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Chakraborty, Subhendu; Ramesh, A.; Dutta, Partha Sharathi

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Toxic Phytoplankton as a Keystone Species in Aquatic Ecosystems: Stable Coexistence to Biodiversity

Subhendu Chakraborty\textsuperscript{a,b}

E-mail: subc@aqua.dtu.dk, Fax: +45-35-883333
\textsuperscript{a} Theoretical Physics/Complex Systems, ICBM, Carl von Ossietzky Universität
PF 2503, 26111 Oldenburg, Germany
\textsuperscript{b} VKR Centre for Ocean Life, National Institute of Aquatic Resources
DTU Aqua, Technical University of Denmark, Charlottenlund Slot
Jægersborg Allé 1, DK - 2920 Charlottenlund, Denmark

Ramesh A

E-mail: aramesh@iitrpr.ac.in, Fax: +91-1881-223395
Department of Mathematics
Indian Institute of Technology Ropar
Punjab - 140001, India

Partha Sharathi Dutta\textsuperscript{†}

E-mail: parthasharathi@iitrpr.ac.in, Fax: +91-1881-223395
Department of Mathematics
Indian Institute of Technology Ropar
Punjab - 140001, India

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\textsuperscript{†} Corresponding author
Abstract

The effect of allelochemicals released by toxic species in plankton community is often taken into account to reveal plankton biodiversity. Using a minimal chemostat model we show that the interaction between toxic and non-toxic phytoplankton species with changing competitive effects among species due to allelopathy helps to promote the stable coexistence of many species on a single resource and hence can solve the paradox of plankton. We emphasize toxic phytoplankton as a keystone species that strongly uncovers its allelochemicals on other non-toxic phytoplankton and enhances the species persistence and diversity in aquatic ecosystems. In addition, we analyze the consistency of ecosystem functioning and species diversity using a number of approaches, such as sampling hypothesis with selection and complementarity effects, cascading extinction-reinvasion, and examining system dynamics at different enrichment levels and toxicity. Our results suggest that chemostats with one toxic and one or more nontoxic phytoplankton species can be used for the experimental verification of the stable coexistence of many species on a single resource in aquatic ecology.
Introduction

Plankton biodiversity has fascinated ecologists for the past few decades. It has become a major topic of research in plankton ecology. Although the principle of competitive exclusion (Hardin 1960) tells us that the number of surviving species cannot exceed the number of limiting resources at equilibrium, still many plankton species coexist depending on a limited number of resources. In the famous paper “The paradox of plankton”, Hutchinson (1961) first posed the classical problem “... how it is possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sorts of materials”. Numerous attempts have been made to explain this paradoxical diversity. In isotropic environments, non-equilibrium dynamics (i.e. limit cycle or chaotic oscillations) has been suggested to play a leading role in maintaining the desired coexistence of many species on limited variety of resources (Huisman and Weissing 1999, Czárán et al. 2002, Revilla and Weissing 2008, Dutta et al. 2014). Though the approach of non-equilibrium dynamics as a possible solution of the paradox of plankton is fascinating, these findings are still debated in literature (Schippers et al. 2001, Shoresh et al. 2008, Barton et al. 2010). The explanation of the paradoxical diversity of phytoplankton in isotropic environments governed by equilibrium dynamics is still an unachieved goal. A detailed review of various factors responsible for the plankton biodiversity has been given in the papers by Scheffer et al. (2003) and Wilson (2011).

Allelopathy has a great impact on plankton ecosystems. By definition, allelopathy describes any inhibitory or stimulatory effect of one species on another species mediated by the production of some chemical compounds (Rice 1984). Nowadays, however, the term allelopathy is often used to represent an inhibitory effect of biochemical compounds (Cembella 2003). Allelochemical interactions between different populations can have different ecological consequences (Rice 1984). Regarding species distribution in aquatic ecosystems, allelochemicals produced by some species of phytoplankton (generally known as toxin producing phytoplankton) play a crucial role in shaping...
ecological communities (Macias et al. 2008). In fact, these toxic species act as keystone species where keystone species can be defined as the one whose impact is large on the ecosystem and its communities, disproportionate with its abundance and plays a structural role in the ecosystem (Mills et al. 1993). As a keystone species, toxic phytoplankton negatively affect species interactions by producing toxic chemicals and hence influence other competing species and grazers. However, laboratory experiments and modeling results suggest that allelopathy becomes important and effective only when toxic species abundance is very high (Schmidt and Hansen 2001, Solé et al. 2005) and thus allelopathy can have a profound role in toxic bloom prolongation. Recently, Van de Waal et al. (2014) studied the stoichiometric regulation of phytoplankton toxins which may help us to predict toxin-producing phytoplankton blooms and also can manage to control the overall toxicity in natural waters.

However, the role of allelopathy (i.e. the presence of a toxic species in the community) in maintaining the large scale biodiversity of phytoplankton is not well established. Although, most of the competition results demonstrate the exclusion of one of the species (Adams et al. 1979, Chao and Levin 1981), only a few empirical data reported the coexistence of toxin producing and sensitive strains in natural and laboratory systems (Abranches et al. 1997, Ruiz-Barba et al. 1994). Such production of toxins is known to reduce the competitive effectiveness among different species either by reducing the abundance of other species (Folt and Goldman 1981, Solé et al. 2005) or by creating spatial separation between species (Frank 1994), which ultimately results coexistence. Previous theoretical studies show the coexistence in non-homogeneous environments or governed by non-equilibrium dynamics (Tilman 1982, Huisman and Weissing 1999, Czárán et al. 2002, Revilla and Weissing 2008, Dutta et al. 2014). In the present work, using a minimal mechanistic modelling approach, theoretically we explore the role of allelopathy on the biodiversity of phytoplankton in a homogeneous environment governed by equilibrium dynamics.

We extend the well known Droop model (Droop 1973) in the context of multiple species...
and single resource in the presence of allelopathy among phytoplankton. The Droop model is the standard quota model of resource competition (Grover 1991, Grover and Wang 2014). Previously, Revilla and Weissing (2008) studied the effects of resource storage by considering a similar model for multiple resources. In a homogeneous environment, they found the coexistence of many species on few resources governed by non-equilibrium dynamics and this is possible only when the number of limiting resources is either three or more. There has been a long-standing debate to identify the relationship between stability and diversity. Trait based approach plays a key role in predicting the diversity effects on the stability of ecosystems (Loreau and Mazancourt 2013). In order to get a stable ecological community, species traits must differ in specific ways (Clark et al. 2007). In the present paper, considering allelopathy as an important trait, we investigate how the presence of a single toxic species promotes the biodiversity of phytoplankton community under single resource. Using chemostat approach, first we find the species diversity by simulation technique and then check the stability of those existing species. Secondly, to identify the impact of allelochemical interactions on phytoplankton biodiversity, we find the supersaturation region for different toxicities and biodiversity measures like net effect, selection effect, complementarity effect and resource use efficiency. Further, cascading extinction patterns following the removal and the reinvasion of a species are also investigated. In addition, we discuss species diversity in fluctuating environmental conditions by varying the nutrient concentration at different levels.

2 Model description

The model we analyze here is derived from the standard Droop model of resource competition (Droop 1973). The Droop model describes a well-mixed bioreactor (i.e. a chemostat) that contains phytoplankton cells with density $N_i \ (10^9 \text{ cells} \ \text{L}^{-1})$ in a growth medium of a limiting nutrient with concentration $R \ (\mu \text{mol L}^{-1})$. Each phytoplankton cell is assumed to possess an internal pool of stored
nutrients (the so-called cell quota) $Q_i \ (10^{-9} \ \text{μmol cell}^{-1})$. The chemostat is supplied with nutrients
at an input concentration $S \ (\text{μmol L}^{-1})$ from an external medium. The outflow contains
both medium and phytoplankton cells. Inflow and outflow are characterized by the dilution rate $D \ (\text{day}^{-1})$. In the present model, one of the species $(N_1)$ produces toxin that induces additional
mortality in all the other non-toxic species $(N_2, N_3, ..., N_n)$ via allelopathy parameter $\theta_i \ (10^9 \text{cells}^{-3} \text{day}^{-1})$. Specifically, here $\theta_i$ measures the strength of allelopathic effect of $N_1$ on
the $i$-th species. Under these assumptions, the modified Droop model for $n$-species and one-resource
takes the following form:

$$\frac{dR}{dt} = D(S-R) - \sum_{i=1}^{n} f_i(R) N_i, \quad i=1, ..., n, \quad (1a)$$

$$\frac{dQ_i}{dt} = f_i(R) - \mu(Q_i) Q_i, \quad i=1, ..., n, \quad (1b)$$

$$\frac{dN_1}{dt} = [\mu(Q_1) N_1 - m_1] N_1, \quad (1c)$$

$$\frac{dN_i}{dt} = [\mu(Q_i) - m_i] N_i - \theta_i N_1^2 N_i^2, \quad i=2, ..., n, \quad (1d)$$

with

$$f_i(R) = \frac{\nu_i R}{K_i + R} \quad \text{and} \quad \mu(Q_i) = r_i \left(1 - \frac{q_i}{Q_i}\right).$$

Uptake of the resource $R$ from the external medium is assumed to be an increasing and a
saturating function $f_i(R)$ of the external resource concentration, where $\nu_i \ (10^{-9} \ \text{μmol cell}^{-1} \ \text{day}^{-1})$ is the maximum uptake rate and $K_i \ (\text{μmol L}^{-1})$ is the half-saturation constant for the $i$-th
species. It is assumed that the phytoplankton growth rate $\mu(Q_i)$ depends entirely on the cell quota
$Q_i$, where $r_i \ (\text{day}^{-1})$ is the maximum growth rate under quota saturation and $q_i \ (10^{-9} \ \text{μmol}$
cell\(^{-1}\)) is the minimum amount of subsistence quota per phytoplankton cell. For \(Q_i > q_i\) the
growth rate is positive. However, it is set to zero if \(Q_i < q_i\). The species specific mortality rate is
given by \(m_i\) (day\(^{-1}\)), which is considered to be same with the dilution \(D\) for all the species.

It is evident from Eqs. (1) that we introduce additional mortality to the non-toxic
phytoplankton due to allelopathy. From a theoretical point of view, allelopathic interaction was first
introduced in a two species Lotka-Volterra competition model by Maynard-Smith (1974). He
considered a constant release of toxic chemicals by one species appearing harmful to the other one.

After necessary modifications, this model was applied in several directions including allelopathy in
bacterial (Nakamaru and Iwasa 2000) and plant community (Dubey and Hussain 2000, An et al.
2003). Concerning allelopathy among phytoplankton, Chattopadhyay (1996) applied this concept
in a general two species competition system where each species produces a substance toxic to the
other, but only when the other is present. Mathematically, if \(N_1\) and \(N_2\) are the population
densities of the toxic and the non-toxic phytoplankton respectively, then the allelopathic interaction
term was considered as \(\theta_2 N_1 N_2^2\), where \(\theta_2 (> 0)\) is the allelopathy parameter representing the
strength of the allelopathic effect of species \(N_1\) on \(N_2\).

There is evidence that allelopathy among phytoplankton becomes important only when the
toxic species reaches high density. Maestrini and Graneli (1991) suggested that at a relatively low
population density (10\(^4\) cells/l), the toxin produced by \(C.Polylepis\) helps only in repealing grazers,
but at high cell density (> 10\(^6\) cells/l), the concentration of accumulated toxin becomes high and
starts affecting competitors. Based on 21 published experimental works, Jonsson et al. (2009)
performed a meta-analysis and detected significant allelopathic effect only in studies using high but
not low Chlorophyll \(a\) content. Allelopathic term used by Chattopadhyay (1996) does not exactly
satisfy the above mentioned criteria and shows proportional allelopathic effect with all
concentrations of cell density of the toxic species. Solé et al. (2005) verified the expression
representing allelopathic interaction used by Chattopadhyay (1996) with the help of experimental data taken from a laboratory study where total 15 species of marine phytoplankton were cultured together with the toxic *Chrysocromulina polylepis* (Schmidt and Hansen 2001). They found that the model with the allelopathic term similar to Chattopadhyay (1996) showed discrepancies at low initial concentrations of the toxic species and suggested that the allelopathic effect should be a nonlinear function of the amount of toxic cells present in the medium rather than the linear one. While modifying the expression by $\theta_i N_1^2 N_i^2$, they found a better agreement between the theoretical outcome and the experimental data at both high and low concentrations of toxic cells. In accordance with the previous findings, we also consider a similar type of expression to represent the allelopathic effect. In our model, the first species, $N_1$, is assumed to be toxic and it produces toxin which increases the mortality of all the remaining species by the quantity $\theta_i N_1^2 N_i^2$, $i=2,\ldots,n$. It is natural that the amount of toxin around the toxic phytoplankton would be greater than that of the non-toxic phytoplankton, suggesting that the toxic phytoplankton must have evolved resistance to the toxin and that’s why it is having no extra mortality due to the toxin. This argument also leads to the fact that there is a strong selection pressure on the non-toxic phytoplankton to evolve resistance to the toxin after coexisting with the toxic phytoplankton for some period (Hairston et al. 2001). However, the effect of evolution on biodiversity falls outside the scope of this paper.

3 Results

3.1 Allelopathy and the coexistence of many species on single resource

We use the modified Droop model with allelopathy to explore the possibility of stable equilibrium coexistence of many species on limited number of resources. For this reason, a series of simulations was carried out considering randomly chosen half-saturation constants $K_i$ for different species.
$N_i$ ($i = 1, \ldots, 8$) and random allelopathic effects $\theta_i$ ($i = 2, \ldots, 8$) of $N_1$ on the other species $N_i$ ($i = 2, \ldots, 8$). Apart from the values of $\theta_i$, all the other parameter values and their ranges of variations are taken from the paper by Klausmeier et al. (2004). The set of all the parameter values corresponding to each of the figures presented in this paper is given in Online Supplementary Material I.

For the model given in Eqs. (1) altogether, 100 simulations are performed, each starting with a single resource and 8 species with initial species density 0.01. We carry our numerical simulations for 20 consecutive years to exclude the possibility of transient steady states (Göthlich and Oschiles 2012). In Fig. 1(a), we plot the number of surviving species (along the $y$-axis) for each of the 100 different simulations (along the $x$-axis). Clearly, each simulation corresponds to different parametric setup thus representing different species combinations. The positions of the circles represent the number of surviving species for different simulation runs. In the case of the survival of a single non-toxic species (depicted by the plus signs in Fig. 1(a)), the toxic species extinct from the system and thus leaving the possibility of the coexistence of more species on a single resource in equilibrium.

The red circles represent the situation when the toxic species survives in the community and hence can promote the possibility of the stable equilibrium coexistence of many species only on one resource (see Online Supplementary Material II for the mathematical derivation which shows the coexistence of two species on a single resource and can be extended for more species).

Interestingly, in most of the cases (almost 70 – 80% of our simulations with stochastically chosen parameters), the number of surviving species exceeds the number of resources (remember only one resource in our case) in the presence of the allelopathic effect by toxic phytoplankton (see for example Fig. 1(b)). One should note that the existence of the toxic species in an initial community does not always guarantee the stable equilibrium coexistence of many species on less number of resources. The principle of competitive exclusion holds for some of the cases even in the presence of toxic species. Approximately, in 20 – 25% of our simulations, toxic species is the winner (by
outcompeting all other non-toxic competitors) when many species are competing for a common resource (see Fig. 1(b)).

Considering a particular species composition (i.e. only one specific set of parameter values) where eight species coexist in one resource, we check the stability of the existing species. Whether the existing species reach an equilibrium state or not, that can be identified by the trajectories using simulation or by the stability analysis. Here we adopt the first one. In this model, the stable coexistence occurs for both toxic and non-toxic species which is shown in Fig. 2. Since eight species are competing for a single resource, the allelopathic interactions between the toxic and the non-toxic species help in the coexistence of many non-toxic species with equilibrium density (Chesson 2000). The allelopathic interactions shape the community structure and also decide the species richness and abundances of the populations. Here the toxic one always dominates in the chemostat. There are evidences that the toxic species exists with high biomass in ecosystems due to the defense mechanism against predation and the availability of rich nutrition (Irigoien et al. 2005). Toxic bloom occurs due to the growing environmental conditions and the relationship between the cell size and nutrient helps the toxic species to dominate in the ecosystem. Subsequently, these blooming species strongly influence the coexistence of many non-toxic species and further shape the ecosystem processes. In Moncheva et al. (2001), the blooming species is considered as *keystone species* or *ecological engineers* that helps to maintain local biodiversity within a community. In our model, the toxic species density settles in higher equilibrium density (approx $5.4 \times 10^6$ cells/ml in Fig. 2) in comparison to the non-toxic species equilibrium densities. In addition, the non-toxic species density initially increases, but once the toxic species reaches high density, allelopathic effect comes into play which suppresses the non-toxic species abundances and finally all the non-toxic species settle down in equilibrium with low population densities. Therefore, we also find a similar kind of dominance and high abundance of the toxic species in the presence of allelopathic effect on other species. Moreover, the biodiversity is maintained for a longer time scale.
by most of the species, even in an equilibrium state.

To study how allelopathy influences the coexisting steady state with changing environments, first we consider a two species-one resource system where one of the species is toxic and we perform an analysis in a two-dimensional parameter space spanned by the two experimentally accessible parameters, the dilution rate $D$ and the concentration of the resource $S$ in the inflow medium. In literature, the coexistence of more species in less number of resources is termed as supersaturated coexistence (Revilla and Weissing 2008). The regions of supersaturation in the $S-D$ parameter space for three different values of the allelopathy parameter $\theta$ ((a) $\theta = 8 \times 10^{-5}$, (b) $4 \times 10^{-4}$, and (c) $5 \times 10^{-3}$) are shown in Fig. 3 (marked by gray shaded regions). The other parameter values are same as in Fig. 1, whereas the values of $q$, $\nu$ and $K$’s are given in Table 3 of Online Supplementary Material I. We consider that a species exists if its concentration is greater than $10^4$ cells/ml (Prince et al. 2008). Here, the break-even resource concentrations for each species above which the toxic and the non-toxic species survive in isolation, are not indicated in Fig. 3 as they are very small. **Therefore, in the white region, either of the species survives depending on the initial conditions.** In Fig. 3, in the white regions, either of the species survives depending upon the initial conditions whereas in the blue shaded regions both the species are extinct from the system due to high dilution rate. Interestingly, the presence of high allelopathy helps in the coexistence at low resource supply concentrations. However, an increase in the resource input concentration increases the toxic species abundance, which suppresses the non-toxic phytoplankton abundance. As a result, at a very high resource input concentration, the non-toxic species extinct from the system (not shown in Fig. 3). Therefore, very high allelopathy again disturbs the coexistence of toxic and non-toxic phytoplankton. Such coexistence in the presence of allelopathy is pointing towards the fact that the interactions between phytoplankton species may be linked to the evolution of a mechanism for maintaining stable supersaturated biodiversity. By considering more species and one resource in the presence of allelopathy, it is also possible to find a robust region of supersaturation.
3.2 Impact of phytoplankton diversity on biomass production

The relationship between biodiversity and productivity (i.e. biomass production) of species can be explained by two hypotheses, called “sampling effect” and “niche complementarity” (Loreau 1998, Loreau 2000, Loreau and Hector 2001, Fox, 2005, Fargione and Tilman 2005). The sampling effect reveals the possibility of biodiversity due to one or few dominant high biomass species or species with particular traits being present in the polyculture. On the other hand, the niche complementarity hypothesis suggests that resource partitioning interactions between species lead to an increase in total resource use. These effects can be observed by identifying the species biomass in monoculture and polyculture. Here, the coexistence of both the toxic and non-toxic species is considered as polyculture, whereas monoculture represents the presence of either only the toxic or only the non-toxic species. Using sampling hypothesis, we calculate the diversity effects such as net effect, selection effect and complementarity effect as a function of nutrient supply ($S$) using the technique of Loreau and Hector (2001).

The net effect of biodiversity is defined as $\sum Y - \overline{M}$, where $Y$ is the observed biomass of each species in polyculture ($\sum Y = \sum_{i=1}^{n} Y_i$ is the biomass of all the species in polyculture) and

$\overline{M} = \frac{1}{n} \sum_{i=1}^{n} M_i$ is the average monoculture biomass of all the species, where $M_i$ is the monoculture biomass of the $i$-th species. The net effect of biodiversity can be partitioned into two additive components: the selection effect and the complementarity effect. It is given by:

$$\sum Y - \overline{M} = \overline{D \times cov(M, \Delta RY)} + \overline{D \times \overline{M} \times \Delta RY}.$$  

= Selection effect + Complementary effect,
where $\bar{D}$ is the number of species in polyculture, ‘cov’ is the covariance operator, $M$ represents the biomass of a species in monoculture, $\Delta RY$ is the difference between the observed relative yield $Y/M$ and the expected relative yield $1/\bar{D}$, and $\bar{\Delta RY}$ is the average $\Delta RY$ of all the species in the considered community. The relative yield and the performance of the species both in monoculture and polyculture depend on species growth. This species growth can be described in many ways. It can be a function of the nutrient supply, proportion of resources used by species, and the efficiency with which a species uses resources (Binkley et al. 2004). Resource use efficiency is defined as the amount of biomass produced per unit resource (Pastor 2008). Here, we find the efficiency with which a resource is used by the species in polyculture—in the presence and absence of allelopathic effect. The resource use efficiency (RUE) is calculated by the sum of biomass of all the species over nutrient supply (i.e. $\text{RUE} = \frac{\sum_{i=1}^{n} N_i}{S}$).

In Fig. 4, we plot the total biomass, total resource, resource use efficiency, net effect, selection effect and complementary effect as a function of nutrient supply concentration for two species—one resource system where one of the species is toxic. Here we choose $\theta = 1 \times 10^{-3}$ and the other parameter values are same as in Fig. 3. The solid (dashed) curve represents the measures in the presence (absence) of allelopathic effect. Clearly, total biomass of the species and RUE increase (see Fig. 4(a) and (c)) in the presence of allelopathic effect. Also, the net effect and the selection effect increase during the increase of nutrient supply (see Fig. 4(d) and (e)). A positive selection indicates that a species achieves greater abundance in polyculture than in monoculture. A change in average relative yield is measured by the complementarity effect. Negative complementarity effect in Fig. 4(f) indicates that there might be a lower yield on average than expected in monoculture. Since, the net effect is the addition of selection and complementarity effects, overall it is positive even though complementarity has small negative values. Without allelopathy, an increase in the nutrient supply results in decrease in the selection effect.
3.3 Chance of cascading extinction and reinvasion

Due to complex physical and chemical interactions between species in communities, the event of sudden extinction of one species can lead to a cascade of additional species loss (Lundberg et al. 2000, Fowler 2010). Here, we investigate how the diversity promoted by allelochemicals affects the pattern of cascading extinction following the removal of a non-toxic species in the supersaturated community. Similar to the situation in Fig. 1, here also we maintain the same parametric setup and construct 50 communities for each size of surviving species $j$ ranging from 2 to 8. In total, we collect 350 communities. From each such community, a surviving species (assuming the surviving species density is more than $10^2$ cells/ml) is randomly removed (except the removal of the toxic species in order to take into account the effect of allelopathy and hence to retain the supersaturation). Moreover, we set the corresponding maximum uptake rate $(v)$ and the maximum growth rate $(r)$ of the removed species equal to zero and the remaining system is allowed to run for another 20 years. Before removing the species randomly, all the existing species densities are maintained in an equilibrium state. We check the new community sizes and plot them (along $y$-axis) with respect to their initial community sizes before random removals take place (along $x$-axis) (see Fig. 5(a)). The open circles represent the expected number of species $(j-1)$ just after the removal of one of the surviving species and filled circles indicate the number of species after cascading extinctions, i.e. when the resulting community consists of less than $j-1$ species. Here, the size of the circles corresponds to the number of communities for each community size. It is clear that, in general, for small $j$, the final community size is same as the expected community size, $j-1$, just after the removal of one surviving species. Moreover, for higher community sizes, generally cascading extinction occurs, but just only one more species than the target species extinct for each case, although the total number of cases of cascading extinction is also very less (0.9% approximately out of 350 communities).
We also investigate the cascading extinction patterns (Lundberg et al. 2000) for reinvasion of the previously removed species into a new community formed after its disappearance (see Fig. 5(b)). For this reason, first we reach at the equilibrium after the removal of the species and then reintroduce it to the system with a population density of $10^4$ cells/ml. Note that, here we assume if one species goes extinct (density less than $10^2$ cells/ml), it cannot return to the system, and we assign a zero value for that extinct species. The result shows that only 0.3 % of the former member of the community were unable to reinvade and interestingly no cascading extinction occurs. Also, the removal and the reintroduction of species do not affect the stability of the remaining coexisting species at the end of the simulation (Rohr et al. 2014). Hence, here the species loss and the appearance of new invaders do not lead to community closure.

### 3.4 Role of increased nutrient supply on species richness

Now, we investigate the role of nutrient supply on the number of surviving species. In an ecosystem, environmental conditions change due to seasonal succession and many other factors. These environmental fluctuations or changes in the season result in the variation of nutrient level in ecosystems. To take such variations of environment into account, we change the nutrient supply into the system with fixed half saturation constants and fixed allelochemical effects of species. Initially we set all the parameters in such a way that 8 species coexist for constant nutrient supply (i.e. $S=19$ μmol/l). Now, nutrient supply is increased (i.e. at a certain time, change the constant nutrient supply) and the system is allowed to run for another 20 years. We find that the number of coexisting species in the community (i.e. species richness) decreases with increasing nutrient supply (see Fig. 6). Note that, again if we reintroduce the extinct species in the chemostat by changing the nutrient supply to its previous rate ($S = 19$ μmol/l), the species survives. Specifically, the extinct species reinvades in the system during the same amount of nutrient supply. Therefore, species coexistence depends on the supply of nutrient in the chemostat model. In Fig. 6, the blue and the red circles
represent two communities with 8 and 6 species in the initial stage, respectively, for the nutrient
supply $S = 19 \, \mu$mol/l for the fixed $K_i$’s and fixed $\theta_j$’s. The parameter values used are given in
Table 4 of Online Supplementary Material I. We further increase the nutrient supply in the
chemostat and find the nutrient supply where the number of coexisting species changes. We
marked those nutrient supply rates where exactly one species becomes extinct from the previous
community size. Clearly, the number of coexisting species in the community (i.e. species richness)
decreases with increasing nutrient supply (see Fig. 6). Therefore, we see biodiversity loss due to the
increase in the supply of nutrient in the chemostat.

As an explanation we can say that, due to the allelopathic effect, the abundance of all non-
toxic phytoplankton decreases which results in decrease in competition between species and helps
in species coexistence. However, an increase in nutrient supply in the system favors the growth of
those species which are superior competitor for resource and results in the extinction of inferior
resource competitors. Specifically, the benefit provided by allelochemicals in reducing differences
in competitive abilities between different species nullifies under high nutrient input in the system.
As a result, an increase in nutrient supply exhibits the loss of species diversity and hence shows the
paradox of enrichment (Rosenzweig 1971). Although several other environmental factors affect the
diversity, this eutrophication (changing the system from oligotrophic to eutrophic through resource
enrichment) actually weakens the species diversity with an equilibrium density of the existing
species (Sperfeld et al. 2010).

3.5 Effect of variations in allelopathy on plankton biodiversity
The competition for resources and trait variations among species are the major selective forces to
maintain species diversity. The variations in the allelopathic effect influence the competitive ability
of species and also the species composition. By varying the strength of allelopathic effect in the
community, it largely reflects in species richness. The relationship between the interspecific
competition and the evolutionary dynamics of allelopathy can be explained by phenotypic plasticity (Mougi 2013). Due to changing environmental conditions, species phenotype makes an impact in the community (i.e. changes in the growth, morphology, behaviour, etc.) as well as in the ecosystem.

Here, we change the allelochemical effects \((\theta_i)\) on each species proportionally, i.e. each \(\theta_i\) is multiplied by a unique constant, say \(\theta_c\). We identify the changes in the species diversity by varying the constant \(\theta_c\). Initially we start with a community of eight species where one is toxic and rest of the species are non-toxic. At first, \(\theta_c\) is varied between 0 and 1 which captures the scenario of the reduction in allelopathic effect on each species. As we decrease \(\theta_c\) from 1 to 0, the number of species suddenly drops down from 8 to 1 as \(\theta_c\) crosses 0.46 (cf. Fig. 7). On the other hand, to take into account the effects of an increase in the strength of allelopathy, \(\theta_c\) is varied from 1 to 200. Clearly, the increase in \(\theta_c\) gradually decreases the number of coexisting species.

4 Discussion

Allelopathy is one of the important biological factors that strongly influences the structure of plankton community. In this paper, we investigated the role of phytoplankton allelopathy on biodiversity of plankton community. According to the principle of competitive exclusion (Hardin 1960, Armstrong and McGehee 1980), in homogeneous, well-mixed environments, species competing for the same resource cannot coexist, and the final equilibrium consists of a single species. Indeed, simple competition models and laboratory experiments also suggest that the number of coexisting species in equilibrium cannot be greater than the number of limiting factors (Tilman 1977, 1981, Sommer 1985, 1986, Rothhaupt 1988, 1996). In this paper, it is found that the
equilibrium coexistence of more species is possible on just a single resource in the presence of allelopathy. We checked the coexistence up to 8 species on a single resource, although one can further increase the number of coexisting species. In the real world, hundreds of species coexist on a small number of resources. The present study suggests that allelopathy can be one of the factors responsible for the coexistence of many species on a limited number of resources. The robustness of this result was shown in Fig. 1 by plotting the number of coexisting species at different species combinations. To structure plankton community, trait based approach has been used (Litchman et al. 2010) and the trade off among those traits helps to promote the functional diversity of plankton community (Edwards et al. 2013). Considering allelopathic effect as a trait, we found the diversity of species. As long as the toxic species remains present in the community, many species can coexist at equilibrium even in a single resource. So it's a kind of trade-off between the resource limitation and the toxicity with the number of coexisting species.

Another well known trade-off associated with allelopathy is the trade-off between benefits in competition or nutrient availability and the metabolic costs of toxin production (Lewis 1986). Allelopathy reduces competitor abundance either by resulting direct mortality to the competing cells or by reducing competitor population growth rate, which ultimately provides a relief mechanism to the toxin producing species from resource competition. Moreover, the toxins produced by some species, make holes in the cell membrane of other species, resulting in a transient nutrient leakage (known as dasmotrophy), which is then taken up by the toxin producing species via osmotrophic uptake. Furthermore, toxins produced by some mixotrophic species, helps in prey capture by paralyzing the prey species. However, according to the evolutionary theory, no benefit comes without a cost. Since, the production of toxin needs allocation of acquired nutrient as well as the maintenance of the synthetic machinery for toxin production, it results in a lower competitive ability or the lower growth rate of the toxin producing species. Moreover, auto-toxicity bears the risk of damaging the toxin producing cells from its own released toxic compounds and thus may
involve some further costs to acquire immunity. In addition to this, the release of toxins in the surrounding water increases the risk of predation as most planktonic grazers use chemical cues to locate their prey. Some recent modeling studies with toxin production by phytoplankton incorporated such trade-offs (Grover and Wang 2014, Chakraborty and Feudel 2014). In the present paper, although the cost-benefit analysis is not taken into account explicitly, but a trade-off is assumed between the growth advantage due to allelopathy and the competitive ability of the toxin producing species. However, the toxin production as well as the trade-offs associated with allelopathy varies with species and several other factors (Brönmark and Hansson 2012). Thus, the robustness of our result were examined by plotting the number of coexisting species at different species combinations (see Fig. 1). Because of this trade-off, the $R^*$ value of the non-toxic species becomes lower compared to the $R^*$ value of the toxic species. Here, $R^*$ value of a species is the break-even resource concentration where the mortality rate equals its reproduction rate and resource concentration below this value forces the species to get extinct. During the competition for a single resource, at equilibrium, theory predicts that the single species with lowest $R^*$ value (lowest requirement for resources) would displace all other species and win the competition (Tilman 1977, 1982). According to our formulation, the non-toxic species would win the competition in the absence of allelopathy because of its lower $R^*$ value. However, the presence of allelopathy suppresses the growth of the non-toxic species, which increases equilibrium resource concentration and sets it to the $R^*$ value of the toxic species. This opens the possibility of the stable coexistence of both the non-toxic and the toxic species on single resource. In the presence of sufficient allelopathic effect, more species can coexist on single resource. In other words, the presence of allelopathy manages to keep the resource equilibrium level high, thus enables the invasion of more species on less number of resources.

The role of competition on phytoplankton biodiversity was previously investigated by many researchers. With the help of a resource competition model (based on a standard model for
phytoplankton competition), Huisman and Weissing (1999) showed that the number of coexisting species can exceed the number of resources in a constant and homogeneous environment via non-equilibrium conditions, only if the number of resources is either three or more. A modified version of the model of Huisman and Weissing (1999) by incorporating cell quota also gives similar kind of results (Revilla and Weissing 2008). In the current paper, we examined the diversity of plankton ecosystems considering a similar model like Revilla and Weissing (2008), but with a single resource in the presence of allelopathy. On one hand, the presence of allelopathic interaction relaxed the restriction of the number of minimum resources needed for the coexistence of more species than the number of resources. On the other hand, allelopathy allows more species to coexist in stable equilibrium. However, such stable coexistence always needs the presence of toxic phytoplankton at high density. A high abundance of the toxic species reduces the competitive interaction between different species of phytoplankton by reducing other species abundances and helps to maintain phytoplankton biodiversity. All the other species remain in the system in a stable steady state with low abundances. In natural ecosystems, there are evidences of the dominance of toxic species in a region for a long time period. For example, toxic *Aureoumbra lagunensis* (Buskey and Hyatt 1995) formed a massive long-lasting bloom event in the Laguna Madre, Texas (USA) from the time period 1990 to 1997 (Buskey and Stockwell 1993, Buskey et al. 2001). According to our study, we expect that all the other species of phytoplankton coexist with the toxic species at low abundances during that time period and maintain relatively stable abundances.

Although, frequent in natural systems, the amount of empirical data showing the coexistence of toxic and non-toxic species is limited. Only a few experiments from various communities are documented showing the coexistence in the presence of toxic effect. The coexistence in the experiments with killer toxin producing yeasts and toxin sensitive yeasts can occur because of the reduction in competition due to the spatial separation in microhabitats or temporal separation in different stages of successions (Abranches et al. 1997, Starmer et al. 1987). Although, our result
also shows a similar kind of reduction in competition between toxic and non-toxic phytoplankton, such reduction occurs due to the suppression of the abundance of the other non-toxic competitors in response to allelopathic interaction. As a result, the toxic phytoplankton dominates and coexists with other non-toxic species (with low abundance) in the system. Similar kind of dominance of the toxin producing species over the non-toxic competitors and their stable coexistence has been observed in an experiment with different bacterial strains. Ruiz-Barba et al. (1994) evaluated Bacteriocin-producing *Lactobacillus plantarum* LPCO10 and its non-bacteriocin-producing, bacteriocin-immune derivative, *L. plantarum* 55-1, to investigate their growth and persistence in natural Spanish-style green olive fermentation. During the experiment, they found the stable coexistence of both the strains and the dominance of bacteriocin producing strain with high population levels. A spatially explicit model of a multispecies bacterial community in the presence of antibiotic interactions within the community also found the stable coexistence of huge numbers of different species even in a temporally constant and spatially homogeneous environment (Czárán et al. 2002). Such stable coexistence occurred due to the local interference competition resulting from the excretion of antibiotic compounds and the resource competitive effects caused by the associated metabolic costs. However, the basic difference with our system is that the previous study was based on a spatially explicit game theoretical model with multiple cyclic dominance structures of different strains within a species where one of the strains are resistant to toxin and the self-organized spatial pattern of the system played a crucial role in determining the dynamics of the system. In comparison, the present system considers competition between two different species of phytoplankton in the presence of allelopathy and no immunity factor is involved.

Not only stabilizing effects, allelopathy can also result in the destabilization of the coexistence steady state. Recently, Grover and Wang (2014) studied a competition model where two toxin producing phytoplankton species are competing for two essential resources with cell quota for each species and found the destabilization of the coexisting equilibria in the presence of
allelopathic interactions become significant only when the abundance of the toxic species becomes very high and shows stabilizing effects of allelopathy. In natural ecosystems, several factors influence toxin production and different species produce toxins at different rates at different environmental conditions. Thus, depending on the conditions, allelopathy can have different effects, or no effects on the coexisting steady state (Czárán et al. 2002, Grover and Wang 2014, Chakraborty et al. 2008).

Furthermore, in this study, we examined how the resource supply affects the ecosystem functioning in the presence and the absence of allelopathy using biodiversity measures (Loreau and Hector 2001). These biodiversity measures show a positive effect of allelopathy on plankton ecosystems. We find positive measures in selection and net effects. Moreover, in the presence of different species combinations in the polyculture, these biodiversity effects may suggest which species dominance affects the relative yield and which one helps to maintain the species composition and diversity (Loreau 1998, Loreau et al. 2009, Turnbull et al. 2012). In general, separating the selection and the complementarity effects in biodiversity experiments, it is possible to assemble the communities with relative performing species. Although negative effects do not contribute to ecosystem functioning, but dominant species significantly identify the species performance with lack of efficiency in resource usage. These effects provide a clear evidence for the importance of toxic species in aquatic ecosystems. The strong relationship between diversity and stability of interacting species makes a positive effect to the ecosystems (Ives and Carpenter 2007).

The patterns of cascading extinction are examined following the removal and invasion of species. We find that the rate of cascading extinction is very less in the presence of allelopathy, even for an extremely diverse system. Moreover, community restoration can also be possible if we reintroduce the species in the ecosystem with appropriate environmental conditions. On the other hand, the removal of a species from a community happens due to the inability of the species to
make use of the available resources at some particular environmental condition. Removal of species can also occur in nature through selective predation by grazers or seasonal changes in the environment. However, the presence of allelopathy or negative competitive effect of species weakens the interspecific interaction among phytoplankton and helps to maintain the biodiversity for longer time scale. Therefore, we can suggest that environmental factors those are having negative effects on the competition between different phytoplankton species can also have a deep impact on plankton biodiversity.

In this study, the nutrient enrichment explains the relationship between ecosystem functioning and species diversity through the competition among phytoplankton species. The strength of the ecological community is explained by considering the toxic phytoplankton as a keystone species. In addition, how nutrient enrichment can influence the structure and pattern of phytoplankton species diversity are illustrated using this model. Although, an increase in the nutrient input enhances species abundances, the competition among species leads to species extinction and results in the loss of biodiversity. The existence of multiple species in different environmental conditions emphasizes the possibility of supersaturation coexistence although the number of existing species varies with the degree of nutrient enrichment (Sperfeld et al. 2010). Further, the invasion of species shows the diversity-stability relationship among communities (Ives and Carpenter 2007).

Similar to the nutrient enrichment scenario, the loss of biodiversity can also occur due to the increase in allelopathic interactions among species in the ecosystem. More toxicity in the community leads to loss of species abundance. Therefore, from the biodiversity point of view, nutrient enrichment and allelopathic interactions among different species of phytoplankton can have similar kind of impact on plankton community although they affect plankton populations differently. Nutrient enrichment increases competition among phytoplankton by increasing their abundances (Tubay et al. 2013) whereas allelopathy decreases competition by reducing population
abundances of non-toxic phytoplankton (Prince et al. 2008). However, in natural plankton systems, these two factors are interrelated. In general, allelopathy is not effective when the abundance of the toxic phytoplankton remains low. However, under nutrient enrichment, when the abundance of toxic phytoplankton become very high, the aggregated amount of toxin in the water column becomes significant and as a result, allelopathy appears important. Thus, nutrient enrichment increases the competition between phytoplankton species which can be neutralized by an increase in allelopathy, and this can open the possibility for the coexistence of many species. As a consequence of this study, we consider an analogy related to the toxicity. If we consider zooplankton as a keystone predator, we can easily compare the grazing on phytoplankton by the zooplankton with the allelopathic effect. Due to their grazing activity, the zooplankton suppress phytoplankton abundances which helps in the biodiversity of plankton (Leibold 1996). Similarly, we argue that allelopathy also acts in the same way and plays an important role in maintaining biodiversity in plankton systems. However, it is not only grazing rate or allelochemical effects, overall, any kind of negative effect on phytoplankton helps to maintain a stable as well as diverse ecological communities. Further, these diversity-stability relationships can be maintained for a longer time scale in the presence of negative effect. Moreover, variation in the environmental factors can also be responsible for the plankton biodiversity (Stomp et al. 2011). We suggest that, in the low-seasonality subtropical oceans, where non-equilibrium dynamics are less important, allelopathy can play a major role in maintaining the plankton biodiversity.

To summarize, number of approaches have been used to find the effects of toxic phytoplankton on a community of different phytoplankton. Each approach suggests that the toxic phytoplankton as a keystone species contributes to have diverse ecological communities. However, still we need to identify the mechanisms to control the toxic phytoplankton bloom with diverse plankton communities. Further extension has to be identified across multiple trophic levels controlling the toxic phytoplankton blooms by predation techniques and food-web patterns.
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References


Figure captions

Figure 1: (a) Number of surviving species in 100 different communities after 20 years of simulations. For each simulation, half-saturation constants, $K_i$, for the different species $N_i$ ($i = 1, \ldots, 8$) and allelopathic effects, $\theta_i$, of $N_1$ on other species $N_i$ ($i = 2, \ldots, 8$) are chosen as random and the other parameter values are given in Online Supplementary Material I in Table 1. This figure depicts that the number of coexisting species varies from 1 to 8 only depending on single resource. (b) Histogram showing the frequency of occurrence of different communities with equal number of coexisting species out of the 100 different communities.

Figure 2: Trajectories of the toxic and the non-toxic species. All the 8 species are coexisting together in a stable equilibrium state only depending on one resource. Parameter values are given in Online Supplementary Material I in Table 2.
**Figure 3:** The region of supersaturations (shaded in gray) at (a) $\theta = 0.00008$, (b) $\theta = 0.0004$, and (c) $\theta = 0.005$ when one toxic and one non-toxic species are competing for a single resource. In the white region, either of the species exists depending on the initial conditions. In the white regions, either of the species exists and in the blue shaded regions both the species are extinct. Clearly, the coexistence of both the species starts to occur at comparatively lower values of the resource supply under high toxic effects. Parameter values are given in Table 3 in Online Supplementary Material I.

**Figure 4:** Plot of (a) total biomass of the species, (b) resource, (c) resource use efficiency, (d) net effect, (e) selection effect and (f) complementary effect as a function of the nutrient supply concentration ($S$) for two species-one resource system where one of the species is toxic. The solid (dashed) curve represents the measurements in the presence (absence) of allelopathic effect. In this figure, we considered $D = 0.5$ and $\theta = 10^{-3}$ and the other parameter values are same as in Fig. 3.
Figure 5: (a) Pattern of cascading extinction following the removal of a randomly chosen species (50 communities each of all sizes of surviving species). The open circles represent the expected number of species \((j - 1)\) just after the removal of one of the surviving species and filled circles indicate cascading extinctions. The size of the circles corresponds to the number of communities for each community size. For higher community sizes cascading extinction occurs but only one more species than just the target species extinct. (b) Reinvasion of the removed species with density \(10^4\) cells/ml into the new community formed by its disappearance. The open circles represent the expected number of species just after the reinvasion and filled circles indicate cascading extinctions. The size of the circles corresponds to the number of communities for each community size.

Figure 6: Blue and red circles represent two communities with 8 and 6 species in the initial stage for the nutrient supply \(S = 19\), respectively. Increase in the nutrient supply (along \(x\)-axis) to find where one coexisting species (along \(y\)-axis) extincts. Each point represents the nutrient supply rate where the number of species decreases by one from the larger number. Parameter values are given in Online Supplementary Material I in Table 4.

Figure 7: Diagram shows the proportion of allelochemical effect in the chemostat model (along \(x\)-axis) with the number of coexisting species(along \(y\)-axis). Parameter values are given in Online Supplementary Material I in Table 5.
Figures

Figure 1

Figure 2
Figure 3

(a) \( \theta = 0.00008 \)  
(b) \( \theta = 0.0004 \)  
(c) \( \theta = 0.005 \)

![Figure 3](image)

Figure 4

(a) Total Biomass
(b) Total Resource
(c) Resource Use Efficiency
(d) Net Effect
(e) Selection Effect
(f) Complementary Effect

![Figure 4](image)
Figure 5

Figure 6
Figure 7

- Number of coexisting species vs. Toxicity multiplier ($\theta_c$)