Harmful Algae 51 (2016) 16–25

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Nutrient quotas and carbon content variability of *Prorocentrum minimum* (Pavillard) Schiller, 1933

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ARTICLE INFO

Article history:
Received 24 May 2015
Received in revised form 7 October 2015
Accepted 14 October 2015
Available online 27 November 2015

Keywords:
Nitrogen quota
Phosphorus quota
Carbon content
Phytoplankton model
Stoichiometric constraints
Prorocentrum minimum

ABSTRACT

Frequency, severity, and geographic range of harmful blooms caused by a dinoflagellate *Prorocentrum minimum* have been increasing significantly over the past few decades. The ability to adapt nutrient quotas and carbon content to a wide range of environmental conditions is one of the key factors for the proliferation of *P. minimum*. Understanding the limits of stoichiometric variability in terms of nutrient quotas and carbon content would help explain the observed trends and assist in *P. minimum* growth model creation. This manuscript aggregates information from 15 studies to investigate variability in nutrient quotas and carbon content for a broad range of *P. minimum* isolates and clonal lines. Nitrogen quota, phosphorus quota, and carbon content in the studies varied between 11–107.5 pgN cell^{-1}, 1.45–17.58 pgP cell^{-1}, and 70–656.36 pgC cell^{-1}, respectively. Regression analysis was used to estimate average nitrogen and phosphorus quotas as functions of carbon, and to show that carbon content variability explains 55% of nitrogen and 23% of phosphorus quota variability. Confidence intervals for data (CID) found during the analysis were used to define maximal and minimal nutrient quotas as functions of carbon content. The ratios of the upper and lower CID ranges can, therefore, be used to estimate nutrient storage capacity as a function of carbon content. The new results and comparison with other species show that, at least for *P. minimum*, carbon-based quotas are more suitable for modelling than cell-based quotas. Finally, results indicate that environmental nutrient availability affects quotas more than light does: while quota variability due to light remains within 80% CID, nutrient variability covers the 95% CID.

1. Introduction

Prorocentrum minimum is a harmful bloom-forming dinoflagellate with a significant and increasing environmental impact. The extent of direct toxicity of *P. minimum* is still a matter of some debate (Heil et al., 2005), but *P. minimum* has been implicated in shellfish toxicity (Nakajima, 1965a,b,c, 1968; Kat, 1979; Grzebyk et al., 1997; Denardou-Queneherve et al., 1999), fish kills (Silva, 1980; Tseng et al., 1993), reduction in egg production, growth, and survivorship of invertebrates (Wikfors and Smolowitz, 1993, 1995; Luckenbach et al., 1993; Hégaret and Wikfors, 2005), and has been associated with oxygen depletion in the water column (Rabbani et al., 1990; Moncheva et al., 1995; Azanza et al., 2005). Blooms of *P. minimum* are of particular concern because they occur in coastal and estuarine regions, thus threatening both the environment and industries such as fisheries and aquaculture. Severity, frequency, and geographical range of *P. minimum* blooms has been increasing over the past few decades (Heil et al., 2005). Since *P. minimum* blooms are primarily driven by anthropogenic phosphorus and nitrogen inputs (Glibert et al., 2008, 2012), and the trend of inputs is not likely to be reversed in the near future, the threats may escalate. Understanding the causes and quantifying the dynamics of *P. minimum* blooms can help predict the threats and develop mitigation measures and, consequently, reduce both environmental and economic risks.

Understanding of the causes and dynamics of *P. minimum* blooms requires quantitative links of environmental factors (e.g. nutrients, temperature and light) to *P. minimum* growth. Linking environmental factors to growth, however, requires knowledge of stoichiometric constraints of *P. minimum*. Research has shown that stoichiometric relationships between carbon, nitrogen and phosphorus (*C*, *N* and *P*, respectively) are of particular importance: (i) stoichiometry determines the biomass (in units of carbon) that can be reached by a given nutrient availability; (ii) the relationship between cellular and environmental *N*:*P* ratios indicate which

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http://dx.doi.org/10.1016/j.hal.2015.10.010
1568-9883/

species might dominate the bloom (Geider and LaRoche, 2002; Klausmeier et al., 2008; Edwards et al., 2011); and (iii) knowing phytoplankton *C*, *N* and *P* stoichiometry is typically a prerequisite for creating and parameterizing biogeochemical environmental models that incorporate phytoplankton growth (Geider et al., 1998; Flynn, 2001; Flynn et al., 2001; Klausmeier et al., 2004; Ross and Geider, 2009; Pahlow and Oschlies, 2009).

Most algae have, despite variable concentrations of nutrient storage pools, a species-specific, fairly uniform stoichiometry that reflects the composition of biomolecular functional machinery (Sterner and Elser, 2002; Klausmeier et al., 2004). *P. minimum* exhibits a marked variety in physiology due to ecophysiological adaptations enabling it to inhabit a wide range of environments, reflected in a high number of taxonomic synonyms, isolates, and clonal lines. This variety, in turn, implies a high potential for differences in the underlying stoichiometry that has insufficiently been investigated on the species level.

Here, the overall stoichiometric variability within *P. minimum* is determined, and (to the extent possible from the existing data) stoichiometric variations due to light are distinguished from those due to nutrients. Carbon content and cellular quotas of *N* and *P* for *P. minimum* were compiled and analyzed (194 data total). Data included a spectrum of distinct natural and laboratory conditions, and a broad range of *P. minimum* isolates and clonal lines. Regression analysis was used to determine (cor)relation between *N* and *P* quotas relative to cellular biomass quantified by *C* content. The analysis helped determine the variability attributable to changes in biomass, compared to that attributable to the differences in environmental conditions and other factors. Using statistical tools, the stoichiometric constraints for *P. minimum* were determined from the calculated minimal and maximal expected quotas of *P. minimum* relative to *C* content. The findings were then used to analyze whether the data relate to the newly determined constraints, and whether environmental factors e.g. nutrient conditions, light availability, or temperature deprivation, affect the *N* and *P* quotas.

2. Methods

2.1. Multivariate data set

Data on *P. minimum* *C* content and *N* and *P* quotas were aggregated from 15 experimental studies published between 1983 and 2014 (see Table 1 for details). Studies covered a broad range of environmental conditions, including reactions of *P. minimum* to nutrient deprivation, light and temperature variations, optimal growth conditions, and natural environment. More than 10 different clone lines, isolates, and natural samples were included. Most studies (10) used batch reactors in their experimental setups, while three studies used semi-continuous or pulse batch reactors. In 12 studies, cells were harvested during the exponential growth phase; two included cells harvested in initial and/or late stationary phases as well.

The complete multivariate data set consists of 79 measurements: 37 simultaneous measurements of *C*, *N* and *P*; 41 simultaneous measurements of *C* and *N*, and one measurement of *C* alone. All data were treated equally, with equal weights assigned in all analyses. The differences introduced by variability in *P. minimum* clone lines and growth conditions were assumed to be much greater than any potential bias stemming from analytical methods used.

2.2. Data analysis

Descriptive statistics of the data Descriptive statistics of *C*, *N* and *P* distributions and ratios were used for data overview: central tendency (mean, median) and dispersion (range, quantiles of the data-set, standard deviation). All statistical analysis were conducted using MathWorks Matlab2011b built-in statistical functions.

Data correlation All measurements were $\log_{10}$ transformed prior to analysis to satisfy statistical assumptions of normality and homoscedacity, as well as partially reduce the effects of outliers. The Pearson product–moment correlation coefficient was calculated for pairs of *C*, *N* and *P* variables to measure the degree of linear dependence between the variables. The sample estimates of correlation and 95 percent confidence intervals were obtained by R software. Significances of correlations were estimated using the Student's *t*-test.

Regression analysis Ordinary least square regression analysis (OLS) (Niklas, 1994; Zar, 1999) was used to determine allometric relationship $Y = aC^b$ converted to a log-normal form:

$$\log_{10} Y = \log_{10} a + b \log_{10} C, \tag{1}$$

where *C* represents *C* content (pgC cell^{-1}) and *Y* represents *N* or *P* quotas (pg Y cell^{-1}). MathWorks Matlab 2011b curve-fitting toolbox (CFT) was used to construct the regression models and assess the confidence intervals for the estimated parameters. The coefficient of determination (R^2) indicated the goodness of fit, and an *F*-test provided a test of statistical significance of the overall fit. The parameter *b* was tested against two null hypotheses ($b = 0$ and $b = 1$) using the two-tailed Student's *t*-statistics (Zar, 1999).

Expected minimal and maximal nutrient quotas 'Confidence interval for function' (CIF) is the prediction range where the expected mean of future observations is most likely to reside due to uncertainty of the estimated coefficients. 'The Confidence interval for data' (CID) is the prediction interval in which future observations are most likely to occur. Regression analysis implemented in MathWorks Matlab 2011b CFT was used to estimate CIF at 95% confidence level, and CID at 80%, 90%, and 95% confidence levels. Since CID includes both the observation variance (noise), and the uncertainty of coefficient estimates, CID is expected to be wider than CIF.

Boundaries of CIDs were set to minimal and maximal nutrient quotas with respective confidence levels (80%, 90%, or 95%). At each confidence level, the ratio of maximal to minimal quotas as a function of *C* content was calculated. The obtained ratios were then used to calculate minimal, mean and maximal ratio values (at each confidence level).

2.3. Comparison and applicability of regression relationships to P. minimum

The regression relationships between nutrient quotas and *C* content obtained for *P. minimum* were compared to: (a) similar regression models found in the literature (Shuter, 1978); and (b) regression models developed from existing data on other phytoplankton species (Taguchi, 1976; Blasco et al., 1982; Verity et al., 1992; Menden-Deuer and Lessard, 2000). In the latter case, regression analysis was performed using the methods described above. The root mean square error (RMSE) was used as a measure of model accuracy when comparing forecasting errors of different models. RMSE quantifies the difference between nutrient quotas predicted by the model, and the actual observed values for *P. minimum*:

$$\mathrm{RMSE} = \sqrt{\sum_i \frac{(\log_{10} Y_i - \log_{10} a - b \log_{10} C_i)^2}{n}} \tag{2}$$

where Y_i is the observed *N* or *P* quota of *P. minimum*, C_i is the *C* content, *a* and *b* are regression coefficients of a particular model.