins can also be degraded by bacteria (e.g. microcystin, Christoffersen et al., 2002, Hagström et al., 2007). The same degradation processes could occur to allelochemicals. Allelochemicals may also be removed from the system by, for example, binding to cell membranes (Tillmann, 2003). All these results indicate that allelochemicals are not persistent, and thus, one filtrate addition may not be representative of natural conditions. Under natural conditions, allelochemicals are presumably constantly released to the environment. Therefore, experiments using repeated filtrate additions probably give a better representation of natural conditions. In most studies on phytoplankton allelopathy, only one filtration addition is usually employed, and thus the allelopathic effect may have been underestimated. Repeated filtrate additions demonstrate an increase in the allelopathic effect compared to one filtrate addition (Suikkanen et al., 2004; Fistarol et al., 2005).

4. Allelochemicals – Which Substances are they?

The allelochemicals are for the great majority of the algal species unknown, however some allelochemicals have been identified (see Table 1) (Granéli and Weberg, in press). The strongest allelochemicals however, have haemolytic capacity, perforating holes on the cell membranes of other algal species, as in the case of *C. polylepis* and *P. parvum* (Johansson and Graneli, 1999a, 1999b; Schmidt and Hansen, 2001; Fistarol et al., 2003; Fistarol et al., 2004a). As a consequence, there are associated fish-kills, as the delicate gills cell membranes are affected as well by the action of the haemolytic compounds (Igarashi et al., 1995, 1999). The majority of the allelochemicals however, have a mild mode of action, as e.g. inhibition of photosynthesis and growth (Legrand et al., 2003). Studies that have tested if some known algal toxins (e.g. okadaic acid –OA, paralitic shelfish posion – PSPs- toxins, nodularin) caused allelopathic effects have shown a negative result, i.e. the algal toxins were not the compounds causing allelopathy (Sugg and Van Dolah, 1999; Tillmann and John, 2002; Fistarol et al., 2003; Suikkanen et al., 2004, 2006).

TABLE 1. Allelopathic harmful algae species, their allelochemicals and allelopathic effect.

Species	Allelochemicals[a]	Reference
Bacillariophyceae		
Pseudo-nitzschia pungens	U	(Legrand et al., 2003)
Skeletonema costatum	U	(Yamasaki et al., 2007)
Coscinodiscophyceae		
Rhizosolenia alata	U	(Legrand et al., 2003)
Cyanophyceae		
Anabaena sp.	U	(Suikkanen et al., 2005)
A. cylindrica	EP	(Legrand et al., 2003)
A. flos-aquae	HX, A, M, U	(Murphy et al., 1976; Kearns and Hunter, 2001; Legrand et al., 2003)
A. lemmermannii	U	(Suikkanen et al., 2004)
Aphanizomenon sp.	U	(Suikkanen et al., 2005)
A. flos-aquae	U	(de Figueiredo et al., 2004; Suikkanen et al., 2004; Suikkanen et al., 2006)
A. gracile	U	(Legrand et al., 2003)
Cylindrospermopsis raciborskii	U	(Figueredo et al., 2007)
Gomphosphaeria aponina	U	(Legrand et al., 2003)
Hapalosiphon fontinalis	Hapalindole A	(Moore et al., 1984)
Fischerella sp.	U	(Bagchi and Marwah, 1994)
Fischerella muscicola	Fischerellin	(Gross et al., 1991; Legrand et al., 2003)
Microcystis sp.	U, Microcystin	(Sukenik et al., 2002; Vardi et al., 2002)
Nodularia spumigena	U	(Suikkanen et al., 2004; 2005; Suikkanen et al., 2006)
Nostoc sp.	U	(Schagerl et al., 2002; Legrand et al., 2003)
Nostoc spongiaeforme	Nostocine A	(Hirata et al., 1996; Hirata et al., 2003)
Oscillatoria sp.	FA	(Chauhan et al., 1992)
Oscillatoria spp.	U	(Legrand et al., 2003)
Oscillatoria laetevirens	U	(Ray and Bagchi, 2001)
Dinophyceae		
Alexandrium catenella	U	(Arzul et al., 1999)
A. minutum	U	(Arzul et al., 1999; Fistarol et al., 2004b)

(Continued)

TABLE 1. *(Cont.)*

Species	Allelochemicals[a]	Reference
A. ostenfeldii	U	(Tillmann et al., 2007)
A. tamarense	U	(Arzul et al., 1999; Fistarol et al., 2004a; Fistarol et al., 2004b; Wang et al., 2006)
Amphidinium klebsii	U	(Sugg and VanDolah, 1999)
Ceratium sp.	U	(Legrand et al., 2003)
Coolia monotis	U	(Sugg and VanDolah, 1999; Legrand et al., 2003)
Gambierdiscus toxicus	U	(Sugg and VanDolah, 1999)
Karenia brevis (Gymnodinium breve)	U	(Kubanek et al., 2005)
K. mikimotoi (Gymnodinium mikimotoi)	U	(Uchida et al., 1999; Fistarol et al., 2004a)
Ostreopsis lenticularis	U	(Sugg and VanDolah, 1999)
Peridinium aciculiferum	U	(Rengefors and Legrand, 2001)
Prorocentrum lima	U	(Sugg and VanDolah, 1999)
Prymnesiophyceae		
Chrysochromulina polylepis	U	(Myklestad et al., 1995; Schmidt and Hansen, 2001; Fistarol et al., 2004a)
Phaeocystis pouchetii	U, PUA	(Hansen et al., 2004; Hansen and Eilertsen, 2007; van Rijssel et al., 2007)
Prymnesium parvum	U, Prymnesin	(Igarashi et al., 1998; Fistarol et al., 2003; Granéli and Johansson, 2003; Barreiro et al., 2005; Fistarol et al., 2005)
Raphidophyceae		
Chattonella antiqua	U	(Matsuyama et al., 2000 cited in Gross, 2003)
Heterosigma akashiwo	U	(Matsuyama et al., 2000 (cited in Gross, 2003; Pratt, 1966; Yamasaki et al., 2007)

a) A anatoxin, EP extracellular peptides, F fatty acids, HX hydroxamates chelators, M microcystin, OA okadaic acid, PUA polyunsaturated aldehyde, U unknown. Source: Granéli and Weberg, (in press)

5. Abiotic and Biotic Factors Regulating Allelopathy

Many abiotic and biotic factors have been investigated in the context of toxin production, however, as allelopathy is a relatively new topic of research in

aquatic ecosystems, fewer studies and information exists. There are to our knowledge only a handful of published studies showing how the production of an allelochemical is influenced by abiotic or biotic factors. This is no surprise since most allelochemicals have not yet been isolated and structurally determined (Table 1) (Granéli and Weberg, in press). Furthermore, potentially allelopathic compounds have been characterized from intracellular extracts but these cannot be regarded as a allelochemicals until a mode of release into the surrounding environment and a correlation to allelopathic effect have been shown (Willis, 1985). However, some work on factors affecting allelopathic effects rather than quantifying the produced allelochemicals has been done and are presented.

5.1. INFLUENCE OF ABIOTIC FACTORS

5.1.1. *Light*

Allelopathic compounds released by some phytoplankton species seem to be effective only in a relative short time period. Cell-free filtrates of *Prymnesium parvum* added to cultures of *Thalassiosira weissflogii*, *Rhodomonas cf. baltica* and *Prorocentrum minimum* had a great negative impact on cell numbers, but within a few days the exposed species' began to recover (Graneli and Johansson, 2003; Suikkanen et al., 2004; Fistarol et al., 2005). However, when exposed to repeated additions of cell-free filtrate no recovery was possible (Fistarol et al., 2003; Suikkanen et al., 2004; Fistarol et al., 2005). Similar findings were observed for the dinoflagellate *Scrippsiella trochoidea* which managed to recover from *Alexandrium ostenfeldii* exudates (Tillmann et al., 2007). These findings suggest that one or several mechanisms reduce the allelopathic effect. Exposure from UV light at 255 nm and visible light between 400 and <520 nm completely inactivated extracellular ichthyotoxins from *P. parvum* within 90 min (Parnas et al., 1962). Another prymnesiophyte, *Phaeocystis pouchetii*, enhanced its haemolytic activity when incubated at higher light intensities (van Rijssel et al., 2007), thus showing a positive stimulation from light.

5.1.2. *Temperature*

Not much is known how changes in temperature affect phytoplankton allelopathy. An example is that the haemolytic activity of *Phaeocystis pouchetii* increased in higher temperatures going from 4°C to 15°C (van Rijssel et al., 2007). On the other hand, raising the culturing temperature from 14°C to 20°C of *Alexandrium tamarense* gave no difference in allelopathic effect to *Scrippsiella trochoidea* or *Heterocapsa triquetra* (Fistarol et al., 2004a).

5.1.3. *pH*

Coastal surface waters may reach high pH levels (Pegler and Kempe, 1988; Emery, 1969 cited in Hinga, 1992, Hansen, 2002) and the high pH can be confounded with the effect seen from allelopathic interactions. For example, raised pH level reduced motility of the dinoflagellate *H. triquetra* when mixed with low cell densities of *C. polylepis* (Schmidt and Hansen, 2001). Increasing the pH from 8 to 9 resulted in more than double numbers of non-motile *H. triquetra* cells. The toxicity of high-density cultures of *C. polylepis* increased from pH 6.5 and leveled out at pH 8 rendering circa 90 percent of the *H. triquetra* cells non-motile. So, in these studies the authors found that high pH have a negative effect on the motility of *H. triquetra*, and together with the allelochemicals increased allelopathy even further (Schmidt and Hansen, 2001). Similar results have also been found for the freshwater cyanobacterium *Oscillatoria laetevirens*, i.e. the production of algicides almost four folded when pH was elevated from 8 to 9 and at neutral or lower pH the algicide was found in lower concentrations (Ray and Bagschi, 2001).

5.1.4. *Nutrients*

Toxicity in phytoplankton usually increases under nutrient limitation (Edvardsen et al., 1990; Reguera and Oshima, 1990; Granéli and Johansson, 2003; Granéli and Flynn, 2006). If the algae need nitrogen in their toxins (as saxitoxins, nodularin, domoic acid e.g.), this will happen only under phosphorus limitation (excess nitrogen in the medium). For the algae which toxins do neither contain P nor N, toxicity increases under both P and N limitation (Johansson and Granéli, 1999a, 1999b; Granéli and Flynn, 2006). This indicates that the motive behind the increased toxicity is actually stress, for not having enough of the limiting nutrient to provide for cell division.

There are very few studies on allelopathy in aquatic systems dealing with nutrient limitation. Granéli and Johansson (2003) found that starved *Prymnesium parvum* cells from N or P increased dramatically their production of allelochemicals while and NP sufficient conditions allelopathy was nearly nil. Thus, this results support the assumption that production of allelochemicals by algae is similar to their production of toxins, i.e. physiological stress is the cause for it.

The target species also respond differently to the allelochemicals if they are grown under nutrient sufficient or deficient conditions. *Thalassiosira weissflogii* grown under N and P limitation was significantly more sensitive to *Prymnesium parvum* allelochemicals than when it was grown under sufficient nutrient conditions. (Fistarol et al., 2005). Target organisms would

be sharing the same stress environment as the allelopathic species, and it has been suggested that stress would make them more sensitive to allelochemicals (Einhellig, 1995; Tang et al., 1995; Reigosa et al., 1999; Fistarol et al., 2005; Granéli and Pavia, 2005; Granéli and Hansen, 2006). Thus, under stress conditions the allelopathic effect may be higher due to both the increase in the production of allelochemicals and in the sensitivity of the target species, increasing the competitive advantaged of allelopathic algae.

5.2. INFLUENCE OF BIOTIC FACTORS

5.2.1. *The organisms involved*

The main factor influencing allelopathic interactions are the organisms involved. The allelopathic effect depends on both the allelopathic (i.e. donor) species and the target species (Figure 1). Usually, allelopathic species affect several, but not all target species, and target species are sensitive to several, but not to all allelochemicals (Fistarol et al., 2003; Fistarol et al., 2004; Suikkanen et al., 2004; Fistarol et al., 2005). Furthermore, the allelopathic effect also depends on the cell concentration of the donor and the target species (Schmidt and Hansen, 2001; Tillmann and John, 2002; Tillmann, 2003).

The different effect of allelopathic species on different target organisms, as well as the different responses of target organisms to allelochemicals from different allelopathic algae are reported in Fistarol et al. (2003, 2004a and 2005), Suikkanen et al. (2005). For example, in Fistarol et al. (2003 and 2004a), it was found that diatoms (in Figure 1, it could be, e.g. target 1) can be highly inhibited by *P. parvum* (e.g. allelopathic species, AS, 1), but they are only moderately inhibited by *A. tamarense* filtrate (e.g. AS 2). *A. tamarense,* on the other hand, has a higher effect on nanoflagellates (e.g. target 2) than on diatoms. The differentiated effect of allelopathic species on different targets, as well as the resistance shown by some target species, might have implications for the evolution of aquatic microalgae. Evolutionary constrains will be discussed further along in the text.

Since allelopathy is mediated by chemicals released into the medium, its effect depends on the cell concentration of the allelopathic organism. Increasing the allelopathic effect with increase in the cell concentrations has been shown for some phytoplankton groups, e.g. prymnesiophytes, and dinoflagellates (Schmidt and Hansen, 2001; Tillmann and John, 2002; Tillmann, 2003). Tillmann (2003) also demonstrated that an increase in the cell concentration of the target organism decreases the effect (in this case death of *Oxyrrhis marina*). Since the death is caused by lysis of *O. marina*, the author suggests that the toxic compounds are removed from the system when they bind to the cell membrane of the target organism.

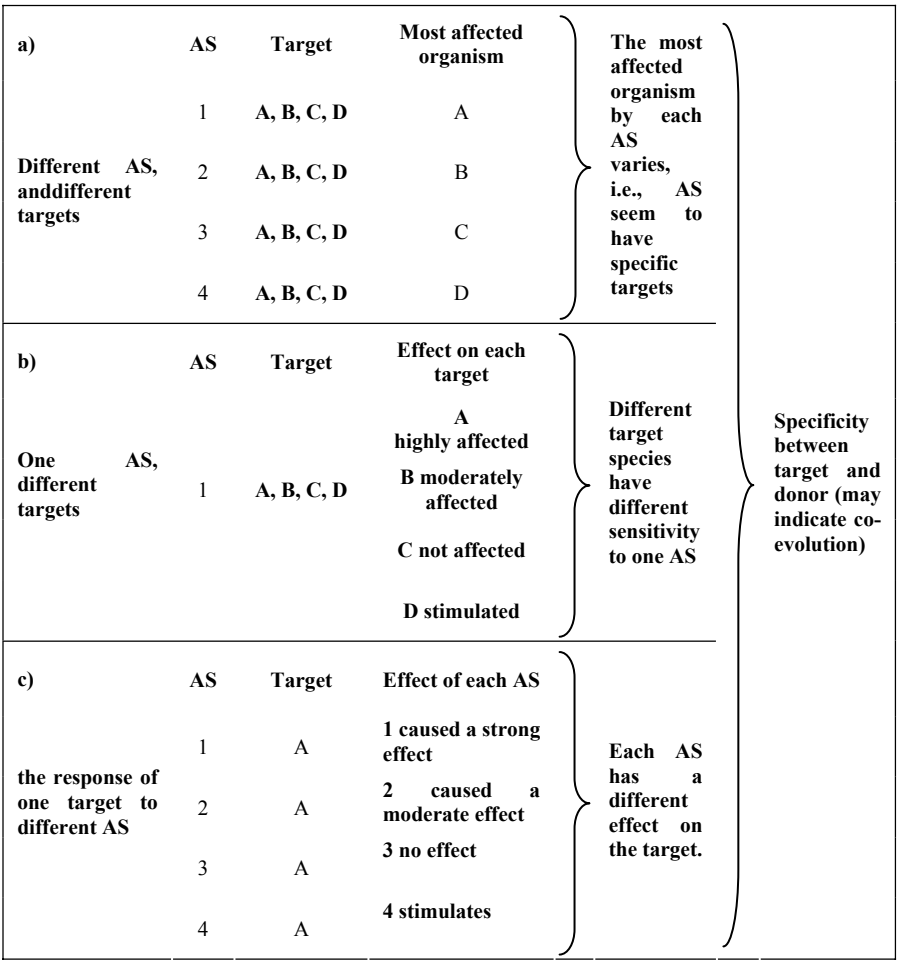

Figure 1. Comparison between the effects caused by different allelopathic species (AS) on one or several target organisms. The most affected organism by different allelopathic species varies (a). An allelopathic species will have different effects on each target (b). The same target organism can be highly affected by one allelopathic species and stimulated by another (c).

There are about 40 harmful phytoplankton species known to exhibit allelopathy (see Table 1). While in marine end estuarine waters the majority of the allelopathic species are found among the dinoflagellates, in fresh-water they are found among the cyanobacteria (Table 1). Nevertheless, it is among the flagellates that are found the allelopathic species producing the allelochemicals with the strongest negative impact on the other algal groups. Blooms of species such as *Chrysochromulina polylepis* and *Prymnesium parvum* are known for killing fauna and flora on the places they occur (Granéli and Johansson, 2003; Legrand et al., 2003).

5.2.2. *Effect of the growth phase*

The intensity of the allelopathic effect depends on the growth phase of the allelopathic species. Schmidt and Hansen (2001) and Suikkanen et al. (2004) demonstrated that allelopathic effect is caused by cells that are growing exponentially, that these effects decrease in the stationary phase, and that senescent cells do not cause allelopathic effects. Since allelopathy is a form of interference competition, it makes sense that the allelopathic species would be more allelopathic during exponential growth, while the cells can benefit from their effects, indicating that these compounds are important to the ecology of allelopathic species.

5.2.3. *Influence of bacteria*

Although most experiments on allelopathy of phytoplankton species were done with non-axenic cultures, it has been demonstrated that the bacteria present in the cultures are probably not responsible for the observed allelopathic effects (Suikkanen et al., 2004; Tillmann and John, 2002).

Tillmann and John (2002) eliminated the influence of bacteria present on *Alexandrium* spp. cultures by removing the bacteria through 0.2 μm membrane filters. They observed that the allelopathic effect of *Alexandrium* spp. did not alter, eliminating therefore the possible bacterial effect.

Suikkanen et al. (2004), tested if bacteria present in the non-axenic cultures of *Nodularia spumigena, Anabaena lemmermannii* and *Aphanizomenon flos-aquae* caused any allelopathic effect. The authors found that bacteria alone caused no effect on the target organism, indicating that the allelopathic effects they observed were indeed caused by cyanobacteria.

Although specific tests should be done for each allelopathic species, the examples above suggest that are indeed the phytoplankton species that cause the allelopathic effects and not bacteria.

6. Ecological Implications of Phytoplankton Allelopathy

Allelochemicals have been suggested to influence phytoplankton competetion, succession, and bloom formation or maintenance (Pratt, 1966; Keating, 1977; Rice, 1984 and references within; Lewis, 1986; Wolfe, 2000; Rengefors and Legrand, 2001; Vardi et al., 2002; Legrand et al., 2003; Fistarol et al., 2003, 2004; Suikkanen et al., 2005). It has been proposed that changes in plankton community structure are caused by the differential effect of allelochemicals on different targets (Mulderij et al., 2003). Target organisms may be completely eliminated, inhibited, resistant to the allelo-chemicals, or be stimulated (Fistarol et al., 2003, 2004a; Suikkanen et al., 2004).

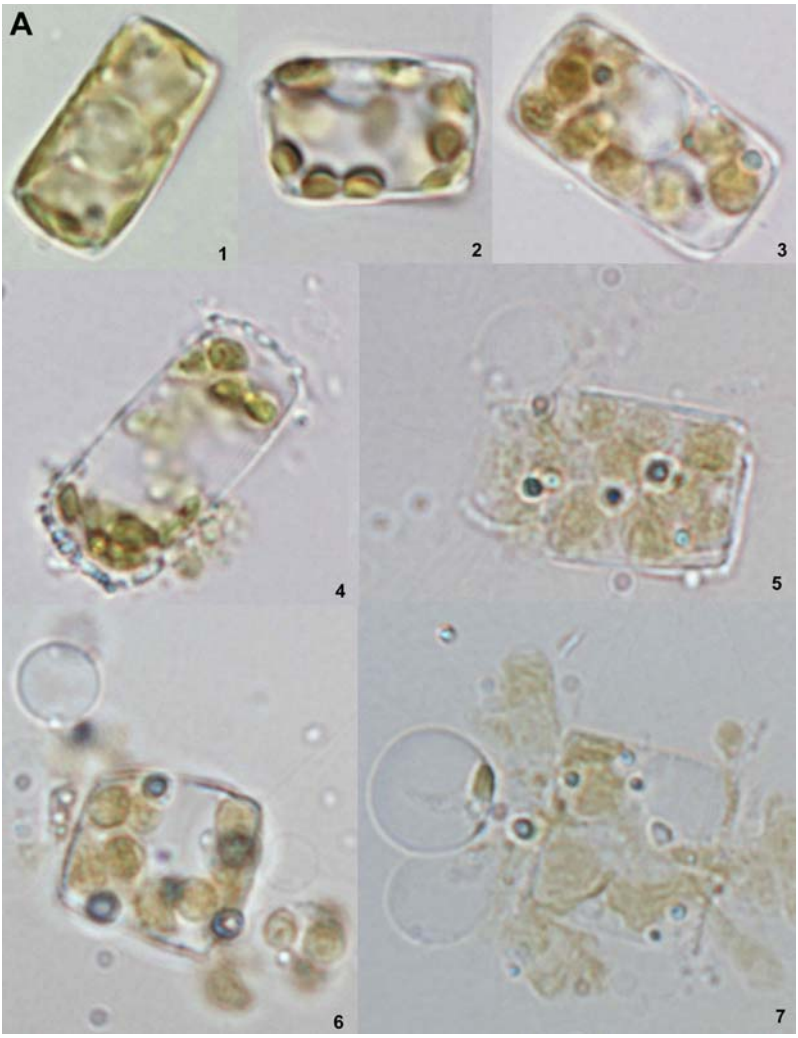

Figure 2. Different stages of cells of *Thalassiosira weissflogii* exposed to *P. parvum* filtrate. The first picture of the sequence shows a normal cell that has not been exposed to filtrate. The pictures are from different cells. Pictures 2 and 3 were after 1 h exposure, picture 4 and 5 after 5 h, and pictures 6 and 7 after 20 h. However, cells completely lyzed could already be observed after 5 h of exposure.

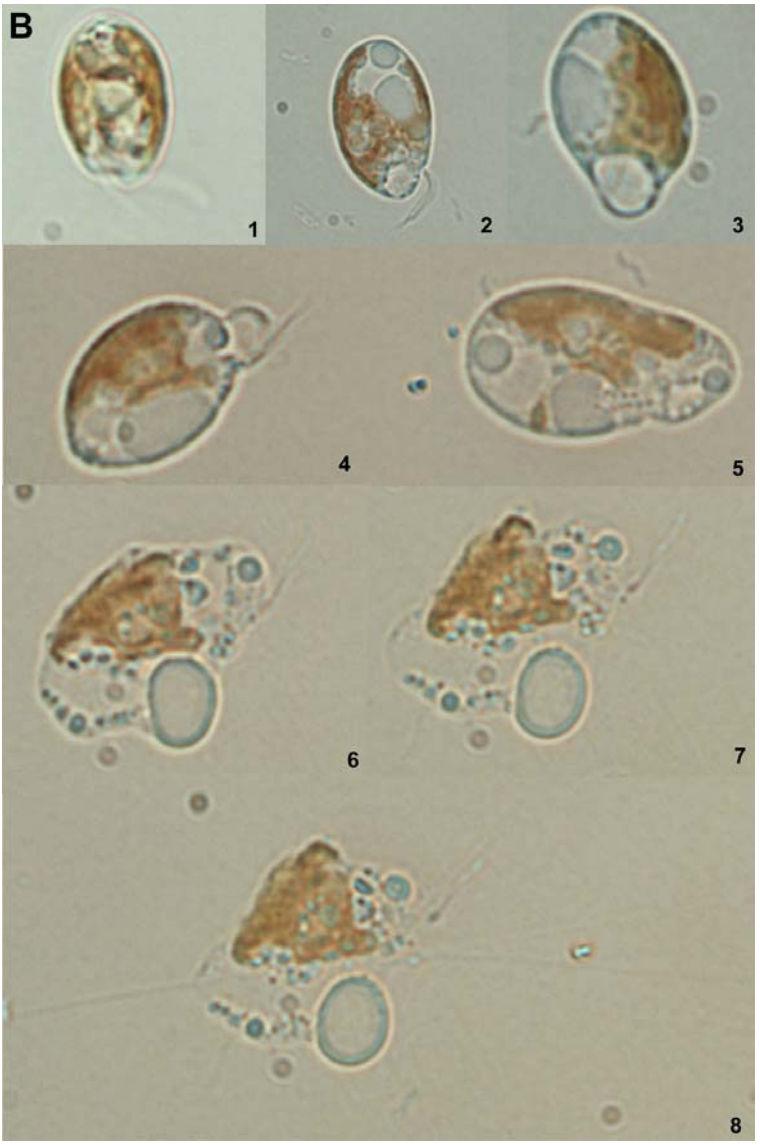

Figure 3. Different stages of cells of *Rhodomonas* sp. exposed to *P. parvum* filtrate. The first picture of the sequence shows a normal cell that has not been exposed to filtrate. The pictures are from different cells, except the three last *Rhodomonas* sp. picture that are all from the same cell. Except for the first *Rhodomonas* sp. picture, all other pictures were taken after 20 h of exposure, and the last three pictures were taken in an interval shorter that 2 min. However, as in the case of *T. weissflogii,* cells completely lysed could be found after 5 h of exposure. Thus, although observations have shown that one cell goes through all the stages depicted in the pictures, some cells are affected before the others.

Selective promotion or inhibition of the growth of individual species will influence succession and competition in aquatic environments.

It is hypothesized that the basic function of allelopathy is to give the donor organism a competitive advantage (Legrand et al., 2003), and the direct effect of allelopathy is shown by killing possible competitors. The fact that *Prymnesium parvum* (Fistarol et al., 2003) had a higher effect on diatoms (predecessor group under natural succession) than over cyanobacteria (group that usually blooms after the highest densities of P. parvum) indicates the potential competitive use of allelochemicals. The effect of *P. parvum* on a natural plankton community (Fistarol et al., 2003) shows how allelopathy can give a competitive advantage to an allelopathic species. The compounds excreted by P. parvum completely eliminated some of the competing phytoplankton groups and kept the biomass of the other groups at low levels (i.e. organisms that were not killed had a lower growth rate, and phytoplankton primary production decreased). Extrapolated to a natural situation these results suggest that *P. parvum* would have less species to compete with, and the ones remaining would show a deficient physiological state.

Usually the lethal effect of allelochemicals involves the lysis of the target organism. This is especially striking when the allelopathic algae caused a strong negative effect, as in *Prymnesium parvum*. Figure 2 and 3 shows different stages of cells of the diatom *Thalassiosira weissflogii* and the cryptophyceae *Rhodomonas* sp., respectively, after exposure to *P. parvum* filtrate. The figures shows how the cells change: how they start to lose pigmentation, that the cytoplasm seems to aggregate in vacuoles, that the cells start to blister, and finally that lyses occurs.

7. Allelopathy and Mixotrophy

Resource competition and grazing are traditionally the main mechanicsms used to explain phytoplankton population dynamics, and allelopathy is rarely taken into account. However, allelopathy, and also mixotrophy, have been shown to affect aquatic communities (Keating, 1977; Vardi et al., 2002; Fistarol et al., 2003, 2004a). It is very likely that these two strategies complement each other. Besides killing possible competitors, allelochemicals may give a further advantage to mixotrophic species, which can use the allelochemicals to help to obtain food mixotrophically, as in the case of *Prymnesium parvum* (Skovgaard and Hansen, 2003; Skovgaard et al., 2003; Tillmann, 2003). Allelochemicals can immobilize the prey, which are then are attacked, or alternatively the allelochemicals can lyse the cells which then release organic material (Skovgaard and Hansen, 2003; Skovgaard et al., 2003; Tillmann, 2003).

8. Conclusions: Evolutionary Aspects

Allelopathy affects phytoplankton succession and competition because it gives a competitive advantage to the allelopathic species and/or the resistant targets organisms. This fact has evolutionary implications because it may favor the selection of the resistant organisms, as it occurs with toxins released by microalgae species, which cause selective pressure on herbivorous organisms (by selecting the resistant ones) (Hairston et al., 2001). Both the tolerance showed by some organisms, and the fact differential effect of allelochemicals on each target species (Fistarol et al., 2003, 2004a; Suikkanen et al., 2004) suggest that co-evolution must be occurring. Furthermore, Fistarol et al. (2004b) shows an example of a behavioral strategy that could be used as a defense mechanism. The tolerance to allelochemicals shown by some target species is especially significant when it is found in successor algae, which then can achieve dominance over the allelopathic algae, as in the case of the resistance of cyanobacteria to *Prymnesium parvum* (Fistarol et al., 2003). Keating (1977) also showed that the phytoplankton groups succeeding the allelopathic species were positively affected, and achieved dominance over the predecessor allelopathic organisms.

Though allelopathic organisms might be good competitors under chemical interactions, they often are poor competitors for nutrients (Huntley et al., 1986). Thus, they will only dominate when their ecophysiological characteristics (Smayda, 1997) make them good competitors, causing a fluctuating selection, where the best competitor under certain conditions will dominate when these conditions occur, but it will be replaced when conditions change.

Enough evidence has been provided showing the ecological importance of phytoplankton allelopathy. The success of a certain microalgae species and the composition of aquatic communities is dependent on a series of abitic and biotic factors, and it is important to consider them all when studying aquatic systems.

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