Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates

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Abstract:	Global change will upset the frequency, scale and distribution of harmful algal blooms (HABs), but we are unable to predict future HAB occurrences due to our limited understanding of how physicochemical changes affect interspecific interactions between HAB and non-HAB species. Trait-based mechanistic modelling is an important tool to unravel such mechanisms and quantify the various direct and indirect interactions within systems. The present study explores whether MacArthur's consumer-resource model can describe resource competition between multiple HAB and non-HAB dinoflagellates. To this end, two batch culture experiments (294 cultures in total) with monocultures and mixed cultures of HAB (Alexandrium minutum, Prorocentrum lima, Protoceratium reticulatum) and non-HAB species (Prorocentrum micans, Scrippsiella trochoidea) were performed. Despite changes to the relative (the N:P ratio) and absolute nutrient availability (dilutions of L1 medium), P. micans continuously outcompeted all other species in mixed cultures. Consumer-resource modelling parameterized using monoculture growth correctly predicted this outcome (R^2 between 0.80 and 0.95). Parameter estimates revealed that P. micans had a faster uptake of nitrogen when compared to its competitors, but did not differ in resource efficiency and natural mortality rate. Yet, while the model accurately predicted community dynamics during the growth phase, it was not able to predict their dynamics beyond the point of quiescence. Overall, consumer-resource modelling was shown to differentiate the roles of resource assimilation, resource efficiency, and natural mortality rates in these common experiments with minimal data requirements.

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Dear Dr. Kudela,

On behalf of my co-authors, I am pleased to deliver the revised manuscript of our original research article - entitled **"Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates."** – to be considered for publication in Harmful Algae.

In this manuscript, we explore the potential benefits of consumer-resource models (CRMs) as a basis for trait-based modelling of community dynamics of dinoflagellates in multispecies cultures. To this end, we adapt MacArthur's CRM – a largely forgotten adaptation of the Lotka-Volterra equations that enjoyed limited success in theoretical ecology – for general use in HAB research, and assess its capabilities by fitting it to the growth data of 294 single and mixed batch cultures. Trait-based modelling is rarely used on lab cultures, but it could advance our understanding of the relative importance of various interspecific interactions (incl. resource competition, allelopathy and grazer deterrence). Here we show that CRMs are well suited to understand and predict the resource competition between dinoflagellates in mixed batch cultures (the most common of culture methods). They are easy to use, require minimal data, and provide key insights into the importance of nutrient uptake, conversion efficiencies and maintenance requirements when comparing various (harmful) algae. We then discuss various novel model improvements that may extend this trait-based approach to studies on allelopathic interactions as well as in situ predictions.

The manuscript was originally submitted to Harmful Algae in Nov. 2019 and came back with "Major Revision" in Feb. 2020. Both reviewers agreed that the manuscript was interesting and found the proposed model to be potentially useful to the HAB community, but raised several points that needed addressing. We have copied these in our Response to the Reviewers.

We remain convinced that CRMs hold a lot of (mostly unexplored) potential for HAB research and, hence, believe that Harmful Algae would be the most appropriate journal for our manuscript. We have no conflicts of interest to disclose. The manuscript is an original work that has not been published, nor is it under consideration for publication elsewhere.

Thank you for your time.

Sincerely,

Maarten De Rijcke; Jan Baert
Natacha Brion; Michiel Vandegehuchte
Frederik De Laender; Colin Janssen

Dear Dr. Kudela,

Thank you for considering our manuscript titled "Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates." (HARALG-D-19-00245) for publication in Harmful Algae. Below you may find the appraisals of both reviewers and point-by-point responses to specific comments made by each. Enclosed, you will find our manuscript that was adapted accordingly. We would be glad to respond to any further questions and comments that may arise and look forward to hearing from you.

Yours sincerely,
Maarten De Rijcke (on behalf of all co-authors).

Reviewer 1 – General appraisal

The manuscript entitled: "Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates." is an innovative research paper which tackles the difficult question of predicting the HAB biomass. The authors used a mechanistic approach to the question by elegantly improving MacArthur's (1970) consumer-resource model with the parameters estimated by the results of the mono-cultures experiment.The model was hereafter used to predict the outcome of the mixed-cultured experiments from which it was partly successful. The procedure was repeated, first, at different N:P ratio treatment, and in second at different N:P ratio and absolute concentration. They did a tremendous experimental work to get the required parameters and to test their newly improved model. I believe to be a really inventive and elegant way forward which will sure gather a lot of interest within the HAB scientist community and even beyond. However, it is clear that the paper mostly tried to promote the model rather than the experimental set-ups, which I believe deserve more credits.

From the materials and methods to the end, I had the sense that the experimental part of each section (M&M, results and discussion) did not have as much attention, scrutiny and clarity than the modelling part. For instance, the M&M section on the first and second experiments (2.2 and 2.3) need to be more fluid. Also, despite mentioning the different statistical analyses, they are not explanation on what they use for. What do they compare with each statistical analysis and why? On the other hand, the "2.5 Development of a community model" section was easy to understand even for me who had never work with such model. As there is a back-and-forth between the experiments and the model I think it would help to have a flow chart illustrating the method design and would greatly help the readers to understand as it is rather complex.

I also had a hard time reading the results section, which to my opinion needs to be rewritten with the appropriate figure or table to illustrate the text. As we don't know what they have compared with each statistical method, we end up with a raw string of p value. Also, it needs more references to the appropriate figure and tables. Fig.3 is not referenced in the results section but only in the discussion which I think it is also useful beforehand. I think the flowchart in M&M could help clarify the needs for the different results and what they are used for.

The discussion suffers the same problem as the M&M section. Even if the authors mention that "The results of our experiments should not be viewed as ecological stoichiometry research" I believe that they should still talk about the problem they encountered during their lab experiments. Why didn't they manage to get the P.lima carrying capacity? Can they provide an explanation? Also, why do they think there was no cell growth in CF1 and CF0.1? What is their explanation? How would they've done it differently? As they provided new insight upon their model, I think they should make the same effort with the experimental section.

After my review I think that this work should be published after major revision as, despite the problem mentioned above, it has a great potential.

We would like to thank Reviewer 1 for the detailed comments that have helped us improve the manuscript. We appreciate the feedback that the model section was easy to understand as it has indeed received most scrutiny to that end. With the help of the comments of Reviewer 1, which we copied below, we hope to have improved the manuscript to similar effect.

Reviewer 1 – Specific comments

Introduction

l. 31 replace "upset" by "disturb"

A: We replaced “upset” by “disturb” as suggested (l. 31).

l. 71 "the succession of groups of phytoplankton" or "the phytoplankton groups succession".

A: We changed “the succession of phytoplankton groups” to “the succession of groups of phytoplankton” as suggested (l. 72).

l. 79 "appeared" or "seemed to be linked"

A: We replaced “can be linked” by “seem to be linked” to accommodate this remark (l. 80).

l. 83 "altering every biological interactions"

A: We used “every biological interaction” as suggested (l. 86).

l. 88 "In order to coop"

A: We replaced “to coop” with “In order to cope” to incorporate the suggestions of both reviewers (l. 91).

l. 91 "Their ability"
&
ll. 91 - 95 a bit confusing and the end of the sentence is not clear

A: We agree that the sentence could have been more concise. We have combined and rephrased this section as follows:

“Toxins, grazer deterrents and allelochemicals - i.e. exudates that cause nutrient leakage, inhibit photosynthesis, arrest the cell-cycle, or affect other enzymes of competing algae (Granéli and Hansen, 2006; Legrand et al., 2003; Reigosa et al., 1999) - reduce the long-term extinction risk of toxic algae and may help maintain toxic blooms (Granéli et al., 2008a; Ianora et al., 2011; Smayda, 1997; Smayda, 2008; Turner, 2006; Xu and Kiørboe, 2018).” (ll. 95-99).

ll. 100-104. "Because of the variable .... during bloom initiation." The sentence is unclear and too long.

A: We have rephrased and split the sentence to improve this section. It now reads:

“Toxic effects are variable or inducible in nature (e.g. Dam and Haley, 2011; Poulin et al., 2018), mostly occur at bloom-level densities (Jonsson et al., 2009), and can have a high individual cost for toxic species while providing collective benefits to others (Driscoll et al., 2016; Flynn, 2008a). For these reasons, there is doubt that these chemical interactions play a crucial role during bloom initiation.” (ll. 102-106).

l. 104-105 I suggest "More recently, Blossom et al. (2019)..."
&
l. 106 "low cell concentration. Moreover, they also suggested"

A: The sentences were changed according to these suggestions (ll. 106-109).

ll. 107-109 "The processes..., so to better understand the non-deterministic"

A: The proposed sentence felt disconnected from the prior text due to the changes detailed above. We have changed the sentence to “Overall, these studies demonstrate that the processes behind allelopathy need to be unravelled further to understand the non-deterministic nature of HABs during windows of opportunity.” instead (ll. 109-111).

l. 118-121 Split the sentence

A: We split the sentence as well the subsequent long sentence. The section now reads:

“This issue is often addressed by use of the dilution method. By increasing the number of target cells relative to a constant density of an allelopathic species, the amount of allelochemicals per target cell decreases. This should lead to increased growth of the target species. If the growth rate remains constant or decreases due to an increase in competition, allelopathy is considered to be absent (Weidenhamer, 2006).” (ll. 120-124).

l. 124 "(1)..." not clear

A: Poulin et al. (2018) have shown that the allelopathic effects of *K. brevis* on *A. glacialis* varies from strongly inhibitory to strongly stimulatory between strains. When using the dilution method, we would surmise that decreasing growth rates at higher densities of target species are caused by increased intraspecific competition rather than stimulatory allelopathic interactions. To avoid discussing whether allelopathy should be reserved for negative effects (sensu stricto definition) or should include all chemical interactions between cells, we propose to rephrase the sentence.

We have added “between strains” to the sentence and replaced “voiding the null hypothesis” with “obscuring the interpretation of the test” (l. 127).

l. 127 replace "species-species" by "interspecific"

A: Replaced as suggested. (l. 129)

l. 128 remove "aquatic" unless terrestrial phytoplankton exist?

A: The word “aquatic” was removed from the sentence (l. 130).

l. 140 "introduced the resource utilization functions" ?

A: We added “resource” to the sentence as suggested (l. 141).

Material and Methods

l. 168 How did you determine the exponential growth phase?

A: Weekly cell counts were performed on stock cultures. We added the following sentence to the manuscript to clarify this:

“The growth of stock cultures was monitored through weekly cell counts using a Sedgewick-Rafter counting chamber and a Kyowa Optical Biolux-2 light microscope. Both experiments used cells taken from stock cultures that were growing exponentially.” (ll. 172-174)

l. 171 don't start your sentences with "To". Turn the sentence around, you must

A: We have restructured the sentence as suggested. (ll. 177-178)

l. 173 I suggest "Ten algal growth media were prepared so to have ten unique nitrogen-to-phosphorus ratios",

A: We have rephrased the sentence as suggested. (l. 179)

ll. 174 -179 The description of your medium preparation can be simplified. The L1 medium is made of ... NO and ... PO corresponding to a N:P of 24. By only adjusting the NO concentration.... (all the NO concentration with all the N:P ratios).

A: We have shortened the corresponding paragraph (ll. 181-183) as follows:

“By only adjusting the NO_3^- concentrations, ten growth media with different nitrogen-to-phosphorus ratios were prepared: preparations of 294, 368, 441, 478, 515, 551, 588, 662, 735 or 882 µM NO_3^- corresponded to a N:P of 8, 10, 12, 13, 14, 15, 16, 18, 20 or 24, respectively.”

l. 180 replace "in all media" by "in each medium"

A: We replaced “in all media” by “in each medium” as suggested (l. 188).

l. 182 so how many monocultures, mixed culture and in total you had? Show off a bit more the extend of your work

A: We set up 120 monocultures and 30 mixed cultures or 50 treatments. This is now included in the text (ll. 191-192)

l. 184 "a 1 ml sample"

A: This was changed as suggested. (l. 194)

ll. 194 Change the sentence, you must

A: We have rearranged the sentence. Lines 203-204 now read:

“A second experiment was performed to examine whether the interspecific competition between dinoflagellates in batch cultures is affected by larger differences in macronutrient availability.”

ll. 196 It is not like experiment 1 as you had 10 conditions and in the second you have 12

A: We have removed the reference to experiment 1 as suggested. (l. 205)

ll. 197-206 Again I think the explanation of the experimental set up can be simplified. If I haven't misunderstood. L1 with alter N:P ratio (No concentration and respective ratio). Each altered N:P ratio medium were submitted to four different dilution or concentration factors (CFs), so to have 100%,... In the end, 12 unique medium were made and used for monoculture of .... and a mixed culture with triplicates each time. So in total you have 144 cultures including 108 monocultures and 36 mixed cultures. Is that correct? If it is, well done for your job. Maybe a table or an illustration can help the reader to understand your setup

A: The reviewer is right: we made 144 cultures spread across 12 unique media. Taking inspiration from the previous comment on the design of experiment 1, we simplified the description of the setup and summarized the design of experiment 2. The corresponding sections now read:

"Twelve unique algal growth media were made based on regular L1 growth medium. Media were first prepared with 294, 588 or 882 µM NO_3^- to obtain three N:P ratios (8, 16 and 24). All other L1 components (PO_4^{3-}, vitamins and trace elements) were added at the regular dose. Each medium was subsequently diluted by a factor 1, 10, 100 or 1000 to obtain media with 100%, 10%, 1% or 0.1% volume fractions of L1 medium vs. Instant Ocean^{tm} artificial seawater." (ll. 205-209)

"Every treatment was replicated three times, resulting in 144 cultures (108 monocultures and 36 mixed cultures)." (ll. 214-215)

Why not using the same nutrient analysis for experiment 1 and 2?

A: The Analytical, Environmental and Geo-Chemistry research group of the University of Brussels owns the QuAAtro nutrient analyser. We did not have an active collaboration at the time of experiment 1, so we had to resort to a technique that could be performed at our lab.

l. 221 log-logistic? Logarithmic?

A: We used logistic growth models based on the Verhulst equation to describe the growth of our populations. In the earlier version of the manuscript, we incorrectly referred to log-logistic models and even logarithmic models, which has now been corrected throughout the manuscript.

l. 222 Kruskal Wallis and DMC references are missing
&
l. 225 Linear regression Ref?

A: We have added appropriate references for each method to the manuscript (ref. next comment).

l. 222-224 What are you going to compare? Between species? Between treatments? Both?

A: We used pairwise testing to compare the performance of each species to their growth in mixed cultures. Multiple group comparisons were used to compare growth rates and carrying capacities between species as well as to detect differences between treatments within each species. Linear regressions were used to detect linear responses to nutrient stoichiometry. We have rephrased the corresponding section to clarify the use of each test as follows:

"Multiple group comparisons by means of Kruskal Wallis (KW) tests (Kruskal and Wallis, 1952) were used to compare growth parameters (µ and K) between treatments (N:P) and species. Pairwise comparisons using Dunn's multiple comparison (DMC) test (Dunn, 1964) were made to investigate the effects of treatments (CF, mono vs. mixed) on the growth of each species. Linear regression models (LM) were used to detect linear responses to nutrient stoichiometry as described by Wilkinson and Rogers (1973)." (ll. 231-236).

l. 237 "Any excess of prey captured was converted into... into grams of Xi. "

A: We have replaced "any excess prey" by "any excess of prey" as suggested (l. 248).

l. 255 -256 "because nitrogen concentration vary the most in our experiments."

A: This sentence was rephrased in accordance with a comment of reviewer 2. (ll. 266-267)

ll. 272-274 split the sentence, annealing algorithm

A: We have split the sentence at annealing algorithm as suggested. (ll. 283-286).

l. 275 MCMC ref?

A: We have included Hastings (1970) as the appropriate reference as suggested (l. 286).

l. 276 - 277 Turn the sentence, you must

A: We have rearranged the sentence as suggested (l. 287-288).

Results

l. 289 Why 28? Should it not be in the method section?

A: There was no reason beyond the practical. Due to technical problems at the lab, only 1 climate room was available. As all our mixed cultures had reached stationary growth / species dominance, we terminated exp. 1 so that other experiments at a different temperature could take place.

l. 289-301 Where is the carrying capacity of *P. lima*? If you don't have it you should explain why. p should be written in italic capital (*P*)

A: *P. lima* was still growing exponentially by the end of the experiment, so we were unable to determine and report its carrying capacity. We changed the suggested lines emphasize that this species was still growing. The manuscript now states:

“Logistic growth models were used to determine the monoculture growth rates for all species except *P. lima*, which was still growing exponentially at the end of the experiment. Exponential growth models were used to determine the growth rates of *P. lima* instead (Supporting figures SF1-4).” (ll. 300-303).

We have replaced “p” with *“P*” for all statistics as suggested.

l. 302-311 "lost over half of its carrying capacity" where is the information? I also think that there is a figure or a table missing because I don't know what you are describing. The figure 12 in your supporting information (which should S6) has 20 graphs so which one are you describing?

A: The average carrying capacity of monocultures of *P. micans* was reported in the first paragraph of section 3.1. We now reiterate the monoculture value to help readers:

“On average, *P. micans* lost over half of its carrying capacity to competitors: its average carrying capacity decreased from $5.5 \pm 1.4.10^8$ $\mu m^3.ml^{-1}$ in monocultures to $2.1 \pm 0.4\ 10^8$ $\mu m^3.ml^{-1}$ in mixed cultures.” (ll. 316-318).

The statement related to SF12 refers to the declining nutrient concentrations. These can be found in the right graph for each treatment, which was only apparent in the figure caption. We added legends to each plot within the figure. In addition, we removed the asynchronous referencing of supporting figures by modifying their order and fixing their references, making sure that they are now uniformly called Supporting Figure or SF. The modified section now reads:

“Nitrogen and phosphorus concentrations from the first experiment can be found in supporting figures SF6-10. In mixed cultures, nutrients were depleted in all but the highest N:P ratio by day 14 (Fig. SF10).” (ll. 323-325)

l. 314 So CF 0.1 and 1 did not work out am I right? Need to write it

A: We observed between 1 and 3 cell divisions (population doublings) before the growth stopped. We added this observation to the text. Section 3.2 now starts with:

"The second experiment lasted 56 days, but the lowest concentration factors (CF0.1 and CF1) did not support prolonged growth. We observed between 1 (the lowest belonging to *P. reticulatum*) and 3 (found for *A. minutum*) population doublings before growth stalled. These treatments were no longer sampled after 39 days." (ll. 328-331)

l. 317 remove "again"

A: We have removed "again" from line 333 as suggested.

l. 320-321 rewrite the sentence

A: We have rephrased the sentence. It now states:

"The N:P ratio did have a significant (LM $P < 0.01$) positive effect on the carrying capacities of the three dinoflagellates at both CF10 and CF100." (l. 335-337).

l. 322 remove "again"

A: We have removed "again" from line 338 as suggested.

ll. 323-324 sentence not clear

A: We have split and rephrased the sentence:

"The growth rates of each species were determined by logistic growth models for CF10 and CF100. No significant differences were found between the growth rates of monocultures and mixed cultures for any of the three species (KW $P > 0.05$)." (ll. 338-341)

l. 351 Isn't there something missing between the brackets?

A: The bracket was complete but too brief. We found that increased growth rates are significantly linked to higher nutrient uptake rates in both experiments through linear regression models. We changed the bracket to "(LM: $P < 0.001$ for exp. 1; LM $P < 0.01$ for exp. 2)" to reflect this (l. 369).

Discussion

l. 361 "While many studies have" which one have you read?

A: There are numerous papers that investigate the effect of one or more parameters on the growth HAB species available in literature. We have added references to examples from the references that were already present in the study in lines 379-382. These are Chang and McClean (1997), Cooper et al. (2016), Gallardo Rodríguez et al. (2009), Guerrini et al. (2007), Ignatiades et al. (2007), John and Flynn (2000), Nascimento et al. (2005), Peperzak (2003), Sala-Pérez et al. (2016), Varkitzi et al. (2010), Wang et al. (2014), Zhengbin et al. (2006)

l. 363 "only a few have" where are the ref?

A: We now refer to Ji et al. (2011), Li et al. (2012), Poulin et al. (2018), Riegman et al. (1996) and Wang and Tang (2008) as examples. All these references were part of the original reference list. (ll. 383-384)

ll. 378 -379 "the data shown" what table and/or graph?

A: Growth rates of this study can be found in section 3.1 and Table 2. We included a reference to these sections (l. 400).

l. 379 Modification of the macro(nutrient) concentration in the growth media

A: We have rephrased the sentence as suggested. (line 401).

l. 394 I suggest "the dinoflagellates community structure" instead of hierarchy

A: We replaced “the hierarchy of dinoflagellates” with "the dinoflagellates’ community structure" as suggested. (line 417).

l. 398 CRM you might want to introduce it as consumer-resource model I presume?

A: The reviewer is right to point out that the abbreviation CRM was not introduced in the discussion (only in the introduction). It is now written “consumer-resource model (CRM)” in full (line 421).

ll. 397-400 Split the sentence

A: We have split the sentence as requested. The section now reads:

“According to the mean parameter estimates of our consumer-resource model (CRM), the success of *P. micans* should be attributed to its ability to capture resources rather than a high resource efficiency or low natural mortality rates. The uptake probability of both nitrogen and phosphorus of *P. micans* were (among) the highest observed.” (lines 420-424).

ll. 401-402 replace by "in the two experiments"

A: We have replaced “during both the experiments” by “in the two experiments” as suggested. (line 419).

ll. 400-404 split the sentence

A: We have split the sentence as suggested. The section now reads:

“All pelagic dinoflagellates grew at roughly the same rate relative to their monocultures in the first days of the two experiments. By sequestering nitrogen and phosphorus more rapidly, thereby denying its competitors access to these nutrients, *P. micans* was eventually able to outgrow all other species in mixed cultures”

l. 405 "in mixed cultures" instead of "in competition"

A: We replaced “in competition” by “in mixed cultures” as suggested. (line 428).

l. 410 "Luxury consumption" definition? Ref?
&
ll. 410-412 rewrite the sentence for better clarity

A: The manuscript now references the original paper that coined the term “luxury consumption” as well as a recent review that covers its potential as a functional trait. We have also rewritten the next sentence as suggested. The entire section now reads as follows:

“Another unknown is whether the success of *P. micans* can be attributed to “luxury consumption”. The rapid acquisition and storage of excess nutrients may be used to pre-emptively reduce the availability of resources for competing species (Droop, 1973; de Mazancourt & Schwartz, 2012). This trait has not been studied in *P. micans* to our knowledge, but its carrying capacity is known to positively correlate with nitrogen concentrations (Zhengbin et al., 2006; Zheng-fang et al., 1995). Similar results were found in this study.“ (ll. 432-437)

ll. 422-425 split the sentence

A: We have split the sentence as suggested. The section now reads:

“We set out to determine the efficacy of consumer resource modelling. Starting with the simplest setup available, which is the batch culture, we found that CRM’s could be used to predict species dominance resulting from interspecific competition between dinoflagellates in mixed cultures.” (ll. 447-450).

l. 460 "However, as shown here" which graph table?

A: The population decline can be observed in the density date of both experiments, as shown in supporting figures SF5 and SF12. We have added a reference to these figures in the statement. (line 484)

Reviewer 2 – General appraisal

This study combines batch-culture experiments testing the growth of five different dinoflagellates in monoculture and in mixture under different nutrient regimes (varying N:P concentrations and ratios) with a consumer-resource model (CRM). This model was parameterized from monoculture growth and was used to predict the outcome of competition in species mixtures. Overall, *Prorocentrum micans* outcompeted all other species in mixed cultures irrespective of nutrient regime, apparently based on the dinoflagellate's faster uptake of nitrogen. The CRM correctly predicted this outcome, at least during the growth phase of the dinoflagellates. The authors claim that CRMs provide a useful tool to predict dominance of HAB versus non-HAB species and that their model may improve our understanding of HAB dynamics.

Overall, this manuscript yields interesting information and a potentially useful model approach for investigating competition of phytoplankton including HAB dinoflagellates. However, the study does not live up to its promises: The introduction strongly focuses on allelopathy and direct interactions of HAB species with competing non-HAB species. The authors distinguish between toxic and non-toxic dinoflagellates in their experiments at first, misleading the readers to expect a study explicitly testing for competitive interactions between allelopathic HAB and non-HAB species. However, neither the experiments nor the model were designed to explicitly investigate competition between HAB and non-HAB species, or the effect of allelopathy in competitive interactions. Rather, the authors seem to use a random set of dinoflagellates, some of which may also produce toxins and all of which have been shown to produce allelochemicals (which does not become clear until the end of the discussion). Allelopathic interactions were neither measured for the species, nor were they included in the model; therefore, it is not known whether allelopathy played any role at all in the experiments.

The authors should make very clear from the beginning on that this study investigates competition of dinoflagellates in general without taking direct interactions such as allelopathy into account, and that the model can be used for all competing phytoplankton species, while it is not specifically designed to investigate interactions of HAB versus non-HAB species.
As allelopathy or HAB versus non-HAB species do not play any role in either the experiments or the model, the introduction and parts of the discussion need to be completely rewritten, focusing on nutrient competition, stoichiometry and traits of the dinoflagellates used in this study. The major point in the discussion should be that a model that is solely based on nutrient concentrations, uptake probability and conversion efficiency can predict the competitive outcome of dinoflagellates, at least in the growth phase, while direct interactions may play a larger role at high bloom concentrations, which could be included in the hybrid model that the authors propose at the end of the discussion.

I recommend publication of this manuscript after a major revision setting the study into the right context, clearly motivating its intention and emphasizing its potential relevance. However, I leave it to the editor to decide whether this general competition study is suitable for publication in Harmful Algae.

We would like to thank Reviewer 2 for his/her valuable input that helped us improve our work. We agree with the reviewer that our experiments do not adequately address HAB vs. non-HAB competition and have removed most references to HAB vs. non-HAB and toxic vs. non-toxic from the manuscript. The main conclusion of this pilot study is that CRMs provide a valuable basis for trait-based modelling of species interactions in mixed cultures. We believe that the use of mixed cultures coupled to improved CRMs (ref. discussion) can expand our understanding of allelopathic interactions. Lessons should be drawn from this study to design experiments to that end. For this reason, we introduce allelopathy in the introduction and conclude on allelopathy in the discussion.

Some of the reviewers' comments are related to our geographical bias. The research presented here was part of a larger project that aimed to investigate the present and future risk of HABs in the Belgian Part of the North Sea. The seemingly random set of dinoflagellates are all species occurring within our EEZ. Likewise, the N:P ratios and light treatment we used are based off of in situ observations. We provided further clarification below.

Reviewer 2 – Specific comments

Abstract & Highlights

The information that is provided in the highlights should also be included in the abstract

A: We have modified the highlights to adhere to the abstract.

Please clarify that the experiments were performed with multispecies mixtures (as opposed to 2-species mixtures)

A: l. 38 now refers to “multispecies cultures” instead of “mixed cultures”

The authors should clarify the most important findings of their study (see also general comment)

A: The abstract now contains our main finding that CRMs are a potentially valuable basis for trait-based modelling that needs further development (ll. 49-50)

Introduction

l. 68: please add an "a" or "the" before "…major goal…."

A: l. 69 was changed as suggested (“a major goal”).

Line 80 - 82: please add that in addition to similar nutrient requirements and uptake kinetics to non-HAB species, there is a huge variability within and among different HAB groups in these parameters, hampering our predictive capability of where and when HABs will occur

A: We have rephrased ll. 80-82 to include reference to the natural variability within species. The section now reads as follows:

“While both chronic and episodic eutrophication seem to be linked to HABs, there is no clear evidence that nutrients promote HABs by themselves. Nutrient uptake kinetics and resource preferences vary greatly within and between HAB species (Glibert and Burkholder, 2006) and cannot be distinguished from those of closely related non-HAB species (Anderson et al., 2002; Heisler et al., 2008; Wells et al., 2015). This hampers our ability to predict where and when HABs will occur based on resource abundance alone” (ll. 80-85).

Line 88: please exchange "coop" by "cope"

A: This was changed as suggested (ll. 90).

Line 90: allelopathy is not necessarily toxin-mediated; e.g. for Alexandrium it has been shown that allelopathic substances are not related to the production of PSP toxins (Tillmann & John 2002, MEPS 230)

A: We agree with the reviewer, that is why the original sentence listed allelochemicals, toxins and grazers deterrents as separate entities, but the sentence was unclear (see also reviewer 1). We have rephrased the sentence. The manuscript now reads:

“Toxins, grazer deterrents and allelochemicals - i.e. exudates that cause nutrient leakage, inhibit photosynthesis, arrest the cell-cycle, or affect other enzymes of competing algae (Granéli and Hansen, 2006; Legrand et al., 2003; Reigosa et al., 1999) - reduce the long-term extinction risk of toxic algae and may help maintain toxic blooms (Granéli et al., 2008a; Ianora et al., 2011; Smayda, 1997; Smayda, 2008; Turner, 2006; Xu and Kiørboe, 2018).” (ll. 95-99).

Line 114 - 115: not necessarily, this is very dependent on the HAB species

A: The reviewer is right to point out that toxicity is not necessarily increased by direct contact. We have rephrased the sentence to be more cautious:

"The toxicity of intact cells can sometimes be increased by or be dependent on direct contact with targets (Driscoll et al., 2016)." (ll. 116-117).

Overall, the authors set a strong focus on allelopathy and on how to test for allelopathic interactions; however, neither in their experimental design nor in their model they account for allelopathic interactions. Therefore, the introduction and the actual study presented in Methods and Results seem a bit decoupled

A: As stated above, we believe that multispecies cultures coupled to improved CRMs can improve our understanding of allelopathic interactions. For this reason, we introduce allelopathy in the introduction and conclude on allelopathy in the discussion.

The authors should introduce the dinoflagellate species that they use in their experiment in terms of toxins, allelopathic substances, and especially potential nutrient requirements (have blooms been related to nutrient conditions) as experiments and models are designed to test nutrient effects on competition

A: A detailed introduction on the allelopathic and toxic properties of each species used may create false expectations from the reader. As the reviewer rightfully points out, we did not measure these chemicals during our study. We never measured the resource preferences and uptake kinetics for various N-sources of the strains that we used in the study either. As the reviewer pointed out, these can vary strongly within species. The information also has little bearing on the experiment, as the L1 medium only offers nitrate and phosphate as standardized nutrients.

The authors should motivate why they test different nutrient concentrations and ratios in their experiments - what role does nutrient stoichiometry play for algal competition, how do changes in nutrient ratios effect HAB species etc.

A: As explained in the discussion, our experiments are not suited to study ecological stoichiometry so we want to avoid creating false expectations in that regard. Generally speaking, we only used different nutrient concentrations (be it CF or N:P ratios) to introduce some variability for the model to predict and to try and upset the species dominance in mixed cultures. As the N:P ratios are not controlled throughout the experiment, they will have changed differentially during the experiment depending on the uptake kinetics of each species. Nutrient concentrations should also have been lower / limiting to study the effect of nutrient stoichiometry.

The authors distinguish between HAB and non-HAB species - however, this distinction does not play any role for further aspects of their study, neither for the experiments nor for the model, as the competition of all dinoflagellates in a multi-species mixture are investigated

A: The distinction between HAB and non-HAB has been removed from the manuscript.

l. 151: please specify that dinoflagellates were tested in multispecies mixtures containing "HAB" and "non-HAB" species, and not explicitly testing HAB versus non-HAB species in 2-species mixtures

A: We now refer to multispecies cultures throughout the manuscript and have removed references to HAB and non-HAB dinoflagellates.

Materials and Methods

On what basis were the different N:P concentrations and ratios prepared? What was the intention of such a high resolution of N:P ratios?

A: The research presented here was part of a larger project that aimed to investigate the present and future risk of HABs in the Belgian Part of the North Sea. The N:P ratios used here are centred around 14, which is the mean N:P ratio found between June and September (years 2013-2019) in the marine area designated for aquaculture in Belgium. On an annual basis, the mean N:P ratio for all monitoring stations within the Belgian Part of the North Sea is 22. We have added a short reference to the M&M section to clarify that our nutrient ratios were inspired by our own location:

"The range of N:P ratios that was used was based on N:P ratios that were observed in the Belgian part of the North Sea between 2013 and 2019 (Mortelmans et al., 2019). On average, the Belgian EEZ has a mean N:P ratio of 22 while the average N:P ratio in a local shellfish area is around 14 during summer." (ll. 184-187).

The L1 dilutions were inspired by the work of others, as referenced in the discussion (ll. 401-404).

ll. 181-182: why did the authors chose an additive instead of a substitutive design? Mixed cultures started with a much higher algal biomass than monocultures - are monocultures and mixtures comparable in terms of intraspecific versus interspecific competition?

A: We used an additive design to avoid human error while setting up so many cultures, but both designs would have worked as the consumer-resource model incorporates intra- and interspecific competition through the density-dependency (X_i) of the populations and nutrients (Eq. 7-9).

l. 182: please specify "all resulting treatments" as it is still not clear what and how many mixtures were set up in addition to monocultures

A: As suggested by Reviewer 1, we changed l. 182 to:

"All the resulting 50 treatments were replicated three times for a total of 150 cultures (120 monocultures and 30 mixed cultures)." (ll. 191-192)

Exp. 1: Why did the experiment run at such a high temperature and such a low light intensity? What role could light and temperature have played for dinoflagellate performance and competition? Please comment on that.

A: We had originally planned to compare 20°C to 24°C (as a worst-case IPCC scenario for the Belgian North Sea), so experiment 1 was in fact set up in double (300 cultures). A technical issue with the climate control of the 20°C room left us without reliable data for 20°C. 24°C is, however, representative for current day summer temperatures at a local shellfish area called the "spuikom", but this regional focus is outside the scope of article. The introduced CRM is widely applicable.

Similar to the N:P ratios, light conditions were chosen to mimic the Belgian Part of the North Sea where light is severely limited. Light penetrating the first 20 meters of the wider North Sea has a mean intensity of 75 µmol m^{-2} s^{-1} (Gröger et al., 2013). Due to the presence of high concentrations of light-attenuating cDOM and strong mixing of the photic and euphotic zones within the Belgian EEZ, we typically expose our organisms to 20-40 µmol m^{-2} s^{-1}. We included a reference to summer conditions in the Southern North Sea in our M&M to improve our manuscript (ll. 171-172).

Exp. 2 - please comment on the choice of nutrient concentrations and ratios (see above, from P-limitation to N-limitation, range of concentrations etc.)

A: The N:P ratios were inspired by N:P ratios occurring in the Belgian EEZ , while the L1 dilutions were inspired by the work of others, as discussed later on (ll. 401-404).

Why did the authors use a different temperature (20°C as opposed to 24°C) and a different set of dinoflagellates than in Exp. 1? Only P. micans and P.reticulatum were used in both experiments, and only in the first experiment also non-HAB species were included in addition to HAB species - please clarify. Please also comment on the different experimental durations of Exp. 1 and 2

A: 20°C is more representative for summer temperatures in the Belgian EEZ. As explained above, Exp. 1 was originally designed to have both 20°C (current day) and 24°C (predicted future temp.) but we encountered technical issues with our climate room of 20°C. We used 20°C for Exp. 2 as this has more regional relevance.

Exp. 1 and Exp. 2 were performed months apart. At the time, we did not have sufficient stock of *S. trochoidea* at hand. We are aware that *P. micans* has been implicated with HABs in the past, but we considered the species to be non-HAB in accordance to the Taxonomic Reference List of Harmful Algae. The manuscript no longer refers to HAB vs. non-HAB regardless.

The duration of Exp. 1 was constrained due to practical arrangements as clarified to Reviewer 1. We had a restriction on the use of the climate room due to the problem of the other room. Exp. 2 had no such restrictions, so we could let it run longer.

ll. 204/205: please clarify that a "3-species mixture" was used (see above)

A: We replaced “mixed cultures” with “3-species mixtures” as suggested (l. 213).

ll. 254-256: Please explain why this simplification can be made in this context - the authors substantially vary the N:P ratios, inducing N- and P-limitation in the algal cultures, potentially influencing growth and production of secondary metabolites. Why do the authors think that N is the main driving force in determining dinoflagellate growth? They show in their first experiment that nutrient stoichiometry significantly affected the growth rate of 3 of the 4 dinoflagellates.

A: We agree that the substantial changes in the N:P ratio may have induced N- or P-limitation in our cultures. For this reason, we opted to model each N:P treatment separately rather than coming up with a single mean parameter estimates across all ratios for each species. We did not wish to suggest that nitrogen availability is the main driving force in determining dinoflagellate growth. However, we assume that growth can be adequately described by a single nutrient to predict batch culture growth. This simplification is needed to eliminate the constant of proportionality. The decision to use N-availability is strictly made due to the fact that the experiment was set up with varying nitrate concentrations. Had we modified the N:P ratio through phosphate concentrations, we could have modelled the cultures using P-availability instead. We have rephrased our M&M section to reflect that both nutrients could have been used. (ll. 265-267).

l. 266: what determines the mortality coefficient?

A: The CRM’s mortality coefficient is a constant used to incorporate density-dependent mortality in a simplified manner. It provides a mathematical solution that improves our model predictions in monocultures, but does not account for changes in natural mortality rates throughout the life cycle of each culture. Instead, it represents an average cell loss across the duration of the experiment. Overall, this parameter has little influence on the model predictions (estimates are very small and parameter estimates vary wildly during the convergence of the Markov chains).

Results and Discussion:

Fig 2: please enlarge the Figure as well as axis titles and legends; do the dots represent the experimental observations? Please clarify in the figure legend.

A: We have enlarged Figure 2 and have added that markers are observations as requested. The figure can be found on page 26 of the manuscript. Fig. 1 and Fig. 3 were also reworked to enlarge their axis titles and legends.

ll. 373-375: this is not true - this study was not designed to explicitly test interactions between HAB and non-HAB species, it just tested interspecific competition of a random set of dinoflagellates, some of which can form HABs

A: We removed references to competition between HAB vs. non-HAB throughout the manuscript as discussed above.

ll. 404 - 405: was this also predicted from the model?

A: The CRM we used cannot predict changes in growth rate related to the availability of additional resources that are not part of its structural equations (assuming that organic nitrogen is in fact the driving mechanism here). To better highlight this issue, we have now calculated and included the coefficient of determination for *P. lima* alone (l. 360). The poor fit for *P. lima* is then highlighted in the discussion (ll. 450-454) as follows:

"CRMs can approximate the densities of both winning and losing algal species up to the plateau phase with a high degree of accuracy. Stark changes in growth rate between monocultures and multispecies cultures such as those observed *in P. lima* can, however, lead to poor predictions if the underlying mechanism is not fully understood and included in the structural equations."

ll. 415 - 421: The authors should state that already in the introduction to clarify the motivation of their study

A: The abstract and introduction were changed to put more emphasis on the model. The N:P ratio is now only mentioned once at the end of the introduction to make sure that readers do not get false expectations.

l. 421 - 422: see comment above - please explain why this simplification is suitable here.

A: As discussed above, we rephrased the M&M section (ll. 265-267) to reflect that growth can be described by either nutrient and that we made a choice to use nitrogen. We then further highlight that both are optional in the discussion. The paragraph (ll. 445-447) now reads:

"In this study, the N:P ratios and the CF's were merely used to introduce variability in the nitrate concentrations, which we then chose as the driver of the consumer-resource model used."

l. 424-425: see above - in the context of this study it is completely irrelevant whether the dinoflagellates are HAB or non-HAB species as this is neither considered in the experiments nor in the model. Statements like this mislead the reader to think that this was explicitly tested!

A: We agree with the reviewer and have removed all references to HAB vs. non-HAB throughout the manuscript when discussing our study.

l. 429 - 430: It is not possible for the authors to tell whether direct interactions played a role at all, because none of them were tested. The species / strain specific traits are not known (or have not been introduced)

A: We agree that the previous statement was too bold and have removed the reference to direct interactions from the sentence. The corresponding section now states:

"By using a CRM, this study was able to demonstrate that the presence of a fast-growing species (*P. micans*) had strong, indirect negative effects on the growth of competing dinoflagellates; the growth of competing algae was to a large degree hampered by diminishing nutrient availability due to uptake by *P. micans*." (ll. 454-457).

ll. 431 - 433: This should be stated in the introduction; however, there is a high intraspecific variability in the production of allelochemicals in many dinoflagellates and the fact that all species have been found to potentially produce allelochemicals does not mean that the strains used here were able to do so

A: Considering all comments, we decided not to include an overview of the allelopathic properties of each species in the introduction to avoid creating false expectations from the reader. As the reviewer rightfully points out, we did not explicitly test for allelopathy in the current study. We do, however, believe that we need to discuss allelopathy in the introduction and discussion to highlight the strengths and weaknesses of the CRM, and to provide recommendations for the next iteration of CRMs. To be more clear in the manuscript, we now state that we did not explicitly test the ability of our strains to produce allelochemicals (l. 460).

ll. 433 - 442: this is possible, but cannot be deduced from the present study - see above

A: We have removed the text related to our earlier attempts at modelling our observations using Lotka-Volterra based approaches as, indeed, these faulty analyses were not shown here.

ll. 475 - 477: this should not be stated at the very end of the discussion, but in the introduction to make clear what this study aims at and potentially can provide and what not (now ll. 498-500)

A: As discussed above, the manuscript now puts more emphasis on CRMs being a starting point for future trait-based modelling. By working with our basic CRM on this dataset, we conceptually developed an extended model that includes allelopathy and might be relevant for future research. This theoretical framework did not exist at the time that we designed our experiments, as it is part of the lessons learned during this study, so our data was never intended to suit the model that we propose in the discussion. It is, however, likely that other researchers may come up with different ways of extending the basic CRM in ways that avoid the need for bi-algal cultures.

Highlights

- Five common dinoflagellates were co-cultured under 22 nutrient regimes.
- Monoculture growth was used to parametrize a consumer-resource model (CRM).
- Consumer-resource modelling can predict species dominance in mixed batch cultures.
- CRMs may differentiate resource assimilation, resource efficiency, and natural mortality.

Abstract

Global change will disturb the frequency, scale and distribution of harmful algal blooms (HABs), but we are unable to predict future HABs due to our limited understanding of how physicochemical changes in the environment affect interspecific competition between dinoflagellates. Trait-based mechanistic modelling is an important tool to unravel and quantify various direct and indirect interactions between species. The present study explores whether MacArthur's consumer-resource model can be used as a viable base model to predict dinoflagellate growth in closed multispecies systems. To this end, two batch culture experiments (294 cultures in total) with monocultures and multispecies cultures of *Alexandrium minutum*, *Prorocentrum lima*, *P. micans*, *Protoceratium reticulatum* and *Scrippsiella trochoidea* were performed. Despite changes to the relative (different nitrate concentrations) and absolute nutrient availability (dilutions of L1 medium), *P. micans* outcompeted all other species in mixed cultures. Consumer-resource modelling parameterized using monoculture growth correctly predicted this species dominance (R^2 between 0.80 and 0.95). Parameter estimates revealed that *P. micans* had a faster uptake of nitrogen when compared to its competitors, but did not differ in resource efficiency and natural mortality rate. Yet, while the model accurately predicted community dynamics during the growth phase, it was not able to predict their dynamics beyond the point of quiescence. Consumer-resource modelling was shown to differentiate the roles of resource assimilation, resource efficiency, and natural mortality rates in batch culture experiments with minimal data requirements beyond common measurements. The results suggest that consumer-resource models provide a promising basis for trait-based modelling of interspecific competition between (harmful) algae.

Title: Monoculture-based consumer-resource models predict species dominance in mixed batch cultures of dinoflagellates.

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Declarations of interest: none

Running title:

Nutrient competition in mixed batch cultures of dinoflagellates: species dominance predicted by a simple consumer-resource model

Keywords: Consumer-resource modelling, mixed batch cultures, dinoflagellates

Abstract

Global change will disturb the frequency, scale and distribution of harmful algal blooms (HABs), but we are unable to predict future HABs due to our limited understanding of how physicochemical changes in the environment affect interspecific competition between dinoflagellates. Trait-based mechanistic modelling is an important tool to unravel and quantify various direct and indirect interactions between species. The present study explores whether MacArthur's consumer-resource model can be used as a viable base model to predict dinoflagellate growth in closed multispecies systems. To this end, two batch culture experiments (294 cultures in total) with monocultures and multispecies cultures of *Alexandrium minutum*, *Prorocentrum lima*, *P. micans*, *Protoceratium reticulatum* and *Scrippsiella trochoidea* were performed. Despite changes to the relative (different nitrate concentrations) and absolute nutrient availability (dilutions of L1 medium), *P. micans* outcompeted all other species in mixed cultures. Consumer-resource modelling parameterized using monoculture growth correctly predicted this species dominance (R^2 between 0.80 and 0.95). Parameter estimates revealed that *P. micans* had a faster uptake of nitrogen when compared to its competitors, but did not differ in resource efficiency and natural mortality rate. Yet, while the model accurately predicted community dynamics during the growth phase, it was not able to predict their dynamics beyond the point of quiescence. Consumer-resource modelling was shown to differentiate the roles of resource assimilation, resource efficiency, and natural mortality rates in batch culture experiments with minimal data requirements beyond common measurements. The results suggest that consumer-resource models provide a promising basis for trait-based modelling of interspecific competition between (harmful) algae.

Highlights

- Five common dinoflagellates were co-cultured under 22 nutrient regimes.
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- CRMs may differentiate resource assimilation, resource efficiency, and natural mortality.

1. Introduction

Phycologists have tried to understand and predict the spatiotemporal occurrence of harmful algal blooms (HABs) for decades. Red tides were first considered to be inherently unpredictable due to the dynamic nature of marine ecosystems as well as the vast number of functional properties (e.g. nutrient uptake rates, internal storage, pigment composition etc.) and adaptive strategies (e.g. cyst production, cell shape, motility, thin layer formation) the causative organisms may have (Sweeney, 1978, 1975). Over the years, it was discovered that phytoplankton communities are structured by nutrient competition, species interactions (grazing, allelopathy), abiotic variables (light, temperature, turbulence etc.) and stochastic processes (Armstrong, 1979; Eppley, 1972; Huisman and Weissing, 1994; Legrand et al., 2003; Margalef, 1978; Richerson et al., 1970; Smayda, 2008; Tilman, 1977). Today, it is widely accepted that HAB development results from exceptional successions of phytoplankton that require specific environmental conditions to occur (Stoecker et al., 2008). Identifying the sets of biotic and abiotic conditions that enable the initiation and development of HABs, sometimes referred to as “windows of opportunity”, has been a major goal of HAB research from the start.

Ramón Margalef observed that nutrient availability and the decay of turbulent energy determine the succession of groups of phytoplankton and, hence, the likelihood of toxic bloom development (Margalef, 1978). In his now-famous “mandala”, harmful red tides may develop when the nutrient availability is high and the turbulent energy is restricted. While his mandala was improved through the addition of functional properties, demographic strategies and the inclusion of novel HAB taxa (e.g. Allen and Polimene, 2011; Balch, 2004; Glibert, 2016), neither the original mandala nor the recent renditions were able to resolve the non-deterministic nature of HAB development. Blooms often fail to develop under seemingly ideal conditions. To this day, we are unable to reliably predict how changes in either the relative or absolute availability of nutrients affect the risk of HABs in a given phytoplankton community. While both chronic and episodic eutrophication seem to be linked to HABs, there is no clear evidence that nutrients promote HABs by themselves. Nutrient uptake

kinetics and resource preferences vary greatly within and between HAB species (Glibert and Burkholder, 2006) and cannot be distinguished from those of closely related non-HAB species (Anderson et al., 2002; Heisler et al., 2008; Wells et al., 2015). This hampers our ability to predict where and when HABs will occur based on resource abundance alone. It is, however, clear that eutrophication affects the entire food web, altering every biological interaction (e.g. nutrient competition, grazing, allelopathy) that collectively determines the success of harmful algae (Glibert et al., 2010; Granéli et al., 2008b; Smayda, 2008).

Dinoflagellates are poor competitors for nutrients and, hence, are at risk of competitive exclusion (Smayda, 1997). They also face strong grazing control by microzooplankton, mesozooplankton and benthic filter feeders (Smayda, 2008; Tillmann, 2004; Turner, 2006). In order to cope with both these interspecific interactions, dinoflagellates have evolutionary adaptations such as the production of cysts, mixotrophy, (toxin-mediated) allelopathy and grazer deterrence (Bravo and Figueroa, 2014; Chakraborty et al., 2015; Crane and Grover, 2010; Roy and Chattopadhyay, 2007). Toxins, grazer deterrents and allelochemicals - i.e. exudates that cause nutrient leakage, inhibit photosynthesis, arrest the cell-cycle, or affect other enzymes of competing algae (Granéli and Hansen, 2006; Legrand et al., 2003; Reigosa et al., 1999) - reduce the long-term extinction risk of toxic algae and may help maintain toxic blooms (Granéli et al., 2008a; Ianora et al., 2011; Smayda, 1997; Smayda, 2008; Turner, 2006; Xu and Kiørboe, 2018). Allelopathy and grazer deterrence should allow increasingly dominant organisms to overpower their competitors during HAB initiation. Yet, to date, their role during the first stages of HAB development remains unclear. Toxic effects are variable or inducible in nature (e.g. Dam and Haley, 2011; Poulin et al., 2018), mostly occur at bloom-level densities (Jonsson et al., 2009), and can have a high individual cost for toxic species while providing collective benefits to others (Driscoll et al., 2016; Flynn, 2008a). For these reasons, there is doubt that these chemical interactions play a crucial role during bloom initiation. More recently, Blossom et al. (2019) have demonstrated that allelochemicals can yield significant cell-level benefits at very low cell concentrations. Moreover, they also suggested that

meaningful trade-offs between allelopathy and growth rate (i.e. fitness costs) determine whether allelochemicals are released. Overall, these studies demonstrate that the processes behind allelopathy need to be unravelled further to understand the non-deterministic nature of HABs during windows of opportunity.

Allelopathic interactions between microalgae are usually studied in one of three ways: (1) through the addition of cell-free culture filtrates to competitors; (2) by using caged batch cultures whereby both species are co-cultured, but separated by a permeable mesh or membrane; (3) by means of co-existence experiments that co-culture both species in direct contact. Each method has its own drawbacks. The toxicity of intact cells can sometimes be increased by or be dependent on direct contact with targets (Driscoll et al., 2016). As a result, caution should be used when interpreting the results of the first two methods. Co-existence experiments, on the other hand, do not separate chemical interactions (i.e. allelopathy) from other interactions such as resource competition and mixotrophy (Allen et al., 2016). This issue is often addressed by use of the dilution method. By increasing the number of target cells relative to a constant density of an allelopathic species, the amount of allelochemicals per target cell decreases. This should lead to increased growth of the target species. If the growth rate remains constant or decreases due to an increase in competition, allelopathy is considered to be absent (Weidenhamer, 2006). Crucially, this approach fails to address two key aspects of allelopathic interactions: (1) that they may vary from strongly inhibitory to negligible to stimulatory between strains (Poulin et al., 2018), and (2) that they can be induced by increased nutrient competition (Granéli et al., 2008b), obscuring the interpretation of the test. Mechanistic modelling of culture dynamics may help alleviate these problems and could improve our understanding of interspecific interactions.

The first mathematical description of allelopathy in phytoplankton, where an interaction term was added to a two species Lotka-Volterra model, was proposed by Maynard Smith (1974). Over the years, numerous improvements and refinements were made to the Maynard-Smith function (e.g. Bandyopadhyay, 2006; Chattopadhyay, 1996; Mandal et al., 2014; Mukhopadhyay et al., 2003,

1998; Solé et al., 2005), demonstrating the high potential of Lotka-Volterra derived models. Yet, despite their merits, the Maynard-Smith-based models are completely dependent on direct, density-dependent interactions to assess intraspecific and interspecific competition. Neither the Lotka-Volterra equations, nor the Maynard-Smith equations, include spatiotemporal dynamics of nutrients. Considering that resource competition is a major determinant of blooms (Sourisseau et al., 2017), this study explores whether models that describe how consumers interact indirectly through the use of common resources can be used as an alternative approach.

Macarthur and Levins (1967) introduced resource utilization functions into the Lotka-Volterra's equations, which was later developed into a consumer-resource model (MacArthur, 1970, 1969). In contrast to Maynard-Smith's later model, MacArthur's consumer-resource model (CRM) does not include density-dependent species interactions. Instead, species interact exclusively by using shared resources (section 2.5). This model shares some commonality with the Rosenzweig-MacArthur consumer-resource model, but strives towards a simplification of resource competition dynamics. While it was quickly rejected as a suitable method for understanding niche overlap within natural environments (Abrams, 1975), the model garnered attention as a sound basis for theoretical work (Chesson, 1990). Consumer-resource models have since been used to describe competition dynamics in various organisms.

This study investigates whether consumer-resource models, like Maynard-Smith based models, may function as valuable base models to unravel competition among co-occurring dinoflagellates. Five dinoflagellates that are found in the North Sea (*Alexandrium minutum*, *Prorocentrum lima*, *P. micans*, *Protoceratium reticulatum* and *Scrippsiella trochoidea*) were grown in 294 single and multispecies cultures spread across two experiments. Various nutrient treatments (varying either the N:P ratio, the order of magnitude of nutrient concentrations, or both) were used to determine whether a CRM can reproduce resource competition in multispecies cultures of dinoflagellates under different nutrient regimes. The initial growth and species dominance were then shown to be largely predictable using consumer-resource modelling based on monoculture behaviour.

2. Material and Methods

2.1 Stock cultures

Alexandrium minutum (SCCAP K-0993) and *Protoceratium reticulatum* (SCCAP K-1478) were bought from the Scandinavian Culture Collection of Algae & Protozoa (Copenhagen, Denmark). *Prorocentrum lima* (CCAP1136/9) and *P. micans* (CCAP1136/20) were obtained from the Culture Collection of Algae and Protozoa (Oban, Scotland). *Scrippsiella trochoidea* is an in-house strain, isolated from the Belgian Part of the North Sea one year before the experiments. Stock cultures of all dinoflagellates were grown in L1 medium, prepared from Instant Ocean^{tm} artificial seawater (Belcopet, Belgium) in accordance with Guillard and Hargraves (1993) and replenished (± 80%) every 2 weeks. Cultures were grown at 20°C with a 12-hour light-dark cycle (20-40 µmol.m^{-2}.s^{-1}), similar to summer conditions in the photic zone of the Southern North Sea (Gröger et al., 2013; Mortelmans et al., 2019). The growth of stock cultures was monitored through weekly cell counts using a Sedgewick-Rafter counting chamber and a Kyowa Optical Biolux-2 light microscope. Both experiments used cells taken from stock cultures that were growing exponentially.

2.2 Experiment 1: 4 species, 10 N:P ratios

A first experiment was set up to investigate whether small variations in nutrient availability and nutrient stoichiometry affect the interspecific competition among dinoflagellates in batch cultures. Ten algal growth media were prepared so to have ten unique nitrogen-to-phosphorus ratios. Regular L1 medium contains 882 µM NO_3^- and 36.2 µM PO_4^{3-}, corresponding to a N:P ratio of 24. By only adjusting the NO_3^- concentrations, ten growth media with different nitrogen-to-phosphorus ratios were prepared: preparations of 294, 368, 441, 478, 515, 551, 588, 662, 735 or 882 µM NO_3^- corresponded to a N:P of 8, 10, 12, 13, 14, 15, 16, 18, 20 or 24, respectively. All other components of L1 medium (PO_4^{3-}, trace metals, vitamins) were added at the regular dose. The range of N:P ratios that was used was based on N:P ratios that were observed in the Belgian part of the North

Sea between 2013 and 2019 (Mortelmans et al., 2019). On average, the Belgian EEZ has a mean N:P ratio of 22 while the average N:P ratio in a local shellfish area is around 14 during summer. Monocultures of *P. micans*, *P. lima*, *S. trochoidea*, and *P. reticulatum* were set up in each media by adding 100 cells ml^{-1} to Erlenmeyer flasks filled with 50 ml of medium. Mixed cultures were set up in each medium by adding 100 cells ml^{-1} of each of the four algae to 50 ml of medium. All the resulting 50 treatments were replicated three times for a total of 150 cultures (120 monocultures and 30 mixed cultures).

Cells were grown for 28 days at 24°C with a 12-hour photoperiod of 30±5 µmol m^{-2} s^{-1}. Twice a week, a 1 ml was taken from each flask, fixed with 100 µl of 12% formaldehyde, and counted using a Sedgewick-Rafter counting chamber and light microscopy. Additional samples (7 ml) were taken on day 14 and day 28 for nutrient analyses. During the first experiment, the NO_3 and PO_4 concentrations were determined using spectrophotometric test kits (Merck Millipore, Darmstadt, Germany) that need large volumes (20 ml). For this reason, replicates were pooled and filtered with Millex-GV 0.22 µm PVDF syringe filters (Merck Millipore). Filtrates and initial media (day 1) were then analysed using an Aquamate spectrophotometer (Thermo Scientific, San Jose, USA).

2.3 Experiment 2: 3 species, 3 N:P ratios, 4 orders of magnitude

A second experiment was performed to examine whether the interspecific competition between dinoflagellates in batch cultures is affected by larger differences in macronutrient availability. Twelve unique algal growth media were made based on regular L1 growth medium. Media were first prepared with 294, 588 or 882 µM NO_3^- to obtain three N:P ratios (8, 16 and 24). All other L1 components (PO_4^{3-}, vitamins and trace elements) were added at the regular dose. Each medium was subsequently diluted by a factor 1, 10, 100 or 1000 to obtain media with 100%, 10%, 1% or 0.1% volume fractions of L1 medium vs. Instant Ocean^{tm} artificial seawater. Hereafter, these dilutions will be referred to as “concentration factors” (CFs), so that the medium with a N:P ratio of 24 and a CF of 100 reflects actual L1 medium, while the medium with a N:P ratio of 8 and a CF

of 0.1 corresponds to 0.033% of L1 medium. In each of the twelve resulting media, monocultures of *A. minutum*, *P. reticulatum* and *P. micans*, as well as 3-species mixtures of these algae, were made by adding 100 cells.mL^{-1} (each) to 75 ml of medium in Erlenmeyer flasks. Every treatment was replicated three times, resulting in 144 cultures (108 monocultures and 36 mixed cultures). Cultures were placed at 20°C with a 12-hour photoperiod of 33±6 µmol.m^{-2}.s^{-1} for 56 days. Twice a week, cell counts were made using a Sedgewick-Rafter counting chamber and light microscopy. Once a week, 2 ml samples of each flask were taken for nutrient analysis. For this experiment, we used a QuAAtro segmented flow analyser to determine the N-NO_3 and P-PO_4 concentrations using the colorimetric methods found in Hansen and Koroleff (1999). Around 5 ml was needed for both analyses. To this end, replicates were filtered and pooled as described for experiment 1. The filtrates were stored at 4°C in 15 ml falcon tubes prior to their analysis.

2.4 Simple growth models

Growth rates (μ; d^{-1}) and carrying capacities (K; µm^3.ml^{-1}) were determined to assess the overall growth of each dinoflagellate. Cell counts (N_t) were transformed to biovolume (µm^3.ml^{-1}) using size measurements and the geometric formulas of Olenina et al. (2006). The conversion factors (µm^3.cell^{-1}) used were: 7299 (*A. minutum*), 7580 (*S. trochoidea*), 12596 (*P. reticulatum*), 20293 (*P. micans*), and 43960 (*P. lima*). Depending on whether or not the stationary growth phase was reached, the biovolumes of each flask were fitted with exponential or logistic growth models using least square optimisation in the 'nls' function in R (Baty et al., 2015). Multiple group comparisons by means of Kruskal Wallis (KW) tests (Kruskal and Wallis, 1952) were used to compare growth parameters (μ and K) between treatments (N:P) and species. Pairwise comparisons using Dunn's multiple comparison (DMC) test (Dunn, 1964) were made to investigate the effects of treatments (CF, mono vs. mixed) on the growth of each species. Linear regression models (LM) were used to detect linear responses to nutrient stoichiometry as described by Wilkinson and Rogers (1973).

2.5 Development of a community model

An adaptation of MacArthur's (1970) consumer-resource model for non-interacting resources was used to predict competition between dinoflagellates in mixed batch cultures using only the uptake and conversion of nutrients by individual species. According to the original model, predators (*n*) interact solely by consuming common, non-interacting prey species (*k*). As a result, the per capita growth rate of a predator (*i*) can be described by the following equation (Eq. 1):

$$(1)\ \frac{1}{X_i}\cdot\frac{dX_i}{dt} = C_i\cdot\left(\sum_{k=1}^{n} a_{i,k}\cdot w_k\cdot R_k - T_i\right)$$

Where X_i is the population density of the predator *i*; R_k is the population density of prey species *k*; $a_{i,k}$ is the probability that predator *i* captures prey species *k*; w_k is the weight of prey species *k*; and T_i is the threshold weight that the predator needs to capture per capita to get a net population growth of 0 (MacArthur, 1970). Any excess of prey captured (i.e. the result of the sum) is converted to population growth by a constant of proportionality C_i that governs the conversion of grams of resource captured to grams of X_i .Because of predation, the logistic population growth of prey species *k* is reduced by consumer-imposed mortality (Eq. 2), with r_k being the growth rate of prey species *k* and K_k being the carrying capacity of its environment.

$$(2)\ \frac{1}{R_k}\cdot\frac{dR_k}{dt} = r_k\cdot\left(1-\frac{R_k}{K_k}\right) - \left(\sum_{i=1}^{n} a_{i,k}\cdot X_i\right)$$

Here, we propose that MacArthur's consumer-resource model can be adapted to the uptake of non-interacting abiotic nutrients by describing resource abundance as:

$$(3)\ \frac{1}{R_k}\cdot\frac{dR_k}{dt} = I_k - \left(\sum_{i=1}^{n} a_{i,k}\cdot X_i\right)$$

Where I_k is the renewal of resources by riverine discharge, submarine weathering, atmospheric exchange, and biological activity (remineralisation, nitrogen fixation etc.). In closed environments like our batch cultures the short-term renewal of resources was assumed to be negligible (I_k = 0).

When applied to the present setup, i.e. dinoflagellates interacting through the consumption of nitrate and phosphate, the following equations were derived from the model:

$$(4)\ \frac{1}{X_i}\cdot\frac{dX_i}{dt} = C_i\cdot\left(a_{i,NO3}\cdot w_{NO3}\cdot[NO_3^-] + a_{i,PO4}\cdot w_{PO4}\cdot[PO_4^-] - m_i\right)$$

$$(5)\ \frac{1}{[NO_3^-]}\cdot\frac{d[NO_3^-]}{dt} = -\sum_{i=1}^{n} a_{i,NO3}\cdot X_i$$

$$(6)\ \frac{1}{[PO_4^-]}\cdot\frac{d[PO_4^-]}{dt} = -\sum_{i=1}^{n} a_{i,PO4}\cdot X_i$$

The model was simplified to a prototypical consumer-resource model by assuming that growth can be adequately described by either nutrient. Here, nitrogen was used (i.e. W_{PO4} was assumed to be 0) since the experimental design included most variability in nitrogen concentrations. Next, the constant of proportionality C_i was merged with the parameters w_{NO3} and m_i. In the end, the uptake and conversion of nitrogen was used to predict the growth of each dinoflagellate (Eq. 7, Eq. 8). Phosphorus measurements were used to estimate the uptake of phosphorus (Eq. 9) using the predicted per capita growth. The final model is:

$$(7)\ \frac{dX_i}{dt} = X_i\cdot\left(U_{i,NO3}\cdot W_{NO3}\cdot[NO_3^-] - M_i\right)$$

$$(8)\ \frac{d[NO_3^-]}{dt} = -[NO_3^-]\cdot\sum_{i=1}^{n} U_{i,NO3}\cdot X_i$$

$$(9)\ \frac{d[PO_4^-]}{dt} = -[PO_4^-]\cdot\sum_{i=1}^{n} U_{i,PO4}\cdot X_i$$

Where X_i is the density (in biovolume) of dinoflagellate *i* ($\mu m^3.l^{-1}$); $U_{i,\ NO3}$ is the probability of uptake of NO_3 per dinoflagellate *i* per time unit (d^{-1}); W_{NO3} is the conversion efficiency, i.e. the biovolume formed by dinoflagellate *i* per unit NO_3 taken up ($\mu m^3.\mu g^{-1}$); M_i is a mortality coefficient (i.e. the fraction of biovolume dinoflagellate *i* loses daily; $\mu m^3.d^{-1}$); $[NO_3^-]$ is the abundance of NO_3 (µg); C_{PO4} is the abundance of PO4 (µg); $U_{i,\ PO4}$ is the probability of uptake of PO_4 per unit of dinoflagellate *i* per time unit (d^{-1}).

281

282 2.6 Applying the model

283 Monoculture data was used to estimate the parameters (U_{NO3}, U_{PO4}, W_{NO3}, M_i) per treatment and
284 dinoflagellate with a simulated annealing algorithm. The mean absolute percentage error was
285 used as an objective function to ensure an equal fit across the different magnitudes of species'
286 densities. Markov chain Monte Carlo (MCMC) simulations (Hastings, 1970) were then used to
287 generate the joint posterior distributions for each parameter. The parameter space was restricted
288 to 50% deviation of the initial estimates to get fast parameter convergence. Convergence of the
289 posterior distributions of three parallel Markov chains was assessed based on the Gelman-Rubin
290 convergence criterion (Gelman and Rubin, 1992) and plotted to manually optimize burn-in.
291 Predictions for both monocultures and mixtures cultures (densities and nutrients) were obtained
292 using a 1000 Monte Carlo simulations, each randomly drawing parameter estimates from the
293 posterior distributions. Predictions of each simulation were stored. Model performance was
294 assessed by comparing the observed species densities to the median predicted densities. All
295 calculations were done in the statistical software R using the deSolve (Soetaert et al., 2010),
296 abind (Plate and Heiberger, 2011), and GenSA (Xiang et al., 2013) packages.

297

298 **3. Results**

299 3.1. Relative resource availability

300 During the first experiment all cultures were grown for 28 days. Logistic growth models were used
301 to determine the monoculture growth rates for all species except *P. lima*, which was still growing
302 exponentially at the end of the experiment. Exponential growth models were used to determine
303 the growth rates of *P. lima* instead (Supporting figures SF1-4). Overall, *P. micans* had the highest
304 growth rate (0.46±0.07 d^{-1}; μ±σ), followed by *S. trochoidea* (0.37±0.04 d^{-1}), *P. reticulatum*
305 (0.28±0.04 d^{-1}), and *P. lima* (0.04±0.01 d^{-1}). Nutrient stoichiometry significantly affected the growth
306 rate of *P. micans*, *P. reticulatum* and *S. trochoidea* (KW $P < 0.05$), but not *P. lima* ($P > 0.05$), in a

nonlinear fashion (Fig. 1A). A significant linear relationship (LM $P < 0.001$) was found between the initial N:P ratio and the carrying capacities of *P. micans*, but not between the N:P ratio and the carrying capacities of *P. reticulatum* or *S. trochoidea* ($P > 0.05$). On average, the carrying capacity of *P. reticulatum* ($8.6 \pm 3.9.10^8$ $\mu m^3.ml^{-1}$) was significantly higher than those of *P. micans* ($5.5 \pm 1.4.10^8$ $\mu m^3.ml^{-1}$) and *S. trochoidea* ($4.4 \pm 1.1.10^8$ $\mu m^3.ml^{-1}$; Fig 1B). *P. micans* outcompeted all other species in all mixed cultures (supporting figure SF5) while maintaining growth rates which were similar (DMC $P > 0.05$) to those in monoculture (0.43 ± 0.03 d^{-1}; $\mu \pm \sigma$).

No significant effect of the N:P ratio on the growth rate of *P. micans* in mixed cultures was found (KW $P > 0.05$), but the linear effect of the N:P ratio on the carrying capacity of *P. micans* persisted. On average, *P. micans* lost over half of its carrying capacity to competitors: its average carrying capacity decreased from $5.5 \pm 1.4.10^8$ $\mu m^3.ml^{-1}$ in monocultures to 2.1 ± 0.4 10^8 $\mu m^3.ml^{-1}$ in mixed cultures. *S. trochoidea* and *P. reticulatum* both reached peak density around day 14, after which densities plateaued or declined. Exponential or logistic growth models were used to determine their initial growth rates (up to day 17). These were 0.44 ± 0.10 and 0.31 ± 0.15 (d^{-1}) for *S. trochoidea* and *P. reticulatum*, respectively. Neither were statistically different from monoculture growth rates (DMC $P > 0.05$). Uniquely, *P. lima* grew faster in mixed cultures; it grew at a growth rate of 0.09 ± 0.01 d^{-1} for the duration of the experiment. Nitrogen and phosphorus concentrations from the first experiment can be found in supporting figures SF6-10. In mixed cultures, nutrients were depleted in all but the highest N:P ratio by day 14 (Fig. SF10).

3.2 Absolute (and relative) resource availability

The second experiment lasted 56 days, but the lowest concentration factors (CF0.1 and CF1) did not support prolonged growth. We observed between 1 (the lowest belonging to *P. reticulatum*) and 3 (found for *A. minutum*) population doublings before growth stalled. These treatments were no longer sampled after 39 days. Logistic growth models were used (Supporting Figure SF11) to determine the growth rate and carrying capacity (Table 1) of CF10 and CF100 monocultures. The

mean growth rate of *P. micans* (0.31 ± 0.04 d^{-1}; μ±σ) exceeded the growth rates of *A. minutum* (0.27 ± 0.03 d^{-1}) and *P. reticulatum* (0.19 ± 0.03 d^{-1}). Growth rates were usually higher at CF10 (KW $P < 0.001$), and did not differ between N:P ratios (LM $P > 0.05$). The N:P ratio did have a significant (LM $P < 0.01$) positive effect on the carrying capacities of the three dinoflagellates at both CF10 and CF100.

P. micans dominated all multispecies cultures (Supporting figure SF12). The growth rates of each species were determined by logistic growth models for CF10 and CF100. No significant differences were found between the growth rates of monocultures and mixed cultures for any of the three species (KW $P > 0.05$). The N:P ratio had no effect on the growth rate of any of the dinoflagellates at neither CF (KW $P > 0.05$; Table 1), but the linear effect of the N:P ratio on the carrying capacity of *P. micans* was again found (LM $P < 0.05$). Nutrients were depleted between day 20 and day 25 in virtually all cultures (Supporting figure SF16).

3.3 Consumer-resource modelling

Overall, our consumer-resource model was able to predict most of the variation in abundance of monocultures of both experiments; the coefficients of determination (R^2) were 0.8981 and 0.9765 for monoculture growth in the first and second experiment, respectively (Fig. 3). During the first experiment, *P. micans* and *S. trochoidea* – the two species that grew fastest and, hence, most in mixed cultures – were found to have similar nitrogen conversion efficiencies and likelihoods of nitrogen uptake (Table 2). By contrast, *P. reticulatum* exhibited a markedly lower likelihood of nitrogen uptake and a higher nitrogen conversion efficiency. *P. lima* had a nitrogen conversion efficiency that far exceeded those of all other species, which could be a computational artefact. When used to predict abundances for the entire duration of the first experiment, the goodness-of-fit of the model was generally poor ($R^2 = 0.3581$). Yet, when looking at the data up to quiescence, which we isolated by first identifying the highest density per species and then removing all counts after t_{max} which were smaller than 80% of the peak abundances, we found that the model generally

produced good predictions for the exponential growth of mixed cultures (R^2 = 0.8191; all species). Densities of *P. lima* in mixed cultures were, however, predicted poorly (R^2 = 0.12).

The increased temporal resolution of nutrient data during the second experiment greatly improved the model's performance in mixed cultures. When used to predict the growth of mixed cultures of the two highest concentrations factors (CF10 and CF100), a coefficient of determination of 0.8910 was found for all data. Using the same quiescence filter as before to remove the death phase, the goodness-of-fit improved even further (R^2 = 0.9289; Fig. 2-3). Overall, the natural mortality rates during exponential growth were negligible for all dinoflagellates and did not differ greatly between species and experiments. In addition, we generally found that changes in growth rates – such as those linked to CF's and NP ratios – are coupled to differences in the likelihood of nutrient uptake (LM: $P < 0.001$ for exp. 1; LM $P < 0.01$ for exp. 2), but not to changes in the nitrogen conversion efficiencies ($P \geq 0.05$ for both experiments).

4. Discussion

Despite decades of experimental and observational research, much can still be learned of the key biological processes that influence HAB development. Interspecific competition between (closely) related species, in particular, is far from fully understood (Wells et al., 2015). Even though several key biological processes are known to affect HAB development (e.g. grazer resistance, nutrient competition, allelopathy), we do not understand the relative importance of these elements during all stages of a bloom cycle. To this end, co-culturing of HAB and non-HAB species (plus grazers) needs to become more prevalent. While many studies (e.g. Chang and McClean, 1997; Cooper et al., 2016; Gallardo Rodríguez et al., 2009; Guerrini et al., 2007; Ignatiades et al., 2007; John and Flynn, 2000; Nascimento et al., 2005; Peperzak, 2003; Sala-Pérez et al., 2016; Varkitzi et al., 2010; Wang et al., 2014; Zhengbin et al., 2006) have investigated the physiological responses of individual HAB species to environmental conditions, only a few (e.g. Ji et al., 2011; Li et al., 2012; Poulin et al., 2018; Riegman et al., 1996; Wang and Tang, 2008) have added environmental

variability when looking at interactions between two or more species. Here, we used co-cultures to investigate how naturally co-occurring dinoflagellates are affected by changes in macronutrient availability and illustrate how consumer-resource models can be used to predict resource competition between multiple species in mixed batch cultures. This study demonstrates that consumer-resource modelling is a viable trait-based approach to understanding the dynamics of multiple species in mixed communities.

4.1 Growth and competition

Two large batch culture experiments, for a combined total of 294 single and mixed cultures of five common dinoflagellates (*Alexandrium minutum*, *Prorocentrum lima*, *P. micans*, *Protoceratium reticulatum* and *Scrippsiella trochoidea*) spread across different nutrient regimes, were set up to explore whether consumer-resource modelling provides a good basis to understand interspecific interactions between dinoflagellates. As all the monoculture growth rates fell within the ranges expected from literature (Chang and McClean, 1997; Guerrini et al., 2007; Ignatiades et al., 2007; Lee et al., 2005; Nascimento et al., 2005; Peperzak, 2003; Sala-Pérez et al., 2016; Varkitzi et al., 2010; Wang et al., 2014), the growth rates reported here (ref. section 3.1 and Table 2) were considered representative for batch culture experiments. Modifications of the (macro)nutrient concentration in growth media are commonly used to study the effect of nutrient availability and stoichiometry on the growth of dinoflagellates (e.g. Cooper et al., 2016; Gallardo Rodríguez et al., 2009; Guerrini et al., 2007; Varkitzi et al., 2010; Zhengbin et al., 2006). This study occasionally found differences in growth rates between N:P ratios (experiment 1), but no linear or unimodal relationships were detected. Given that other studies have failed to find a relation between the growth rates of dinoflagellates and the relative availability of nutrients (John and Flynn, 2000; Li et al., 2012; Rhee, 1978; Varkitzi et al., 2010), the differences found here might be caused by intraspecific variation. It should, however, be noted that a small range of resource ratios was used, and that far more extreme N:P ratios are found in natural environments. Whether or not extreme

N:P ratios have significant effects on the growth of dinoflagellates cannot be deduced from our results. The increase in growth rate between orders of magnitude of nutrient availability (i.e. CF100 and CF10) should also be interpreted cautiously; the CF10 growth rates may have been overestimated due to the lower number of time points between the lag phase and the stationary phase for these treatments.

Shifts in growth rates caused by changes in either the relative (N:P) or the absolute (CF) nutrient concentrations did not change the dinoflagellates' community structure; *P. micans* attained the highest growth rate in all cultures. As interspecific competition in discontinuous cultures tends to favour whichever species grows fastest under the conditions used (Riegman et al., 1996), it is normal that *P. micans* dominated all mixed cultures. According to the mean parameter estimates of our consumer-resource model (CRM), the success of *P. micans* should be attributed to its ability to capture resources rather than a high resource efficiency or low natural mortality rates. The uptake probability of both nitrogen and phosphorus of *P. micans* were (among) the highest observed. All pelagic dinoflagellates grew at roughly the same rate relative to their monocultures in the early stages of both experiments. By sequestering nitrogen and phosphorus more rapidly, thereby denying its competitors access to these nutrients, *P. micans* was able to outgrow all other species in mixed cultures. Conversely, the benthic dinoflagellate *P. lima* was able to significantly increase its growth rate in mixed cultures. The difference in growth characteristics between its monocultures and the mixed cultures might have been caused by the release of organic nutrients by decaying cells of pelagic competitors. Sahraoui et al. (2013) have proposed that the growth of *P. lima* inside a lagoon can be triggered by organic matter, but little is known about the growth of this species on organic substances. Another unknown is whether the success of *P. micans* can be attributed to "luxury consumption". The rapid acquisition and storage of excess nutrients may be used to pre-emptively reduce the availability of resources for competing species (Droop, 1973; de Mazancourt & Schwartz, 2012). This trait has not been studied in *P. micans* to our knowledge,

436 but its carrying capacity is known to positively correlate with nitrogen concentrations (Zhengbin et
437 al., 2006; Zheng-fang et al., 1995). Similar results were found in this study.
438
439 4.2 CRM: use and considerations
440 The results of our experiments should not be viewed as ecological stoichiometry research; testing
441 the effect of nutrient stoichiometry on the growth of dinoflagellates requires the use of continuous
442 cultures with controlled dilution rates (cfr. van de Waal et al., 2014). Only chemostats can be used
443 to determine the resource requirements of each species at the same net population growth rate.
444 In batch culture, the relative availability of external nutrients will rapidly change over the course
445 of the experiment, thus altering the intended treatment. In this study, the N:P ratios and the CF's
446 were merely used to introduce variability in the nitrate concentrations, which we then chose as
447 the driver of the consumer-resource model used. We set out to determine the efficacy of consumer
448 resource modelling. Starting with the simplest setup available, which is the batch culture, we found
449 that CRMs could be used to predict species dominance resulting from interspecific competition
450 between dinoflagellates in mixed cultures. CRMs can approximate the densities of both winning
451 and losing algal species up to the plateau phase with a high degree of accuracy. Stark changes
452 in growth rate between monocultures and multispecies cultures such as those observed in *P. lima*
453 can, however, lead to poor predictions if the underlying mechanism is not fully understood and
454 included in the structural equations. By using a CRM, this study was able to demonstrate that the
455 presence of a fast-growing species (*P. micans*) had strong, indirect negative effects on the growth
456 of competing dinoflagellates; the growth of competing algae was to a large degree hampered by
457 diminishing nutrient availability due to uptake by *P. micans*.
458 All dinoflagellates used here may produce allelochemicals that affect algal growth in one way or
459 another (Arzul et al., 1999; Fistarol et al., 2004; Ji et al., 2011; Sala-Pérez et al., 2016; Wang and
460 Tang, 2008; Yang et al., 2008), but we did not explicitly test our strains ability to do so here. By
461 using a CRM, we managed to accurately predict the community dynamics throughout the growth

462 phase of each mixed culture using the nutrient uptake rates, conversion efficiencies and natural
463 mortality rates of each species (Fig. 3). That is not to say that allelopathic interactions could not
464 have occurred here. More likely than not, nutrient stress coupled to higher cell densities caused
465 increasingly significant allelopathic interactions by the end of the experiments but, as it stands,
466 the prototypical CRM cannot mimic quiescence and transient community dynamics. For starters,
467 the model is prone to underestimate maximum densities as the predicted cellular growth is
468 coupled to external nutrient concentrations and, hence, stops once nutrients are depleted. In
469 reality, cell growth is based on internal nutrient concentrations (Droop, 1974), thus allowing
470 population growth to continue in the absence of external nutrients. A common solution is to use
471 cell-based nutrient quota to establish relationships between the growth rate, internal nutrient
472 reserves, and external resource availability (Flynn, 2008b). Yet, while Droop's cell-quota model
473 (1974) is a good descriptor of growth in laboratory cultures, it is not well suited for competition
474 modelling due to the need to distinguish cell quota per species (in addition to other concerns; see
475 Flynn, 2008b). An alternative solution could be to add a discrete time lag (ε) to the growth and
476 external nutrient relation (cfr. the delayed allelopathic interactions of Mukhopadhyay et al., 1998).
477 The time lag (ε) of each species should correspond to the difference between its time of peak
478 density and the time of nutrient depletion. Population growth would then be described by:

479 $$(10)\ \frac{dX_i}{dt} = X_i \cdot \varepsilon - M_i$$

480 $$with\ \varepsilon = t_{X_{max}} - t_{[NO_3^-]_{min}}$$

481 In addition to this time lag, the inclusion of allelopathy would likely improve the predictions beyond
482 the growth phase. In the current model, population decline can only occur as a result of natural
483 mortality as observed in monoculture. However, as shown here, the population decline in mixed
484 cultures is far steeper than in monoculture (supporting figures SF5 and SF12), resulting in poor
485 predictions of species abundance after some time. Interspecific interactions – be it allelopathy or
486 mixotrophy – can be added to the model by introducing density-dependent parameters. Similar to

the work on Lotka-Volterra models (cfr. Ji et al., 2011; Qiu et al., 2012; Tameishi et al., 2009; Wang et al., 2013), the CRM could be modified as follows (equation 11). This hybrid model would bring together both direct and indirect interactions between competing microalgae.

$$(11)\ \frac{dX_i}{dt} = X_i \cdot \varepsilon - M_i - \sum_{j=1}^{n} \alpha_{ij} X_j$$

With α_{ij} being the coefficient of interaction between species i and species j.

Note that this approach assumes both constant and linear relationships between the densities of the allelopathic species and allelopathic interactions, which is an oversimplification given that the excretion of allelochemicals and their effects are heavily context-dependent (Poulson et al., 2010). The approach will also capture, confound or conceal other interactions (e.g. mixotrophy, induced cyst formation, stimulatory interactions, pH change) if these facets are not specifically measured. Unfortunately, the data generated here are not well suited to test this proposed model. In order to determine the interaction between two species, the experimental design should include bi-algal cultures of all competitors and account for the aforementioned pitfalls. Regardless, CRMs could become key instruments for understanding various species-species interactions in HAB ecology, and should be developed further to that end. Given the success of the Maynard-Smith function (e.g. Bandyopadhyay, 2006; Chattopadhyay, 1996; Mandal et al., 2014; Mukhopadhyay et al., 2003, 1998; Solé et al., 2005), we believe that CRMs and hybrid models still hold a great, mostly unexplored potential to improve our understanding of HAB dynamics. Even if CRM-based in situ modelling proves ineffective, simple CRMs should become a staple analysis when conducting multispecies lab experiments; they provide enhanced insights in competition dynamics with minimal data requirements. Going forward, it is recommended that the findings are tested further by applying the basic CRM to comparable datasets, that the model improvements suggested here (time delay, allelopathy, or others) are explored using fit-for-purpose experimental designs, that

511 the virtue of CRMs to understand continuous multispecies cultures (incl. grazing) is explored, and
512 that additional nutrient sources (e.g. Si) are reintroduced into the model.
513
514 **5. Conclusions**
515 Consumer-resource modelling is a simple trait-based approach that has been used to understand
516 coexistence dynamics in fields ranging from plant ecology to oncology. To date, however, CRMs
517 are not commonly used in HAB research. This study shows that consumer-resource models can
518 be used on the most common growth setup – the batch culture - with minimal data requirements,
519 and that they provide key benefits to understanding resource competition between dinoflagellates.
520 Based on our results and the success of Lotka-Volterra-based modelling approaches, we believe
521 that the application of CRMs and derivatives should be explored further, both as a lab-tool as well
522 as for in situ HAB modelling.
523
524 **Author contributions**
525 M.V. and C.J. acquired the main funding for this study. M.D.R. and C.J. designed the experiments.
526 J.B. and F.D.L. provided the framework of the consumer-resource model. M.D.R. carried out the
527 experiments and implemented the CRM with the help of J.B. N.B. performed the nutrient analyses.
528 M.D.R. wrote the manuscript with input from all authors.
529

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Supporting Figures

SF1-SF4: Monoculture growth of *P. lima*, *P. micans*, *P. reticulatum*, and *S. trochoidea* at various N:P ratios (experiment 1) fitted with exponential or logistic growth models.
SF5: Growth of *P. lima*, *P. micans*, *P. reticulatum*, and *S. trochoidea* in multispecies cultures at various N:P ratios (experiment 1).
SF6-SF9: Monoculture growth of *P. lima*, *P. micans*, *P. reticulatum*, and *S. trochoidea* at various N:P ratios (experiment 1), densities and nutrients fitted with a consumer-resource model.
SF10: Growth of *P. lima*, *P. micans*, *P. reticulatum*, and *S. trochoidea* in mixed cultures at various N:P ratios (experiment 1): nutrients and densities of each species fitted with a consumer-resource model based on parameter estimates from monocultures.
SF11: Monoculture growth of *A. minutum*, *P. micans* and *P. reticulatum* at three N:P ratios and four concentration factors (CFs). Black = CF100; Blue = CF10. Data from the two highest CFs was fitted with logistic growth models.
SF12: Growth of *A. minutum* (red), *P. micans* (black) and *P. reticulatum* (blue) in mixed cultures at three N:P ratios and four concentration factors (CFs): Data of *P. micans* was fitted with logistic growth models.
SF13-15: Monoculture growth of *A. minutum*, *P. micans* and *P. reticulatum* in mixed cultures at three N:P ratios and two concentration factors (CFs) fitted with a consumer-resource model.
SF16: Growth of *A. minutum* (red), *P. micans* (black) and *P. reticulatum* (blue) in mixed cultures at three N:P ratios and two concentration factors (CFs): nutrients and densities of each species fitted with a consumer-resource model based on parameter estimates from monocultures.

559 Table 1: Mean growth rates and carrying capacities of *A. minutum*, *P. reticulatum* and *P. micans*
560 grown in either single or mixed cultures at different N:P ratios and two concentration factors (CF),
561 representing 100% or 10% v/v dilutions of L1 medium and artificial seawater at a N:P ratio of 24.
562 Results shown are from the second experiment. Values represent µ±s.d.
563

Species	**N:P**	**CF**	μ_{mono} (d^{-1})	K_{mono} (10^8 µm^3.ml^{-1})	μ_{mix} (d^{-1})	K_{mix} (10^8 µm^3.ml^{-1})
A. minutum	8	100	0.28±0.01	7.09±0.15	0.25±0.01	1.03±0.26
		10	0.31±0.02	0.99±0.06	0.35±0.02	0.13±0.01
	16	100	0.25±0.00	12.3±0.56	0.24±0.09	0.77±0.22
		10	0.32±0.02	1.36±0.01	0.36±0.01	0.13±0.02
	24	100	0.25±0.01	15.6±0.40	0.23±0.09	0.81±0.17
		10	0.33±0.02	1.59±0.05	0.35±0.02	0.19±0.02
P. micans	8	100	0.28±0.01	4.73±0.33	0.26±0.03	4.10±0.77
		10	0.36±0.01	0.67±0.04	0.33±0.04	0.52±0.07
	16	100	0.28±0.02	6.52±0.73	0.24±0.03	5.61±0.03
		10	0.38±0.02	0.88±0.06	0.33±0.00	0.67±0.02
	24	100	0.30±0.00	6.86±0.02	0.27±0.01	5.25±0.28
		10	0.37±0.02	1.02±0.02	0.30±0.04	0.69±0.04
P. reticulatum	8	100	0.18±0.01	3.29±0.21	0.25±0.12	0.27±0.20
		10	0.21±0.03	0.47±0.01	0.28±0.06	0.08±0.07
	16	100	0.17±0.01	6.75±0.10	0.16±0.09	0.71±0.40
		10	0.19±0.02	0.65±0.04	0.28±0.06	0.07±0.00
	24	100	0.19±0.01	7.76±0.20	0.16±0.03	0.74±0.11
		10	0.28±0.03	0.48±0.02	0.15±0.03	0.12±0.07

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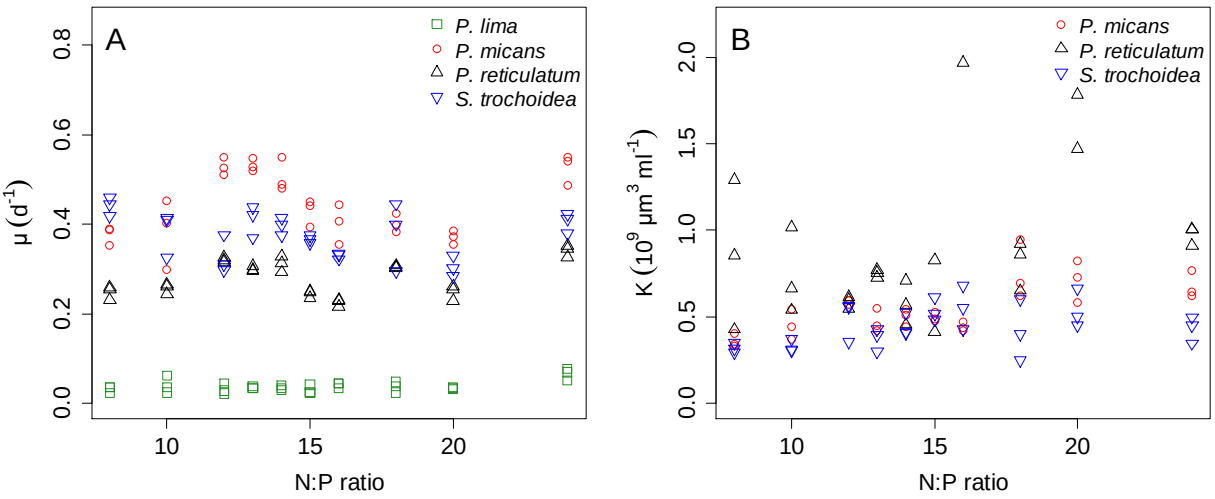
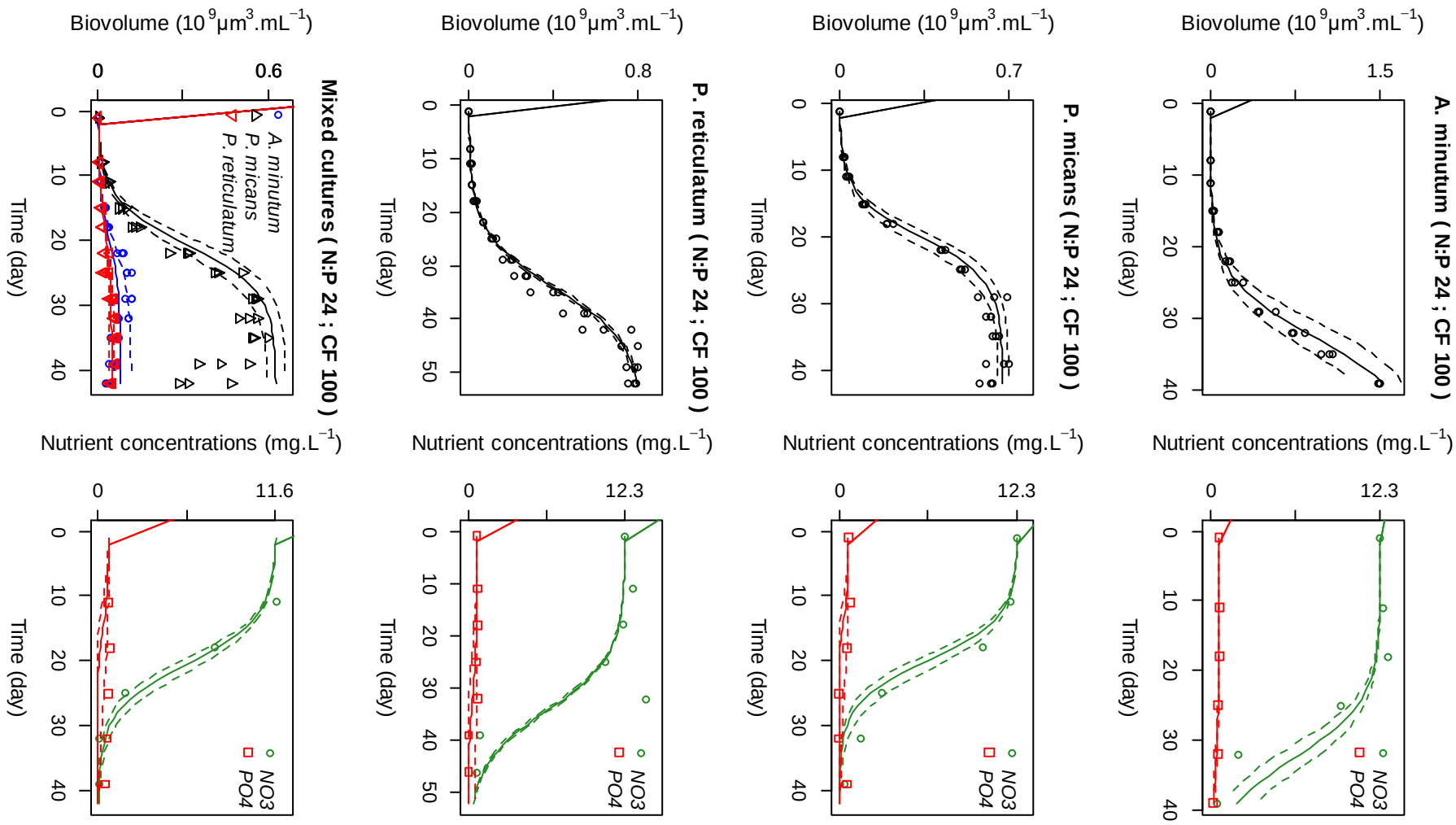
569 Table 2: Mean parameter estimates derived from monocultures and used to predict cell growth in
570 multispecies cultures with a simplified version of MacArthur's consumer-resource model (1970).
571 The results are calculated based on a 1000 Monte-Carlo simulations, each randomly drawing
572 from the prior distributions generated by Markov chain Monte Carlo (MCMC) simulations. U_{NO3} is
573 the uptake probability of NO_3 per unit biovolume of a dinoflagellate per time unit; U_{PO4} is the uptake
574 probability of PO_4 per unit biovolume of a dinoflagellate per time unit; W_{NO3} is the efficiency at
575 which nitrogen is converted into biovolume; *M* is the fraction of biovolume lost daily due to natural
576 mortality. Values shown are the averages (±s.d.) per experiment (Exp) across all N:P ratios.
577

Species	**Exp**	**CF**	**U_{NO3}** **(10^{-10} µm^{-3}.d^{-1})**	**U_{PO4}** **(10^{-10} µm^{-3}.d^{-1})**	**W_{NO3}** **(µm^3.pg^{-1})**	**M** **(10^{-6} d^{-1})**
P. lima	1	100	1.7±3.1	8.2±3.9	0.26±0.38	4.9±0.6
P. micans	1	100	11±3.5	7.9±11	0.06±0.01	4.6±0.7
P. reticulatum	1	100	3.7±1.4	2.6±1.8	0.10±0.04	5.2±0.4
S. trochoidea	1	100	13±5.6	2.9±7.7	0.04±0.01	5.1±0.7
A. minutum	2	100	2.2±1.0	0.3±0.3	0.17±0.02	5.2±0.4
	2	10	26±5.8	32±22	0.18±0.05	5.4±0.8
P. micans	2	100	5.2±1.1	6.2±9.9	0.08±0.02	6.1±0.9
	2	10	49±9.9	11±6.2	0.11±0.03	4.6±0.6
P. reticulatum	2	100	3.5±1.8	2.3±1.4	0.08±0.01	5.0±1.4
	2	10	42±9.2	59±9.8	0.08±0.03	5.7±0.6



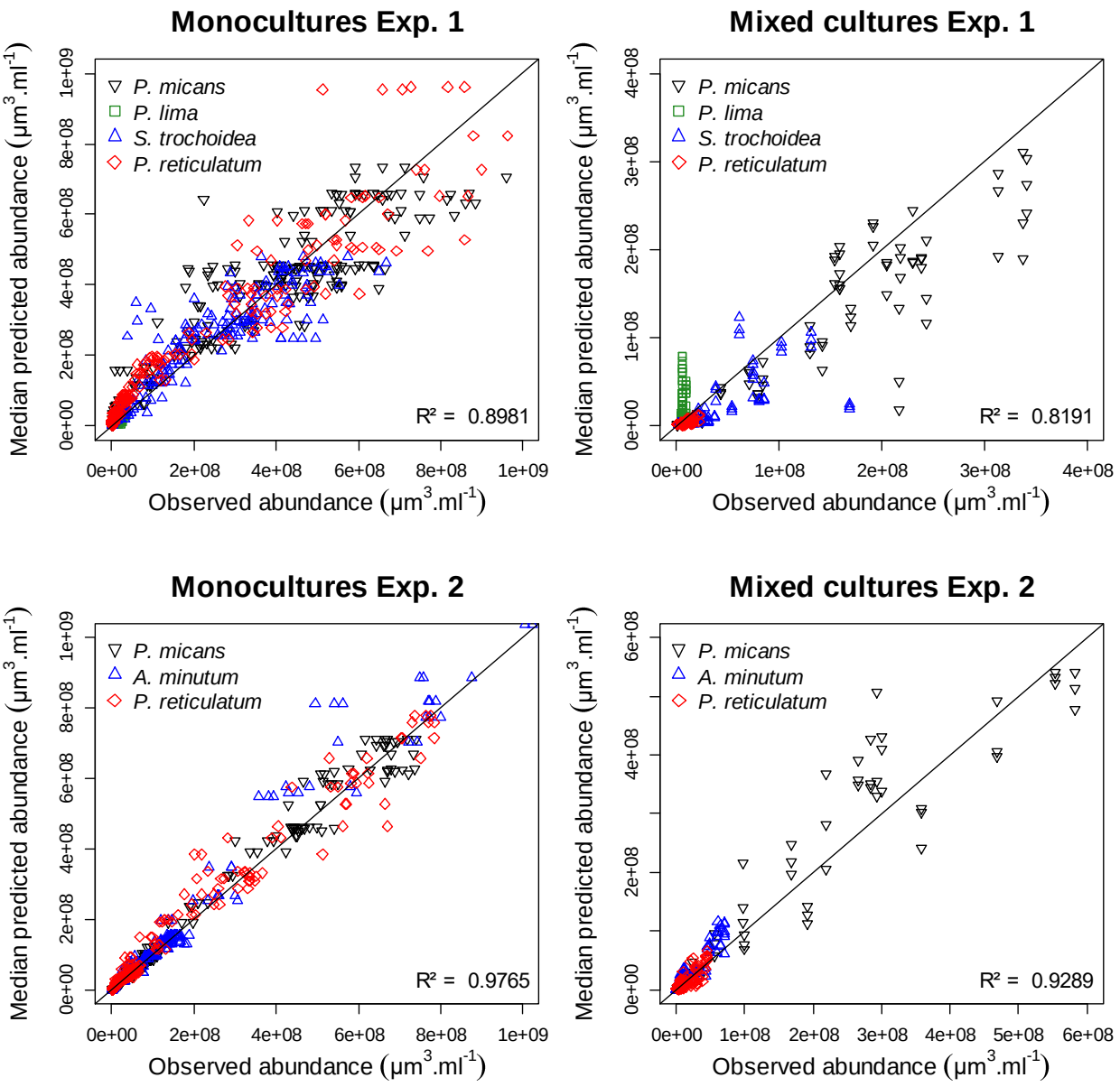
578

579 Fig. 1: (A) growth rates per monoculture of *P. micans* (red), *P. lima* (green), *P. reticulatum* (black)
580 and *S. trochoidea* (blue) across different nitrogen-to-phosphorus ratio's; (B) carrying capacities of
581 monocultures of *P. micans* (red), *P. reticulatum* (black) and *S. trochoidea* (blue) across N:P ratio's.
582 All results were obtained from the first experiment. All cultures, except those of *P. lima*, were fitted
583 with logistic growth models. *P. lima* was fitted with exponential growth models.



584

585 Fig. 2: Monoculture data of *A. minutum*, *P. micans*, and *P. reticulatum* was used to parametrize a consumer-resource model and predict
586 the growth of each dinoflagellate in mixed cultures. The example shown here is from the second experiment, using regular L1 medium
587 (N:P 24; CF100). Full lines are the average predicted abundance of a 1000 Monte Carlo simulations, randomly drawing from posterior
588 parameter distributions made with Markov Chain Monte Carlo methods following simulated annealing. The dotted lines represent the
589 5%-95% confidence interval around these averages. Markers are data as observed.



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591 Fig. 3: Goodness-of-fit of a simplified consumer-resource model of MacArthur (1970), applied to

592 biovolumes from monocultures (left) and multispecies cultures (right) of two growth experiments.

593 Data shown reflect predicted vs. observed abundances up to and including the plateau-phase.

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Supplementary Figure 11

Supplementary Figure 12

Supplementary Figure 13

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Supplementary Figure 15

Supplementary Figure 16

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