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

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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.
- 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.

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

307 nonlinear fashion (Fig. 1A). A significant linear relationship (LM $P < 0.001$) was found between
308 the initial N:P ratio and the carrying capacities of *P. micans*, but not between the N:P ratio and
309 the carrying capacities of *P. reticulatum* or *S. trochoidea* ($P > 0.05$). On average, the carrying
310 capacity of *P. reticulatum* ($8.6 \pm 3.9.10^8$ $\mu m^3.ml^{-1}$) was significantly higher than those of *P. micans*
311 ($5.5 \pm 1.4.10^8$ $\mu m^3.ml^{-1}$) and *S. trochoidea* ($4.4 \pm 1.1.10^8$ $\mu m^3.ml^{-1}$; Fig 1B). *P. micans* outcompeted
312 all other species in all mixed cultures (supporting figure SF5) while maintaining growth rates which
313 were similar (DMC $P > 0.05$) to those in monoculture (0.43 ± 0.03 d^{-1}; $\mu \pm \sigma$).
314 No significant effect of the N:P ratio on the growth rate of *P. micans* in mixed cultures was found
315 (KW $P > 0.05$), but the linear effect of the N:P ratio on the carrying capacity of *P. micans* persisted.
316 On average, *P. micans* lost over half of its carrying capacity to competitors: its average carrying
317 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
318 cultures. *S. trochoidea* and *P. reticulatum* both reached peak density around day 14, after which
319 densities plateaued or declined. Exponential or logistic growth models were used to determine
320 their initial growth rates (up to day 17). These were 0.44 ± 0.10 and 0.31 ± 0.15 (d^{-1}) for *S.*
321 *trochoidea* and *P. reticulatum*, respectively. Neither were statistically different from monoculture
322 growth rates (DMC $P > 0.05$). Uniquely, *P. lima* grew faster in mixed cultures; it grew at a growth
323 rate of 0.09 ± 0.01 d^{-1} for the duration of the experiment. Nitrogen and phosphorus concentrations
324 from the first experiment can be found in supporting figures SF6-10. In mixed cultures, nutrients
325 were depleted in all but the highest N:P ratio by day 14 (Fig. SF10).
326
327 3.2 Absolute (and relative) resource availability
328 The second experiment lasted 56 days, but the lowest concentration factors (CF0.1 and CF1) did
329 not support prolonged growth. We observed between 1 (the lowest belonging to *P. reticulatum*)
330 and 3 (found for *A. minutum*) population doublings before growth stalled. These treatments were
331 no longer sampled after 39 days. Logistic growth models were used (Supporting Figure SF11) to
332 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 $\mu m^3.ml^{-1}$)	μ_{mix} (d^{-1})	K_{mix} (10^8 $\mu m^3.ml^{-1}$)
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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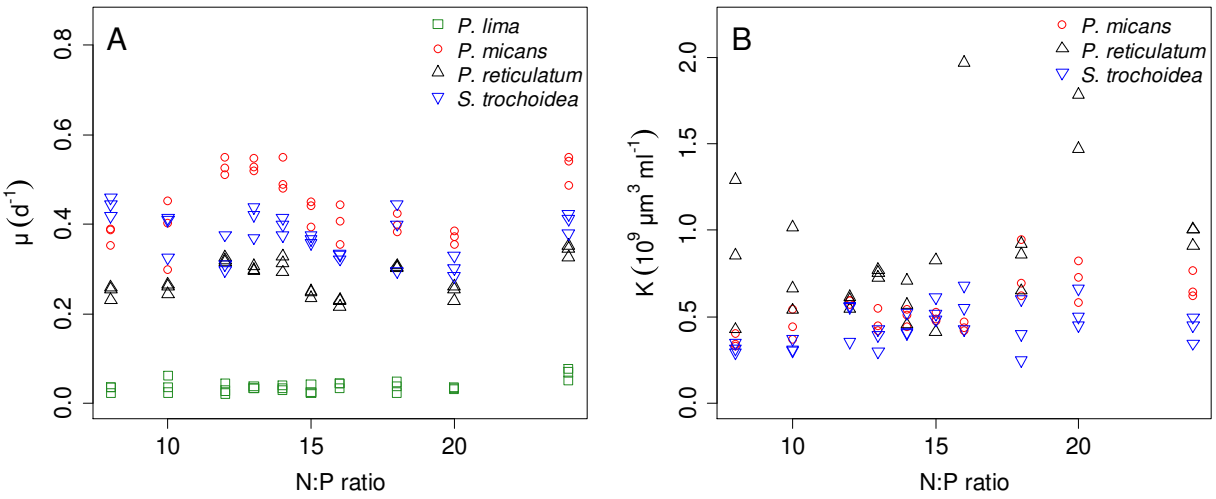
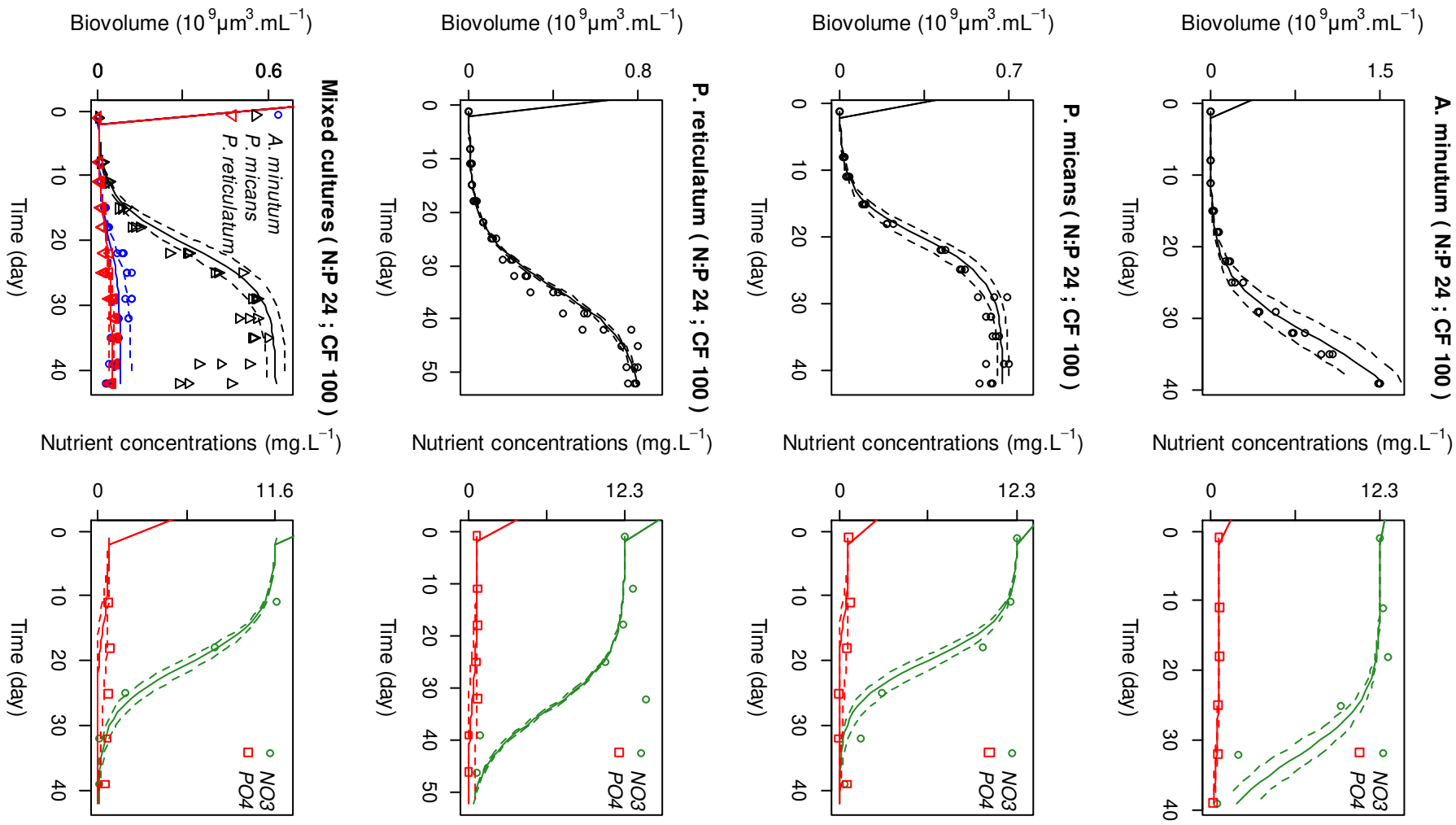
569 Table 2: Mean parameter estimates derived from monocultures and used to predict cell growth in
570 multispecies cultures with a simplified version of MacArthur's consumer-resource model (1970).
571 The results are calculated based on a 1000 Monte-Carlo simulations, each randomly drawing
572 from 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



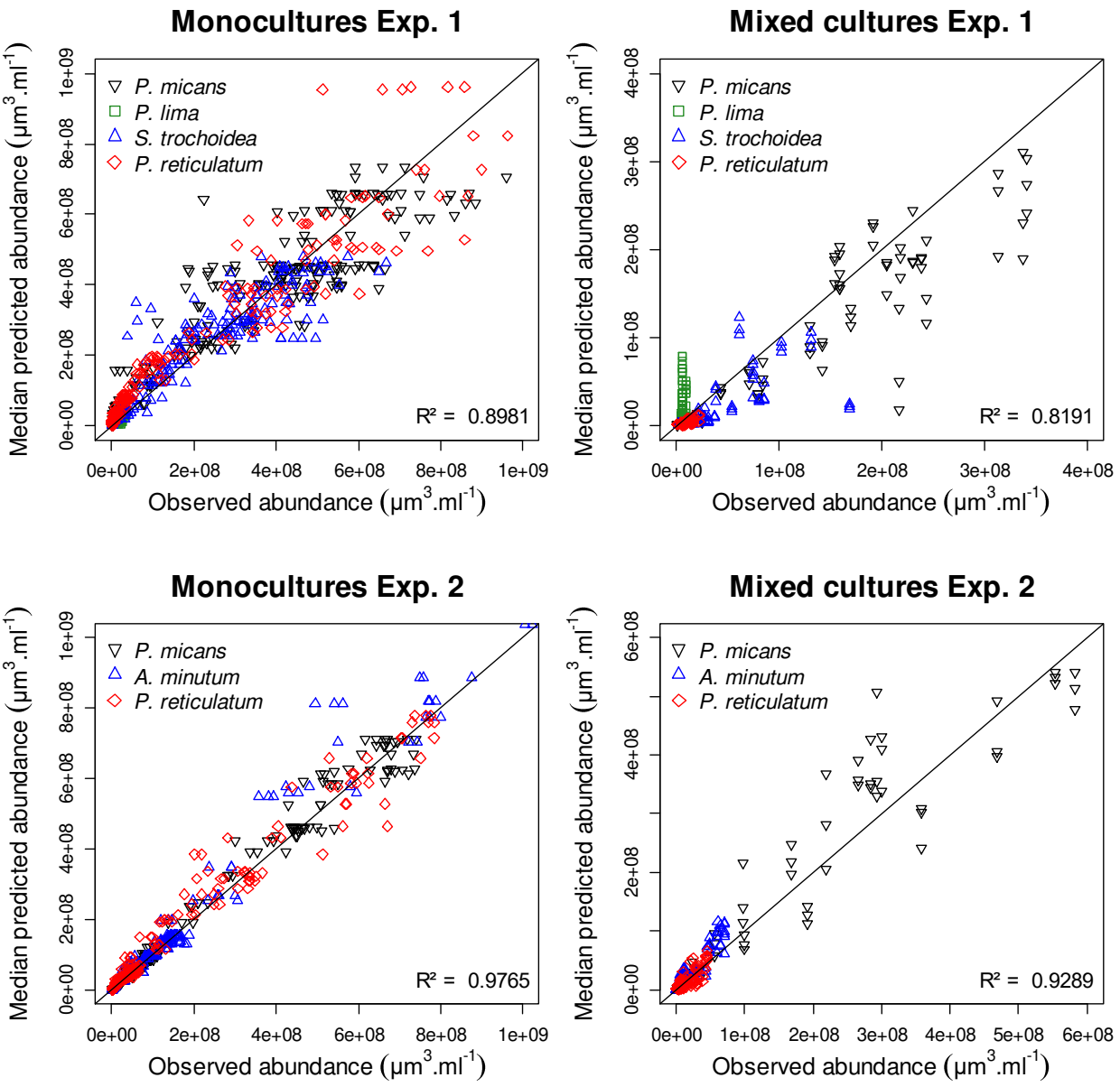
578

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.



590

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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