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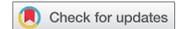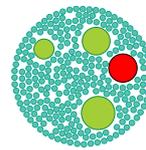
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ABSTRACT
Dinoflagellates are amongst the most abundant and diverse groups of plankton in surface waters and contribute to food web productivity and C:N:P biogeochemistry. Here we analyse the C:N:P of marine, autotrophic, planktonic dinoflagellates compiled from culture data from the scientific literature and test if dinoflagellate C:N:P differs from the Redfield ratio, and whether variability in C:N:P is associated with three traits: cell size, wall structure and toxin production. We find the average C:N:P of dinoflagellates is 90:12:1; higher in C:N, and lower in C:P and N:P than the canonical Redfield ratio. In aggregate the three traits examined here account for between 20–31% while taxonomic order accounts for between 37–38% of the variance in C:N:P. Smaller-sized and thecate taxa are higher in C:N, C:P and N:P than larger-size and athecate taxa. Species known to be able to produce C-rich toxins tend to be higher in C:P and N:P while species known to be able to produce N-rich toxins are lower in C:N, C:P and N:P relative to non-toxic species. These results indicate that any average estimate of dinoflagellate C:N:P will be influenced by the relative number of taxa with these traits.

Introduction

Global changes over the next centuries are projected to alter phytoplankton community structure with consequences for the marine food web and the efficiency of the biological pump function (Finkel *et al.*, 2010; Bopp *et al.*, 2013; Barton *et al.*, 2016; Bindoff *et al.*, 2019). It has been postulated that incorporation of key traits such as cell size, toxin production and elemental stoichiometry (C:N:P) of different phytoplankton groups into ecosystem models could improve our ability to model microbial communities and their influence on biogeochemistry (Litchman *et al.*, 2007; Barton *et al.*, 2013; Meunier *et al.*, 2017). Dinoflagellates are a particularly interesting but challenging group to incorporate into models in part because they include a wide array of cell sizes and shapes (Gómez, 2012; Le Bescot *et al.*, 2016), cell wall types and toxins (Hoppenrath, 2017; Verma *et al.*, 2019) and are amongst the most genetically, morphologically and trophically diverse of the marine plankton (de Vargas *et al.*, 2015; Pierella Karlusich *et al.*, 2020).

Dinoflagellates (Class Dinophyceae) are unicellular eukaryotes with a number of unique characters. The later lineages have amphiesmal vesicles under their plasma membrane (Bujak & Williams, 1981; Janouškovec *et al.*, 2017) that produce cellulosic-like thecal plates of various thicknesses (Hoppenrath, 2017). Dinoflagellates have a unique nuclear architecture: their chromosomes are in a permanently condensed liquid crystalline state (Wisecaver & Hackett, 2011; Murray *et al.*, 2016; Gornik *et al.*, 2019). Dinoflagellates are important members of benthic and planktonic communities, can significantly contribute to primary production, some species produce blooms, and in some cases produce toxins that can be harmful for humans and marine organisms (Verma *et al.*, 2019). Generally dinoflagellates are assumed to have lower growth rates compared with other phytoplankton groups, and are poor competitors for inorganic nutrients (Tilman, 1977; Tang, 1996). Despite their importance, due to their diversity, our current understanding and ability to predict dinoflagellate biogeography, productivity and toxin production is poor (Le Bescot *et al.*, 2016).

There is some indication from past work that dinoflagellates are higher in C:N than other phytoplankton groups (Quigg *et al.*, 2003; Finkel *et al.*, 2016), although Garcia *et al.* (2018) found no relationship between phylogeny and C:N:P in marine phytoplankton. These studies examined a too small number of dinoflagellate taxa to be considered conclusive. The diverse traits within dinoflagellates may be a significant source of variability in C:N:P, making it difficult to generalize about dinoflagellates as a group from studies on a small number of taxa. For example, dinoflagellates with thick theca may be more likely to be C-rich than those with thinner theca, or naked taxa (Menden-Deuer & Lessard, 2000). Similarly, smaller-sized taxa may

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be more likely to have higher C:N and C:P than larger taxa due to their higher surface area to volume ratios. Furthermore, toxin production may alter the elemental stoichiometry of dinoflagellates (Van de Waal *et al.*, 2014*a*). A more extensive analysis of the elemental composition of dinoflagellates that considers traits such as cell size, wall type and toxin production is needed to determine if dinoflagellates, and their traits, influence their C:N:P and whether dinoflagellates have a C:N:P that differs from the Redfield ratio.

The average molar ratio of C:N:P in marine plankton is often assumed to be invariant at 106:16:1 and is referred to as the Redfield ratio (Redfield, 1958; Falkowski, 2000; Martiny *et al.*, 2013). It is commonly recognized that C:N:P stoichiometry varies across taxa and with environmental conditions, affecting the efficiency of the biological pump (Weber & Deutsch, 2010; Martiny *et al.*, 2013). The relative importance of resource supply, community taxonomic composition and environmental conditions such as temperature on the stoichiometry of particulate production and export is an active area of research (Martiny *et al.*, 2013). Here we analyse cellular organic carbon, nitrogen and phosphorus content data from dinoflagellates extracted from lab studies under restricted, non-limiting growth conditions to quantify the C:N:P of marine, planktonic dinoflagellates. In addition we test if taxonomic order, cell size (small, medium, versus larger cell volumes), wall type (thecate versus non-thecate), or toxin formation (species known to be able to produce C-rich or N-rich toxins) have a quantitative influence on dinoflagellate C:N:P.

Methods

Data collection

Data on C:N:P of lab-cultured marine dinoflagellates were identified by searching in the ISI Web of Science using keywords such as elemental composition, elemental stoichiometry, elemental ratios, C:N, C:P or C:N combined with the word dinoflagellate. These studies and suitable studies cited within these sources were evaluated for data collection. If elemental data were only available in figures, ImageJ software was used to extract data (Schneider *et al.*, 2012). Experimental conditions were recorded along with the elemental composition data including culturing methods, the media used, culture temperature and salinity. Cell volume and growth rate data were collected when available.

Taxonomy and trait characterization

The World Register of Marine Species (WoRMS, http://www.marinespecies.org/) was used to identify the currently accepted name and higher-level taxonomy of the species. Species were then assigned the following traits: (1) thecate or athecate; (2) non-toxic or the potential to produce C-rich or N-rich toxins; and (3) small, medium or large cell volume. Contemporaneous, quantitative data on cell volume were often missing. We used cell size data from related work on the same taxa and converted size measurements to size categories to obtain size traits for as many observations as possible. Species with cell volumes reported to range between 100 and 1500 μm^3 were identified as 'small', between 1500 and 10 000 μm^3 as 'medium' and above 10 000 μm^3 as 'large'. These divisions were chosen to create three groups with approximately equal numbers of observations. In the cases where multiple publications reported biovolumes of the same species, we used the average of these values to assign our size category. For the species without any biovolume assigned in any of the manuscripts used in the C:N:P analyses, we extracted cell volumes from Pezzolesi *et al.* (2014), Harrison *et al.* (2015), Munir *et al.* (2015) and O'Boyle *et al.* (2016). Following Hoppenrath (2017), we assumed all species within the orders Amphidiniales and Gymnodiniales are athecate and that all species within the orders Gonyaulacales, Peridiniales, Thoracosphareales and Prorocentrales are thecate. We use the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (http://www.marinespecies.org/hab/), Lassus *et al.* (2016) and Van de Waal *et al.* (2014*a*) to identify species with the potential to produce N-rich or C-rich toxins. Saxitoxin, with a C:N ratio of 1.5 was considered N-rich whereas ovatoxin, yessotoxin, karlotoxin and brevetoxin, with C:N ratios higher than 43, were considered C-rich. For most of the data collected we lacked information on whether the species was actually producing toxin during the study or if the particular strain in the study produced toxins. Our identification of species as non-toxic or as C-rich or N-rich toxin producers was solely based on the potential of the species to produce these toxins based on previous studies.

Analyses

A total of 55 studies including 947 C:N, N:P and C:P observations from a wide variety of culture conditions and orders were collected (see supplementary material). We analysed 259 elemental ratio observations from 36 species from 46 studies under non-limiting exponential growth over a range of nitrogen to phosphorus ratios (9:1 to 59:1 mol:mol) in the media (Table 1) to identify differences in the elemental stoichiometry among dinoflagellate species and traits while minimizing the influence of variation in environmental conditions and sub-optimal, non-steady-state culture conditions. We used C:N:P observations from

Table 1. Sample sizes for each combination of trait and ratio, showing the total number of observations and number of different studies contributing data. The final row is the total number of observations and studies analysed.

Size	Thecate	Toxin	Number of studies	C:N samples	C:P samples	N:P samples
Small	athecate	C-rich	12[1–12]	23	7	7
		Non-toxic	4[5, 13–15]	5	1	1
	thecate	C-rich	13[10, 11, 14, 16–26]	21	4	4
		Non-toxic	5[2, 12, 14, 24, 27]	7	2	2
Medium	athecate	C-rich	5[28–32]	7	7	1
		Non-toxic	8[9, 11, 13–16, 33, 34]	18	7	7
	thecate	N-rich	5[12, 35–38]	12	9	9
		Non-toxic	8[5, 10–12, 16, 38, 39]	23	7	7
Large	athecate	Non-toxic	3[5, 13, 22]	5	0	0
	thecate	C-rich	2[40, 41]	4	3	3
		N-rich	7[5, 20, 21, 42–45]	27	3	3
		Non-toxic	5[5, 10–12,46]	7	1	5
Total			46	159	51	49

[1]Dortch et al., 1984; [2]Keller et al., 1999; [3]Li et al., 2016; [4]Lourenço et al., 2004; [5]Menden-Deuer & Lessard, 2000; [6]Nielsen, 1996; [7]Prioretti & Giordano, 2016; [8]Sakshaug et al., 1983; [9]Johnsen & Sakshaug, 1993; [10]Sakshaug et al., 1984; [11]Paasche et al., 1984; [12]Caruana et al., 2012; [13]Montagnes et al., 1994; [14]Quigg et al., 2003; [15]Moal et al., 1987; [16]Chen et al., 2019; [17]Fu et al., 2005; [18]Fu et al., 2008; [19]Harding et al., 1983; [20]Harke et al., 2017; [21]Hennon et al., 2017; [22]Lomas, 1999; [23]Monti-Birkenmeier et al., 2019; [24]Verity et al., 1992; [25]Coats & Harding, 2008; [26]Johnson, 2015; [27]Gorai et al., 2014; [28]Bercel & Kranz, 2019; [29]Hardison et al., 2014; [30]Hardison et al., 2013; [31]Hardison et al., 2012; [32]Corcoran et al., 2014; [33]Nielsen, 1992; [34]Nielsen et al., 1991; [35]Castro-Bugallo et al., 2014; [36]Yang et al., 2011; [37]John & Flynn, 2000; [38]Meksumpun et al., 1994; [39]Burkhardt et al., 1999; [40]Pezzolesi et al., 2016; [41]Ratti et al., 2011; [42]Zhu & Tillmann, 2012; [43]Van de Waal et al., 2013; [44]Van de Waal et al., 2014b; [45] MacIntyre et al., 1997; [46]Baek et al., 2008.

acclimated, exponential growth conditions in batch and semi-continuous cultures as well as from chemostat experiments. Cultures grown with presence of prey and unrealistic C:N ratios below 2 were not included in the analysis. Statistical differences in the elemental ratios across taxa were assessed by an analysis of variance (ANOVA) and for significant differences ($p < 0.05$), a multiple comparison Tukey's HSD test was conducted with R software version 3.4.4 (R Core Team, 2018).

Estimation of average elemental ratios

We defined an overall mean C:N:P stoichiometry for marine, photosynthetic, lab-grown planktonic dinoflagellates based on our data. There are many ways to compute this ratio, all of which were influenced by the samples available. The range of means resulted from averaging all data; means at the species or order levels varied by about 20%. Since some species had many more samples than others, we first computed a mean for each species, then averaged these species means, using sample size as weights, to get a grand mean for each ratio. We computed a weighted standard deviation similarly, weighting by sample size for each species. The statistical analysis and figures were conducted with R software version 3.4.4 (R Core Team, 2018).

Trait analysis

We estimated the effects of the three traits (cell wall, cell size and toxin) individually and in combination and taxonomic order on the elemental ratios (C:N, C:P, N:P) with a linear model predicting the ratio as a mean plus effects due to each predictor. Since the ratios were strictly non-negative with a somewhat skewed distribution, we used a log transform (log link in the regression model) on

the ratio (response variable). We fitted the model two ways, first using a generalized linear model (R function glm) and then using a Bayesian model (R package brms; Bürkner, 2017). The results were extremely similar, and we present only the Bayesian model results. Unequal sample sizes for various combinations of traits led to a highly unbalanced design, so we were concerned about the interpretability of pairwise differences in the generalized linear model and this was where the largest differences arose between the two modelling approaches. Ratios predicted for each combination of the three traits were computed and quantified by their posterior median and 95% credible interval. Posterior differences in ratios for selected traits were computed and reported as percentage difference from the grand mean. A Bayesian R^2 was computed to quantify the proportion of the variance in each ratio attributable to phylogenetic order, the three traits considered individually, and the three traits combined in one model (R function brms::bayes_R2) following Gelman et al. (2019).

Results

We gathered approximately 3-fold more observations of C:N than C:P or N:P (Table 1). The mean C:N:P ratio of the marine dinoflagellates was 90:12:1, with considerable variability across species (Fig. 1, Table 2). Mean C:N was 7.2 ± 0.5, slightly larger, while C:P (90.3 ± 13.7) and N:P (11.5 ± 1.6) were lower than the canonical Redfield ratio (Table 2). Note we excluded the calcified species *Thoracosphaera heimii* from the order Thoracosphareales (C:N = 17.5; C:P = 112; N:P = 6.4) from Fig. 1.

Taxonomic variability in C:N:P

Nearly 40% of variance in each ratio was explained by taxonomic order (Table 3) with residual variation arising at the genus or species level, or due to differences in

experimental design. The orders Peridiniales and Prorocentrales had higher C:N ratios than Gonyaulacales, Gymnodiniales and Amphidiniales (Fig. 1). The trend found in C:P and N:P ratios is not as conclusive because of the high variability observed in the data. However, the orders Perdidiniales and Prorocentrales had higher C:P and N:P values as well, together with the order Amphidiniales.

C:N

We obtained C:N from five orders of dinoflagellates, belonging to 18 genera and 34 species. Median C:N ratios of the orders Amphidiniales (median C:N = 6.51), Gonyaulacales (median C:N = 6.45) and Gymnodiniales (median C:N = 6.71) were similar or slightly lower than the Redfield ratio. On the other hand, the median C:N ratios of the orders Prorocentrales (median C:N = 7.62) and Peridiniales (median C:N = 7.67) were larger than the Redfield ratio. These two orders had average C:N ratios significantly greater than the other three orders examined (ANOVA, $p < 0.05$).

C:N data in the order Amphidiniales was predominantly provided by *Amphidinium carterae*, with a median C:N ratio of 6.4. Within the order Gymnodiniales, *Lepidodinum chlorophorum* had the highest C:N (median C:N = 8.25), followed by *Karenia brevis* (median C:N = 7.69) and the lowest median C:N was associated with *Karlodinium veneficum* (median C:N = 4.75). Within the order Gonyaulacales most of the C:N data was associated
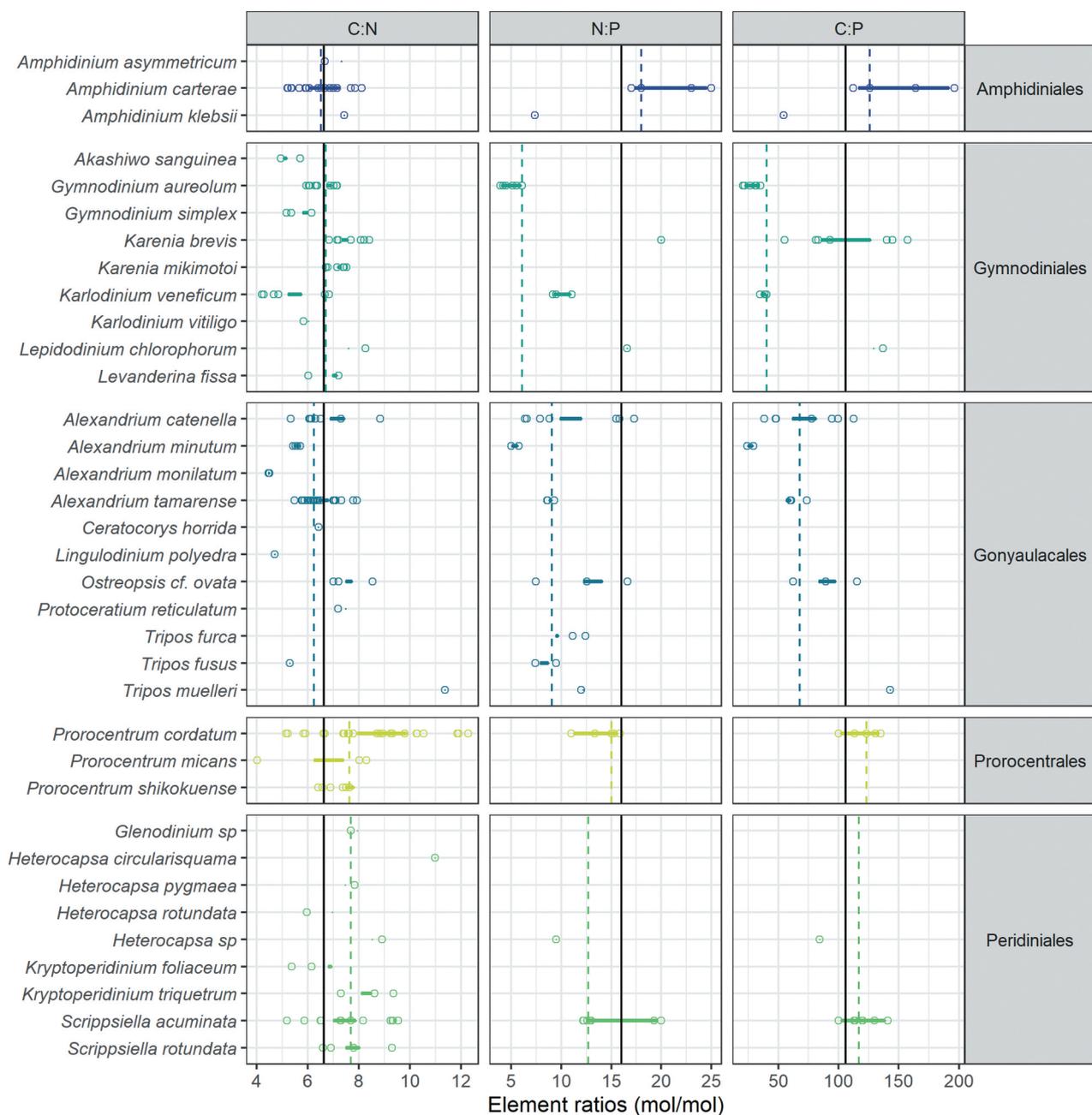

Figure 1. Molar ratios of dinoflagellate species grouped by orders. Vertical dark line represents Redfield ratios and doted lines correspond to the median of each order.

Table 2. Summary of the grand mean stoichiometric ratios (mol:mol) and the differences in ratios between selected levels of each trait. Differences in ratios are reported as a percentage of the grand mean to highlight differences in trait effects for each ratio. Difference labels are presented as arithmetic expressions.

	units	C:N	C:P	N:P
Mean ± 2 SE	mol:mol	7.2 ± 0.5	90.3 ± 13.7	11.5 ± 1.6
Non-toxic-C-rich	%	0.9	-19.1	-25.9
Non-toxic-N-rich	%	15.2	24.6	11
Small-large	%	7.1	17.2	25.6
Thecate-athecate	%	19.1	49.9	29.4

Table 3. Bayesian R^2 (%) reporting the proportion of variance in each stoichiometric ratio explained by regression on taxonomic order, all traits, or each trait separately. A linear model was used with a Gamma distribution and log link for the response variable. R^2 for each trait separately does not add to the total for the model with all three traits unless the uncertainty in each estimate is considered; the standard deviation of R^2 ranges from 2–10%.

Predictor	C:N	C:P	N:P
Taxonomic order	37%	38%	38%
All 3 traits	20%	31%	27%
Toxin	6%	14%	17%
Size	5%	10%	12%
Cell wall	5%	4%	2%

with four species of *Alexandrium* (median C:N = 6.14), representing more than 80% of the observations in the order (39 out of 47). The C:N observations in the order Prorocentrales belonged to three species from the genus *Prorocentrum*. Despite the low number of species within the order, they encompassed the whole range of C:N ratios in the study, having the lowest value, 4.01 for *Prorocentrum micans* as well as the highest, 12.27 for *Prorocentrum cordatum*. In the order Peridiniales, most C:N observations were higher than the Redfield ratio. *Scrippsiella acuminata* was the most represented species of the order with seven studies and 12 observations, with an average C:N ratio of 7.66 ± 1.48 (median C:N = 7.49).

N:P

We gathered N:P data for five orders, 11 genera and 16 species. All orders, except the Amphidiniales (median N:P = 18) had median N:P lower than the Redfield ratio. N:P was lowest in the Gymnodiniales (median N:P = 6.09) and Gonyaulacales (median N:P = 9.04); both were significantly lower than N:P of the Amphidiniales. Within the order Gymnodiniales, there was much variability across the four species observed, *Gymnodinium aureolum* (median N:P = 4.79), *K. brevis* (median N:P = 20), *K. veneficum* (median N:P = 9.48) and *L. chlorophorum* (median N:P = 16.6). High variability in N:P was also found in the order Gonyaulacales, although most of the N:P observations were below the Redfield ratio. The order

Prorocentrales was only represented by *P. cordatum*, with a median N:P ratio of 15. The order Peridiniales had a median N:P ratio of 12.7.

C:P

C:P data were analysed from five orders of dinoflagellates, represented by 11 genera and 14 species. The orders Gymnodiniales (median C:P = 40.4) and Gonyaulacales (median C:P = 68.0) had lower average C:P ratios than the Redfield ratio, and their C:P was significantly different from the other three orders. C:P of the orders Amphidiniales (median C:P = 126), Prorocentrales (median C:P = 123.35) and Peridiniales (median C:P = 116.81) were larger than the Redfield ratio. C:P data for these three orders came from one or two species.

Trait-based variability in C:N:P

Wall structure, cell size and toxin production all influenced C:N, C:P and N:P (Fig. 2, Table 2), accounting for some of the variability across orders and species. Wall structure (thecate versus athecate) had the largest influence on the elemental ratios of all the traits evaluated (Table 2). On average the thecate species had larger C:N (19.1%), C:P (49.9%) and N:P (29.4%) than athecate species and the species-based global means for the dinoflagellates (Table 2). *Alexandrium* species were an exception, with lower elemental ratios than the average for thecates and lower ratios relative to other genera within the Gonyaulacales (Fig. 1). Smaller-sized species were higher in C:N, C:P, and N:P than larger-sized species (7.1, 17.2 and 25.6%, respectively, Table 2). The ability to produce toxins had complex consequences for C:N, C:P and N:P. For example, non-toxic species were similar in C:N but lower in C:P (19.1%) and N:P (25.9%) than species that may produce C-rich toxins while non-toxic species were higher in C:N and N:P than species that may produce N-rich toxins (Table 2). The proportion of variance explained by the three traits is 20%, 31% and 27% for C:N, C:P and N:P, respectively (Table 2). Individual traits were equally predictive of C:N while the toxin trait was most predictive and the thecate trait least predictive for C:P and N:P.

Discussion

The C:N:P of marine, photosynthetic dinoflagellates cultured under non-limiting growth conditions varies considerably across species (Fig. 1), but the average ratio 90:12:1 (Table 2) differs substantively from the canonical Redfield ratio of 106:16:1. Low values of C:P and N:P may be related to luxury phosphorus storage or high nucleic acid content, while high
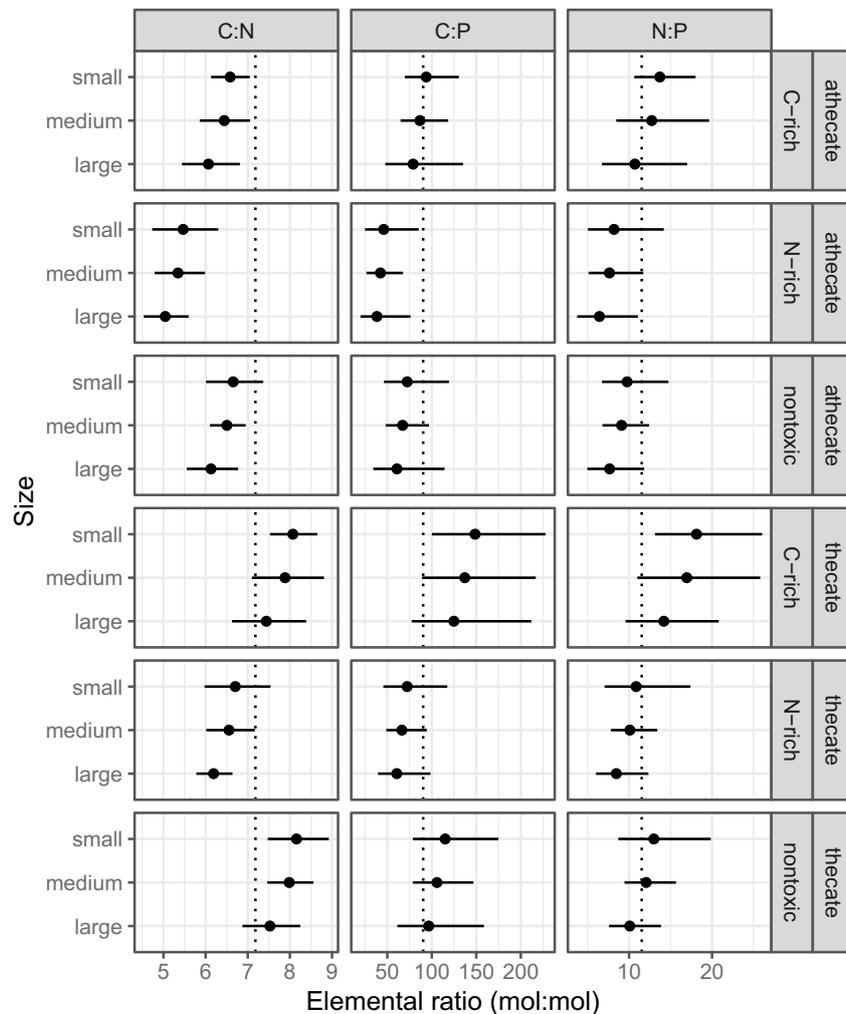
Figure 2. Elemental stoichiometry for combinations of each of the three traits, presented as a median (filled symbol) and 95% credible interval (line) from our Bayesian model. The vertical dotted line in each panel is the grand mean ratio.

values of C:N (average of 7.2) are likely related to the cellulosic-like material that makes up the thick walls of the thecate dinoflagellates (discussed below). These traits are often linked to phylogeny. Taxonomic order accounts for almost 38% of the variance in elemental ratios. Three key traits: cell wall structure, cell size and whether the species can produce C-rich or N-rich toxins have direct consequences for elemental stoichiometry and account for up to a third (21–32%) of the variability in C:N:P within the dinoflagellates.

Low values of C:P and N:P in dinoflagellates are strong indicators that dinoflagellates are high in intracellular phosphorus relative to many other plankton. The largest phosphorus pools in protists are typically in DNA, RNA, polyphosphate and other P-storage molecules, with a relatively small amount in phospholipids (Geider & La Roche, 2002; Sterner & Elser, 2002; Liefer et al., 2019). It has been hypothesized that dinoflagellates may have higher cellular P content due to their large genomes and high DNA content (John & Flynn, 2000; Segura-Noguera et al., 2016). However, some evidence

suggests that dinoflagellates are no higher in DNA than other eukaryotes of similar size (Shuter et al., 1983), despite their large genome (Hackett et al., 2004), and that their unique nuclear architecture is associated with a lower protein:DNA compared with other eukaryotes (Rizzo & Burghardt, 1982; Karpinets et al., 2006; Beauchemin & Morse, 2018). Alternatively, the growth rate hypothesis (Sterner & Elser, 2002) proposes that increasing growth rates are associated with increases in RNA content which are then reflected in decreases in N:P; although it has been shown that this is not always the case for phytoplankton (Flynn, 2010; Hillebrand et al., 2013; Garcia et al., 2018; Moreno & Martiny, 2018). Dinoflagellates are known to have slower maximum growth rates than many other phytoplankton groups (Tang, 1996; Raven et al., 2005), so if the growth rate hypothesis held, then dinoflagellates should be relatively high (not low) in N:P compared with other faster growing plankton. Similarly, larger-sized species often have slower growth rates, and therefore we might expect an increase in N:P with increasing cell

size. In contrast to these expectations, in this study N:P is largest in smaller-sized dinoflagellate species (Table 2).

In aggregate, these results indicate that neither anomalous DNA or RNA content can fully explain the relatively high phosphorus content of the dinoflagellates. Therefore we hypothesize the low C:P and N:P in marine dinoflagellates may be due to the accumulation of polyphosphate under nutrient-replete conditions. Sakshaug et al. (1983) reported that the dinoflagellate A. carterae was capable of incorporating up to 30% of total phosphorus as polyphosphate under non-limiting conditions. Accumulation of phosphorus under non-limiting conditions has been observed in several dinoflagellates, including T. furca and T. fusus (Baek et al., 2008), G. aureolum (Nielsen & Tønseth, 1991; Nielsen, 1992), K. brevis (Hardison et al., 2013) and the freshwater species Peridinium cinctum (Elgavish et al., 1980). It has been hypothesized that the ability to store phosphorus and other nutrients can provide a competitive advantage in certain fluctuating environments and facilitate the formation of blooms (Smayda, 1997). A number of the dinoflagellate species with the lowest C:P and N:P in this study are well-known bloom formers (e.g. Alexandrium).

Cell wall structure (thecate versus athecate), cell size and toxin production all significantly impact C:N:P in dinoflagellates. As a consequence any estimate of the average C:N:P ratio of the dinoflagellates will be significantly influenced by the relative number of taxa with these traits that are included in the analysis (Table 2). As an example, here we find that the thecate taxa are 20% higher in C:N, 50% higher in C:P and 29% higher in N:P than the athecate taxa. Previous studies have established that dinoflagellates are higher in carbon:cell volume relative to other phytoplankton groups and that thecates are more carbon dense than athecates (Menden-Deuer & Lessard, 2000; Segura-Noguera et al., 2016). The relatively high average C:N and C:P of the dinoflagellate species analysed as part of this study reflects the C-rich cellulosic-like material that makes up the wall of the thecate dinoflagellates (Lau et al., 2007). It is unclear why thecates are relatively high in N:P compared with the athecate taxa. Cell size also impacts C:N:P in dinoflagellates. As expected C:N and C:P is higher in smaller relative to larger-sized species, due to the higher surface-area to volume ratio of the smaller species. Surprisingly, N:P is higher in smaller relative to larger species; more effort (data and theory) is required to interpret the variability in N:P in dinoflagellates.

A number of toxic dinoflagellates form harmful algae blooms (HABs) with socio-economic consequences (Smayda & Reynolds, 2003). Our analysis indicates that species with the ability to produce toxins, and toxin type, influences C:N:P in dinoflagellates. For example, the lowest C:N ratios in this study belong to the order Gonyaulacales, despite being thecate. Alexandrium, a genus known to form N-rich neurotoxic saxitoxins (C:N = 1.5) that are responsible for paralytic shellfish poisoning (Anderson et al., 2012), represents 80% of the observations in the order Gonyaulacales in this study. This suggests that the ability to form N-rich toxins may be associated with higher cellular nitrogen content (Van de Waal et al., 2014a; Brandenburg et al., 2020). High concentrations of glutamine and arginine have been reported in Alexandrium species compared with other dinoflagellates (John & Flynn, 2000; Anderson et al., 2012). A second example is the athecate species K. brevis which has a higher C:N than other species within the Gymnodiniales. This species produces C-rich (no nitrogen) brevetoxins and analogues, polycyclic ether neurotoxins, responsible for neurotoxic shellfish poisoning in humans (Landsberg, 2002). However, K. veneficum, another species from the order Gymnodiniales, has a very low average C:N, even though it is known to produce carbon-rich karlotoxins (Place et al., 2012). This discrepancy may be due to the fact that the strains of K. brevis from the studies analysed here were toxin producers (Hardison et al., 2012, 2013, 2014; Bercel & Kranz, 2019), while the toxicity of the K. veneficum strains was not determined.

The data collected here represent a very small fraction (36 species, 20 genera, five orders) of the diversity of dinoflagellates and may not be representative. There are 2500 described species belonging to 238 genera (Hoppenrath, 2017). This estimate of dinoflagellate diversity is likely an underestimate (Le Bescot et al., 2016; Pierella Karlusich et al., 2020; Wu et al., 2020); modern sequencing metabarcoding techniques indicate dinoflagellates diversity may be four times higher (de Vargas et al., 2015). All the samples reported here are for dinoflagellates growing autotrophically, but about half of the free-living dinoflagellates are heterotrophs and among the other half, most of them are capable of phagotrophy (Gómez, 2012). In the last decades, the scientific community has put a great effort to develop a 'mixoplankton paradigm' to be incorporated into biogeochemical models (Ward & Follows, 2016; Chakraborty et al., 2017; Flynn et al., 2019). Mixotrophy in dinoflagellates may provide an advantage for growth over other autotrophic phytoplankton groups in oligotrophic conditions (Barton et al., 2013), with implications for their elemental composition (Moorthi et al., 2017; Golubkov et al., 2019). Further experimental research is needed to better document elemental stoichiometry in dinoflagellates, particularly for mixotrophs and heterotrophs.

Although we show that variability in C:N:P can be related to phylogenetic history and traits, there

is evidence of intraspecific variability of functional traits among strains (Lemmen *et al.*, 2019; Brandenburg *et al.*, 2020). Some of this intraspecific variability is associated with specific adaptations of strains or sub-species to different environmental conditions, as well variability in experimental conditions and analytical methods applied across studies. For example, C:N in *P. cordatum* varies by more than a factor of two; Klanjšček *et al.* (2016) demonstrated some of this variability is strain-specific. In this particular case, data came from 14 studies that included different experimental conditions, including a range of light intensities, light periods, temperatures and salinities. Sampling during the early versus late exponential phase has also been shown to strongly influence elemental stoichiometry. For instance, C:P increases almost three-fold over three consecutive sampling days during the exponential period in a study on *O.* cf. *ovata* (Pezzolesi *et al.*, 2016). There is an extensive literature that shows that nutrient, light regime and temperature can all impact C:N:P (e.g. Johnsen & Sakshaug, 1993; Nielsen, 1996; Finkel *et al.*, 2006; Vidyarathna & Granéli, 2013; Garcia *et al.*, 2018; Chen *et al.*, 2019). In the analysis presented here we attempted to limit the impact of experimental conditions by limiting our analysis to studies to cultures in exponential growth under non-limiting conditions and an intermediate range of nitrogen to phosphorus concentrations in the media.

Our analyses of C:N:P of marine dinoflagellates indicate dinoflagellates are high in C:N and low in C:P and N:P relative to the canonical Redfield ratio. Based on these results and previous studies, we hypothesize that dinoflagellates are on average phosphorus- and carbon-rich relative to many other phytoplankton groups. Further investigations are needed to better understand the biochemical basis for the high phosphorus content of dinoflagellates and determine under what conditions dinoflagellates accumulate phosphorus as polyphosphates or other storage molecules. We show that 38% of the variability in C:N:P within the dinoflagellates can be associated with taxonomic order and 20–31% with the combination of three traits: cell wall structure (thecate versus athecate), cell size, and toxin production and toxin type. These results indicate that any average estimate of dinoflagellate C:N:P will be influenced by the relative number of taxa with these traits.

Particulate C:N:P in the surface ocean varies across biogeographic regions and with latitude (Martiny *et al.*, 2013). These patterns in C:N:P may be caused by differences in phytoplankton community structure and may influence the strength of the biological pump (Weber & Deutsch, 2010). There is some evidence that climate warming will result in a relative shift in the abundance of dinoflagellates relative to diatoms (Leterme *et al.*, 2005; Xiao *et al.*, 2018), affecting particulate C:N:P, food web structure and the carbon cycle in the oceans (Branco *et al.*, 2018; Riebesell *et al.*, 2018; Venuleo & Giordano, 2019). More information is required to fully understand how the trait distribution of dinoflagellates will be influenced by climate change. Our findings highlight that changes in phytoplankton community structure, in particular an increase in the relative biomass of dinoflagellates and dinoflagellates with specific traits: size, wall structure and toxin production, can influence particulate C:N:P in the ocean.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary information

The data that support the findings of this study are openly available in Zenodo at doi:10.5281/zenodo.4419871 and at on the article's online page at https://doi.org/10.1080/09670262.2021.1914860.

Author contributions

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