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Modeling Nitrate Uptake in Freshwater Phytoplankton

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Abstract

Freshwater phytoplankton are typically limited by nitrogen or phosphorus. Additions of these nutrients can lead to blooms, so understanding nutrient metabolism is important for predicting ecosystem responses. Nitrate metabolism was studied in an urban pond and large lake using controlled incubations of whole-water cultures. Cultures were either not enriched, or enriched with phosphate, and nitrate or ammonium and followed for two weeks, measuring nutrients, chlorophyll, particulate C and N, the activity of a key enzyme, nitrate reductase (NR), and kinetics of nitrate uptake and NR activity. Pond phytoplankton responded rapidly to enrichments; added nitrate disappeared in 5 d. Lake phytoplankton showed slower growth, with nitrate and phosphate depleted over 1-2 weeks. Only nitrate-enriched cultures had NR activity and it disappeared when nitrate was exhausted or when cells stopped growing. Laboratory nitrate uptake showed novel kinetics; at low external nitrate concentrations uptake rates increased to a plateau but also showed a linear increase in uptake rate with much higher nitrate concentrations. Half saturation constants for uptake were higher (20 μM) than for NR activity (1 μM), suggesting minimal development of intracellular nitrate pools. Ongoing work includes identification of algal taxa in the communities using 18S rRNA gene sequencing and matching to databases. An agent based model (i.e. a model in which parameters are allowed to vary among individuals) was also developed to examine if phytoplankton community responses to nitrate can be predicted from individual species characteristics. The model was developed in Netlogo using Michaelis-Menten kinetic equations for nitrate uptake, and intracellular assimilation through nitrate reduction by NR activity. Ranges of kinetic parameters for cells were selected from our laboratory measurements, and literature values for species. A key component of the model is a cellular N content threshold for cell division. The model did not demonstrate bi-phasic nitrate uptake.

Keywords: phytoplankton, nutrients, modeling

1 Introduction

Phytoplankton are microscopic photosynthetic organisms that play an integral role in biochemical regulation in freshwater and marine environments [3]. In particular this paper will examine phytoplankton populations in Estabrook Pond, a small nitrogen-limited urban pond in Milwaukee, WI as well as a phosphorous-limited area of Lake Michigan local to Milwaukee, WI.

Nitrogen plays an important role on earth because of its abundance in multiple states as well as its biological role as an essential element for organic lifeforms [3]. Both phytoplankton and higher plants require nitrogen for survival [14]. Phytoplankton must uptake inorganic nitrogen compounds and then biochemically change them to a usable form for assimilation into things like amino acids [14] [2]. The uptake and assimilation of nitrogen compounds has physiological and metabolic effects on phytoplankton, but many of the effects are not fully understood [6]. The biological goal of this project was to investigate physiological and metabolic effects in phytoplankton communities caused by the uptake and assimilation of different nitrogen compounds through field and laboratory methods. Complementing that, the mathematical goal was to create a model of nitrate uptake by phytoplankton and seek validation for the physiological effects that were studied in the lab but without hardwiring them into the model.

2 Nitrate Reductase

Nitrate must undergo three steps to become available for incorporation into a phytoplankton [2]. The first step is uptake: the removal of nitrate from the environment and physical transport into the cell [2]. This is a chemical reaction where nitrate is outside the cell then through biochemical means enters the interior of the cell, becoming intracellular nitrate. This reaction is typically modeled by an equation of the form:

$$\text{Rate of reaction} = \frac{V_{max}[S]}{K_s + [S]} \tag{1}$$

where [S] is the concentration of nutrient outside the cell, V_{max} is the maximal rate of uptake when the nutrient is saturating, and K_s is the half-saturation constant: the concentration of nutrient at which half of the V_{max} is reached. Phytoplankton species have heterogeneous nitrate uptake parameters for equation (1) and even within a species there is heterogeneity due to genetic variation [7]. Nitrate uptake gets the nitrate into the cell but it still must be transformed into a compound for assimilation [2]. The primary step for this is reduction from nitrate to nitrite catalyzed by the enzyme nitrate reductase, or simply NR [14]. This process is a reduction reaction where electrons are donated by NADPH [14]. This reaction can also be modeled by equation (1). This is followed by reduction from nitrite to ammonium catalyzed by the enzyme nitrite reductase [14]. This creates the basis for the investigation of the different effects of the interplay between nitrate and ammonium as the presence of ammonium short-circuits nitrate reduction.

3 Nutrient and Enzyme Kinetic Behavior

The rate at which a cell uptakes nutrients as well as the rates at which reactions to change the nutrient into a compound for assimilation occur depend on several factors and can be modeled by equation (1) . When [S] is 0, meaning no nutrient outside the phytoplankton, clearly it cannot uptake any nutrient. Likewise, if there is no nutrient inside of the cell then no reduction of that nutrient can occur. Both of these send equation (1) to 0. Then consider [S] with two fixed parameters: the maximal uptake rate V_{max} and half saturation constant K_s. The uptake rate of nutrient or the reduction of that nutrient is measured at a macro level in the lab and then related back to the amount of biomass that was performing the reaction [7]. This makes sense because these reactions are also dependent upon the number of phytoplankton that are performing the reaction: more phytoplankton means more uptake or more reduction for a given sample [7]. Likewise, equation (1) is maximized when $[S] \to \infty$, assuming that $V_{max}, K_s > 0$, and with some basic calculus the limit is V_{max}. NR kinetics was measured following Berges et al. (1995) and nitrate uptake kinetics was measured following Young et al. (2009) . Each of these methods produces a curve of reaction rates, scaled to biomass, that depend only on the initial concentration of nutrient for that given biomass.

4 Laboratory and Field Methods

Water was collected from Lake Michigan (3m depth) and from a small urban pond at Estabrook Park (43 5'49.47"N, 8754'27.76"W), both located in Milwaukee, Wisconsin, for controlled incubations of whole-water cultures. Water was filtered through a 100 μmeter Nitex screen. Cultures were maintained in carboys of 8L (pond) or 20L (lake). Carboys were aerated with a pump system and aeration stone and were vigorously shaken several times a day to mix them. The samples for analysis were collected near the bottom of the water cultures via mounted tubes and syringes. All carboys were maintained at 18C with 100 μmol/m^2/s irradiance. Duplicate carboys were used for each treatment. All cultures were enriched to the same concentration of phosphate: 6.2 μM PO_4^{3-}, treatments were either enriched with nitrate to concentrations of 100 μM NO_3^-, or enriched with ammonium to 100 μM NH_4^+. The controls were not enriched with any nitrogen source, only phosphate. Because Lake Michigan is phosphorus limited, to obtain the concentrations needed 2.73 mL of $NaNO_3$ was added to the nitrogen treatments (Lake: N1 and N2), 13.04 mL NH_4Cl was added to the ammonium treatments (Lake: A1 and A2), and 5.576 mL KH_4PO_4 was added to the controls (Lake: C1 and C2) as well as the ammonium and nitrate treatments. Estabroook Pond is nitrogen limited so 1.45 mL of $NaNO_3$ was added to the nitrogen treatments (Pond: N1 and N2), 5.16 mL NH_4Cl was added to the ammonium treatments (Pond: A1 and A2), and 2.5mL KH_4PO_4 was added to the controls (Pond: C1 and C2) as well as the ammonium and nitrate treatments.

Over two weeks cultures were sampled to monitor variable chlorophyll a fluorescence. Additional measurements were taken of dissolved nutrients (PO_4^{3-}, NO_3^-, NH_4^+), C and N content, NR activity, and chlorophyll a and phaeopigments extractions. NO_3^- uptake and NR activity kinetics in relation to NO_3^- concentration were measured on selected samples. Nutrient analysis for PO_4^{3-}, NO_3^-, NH_4^+, as well as chlorophyll a and phaeopigments extraction, followed the methods by Parsons et al (1984). NR activity and NR kinetics was measured following Berges et al. (1995), and the nitrate uptake kinetics followed Young et al. (2009). Water samples were filtered onto 13mm Gelman membrane filters. These were frozen for future extraction of total community DNA using rRNA gene sequencing.

Variable chlorophyll a fluorescence was sampled minimum of once daily for both locations. Sampling for dissolved nutrients, CN analysis, chlorophyll a extraction, and NR activity was performed on Estabrok samples on days 1,2,3,6,9, and 14. Estabrook NR kinetics was performed on days 3, 7, and 14. Estabrook nitrate uptake kinetics was performed on day 1 and day 8. Sampling of dissolved nutrients, CN analysis, chlorophyll a extraction, and NR activity was performed on Lake Michigan samples on days 2,3,4,7,10, and 15. Lake Michigan NR kinetics was measured on days 4,8, and 15. Lake Michigan nitrate uptake kinetics was taken on days 2 and 9.

5 Preliminary Biological Results and Conclusions

For Estabrook Pond cultures, Phytoplankton in the controls showed minimal growth. For the nitrate treatment both dissolved PO_4^{3-} and NO_3^- were exhausted by day 5, and growth plateaued around day 7 and then declined. In the ammonium treatment PO_4^{3-} was exhausted after day 8, NH_4^+ was never exhausted but plateaued after day 4, and growth plateaued around day 4.

For Lake Michigan cultures, the controls displayed slow but slight growth until day 7, and dissolved NO_3^- was exhausted by day 7. The nitrate treatment showed steady growth until day 7-10, and dissolved NO_3^- and PO_4^{3-} both declined but were not exhausted. The ammonium treatment also showed steady growth, NH_4^+ was taken up but not exhausted, PO_4^{3-} was exhausted by day 14.

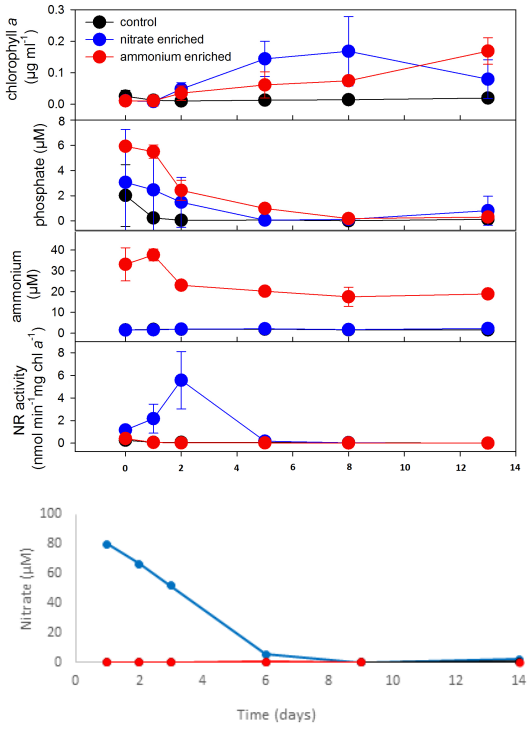

Figure 1: Estabrook Pond data

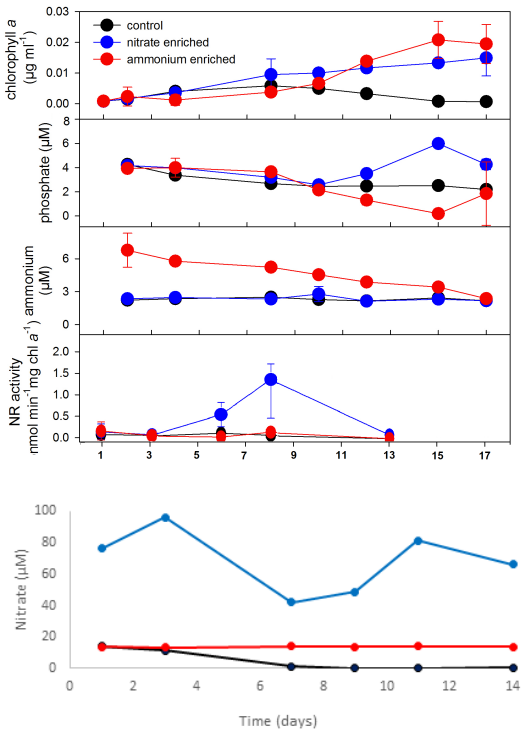

Figure 2: Lake Michigan data

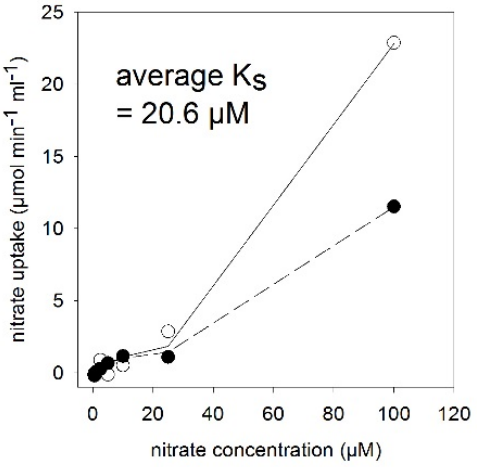

Figure 3: Estabrook NO_3^- uptake kinetics

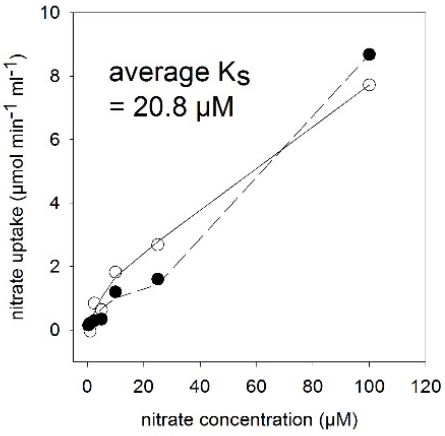

Figure 4: Michigan NO_3^- uptake kinetics

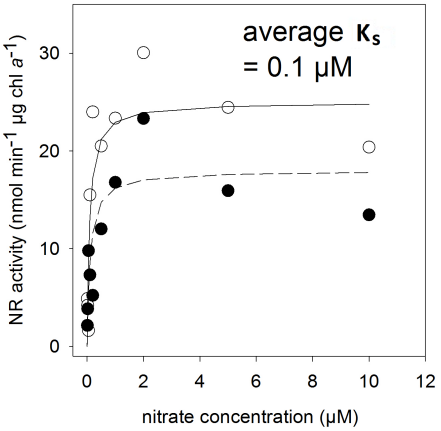

Figure 5: Estabrook NR activity kinetics

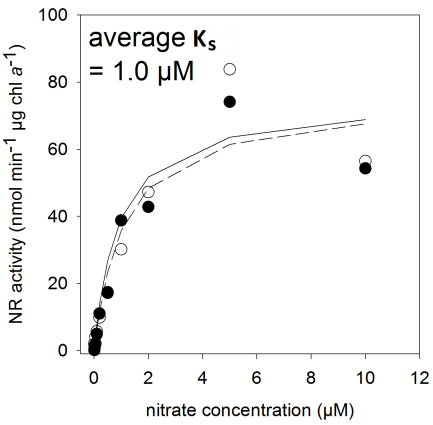

Figure 6: Michigan NR activity kinetics

In both pond and lake experiments NR activity was only observed in NO_3^- enriched treatments suggesting and confirming that NR is an inducible enzyme, meaning that it is only produced when nitrate is present and production is suppressed when no nitrate is present. NR activity in pond samples showed the highest activity on day 3, and in lake Michigan samples NR activity peaked at day 8 (Figure 2). NO_3^- uptake rates of nitrate enriched treatments demonstrated bi-phasic kinetics, with an initial Michaelis-Menten relationship, followed by linear uptake at high external NO_3^- concentration (Figures 3 and 4). Calculated K_s values for NO_3^- uptake in Estabrook Pond samples were 7.8 μM with a V_{max} of 2.7 μmol NO_3^- /min/μg chl a, and Lake Michigan was 10.6 μM, with a V_{max} of 3.0 μmol NO_3^- /min/μg chl a. NR activity kinetics in nitrate enriched treatments demonstrated a Michaelis-Menten relationship with external NO_3^- concentrations and much lower K_s values (Figures 5 and 6).

On day 8, pond and lake samples were inspected microscopically to identify communities. *Actinastrum*, *Synedra*, *Tabellaria*, and *Fragilaria* were viewed as well as rotifers and *Heliozoans*. Bi-phasic nitrate uptake kinetics were observed in Estabrook Pond and Lake Michigan. These properties have been demonstrated in plants and marine phytoplankton communities but have not been commonly established in freshwater phytoplankton [13][15].

6 Estimating Kinetic Parameters

Knowing the parameters V_{max}, K_s for equation (1) that exactly model the phytoplankton populations would be ideal but in biology these parameters must be inferred from data [10]. Data can be corrupted by various

errors making this uncertain [10]. Nonlinear least squares to solve an inverse problem is one of the best methods of fitting models to nutrient kinetic data [1]. Estimating the parameters requires solving a problem with data points d_j for $j \in \{1, 2, ..., n\}$. To do this, assume the data follow an underlying model, equation (1), with some particular set of parameters, $\{V^*_{max}, K^*_s\}$. To find the optimal solution begin by entering a parameter guess into the model to generate a kinetic curve, equation (2). Then calculate the error between the data points and the curve, equation (3). Iterate the parameter guess over the feasible parameter space, ω, searching for the error minimizing parameter set.

$$\text{for } \{V_{max_i}, K_{S_i}\} \in \omega \;\; f([S]) = \frac{V_{max_i}[S]}{K_{S_i} + [S]} \tag{2}$$

$$\text{Minimize } E(V_{max_i}, K_{S_i}) = \sum_{j=1}^{n} \left(f_{V_{max_i}, K_{S_i}}([S]_j) - d_j \right)^2 \tag{3}$$

This measures the parameter set's fit to the dataset consisting of elements $[S]_j$ for $j \in \{1, 2, ..., n\}$ corresponding to the concentrations of nutrient used in the lab to collect the kinetic data. Minimizing this error function over $\{V_{max}, K_S\} \in \omega$ can accurately estimate the true parameters. Solving this inverse problem will identify the underlying model parameters if the number of data points used is sufficiently large and the error committed in measuring those data points is low. However, for this kinetic data there are a few problems. Some of the data scaled to biomass doesn't make biological sense. There are negative values which should not occur. The datasets for nitrate uptake in Estabrook Pond and Lake Michigan are listed in the following tables: Table 1: Estabrook Pond nitrate uptake data

$[NO_3^-]$(μM)	Control 1 (μM/min/μg chl a)	Control 2 (μM/min/μg chl a)
0.5	-0.07416	-0.19541
1	-0.09059	0.04270
2.5	0.88883	0.27507
5	-0.11931	0.67701
10	0.51603	1.15694
25	2.86890	1.09695
100	22.87433	11.53074

Table 2: Lake Michigan nitrate uptake data

$[NO_3^-]$(μM)	Control 1 (μM/min/μg chl a)	Control 2 (μM/min/μg chl a)
0.5	0.12803	0.15217
1	-0.04190	0.21304
2.5	0.84149	0.29360
5	0.63315	0.33835
10	1.81566	1.19587
25	2.68159	1.59866
100	7.71887	8.67897

7 Using Agent Based Modeling

Agent based models, ABMs, and population level models, PLMs, represent two different strategies for mathematically representing a phytoplankton population [9]. ABMs seek to explicitly simulate individuals of a population and their environment [9]. These individuals have basic properties and characteristics and can interact with other individuals and their environment [9]. Helwegger et. al. [9] include a basic example:

> For the wolves and moose of an island, for example, a population-level model (PLM) would take differential equations for the density of wolves and moose and solve them for various times (Lotka-Volterra equations). An IBM would simulate individual wolves and moose, and their behavior (e.g. wolf eats moose), and the population densities would emerge from their cumulative behavior and interaction.

This strategy will be used to model interactions between phytoplankton agents and their nutrient rich environment within the Netlogo modeling environment [11].

8 The Model

The model starts by defining the agents and their playing field in Netlogo. Agents exist on a playing field of "patches" which form a grid across the total environment. Analysis requires knowing how much biomass an agent represents and how much water a patch represents. The method Helwegger et. al. [9] discuss is allowing agents to represent a fixed amount of biomass. The following model is based upon this premise.

The following model assumptions were made. Phytoplankton agents interact with a playing field of 36 patches. Each phytoplankton agent represents 10^5 phytoplankton cells. Each grid patch of the environment represents a fixed volume of water and hosts an initial concentration of nitrate. The model's initial nitrate concentration is equal to the total initial laboratory enrichment divided equally among the 36 patches. Four different phytoplankton species are included. Each species has nitrate uptake parameters drawn from literature. DNA analysis was never completed so it's unclear whether these four are representative of the actual samples. A phytoplankton agent possesses intracellular nitrate as well as incorporated nitrogen which it is assumed is proportional to the predicted lifespan of the organism. Intracellular nitrate is reduced to incorporated nitrogen following equation (1) with a constant V_{max} proportional to the amount of biomass the agent represents calculated from laboratory measurement and a specific K_s from laboratory measurement. [2] An agent uptakes nitrate by sensing the nitrate concentration of the patch that the agent exists upon, and then plugging that concentration into equation (1) with their agent-specific parameters. Nitrate uptake mass-balance is achieved by adding the amount of nitrate that the agent uptakes to its intracellular nitrate pool, then taking away the appropriate amount from the patch's nitrate concentration. Nitrate reduction mass-balance is achieved in a similar fashion with the intracellular nitrate pool and the incorporated nitrogen of the agent. Agents lose a maintenance cost of incorporated nitrogen at each time step. When their incorporated nitrogen reaches zero the agent dies. When it reaches a specific threshold the agent divides into two new agents [5]. To simplify the model a Droop kinetic growth rate like in [5] is not used. The entire environment is mixed at a fixed time interval. Each patch receives a new nitrate concentration equal to the sum of nitrate remaining in the system divided equally amongst the 36 patches. Each agent receives new, pseudo-random, coordinates on the 6 by 6 grid. This is representative of the shaking and mixing performed daily on the samples. Settling of the agents due to gravity is proportional to the square of intracellular nitrogen of the agent following a simplified Stokes' law. NR activity is measured as the sum total of intracellular nitrate reduced to incorporated nitrogen for that time step across all agents. The time step for the model is 1 hour and the rates of uptake etc. have been scaled to that.

These assumptions are based on the biological principles found in [6], and with ideas from [5] and [9]. Comparing these assumptions with [4] the model is adequately connected to the real world. The Netlogo code is available upon request at `kgschuch@ncsu.edu`.

9 Expectations

It is expected that no nitrate means no agent growth. Without any initial nitrate in the patches the agents are expected to reduce their intracellular nitrate pool, survive off of their remaining incorporated nitrogen and then die. Alternatively, with a supply of initial nitrate the agents are expected to uptake the nitrate, reduce the nitrate, and divide, until the nitrate is depleted. Once the supply of nitrate is depleted the agents are expected to stop dividing, survive until their incorporated nitrogen is depleted, then start dying off. Additionally, NR activity is expected to peak at a point in time after the nitrate is depleted but before the population starts dying off.

These expectations will serve as model validation. Anything more specific than these qualitative observations is stretching the limitations of the rigor of the model and the features of the Netlogo environment. The model will be qualitatively compared to the laboratory data to determine whether trends in the lab data are valid.

10 Validation

Two test cases are shown for validation with Figure 7 and Figure 8. No nitrate sends the uptake equations to zero for all phytoplankton based on equation (1). This makes sense: no nitrate means no nitrate uptake. The agents have an intracellular nitrate pool which they reduce to increase their incorporated nitrogen but since they are not adding any more nitrate to their internal pool the NR activity peaks at t=0, validating the NR

activity portion of the model. Since the agents reduce their internal nitrate and survive on their intracellular nitrogen but don't have enough intracellular nitrogen to meet their division quota the cell division threshold parameter is valid since there was no growth in lab cultures that were not enriched. It remains to show that no nitrate means no growth. Once the internal nitrate pools are depleted the NR activity hits zero, at T=28. This makes sense because if there is no intracellular nitrate to reduce there cannot be any reduction activity, again validating the NR activity part of the model. The agent population begins decreasing slightly before the NR activity hits zero, at T=24. This is likely due to the stochastic variation of uptake and reduction parameters as well as the four different agent breeds. One breed might be less robust than the rest and will start dying earlier. In general, after the NR activity hits zero each agent still has incorporated nitrogen that it can use to survive. Once each agent's incorporated nitrogen is depleted they die. Since each agent has heterogeneous uptake and reduction parameters they will die at slightly different times, leading to the stochastic variation in cell death.

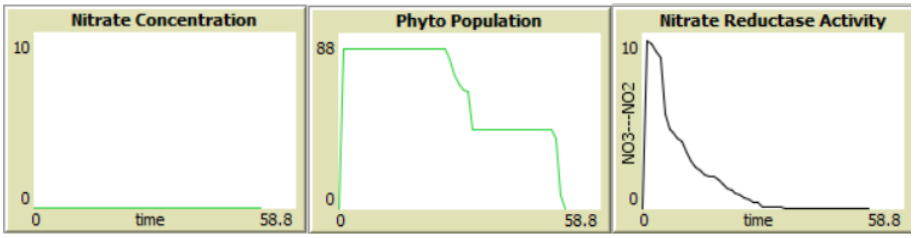

Figure 7: A basic test case where initial nitrate=0

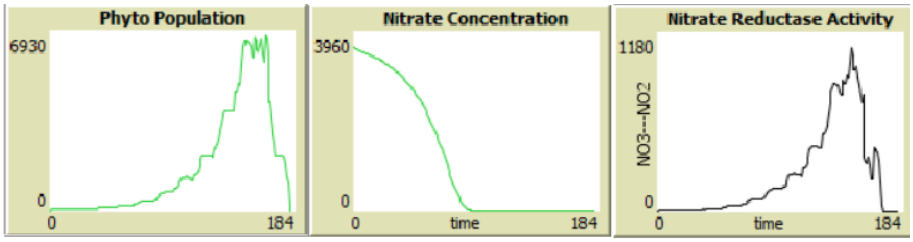

Figure 8: Netlogo simulation of test case phytoplankton population

A test case was run where the reduction V_{max} were scaled back from 100 to 1 in order to create intracellular pooling to ensure that there is always nitrate to be reduced. With the constant presence of nitrate the NR activity constantly increases until the nitrate is depleted and the intracellular pools are depleted. Once the nitrate hits zero the NR activity continues to grow and then declines as the agent population declines, satisfying that validation criteria. Additionally, it can be clearly observed that with the presence of nitrate the agent population increases. This completes validation.

11 Parameters and analysis

The model depends on several parameters which have been sourced from literature, calculated, or taken a thoughtful guess at. The initial biomass represented via the agents is the original biomass present in a fixed volume of aquaculture sampled from the pond and the lake. Chlorophyll a in the sample was measured to determine the concentration of biomass in the sample. From this an approximation for the number of cells in the sample is calculated to get initial conditions:

For Estabrook pond the average initial biomass concentration was 15μg chl a/L

$$\frac{15\mu\text{g chl a}}{\text{L}}\left|\frac{10^6\text{pg chl a}}{1\mu\text{g chl a}}\right|\frac{1\text{ cell}}{0.5\text{pg chl a}}\left|\frac{1\text{ Netlogo agent}}{10^5\text{ cells}}\right. = \frac{300\text{ agents}}{1\text{L}} \tag{4}$$

For Lake Michigan the initial biomass concentration was 0.82μg chl a/L

$$\frac{0.82\mu\text{g chl a}}{\text{L}}\left|\frac{10^6\text{pg chl a}}{1\mu\text{g chl a}}\right|\frac{1\text{ cell}}{0.5\text{pg chl a}}\left|\frac{1\text{ Netlogo agent}}{10^5\text{ cells}}\right. = \frac{17\text{ agents}}{1\text{L}} \tag{5}$$

Equations 4 and 5 were calculated using chlorophyll to cell ratio in data from [12]. Because computational complexity increases dramatically as the number of agents increases, combined with the limitations

of the Netlogo environment, it is desirable to keep the number of agents as low as possible so that the model does not freeze. At the same time, the volume of water being modeled for a given number of agents must be carefully kept track of. Also, the initial value of nitrate for the model depends on the initial concentration of nitrate that the samples were enriched to. The average initial measured nitrate concentration after enrichment was 80-110 μM. It is desirable that each patch has that as its initial concentration. Initial biomass conditions for Estabrook is 80 agents, representing 133mL of pond sample.
Table 3: Model parameters

Incorporated nitrogen cell division threshold	6.1 (validation in section 10).
Incorporated nitrogen maintenance cost	0.21 (validation in section 10)
Initial nitrate condition	2880 and 3600 (from the lab concentrations).
Initial internal nitrate	2.0 (validation in section 10)
Initial incorporated nitrogen	1.0 (validation in section 10)
Initial phytoplankton per breed (1, 2, 3, 4)	20
V_{max} uptake (1, 2, 3, 4)	2, 1, 10, 3.1 [7]
K_s uptake (1, 2, 3, 4)	25, 15, 20, 20.6 [7]
V_{max} reduction	100, (derived below)
K_s reduction (1, 2, 3, 4)	2.0, 0.1, 1.0, 0.1, (chosen from a range of lab data)

V_{max} for NR was calculated from laboratory data as follows. We assume that the amount of nitrate reduced by a sample of water depends both on the V_{max} of the phytoplankton in the sample, as well as the amount of NR enzyme present. The amount of NR is proportional to the biomass in the sample. The estimated best-fit-assay V_{max} =0.0976. To calculate that: 300mL of water was filtered to get 20mL of homogenate of which 400 μL was extracted and placed in a micro-vial, from a sample that had a biomass concentration of $\frac{10.37\mu\text{g chl a}}{\text{L water}}$. This results in $\frac{0.0622\mu\text{g chl a}}{\text{microvial}}$ and then Enzyme $V_{max} = \frac{0.02\mu\text{mol } NO_2^-}{\text{hour} \times \mu\text{g chl a}}$. Converting this back to our model results in $\frac{100\mu\text{mol } NO_2^-}{\text{hour} \times \text{agent}}$. Using this results in a maximum agent population of 504 and a peak biomass concentration of $\frac{0.198\mu\text{g chl a}}{\text{mL}}$ which matches well with the Estabrook biomass peak $\frac{0.2\mu\text{g chl a}}{\text{mL}}$.

Changing the parameters for the initial nitrate value to match Lake Michigan's 100 μM results in: 3600. 80 agents will represent 4.9L of Lake Michigan water with an initial biomass concentration of 0.82 μg chl a/L via equations 4 and 5. A maximum agent population of 612 yielded a biomass concentration of $\frac{0.006244\mu\text{g chl a}}{\text{mL}}$ which also matches somewhat well with the Lake Michigan biomass peak of $\frac{0.02\mu\text{g chl a}}{\text{mL}}$.

NR activity does not match the trends of laboratory data as seen by comparing Figure 10 to Figures 5 and 6. This is probably due to the fact that the agents are reducing their intracellular nitrate at a much faster rate than they are uptaking it in the model, combined with the gravitational settling that is built into the model which leads to patches that are nitrate starved and others that are nitrate rich until the next mixing interval. The model is not entirely representative of the laboratory experiment because DNA analysis for species identification was never completed and aeration stones used in lab cultures contributed to continuous mixing.

A nitrate uptake kinetic analysis was done for the model using 80 agents. The model was initiated with 0.5, 1, 2.5, 5, 10, 25, and 100μM nitrate and the total nitrate taken up at 60,120, and 180 minutes was recorded for each initial concentration. An uptake rate was calculated for each set of 3 time data points, corresponding to the uptake rate at that initial concentration, with a linear regression. Agent growth was artificially halted during this experiment by rewriting the cell growth and division part of the model code to ensure that the biomass remained fixed. This is the same general protocol performed in the lab, just now in silico.

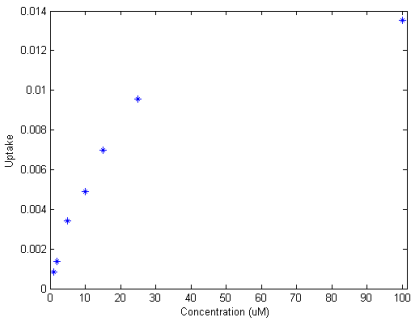

Figure 9: Model nitrate uptake kinetics

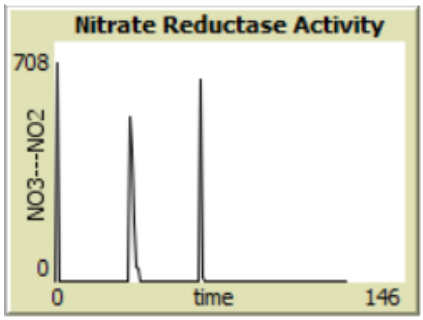

Figure 10: Model NR activity

Comparing Figure 9 with Figures 3 and 4 the model population does not demonstrate bi-phasic uptake kinetics.

12 Model Conclusion

An ABM was created to represent nitrate uptake and reduction in phytoplankton communities. Parameter identification was a challenge for this model. Parameters were chosen from literature and laboratory data when possible. The model with those parameters matched the general growth trends and biomass concentrations from the laboratory experiment, but the NR activity did not match except for a proof-of-concept in model validation testing. Further work should complete parameter identification, whether by laboratory experiment or deeper literature review.
In general, this ABM had several advantages over a population level model:
Table 4: A comparison of ABM and PLM properties

Agent Based Model	Population Level Model
heterogeneity within population	individual behavior is averaged
output is the sum of the individuals	output is the result of averages
environmental heterogeneity	heterogeneity requires PDEs

Some drawbacks to ABM are that the parameters for individuals within a population are difficult to find in literature or measure in a laboratory. Model complexity is limited due to the computational power of the environment. Analysis of ABM is less established than ODE and PDE models and is model specific. In the analysis section rigor was added in the form of calculating the ABM biomass concentration at peak value and compare it to the laboratory data in order to bring the model back to reality.

13 Future Work

A preliminary nitrate uptake kinetic experiment was completed using the agent based model population and did not demonstrate any bi-phasic kinetics. The model simulated a nitrate uptake kinetics experiment which worked very well. Parameters should be established for this data and compared to the laboratory derived parameters. The laboratory data should be recollected with more precision and more data points between the 25μM and 100μM concentrations to determine whether the bi-phasic kinetics were an error or whether it can conclusively be stated that is the behavior. Model parameters should continually be searched for in literature and laboratory values in order to further validate the model. The DNA samples that were collected should be analyzed to determine exactly what species were present in the samples and then include their parameters in the model to genuinely compare the model and the laboratory data. Additionally, inclusion of ammonium nutrient enrichment dynamics should be included in the model.

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