Hindawi Publishing Corporation
International Journal of Ecology
Volume 2011, Article ID 738035, 12 pages
doi:10.1155/2011/738035

Research Article

Mapping the Fundamental Niches of Two Freshwater Microalgae, *Chlorella vulgaris* (Trebouxiophyceae) and *Peridinium cinctum* (Dinophyceae), in 5-Dimensional Ion Space

Terence J. Evens and Randall P. Niedz

USDA Agricultural Research Service, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030, USA

Correspondence should be addressed to Terence J. Evens, terence.evens@ars.usda.gov

Received 26 November 2010; Revised 16 April 2011; Accepted 17 May 2011

Academic Editor: L. Smith

The fundamental niche defined by five ions, NO_3^-, PO_4^{3-}, K^+, Na^+, and Cl^-, was mapped for *Chlorella vulgaris* (Trebouxiophyceae) and *Peridinium cinctum* (Dinophyceae) growth rates and maximum cell densities in batch cultures. A five dimensional ion-mixture experimental design was projected across a total ion concentration gradient of 1 to 30 mM to delineate the ion-based, "potential" niche space, defined as the entire *n*-dimensional hypervolume demarcated by the feasible ranges of the independent factors under consideration. The growth rate-based, fundamental niche volumes overlapped for ca. 94% of the ion mixtures, although the regions of maximal growth rates and cell densities were different for each alga. Both *C. vulgaris* and *P. cinctum* exhibited similar positive responses to cations and negative responses to anions. It was determined that total ion concentration for these five ions, from 1 to 30 mM, did not directly affect either growth rate or maximal cell density for either alga, although it did play an interactive role with several ions. This study is the first that we are aware of to attempt the mapping of a multivariate, ion-based, fundamental niche volume. The implications of the experimental design utilized and the potential utility of this type of approach are discussed.

1. Introduction

The niche concept is elemental to ecological theory and can be simply considered as the science of determining why organisms live where they do and why they do not live where they do not. The origin and evolution of the niche concept have been reviewed at intervals over the past decades (cf. [1]) and will be treated only briefly. Grinnell [2] is generally credited as being the first to define the boundaries of an organism's niche in terms of "environmental conditions." Elton [3] provided the second major advance in the niche concept by focusing on the niche of a species as its functional role within the food chain. In homage to these two pioneers of ecological thought, niches parameterized by "bionomic" variables, that is, something that can be consumed and competed for, are referred to as "Eltonian", while those parameterized by "scenopoetic" variables, defined as environmental conditions, for example, temperature and salinity, for which competition is not generally relevant, are referred to as "Grinnellian" [4, 5]. Hutchinson [6, 7] codified the niche in mathematical terms with his idea of an "*n*-dimensional hypervolume", which he described in the following manner: "consider two independent environmental variables x_1 and x_2 which can be measured along ordinary rectangular coordinates... An area is, thus, defined, each point of which corresponds to a possible environmental state permitting the species to exist indefinitely." Hutchinsonian niches are defined in relation to competition. The niche volume occupied by an organism in the absence of competition is a "fundamental niche", while that occupied in the presence of competition is a "realized niche" [7].

While the niche has been an important conceptual tool in ecology, it has proven very difficult to define and measure [2, 8–11]. These difficulties are primarily due to our inability

to experimentally manipulate organisms, which has forced a reliance on observational data that may or may not be correlated with a given suite of environmental variables [12]. Microalgae have perhaps been given the most attention with regard to experimental niche characterization, because they are relatively easy to grow, they usually exist in an aqueous medium that is amenable to manipulation, and because they play a prominent role as primary producers in many important habitats. However, even the seminal studies with algae that have formed the basis of much ecological theory (e.g., [13, 14]) have been restricted to simplistic manipulations of one or two factors. These types of studies may be useful for illustrating certain aspects of ecological theory but probably do not capture much of "real world" complexities.

For aquatic microalgae, there are many questions surrounding the effects of ions/nutrients in community dynamics. Any given body of water will have ca. 13–25 ions at measurable concentrations. Quantifying the main effects and interactions of these ions, or at the very least, several of the primary "drivers", on algal physiology/ecology has proven to be extremely difficult. However, recent advances in our ability to derive ion-specific media formulations [15] coupled with modern multivariate, experimental designs [16, 17] now facilitate the direct quantification of ion-specific effects and interactions and facilitate the "mapping" of complex, ion-based, fundamental niche spaces. As a first foray into the quantification of ion-specific effects within a multivariate experimental framework, we endeavored to identify and map the ion-based fundamental niche of two common microalgae, *Chlorella vulgaris* (Trebouxiophyceae) and *Peridinium cinctum* (Dinophyceae) in the niche volume delineated by five ions, NO_3^-, PO_4^{3-}, K^+, Na^+, and Cl^-. We name the volume defined by our experimental factors as the "potential niche", that is, the fundamental niche is a subhypervolume of the potential niche.

Within this potential niche space, we endeavored to map the species-specific zonotopes delineated by the points where *C. vulgaris* and *P. cinctum* exhibit positive growth rates. We also mapped the maximum cell densities attained at each of these points. For this effort, we were particularly interested in answering physicochemical, as opposed to nutritional questions in relation to the effects of total ion concentrations and types for these five ions. Specifically, our primary questions were (1) How do the fundamental niche volumes for *C. vulgaris* and *P. cinctum* compare? And (2) What are the general, physico-chemical relationships between these cations (Na^+ and K^+) and anions (NO_3^-, PO_4^{3-} and Cl^-) in relation to the delineation of fundamental niche spaces? In addition, we also wished to address two secondary questions: (1) What role does pH play in defining the fundamental niche? (2) What role does total $[NO_3^-]$ and/or $[PO_4^{3-}]$ concentration play in defining niche space?

2. Materials and Methods

2.1. Experimental Design. The basic experimental design strategy was to (1) define a 5-dimensional experimental design hypervolume parameterized by mixtures of the target ions over a range of total ion concentrations (i.e., a "mixture-amount" design; [17]); (2) grow *C. vulgaris* and *P. cinctum* at several mixture-amount coordinates that representatively sample the potential niche hypervolume in a statistically valid manner; (3) generate prediction equations (i.e., response surface models) that describe growth rates and maximal biomass responses throughout the design hypervolume; (4) validate these models by comparing measured versus predicted *C. vulgaris* and *P. cinctum* responses at design coordinates not included in the model generation.

D-optimality criteria were used to minimize the number of factor/component combinations necessary to provide accurate estimates of the model coefficients of a crossed, that is, "cubic × cubic," polynomial experimental design volume [17, 18]. Specifically, sufficient treatments or "design points" were selected with Design Expert software (v7.0.3, Stat-Ease, Inc. Minneapolis, MN) such that the determinant of the $(X'X)^{-1}$ matrix was minimized, which has the net result of minimizing the volume of the confidence ellipsoid for the coefficients of the selected model [19]. Several design points were added to estimate the lack of fit (LOF) between the response surface models and design points not used to generate the model fits [20]. A number of treatments were duplicated in order to (1) attain sufficient degrees of freedom (df) to estimate pure error across the design space; (2) provide estimates of block effects; (3) to reduce the potential effect(s) of high leverage points. In all, there were 213 design points distributed among three blocks. A 2-dimensional, nonmetric multidimensional scaling plot depicts the design structure and is presented in Figure 1.

2.2. Culture Conditions. *Chlorella vulgaris* (UTEX 1809) and *Peridinium cinctum* (CCAP 1140/1) were obtained from the Culture Collection of Algae at the University of Texas at Austin, USA and the Culture Collection of Algae and Protozoa at Dunstaffnage Marine Laboratory, Argyll, Scotland. These cultures are nonaxenic and were grown in modified Bold's Basal Medium (mBBM) made from a combination of autoclaved and filter sterilized stock solutions. The mBBM constituents kept constant were 185 μM BO_3^{3-}, 170 μM Ca^{2+}, 1.7 μM Co^{2+}, 6.3 μM Cu^{2+}, 171 μM $EDTA^{4-}$, 17.9 μM Fe^{2+}, 304 μM Mg^{2+}, 7.3 μM Mn^{2+}, 4.9 μM Mo^{2+}, 344 μM SO_4^{2+}, and 30.7 μM Zn^{2+}. All ions are referred to in their ultimate valence, that is, PO_4^{3-} versus $H_2PO_4^-$, for convenience only; all specified values are total ionic concentrations within the bulk medium and do not reflect any speciation that may occur in solution. Total ion concentrations of mixtures of NO_3^-, PO_4^{3+}, K^+, Na^+ and Cl^- were varied from 1–30 mM. NO_3^-, and PO_4^{3-} proportions were varied from 5–95% and K^+, Na^+, and Cl^- were varied from 0–90% of the mixtures for these five ions. This assured that there would always be some NO_3^- and PO_4^{3-} in every treatment to serve a nutritive function while we focused primarily on the physico-chemical effects of these ions. All media recipes were derived using the linear programming approach described by Niedz and Evens [15]. Calculations were performed with ARS-Media (Ver. 1.0.1) ion solution calculation software (available as a free download at: http://www.ars.usda.gov/services/software/download.htm?softwareid=148). Although we speak of a "5-ion, fundamental niche", the mBBM used

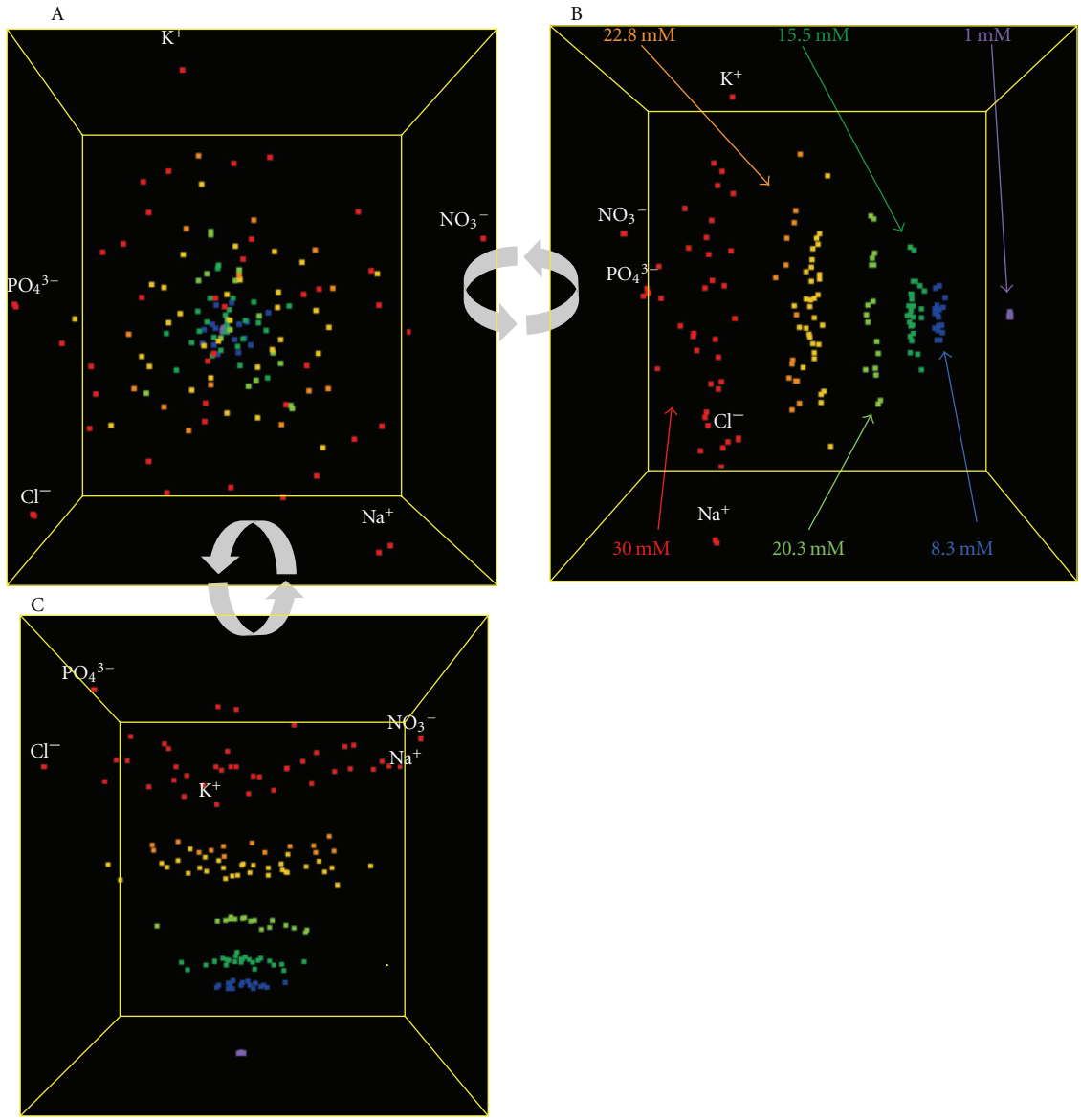

Figure 1: A 3-dimensional (3-D) visualization of the 5-dimensional experimental design used in this study. The concentrations of the individual ions and total ion concentration for each medium were compared via a Euclidean distance-based similiarity index and plotted via multidimensional scaling (MDS; Primer V6.0). The three views, A, B, and C are 90° rotations in the direction indicated by the arrows. The 30 mM vertices for each of the five ions are labeled with NO_3^-, PO_4^{3-}, K^+, Na^+, or Cl^-. The vertices for these ions at each of the other total ion concentrations can be found in the same orientation. While it is impossible to accurately portray a higher-dimensional figure in lower dimensions, that is, some distortions are inevitable, these projections give an indication of the structure and symmetry of the 5-dimensional experimental design. The "clustering" of points with similar total ion concentrations are indicated by the colored arrows.

for this study has 11 other ions at fixed concentrations and, because our flasks are exposed to the atmosphere, there is at least one other ion, CO_3^{2-}, whose concentration is dependent upon the total ionic complement, temperature, the CO_2 partial pressure, and the rate of CO_2/CO_3^{2-} uptake and release by the algae and bacteria in culture. Thus, our 5-ion potential niche space is actually a subzonotope in (at least) 17-ion hyperspace. All subsequent discussions concerning this study should be done with this caveat in mind.

All flasks were initially inoculated to ca. 10^4 cells mL^{-1} from a maintenance culture in standard BBM. Batch cultures were grown in 75 mL of medium in 150 mL Erlenmeyer flasks in a temperature-controlled incubator at 25°C under an 18 : 6 light : dark irradiance regime. Incubator temperature was monitored by an internal digital temperature probe and by a thermometer placed in a 150 mL flask filled with 75 mL of water. Illumination was provided by cool-white fluorescent lights at an irradiance level of 100 μmol photons m^{-2} s^{-1}.

Scalar PAR irradiance (E_o) levels were determined with a 4π spherical micro-quantum sensor (Heinz-Walz US-SQS/B) placed within a stoppered 150 mL flask and immersed in 50 mL of H_2O. Flasks were swirled/shaken by hand at least twice daily, and their positions on the incubator shelves were rearranged randomly at least once each day to mitigate any discontinuities in irradiance or temperature within the incubator. Cultures were acclimated to experimental conditions for a minimum of 5-6 generations before dilution with fresh media and the commencement of the experiments.

Cell counts were carried out microscopically using an improved neubauer hemacytometer. The correlation between cell numbers and optical density at 750 nm (OD_{750}) was determined for *C. vulgaris* and *P. cinctum* (data not shown). OD_{750} was determined daily for each culture and used to estimate cell numbers; these estimates were occasionally checked with hemacytometer counts and were found to deviate no more than ±5% from the predicted values. High-density cultures were always counted with a hemacytometer.

Experimental media formulations that did not exhibit growth were periodically reinoculated with cells from their nearest neighbor(s) in Euclidean space that was exhibiting positive growth. This resulted in the methodical acclimation of the cultures to increasingly extreme ionic conditions and allowed us to find the vertices of the hypervolume for each alga.

2.3. Statistical Analyses. A detailed description of the statistical methods used to analyze the data can be found in Evens et al. [21]. Briefly, all possible models from the mean to cubic polynomial were calculated with Design Expert. Initial model selection was based on a battery of adequacy tests as described by Anderson and Whitcomb [22]. Normality and constant variance were determined graphically; a Box-Cox plot was used to choose the correct transformations [23]. Overly influential data points were identified with DFFITS and DFBETAS plots (cf. [24] for a definition of DFFITS and DFBETAS). Adequate precision of the model was determined by comparing the range of the predicted values at the design points ($\hat{y}$) to the average variance (V-bar) of the prediction [22]. Potential outlier points were checked with externally studentized "outlier-t" [20, 25] and Cook's Distance [26] graphical plots. R^2, adjusted-R^2 (R^2_{adj}), and predicted-R^2 (R^2_{pred}), were estimated for each selected model (cf. [16]).

2.4. Model Validation. A multivariate optimization technique [27, 28] was used to identify the region of high growth rates and high cell densities for *C. vulgaris* and *P. cinctum* by simultaneously maximizing μ and maximum cell density; three validation treatments were grown in and near this region. Cultures were grown at all of these points, and the measured responses were compared to model predictions (data not shown) in order to empirically assess the usefulness of the predictive capabilities of the proposed RSM models. All responses falling within the 95% prediction interval (PI; [29]) were considered as validating the predictions.

2.5. PH Calculations/Measurements. The pH of each medium was measured with an accumet AR25 pH meter fitted with Ag/AgCl gel-filled, temperature-corrected probe. pH values were also calculated with chemical equilibrium software, MINEQL+ (Ver. 4.5; [30]), which is capable of calculating aqueous speciation, solid phase saturation states, precipitation dissolution, and adsorption. Calculations were based on all ions in each medium (i.e., 14 to 17 in total), temperature corrected, and assumed to be open to the atmosphere with a $ppCO_2$ at sea level of 0.04%. All pH values reported and discussed are those of the bulk media before the addition of algal cultures.

3. Results

3.1. Model Fitting. No deviation from normality was detected for any of the measured responses. The variances appeared constant as all points fell within $\pm 3\sigma$ and the scatter of residuals did not reveal any obvious distortions. The predicted versus measured plots indicated very close correlations between modeled and measured data points. Outlier-t and Cook's distance plots did not reveal treatments considered as outliers and, therefore, suspect. DFFITS and DFBETAS plots did not indicate treatments with overly large influences on predictions or regression coefficients, respectively. A summary of ANOVA data, model diagnostics, and coded regression coefficients for *C. vulgaris* and *P. cinctum* is presented in Tables 1 and 2, respectively. Model reduction by backward elimination [31, 32] improved the model fits for *P. cinctum* but not *C. vulgaris*. Therefore, the *P. cinctum* models are referred to as "reduced." The models for all responses were highly significant ($P < 0.0001$), and, with the exception of lack of fit, all diagnostics indicated very close agreement between models and data.

3.2. Specific Growth Rates and Fundamental Niche Volumes. *Chlorella vulgaris* exhibited positive growth rates in 64 of the 174 (37%) unique treatments, while *P. cinctum* grew in 70 of these (40%). The ion-based fundamental niche volumes delineated by positive growth rates for *C. vulgaris* and *P. cinctum* had over 94% of points in common (Figure 2), but there were small regions unique to each alga. Positive specific growth rates ranged from 0.06 to 0.91 d^{-1} for *C. vulgaris* and from 0.03 to 0.59 d^{-1} for *P. cinctum*. Overall, *C. vulgaris* had a greater average growth rate than *P. cinctum* (0.27 versus 0.21 d^{-1}, resp.) for the treatment points where each alga exhibited positive growth. Regression coefficients for individual ions are indicative of the effect of each ion at its vertex in the potential niche hypervolume, which is a geometric term defined as the medium formulation with the maximum proportion of that ion. There was virtually no difference in regression coefficient values between the three anions or between the two cations for *C. vulgaris* and only slight differences for *P. cinctum*. *Peridinium cinctum* exhibited a slightly more positive response to K^+ than Na^+ for growth rate, but this effect was reversed for maximum cell densities (see below). One or both cations were in every significant model term for both *C. vulgaris* and *P. cinctum*, and the concentration factor was common to all of the most

Table 1: ANOVA *P*-values, regression coefficients and model fit diagnostics for *Chlorella vulgaris* specific growth rate, μ (d^{-1}), and relative cell density (percent of maximum) for combined, cubic x quadratic Scheffé polynomial models. Only third order or lower terms significant at $P < 0.05$ for at least one response are presented. Note that the single component *P*-values (i.e., NO_3, PO_4, etc.) are covered under "Linear Mixture". The data were transformed with an inverse square root function before analysis; thus, regression coefficients with lesser values indicate greater effects. C.V.: coefficient of variance, n/s: $P > 0.05$.

	Specific growth rate		Relative cell density	
Source	Regression Coefficients	*P*-values	Regression Coefficients	*P*-values
Model		<0.0001		<0.0001
Linear mixture		<0.0001		<0.0001
NO_3	10.3		9.9	
PO_4	10.4		10.0	
K	1.9		2.0	
Na	2.0		2.4	
Cl	10.3		9.8	
K ∗ Cl	7.2	0.0001	6.7	0.0002
K ∗ [Conc]		n/s	−1.7	0.0005
Na ∗ [Conc]		n/s	−1.4	0.0052
NO_3∗ PO_4∗ K	74.0	<0.0001	73.9	<0.0001
NO_3∗ PO_4∗ Na	84.6	<0.0001	83.9	<0.0001
NO_3∗ K ∗ Na	−74.0	<0.0001	−87.6	<0.0001
NO_3∗ K ∗ Cl	53.9	<0.0001	55.0	<0.0001
NO_3∗ Na ∗ Cl	79.6	<0.0001	74.8	<0.0001
PO_4∗ K ∗ Na	−59.5	<0.0001	−68.4	<0.0001
PO_4∗ K ∗ Cl	60.3	<0.0001	59.1	<0.0001
PO_4∗ K ∗ [Conc]	6.0	0.0086	6.9	0.0015
PO_4∗ Na ∗ Cl	85.8	<0.0001	79.4	<0.0001
K ∗ Na ∗ Cl	−79.6	<0.0001	−81.5	<0.0001
Na ∗ Cl ∗ [Conc]	8.4	0.0006	8.5	0.0002
NO_3∗ K ∗ (NO_3-K)	38.4	<0.0001	39.1	<0.0001
NO_3∗ Na ∗ (NO_3-Na)	39.5	<0.0001	39.2	<0.0001
PO_4∗ K ∗ (PO_4-K)	36.4	<0.0001	37.8	<0.0001
PO_4∗ Na ∗ (PO_4-Na)	37.9	<0.0001	38.5	<0.0001
K ∗ Cl ∗ (K-Cl)	−44.4	<0.0001	−40.2	<0.0001
Na ∗ Cl ∗ (Na-Cl)	−28.5	<0.0001	−27.1	<0.0001
Lack of Fit		<0.0001		<0.0001
Std. Dev.:		1.06		1.00
Mean:		7.54		7.22
C.V.%:		14.12		13.89
R^2:		0.95		0.95
R^2-adj:		0.93		0.93
R^2-pred:		0.88		0.89

influential third order terms. In general, there was very little difference between *C. vulgaris* and *P. cinctum* in negative responses to the anions NO_3^-, Cl^-, and PO_4^{3-}, or in positive responses to the cations Na^+ and K^+ (Figure 3).

3.3. Relative Cell Densities. There was very little correlation between specific growth rates and relative cell densities for either alga (data not shown—see Figures 2 and 3 for a visual depiction). *Chlorella vulgaris* achieved relatively greater cell densities over a much larger portion of its 5-ion fundamental niche than *P. cinctum*. Maximum cell numbers for the densest cultures of each alga were ca. 5.8×10^9 cells L^{-1} for *C. vulgaris* and ca. 3.2×10^8 cells L^{-1} for *P. cinctum*. For discussion purposes, cell densities achieved in each treatment were expressed as a percentage of the treatment with the greatest cell density and, hereafter, referred to as "relative cell densities." The patterns of ion-specific effects on relative cell densities were very similar to the patterns of μ for both

Table 2: ANOVA *P*-values, regression coefficients and model fit diagnostics for *Peridinium cinctum* specific growth rate, μ (d^{-1}) and relative cell density (percent of the maximum) for combined, reduced cubic x quadratic Scheffé polynomial models. Only third order or lower terms significant at $P < 0.05$ for at least one response are presented. Note that the single component p-values (i.e., NO_3, PO_4, etc.) are covered under "Linear Mixture". The data were transformed with an inverse square root function before analysis; thus, regression coefficients with lesser values indicate greater effects. C.V. = coefficient of variance, n/s = $P > 0.05$.

	Specific Growth Rate		Relative Cell Density	
Source	Regression Coefficients	*P*-values	Regression Coefficients	*P*-values
Model		<0.0001		<0.0001
Linear mixture		<0.0001		<0.0001
NO_3	13.0		9.8	
PO_4	13.5		10.3	
K	1.8		2.4	
Na	2.6		2.2	
Cl	12.7		10.1	
K ∗ Na	7.1	0.0257	6.5	0.004
K ∗ Cl	9.3	0.0307		n/s
NO_3∗ PO_4∗ K	99	<0.0001	81.6	<0.0001
NO_3∗ PO_4∗ Na	137.6	0.0002	109.2	<0.0002
NO_3∗ K ∗ Na	−103.3	<0.0001	−89.4	<0.0003
NO_3∗ K ∗ Cl	91.1	0.0047	82.6	0.0003
NO_3∗ K ∗ [Conc]	13.7	0.0013	10.1	0.0008
NO_3∗ Na ∗ Cl	137.1	<0.0001	99.8	<0.0001
NO_3∗ Na ∗ [Conc]	16.3	0.0002	11.1	0.0003
PO_4∗ K ∗ Na	−94.2	<0.0001	−85.4	<0.0001
PO_4∗ K ∗ Cl	134.7	0.0003	92.7	0.0004
PO_4∗ K ∗ [Conc]	10.9	0.0018	6.6	0.0064
PO_4∗ Na ∗ Cl	124.8	<0.0001	77.6	<0.0001
PO_4∗ Na ∗ [Conc]	12.6	0.0017	5.9	0.0339
K ∗ Na ∗ Cl	−129.2	<0.0001	−103.5	<0.0001
Na ∗ Cl ∗ [Conc]	13.5	0.0003	5.2	0.0419
NO_3∗ K ∗ (NO_3-K)	45	0.001	43.9	<0.0001
NO_3∗ Na ∗ (NO_3-Na)	53.5	0.0003	44.8	<0.0001
PO_4∗ K ∗ (PO_4-K)	47.9	<0.0001	38.6	<0.0001
PO_4∗ Na ∗ (PO_4-Na)	54.5	0.0002	42.7	<0.0001
K ∗ Cl ∗ (K-Cl)	−49.1	<0.0001	−37.3	<0.0001
Na ∗ Cl ∗ (Na-Cl)	−57.5	<0.0001	−29.3	<0.0001
Lack of Fit		<0.0001		<0.0001
Std. Dev.		1.63		1.15
Mean		8.77		6.95
C.V.%		18.61		16.57
R^2		0.94		0.94
R^2adj		0.90		0.91
R^2pred		0.80		0.81

Table 3: Media formulations in which only one of the two algae examined in this study exhibited positive growth rates.

			−*C. vulgaris*, +*P. cinctum*			
Ion proportion:					[5-Ion Total]	
NO_3	PO_4	K	Na	Cl	(mM)	Initial pH
0.05	0.65	0.00	0.30	0.00	1.0	3.4
0.65	0.05	0.00	0.30	0.00	1.0	3.4
0.65	0.05	0.30	0.00	0.00	1.0	3.4
0.05	0.05	0.00	0.45	0.45	20.3	2.8
0.05	0.65	0.00	0.00	0.30	10.7	2.2
0.35	0.35	0.00	0.30	0.00	1.0	3.4
0.35	0.05	0.30	0.00	0.30	1.0	3.4
0.05	0.35	0.30	0.00	0.30	1.0	3.4
0.35	0.05	0.30	0.00	0.30	1.0	3.4
0.35	0.05	0.00	0.30	0.30	1.0	3.4
0.05	0.35	0.30	0.00	0.30	30.0	2.2
+*C. vulgaris*, −*P. cinctum*						
0.05	0.05	0.00	0.90	0.00	10.67	9.1
0.05	0.05	0.45	0.45	0.00	20.33	9.3

C. vulgaris and *P. cinctum* (Tables 1 and 2). It is interesting to note that the media formulations conducive to the fastest growth rates for both algae supported relatively low cell densities.

4. Discussion

The effects of specific ions on algal growth have been studied for many decades (cf. [33–37], etc.). Nutrient ratios, osmotic potentials, salinity effects, pH, salt-specific effects, total ion concentrations, and so forth, are all manifestations of the study of ion-specific effects. Algae display variable sensitivities to ions, both in type-effects such as chloride sensitivity and concentration-effects such as salinity tolerance. These sensitivities affect nutrient acquisition, primary productivity, cellular architecture, water relations, and so forth and ultimately define a given alga's ion-based, fundamental niche.

Translating the concept of an ion-based, fundamental niche into a rational experimental design presents several chemical, statistical, and mathematical hurdles. From a chemical perspective, we must overcome both conceptual and practical issues. The two most important of these centers on the appropriate methods for the manipulation of ions in solution and the role of pH in experimental designs. Until recently, the only experimental methodology employed to examine these effects was through the use of salts to make "salt solutions" of varying total ionic strength and ionic complement. However, when salts are used to examine ion-specific effects, the two (or more) ions introduced into solution are covariates and are potentially confounding to any ion-specific quantification [15]. This limitation is somewhat mitigated if the covariate ion is added at small concentrations, for example, μM amounts, to a medium with a large concentration, for example, hundreds of mM or greater, of that ion, such as Na^+ or Cl^- in seawater. A salt-based experimental approach dictates that the only effects that can be quantified in aqueous solutions where added ions have a significant impact on the nutritional and/or physic chemical nature of the solution are the mean effects of all ions specific to the salt in question, that is, ion-specific effects cannot be quantified with salt-based experimentation (cf. [38]).

The primary impediment to manipulating ion concentrations independent of salts has been our inability to calculate feasible ion mixtures using fixed proportionalities of salts. The required calculations are not trivial and require a mathematical technique known as linear programming. The methodology for these calculations has recently been established [15], and a public domain software package that presents the calculations in a graphical user interface has been made available at: http://www.ars.usda.gov/services/software/download.htm?softwareid=148).

From a statistical standpoint, there are considerable difficulties associated with properly designing experiments to quantify the main effects and interactions of ions in complex mixtures (cf. [38] for a detailed discussion). These types of experiments are fundamentally different from standard factorial and regression, or response surface, experiment designs. The calculations required to assure that the n-dimensional hypervolume delineated by n independent factors is adequately sampled to pass a battery of rigorous statistical tests and to support the proposed data analyses are not trivial. Recent advances in computer hardware and software now make it possible to conceive and execute highly efficient experimental designs that systematically sample a given n-dimensional space, identify key response drivers, and generate mathematical equations that describe multiple response variables. The research presented here is the first that we are aware of that has attempted to map the ion-based,

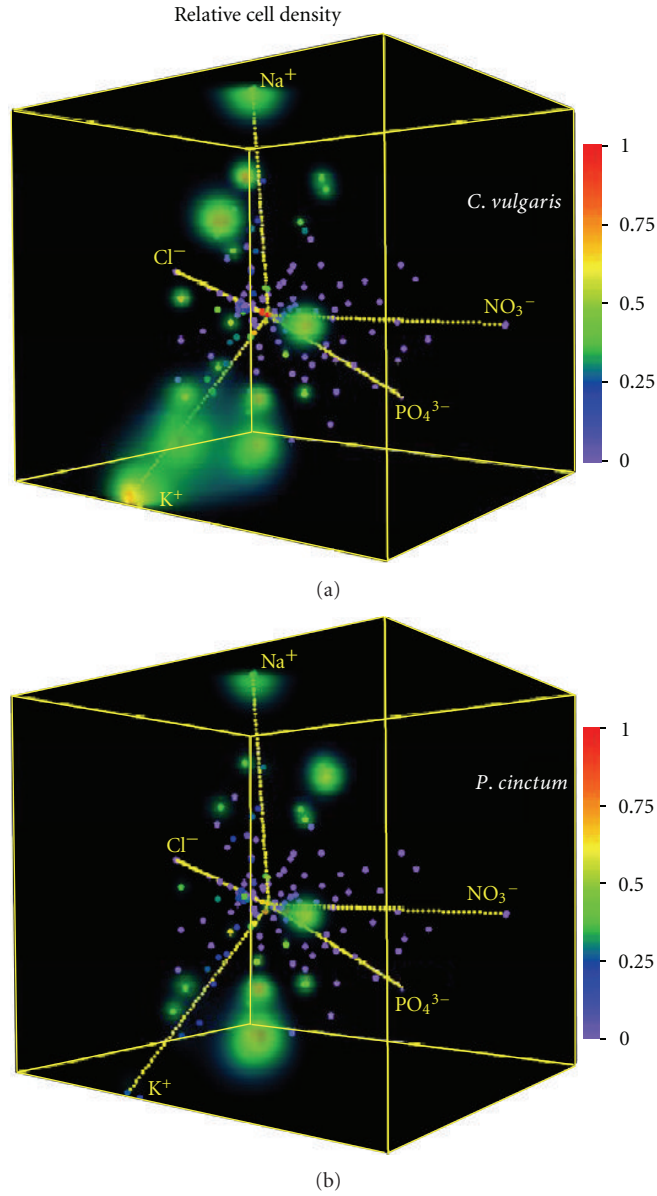

Figure 2: A 3-D visualization of specific growth rates, μ (d^{-1}), for *C. vulgaris* and *P. cinctum*. The individual treatment points are indicated by the colored dots and follow the scale indicated. The "cloudy" regions are volume renderings in which groups of higher-level points combine to increase opacity. Thus, these regions are indicative of multiple treatments, closely related in Euclidean space, where growth was positive.

fundamental niche space of any alga. As such, we are now able to address the set of primary and secondary questions proposed above.

(1) How do the fundamental niche volumes for C. vulgaris and P. cinctum compare? One reason we chose these two algae was because they are very different in evolutionary history, genetics, morphology, and so forth. However, their fundamental niche volumes within the five-ion space explored in this study overlapped by ca. 94% (Figure 2). The regions where *P. cinctum* could grow and *C. vulgaris* could not were characterized by relatively high proportions of anions, which were mostly at the lowest total ion concentrations (Table 3). Conversely, the very small region occupied by only *C. vulgaris* had high proportions of Na^+ and K^+ at relatively high total ion concentrations. Within the fundamental niche volume common to both algae *C. vulgaris* exhibited higher growth rates on average and achieved relatively higher cell densities over a wider range of the space than *P. cinctum* (Figure 3).

(2) What are the general, physico-chemical relationships between these cations (Na^+ and K^+) *and anions* (NO_3^-, PO_4^{3-}, *and* Cl^-) *in relation to the delineation of fundamental niche spaces?* It is clear that, in general, growth rates in relation to physico-chemical conditions were cation/anion specific, as opposed to ion specific, for the five ions explored in this study. This is based on the fact that the regression coefficients were similar among cations/anions. Both algae

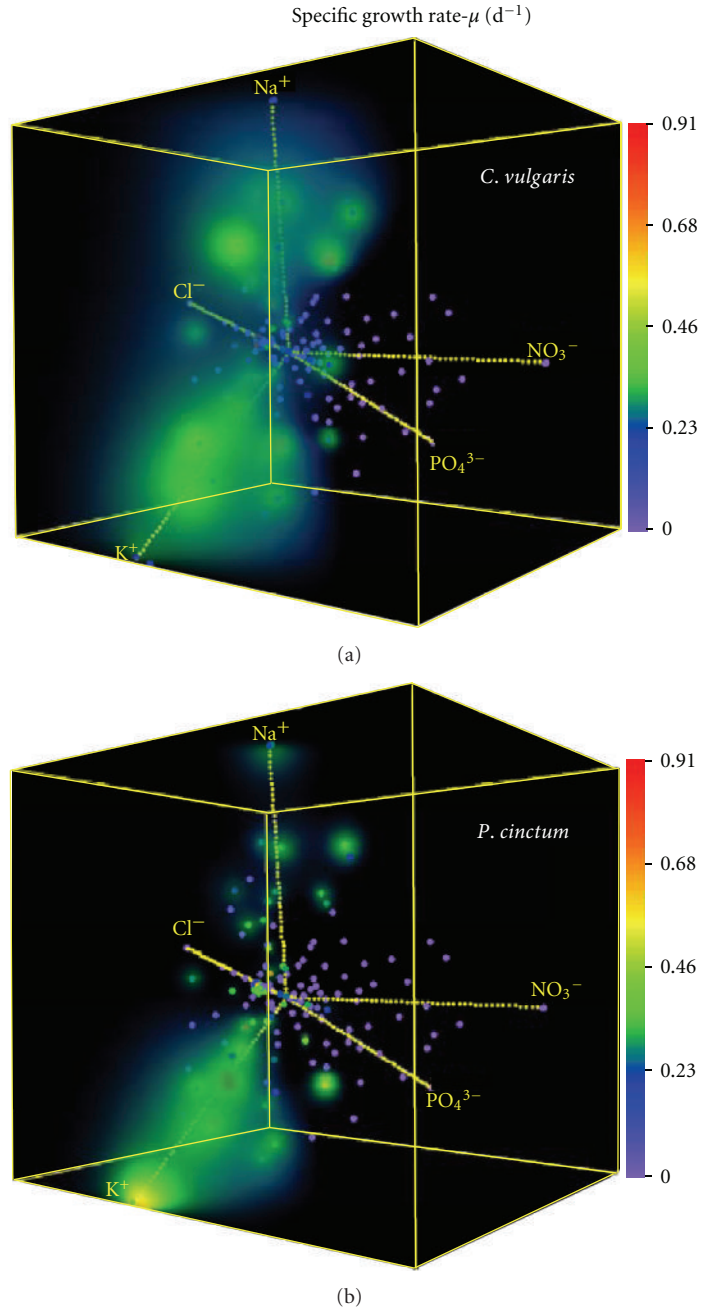

Figure 3: A 3-D visualization of relative, maximum cell densities, for *C. vulgaris* and *P. cinctum*. The individual treatment points are indicated by the colored dots and follow the scale indicated. The "cloudy" regions are volume renderings in which groups of higher-level points combine to increase opacity. Thus, these regions are indicative of multiple treatments, closely related in Euclidean space, where maximum cell densities were higher than the surrounding regions.

were sensitive to greater proportions of anions, *C. vulgaris* more so than *P. cinctum*. Generally, the regions with highest growth rates and cell densities had a greater proportion of Na^+ and/or K^+; both algae exhibited very similar negative responses to greater concentrations of all three anions (Figures 2 and 3). For *C. vulgaris*, it did not appear that these responses were concentration dependent over the range of 1–30 mM used in this study, that is, *C. vulgaris* did not grow well in any ion mixture with greater than 50% of any combination of anions regardless of total ion concentration. This was also true for *P. cinctum* except at 1 mM total ion concentrations. At these lowest concentrations, *P. cinctum* exhibited tolerance for high proportions of NO_3^- and PO_4^{3-} (not Cl^{-1}), but only if there was some Na^+ in the mixture, that is, the N-P tolerance did not occur in the presence of only K^+ (see the regression coefficients in Tables 1 and 2 and Evens and Niedz [39] for a more complete description of these relationships).

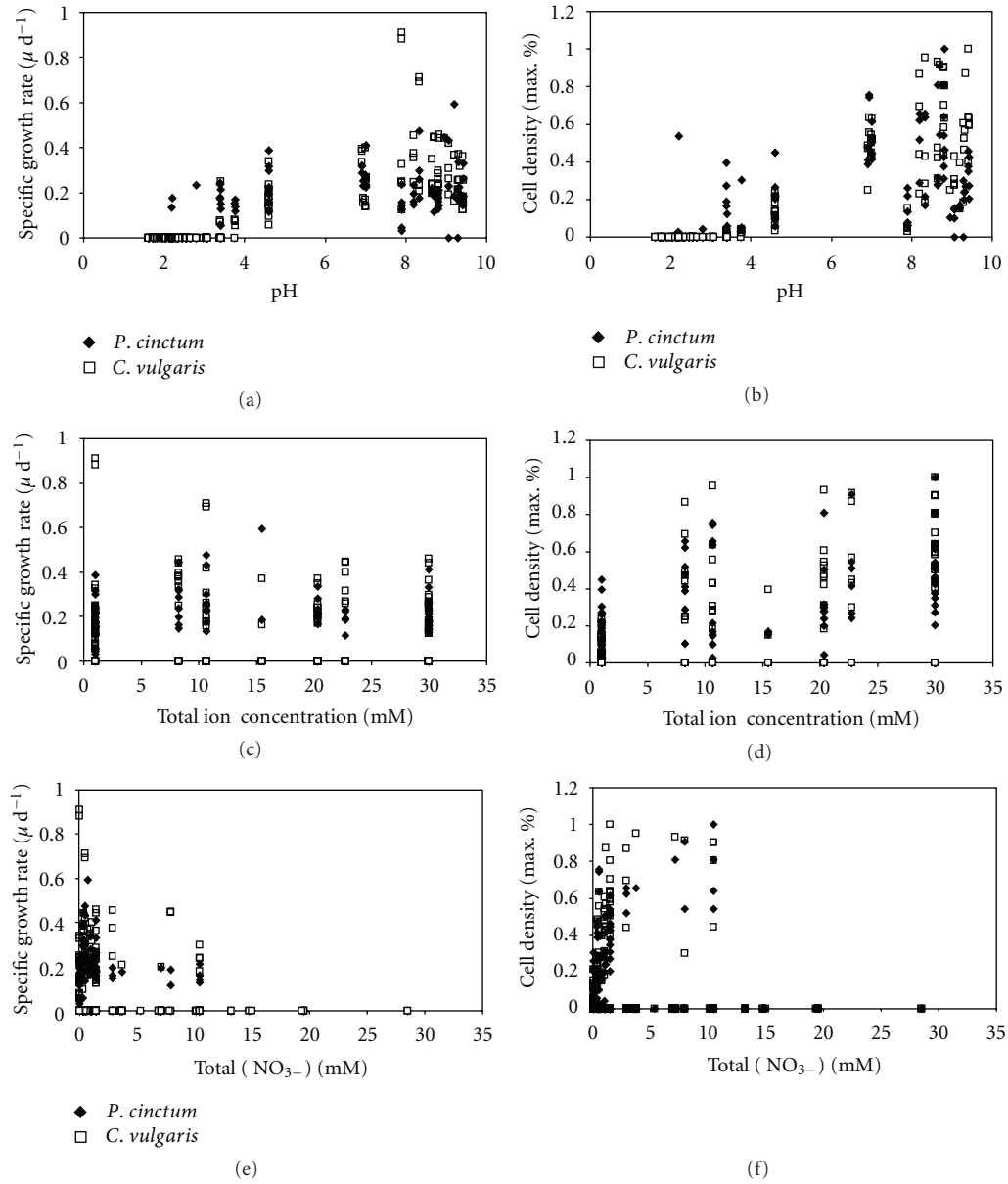

Figure 4: Specific growth rates, μ (d^{-1}) and relative cell densities versus pH (A-B), total ion concentration (C-D), and total NO_3^- concentration (E-F) for *C. vulgaris* (white squares) and *P. cinctum* (black diamonds).

Overall, the responses for both algae to the potential niche space defined by the five ions examined in this study are complex and unique to each alga. Within this context, we also considered two secondary questions.

(1) What role does pH play in defining the fundamental niche? A regression of the media pH against growth rates and cell densities indicated very little linear correlation between these responses (Figures 4(a)-4(b)). The greatest growth rate (μ) for *C. vulgaris*, 0.91 d^{-1}, occurred in a medium with an initial pH of 7.9, but other media combinations with different ion complements and the same pH produced growth rates (μ) ranging from 0.13 to 0.88 d^{-1}. This type of result is expected when pH is placed in proper perspective. As a primary function of specific ions and their concentrations in solution, pH is a *dependent* variable. This means that pH cannot be independently controlled in an experimental setting and, most importantly, cannot be identified as a causal factor. As a dependent variable, the only comparison that can be performed between pH and another response is a correlative analysis. pH-*dependency* can only be established through a preponderance of evidence, which stipulates that any given response must be invariant for any given set of ions that result in the same pH value (cf. [38, 39]). Obviously, this does not hold true for the ions examined in this study for *C. vulgaris* and *P. cinctum* growth rates, cell densities, and, by extension, the mapping of their fundamental niches.

(2) What role does total [NO_3^-] *and/or* [PO_4^{3-}] *concentration play in defining niche space?* One of the primary

difficulties with ion-based research in the context of niche theory lays in the fact that all mineral nutrients are ions, which introduces some level of inherent confounding to these studies and blurs the distinctions between scenopoetic and bionomic factors. At low concentrations, some ions will primarily serve a nutritive role. If the concentration of that nutrient/ion is increased, it will begin to affect bulk solution properties, which in turn will potentially affect nutrient requirements, for example, uptake and utilization rates, for all nutrients in solution. Where this "switch" from a purely nutritional role to having an impact on bulk solution properties occurs is probably ion-specific, and dependent on the types, concentrations, and ratios of the other ions in solution, that is, it is highly complex and multivariate. For example, some important nutrients, for example, NO_3^-, NH_4^+, and PO_4^{3-}, are rarely encountered at concentrations that have a major impact on bulk solution properties and can be considered to play an almost exclusively nutritive role. However, other putative nutrients, for example, K^+, Na^+, and Cl^-, are often primary determinants of bulk solution, physico-chemical, environmental characteristics.

While we have attempted to focus on the physico-chemical aspects of NO_3^- and PO_4^{3-}, there are undoubtedly several important issues associated with the nutritive, or resource, aspect of these ions that are potentially interesting. We are still able to intuit several interesting trends from the data that provide insight into the effects of these ions. The fastest growth rates clustered around the lesser NO_3^- and PO_4^{3-} concentrations, but many other media formulations with the same NO_3-PO_4^{3-} proportions and concentrations performed poorly (Figure 4(e)). The greatest cell densities of *P. cinctum* occurred in media with a $[NO_3^-]$ of 10 mM. However, there were several media blends with $[NO_3^-]$ of ca. 0.5 mM (i.e., 20-fold less) that produced ca. 75% as many cells as the 10 mM NO_3^- medium. Cultures of *C. vulgaris* of greatest density occurred in media with $[NO_3^-]$ of 1.5 mM, but many media formulations with different $[NO_3^-]$ produced almost as many cells. *Peridinium cinctum* produced more dense cultures at lesser NO_3-levels when compared with *C. vulgaris* (Figure 4(f)). We have no way of knowing if or when these cultures became light and/or carbon limited and cannot factor this aspect into our considerations. However, it is very clear that nutritional requirements for the greatest biomass levels were generally met at $[NO_3^-]$ levels below 2 mM for both of these algae.

Ultimately, all of the discussions about niches have the same objective to provide a predictive framework for community ecology. In a time of global climate change, habitat shifts, and threats to endangered and conserved species, an increased understanding of the factors that drive population dynamics is required. This knowledge will aid in the prediction and understanding of natural ecosystems and help to identify the causes of population declines. The research presented here is the first step in quantifying, complex, ion-specific, fundamental niche spaces. The geometries of these niche spaces provide valuable insight into ion-specific effects on algal physiology and facilitate the exploration of primary questions associated with mineral nutrition, salinity effects, competitive fitness, and issues of "bottom-up" control of microalgal community ecology (cf. [40]). Future work will focus on more ions, for example, SO_4^{2-} and NH_4^+, and on using the fundamental niches to predict the outcome of competition in mixed algal assemblages.

Acknowledgment

The authors would like to thank Mr. John Prokop for technical assistance with the culturing aspects of this effort.

Disclosure

Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

[1] J. M. Chase and M. A. Leibold, *Ecological Niches: Linking Classical and Contemporary Approaches*, The University of Chicago Press, 2003.

[2] W. Godsoe, "I can't define the niche but I know it when I see it: a formal link between statistical theory and the ecological niche," *Oikos*, vol. 119, no. 1, pp. 53–60, 2010.

[3] C. Elton, *Animal Ecology*, Sedgwick and Jackson, London, UK, 1927.

[4] G. E. Hutchinson, *An Introduction to Population Ecology*, Yale University Press, New Haven, Conn, USA, 1978.

[5] M. P. Austin and T. M. Smith, "A new model for the continuum concept," *Vegetatio*, vol. 83, no. 1-2, pp. 35–47, 1989.

[6] G. E. Hutchinson, "Limnological studies in Connecticut—part 7. A critical examination of the supposed relationship between phytoplankton periodicity and chemical changes in lake waters," *Ecology*, vol. 25, pp. 3–26, 1944.

[7] G. E. Hutchinson, "Concluding remarks," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 22, pp. 415–427, 1957.

[8] R. K. Colwell and D. J. Futuyama, "On the measurement of niche breadth and overlap," *Ecology*, vol. 52, pp. 567–576, 1971.

[9] M. B. Araújo and A. Guisan, "Five (or so) challenges for species distribution modelling," *Journal of Biogeography*, vol. 33, no. 10, pp. 1677–1688, 2006.

[10] S. H. Hurlburt, "A gentle depilation of the niche: dicean resource sets in resource hyperspace," *Evolutionary Theory*, vol. 5, pp. 177–184, 1984.

[11] H. L. Hooper, R. Connon, A. Callaghan et al., "The ecological niche of Daphnia magna characterized using population growth rate," *Ecology*, vol. 89, no. 4, pp. 1015–1022, 2008.

[12] R. D. Holt, "Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 2, pp. 19659–19665, 2009.

[13] D. Tilman and S. S. Kilham, "Phosphate and silicate growth and uptake kinetics of the diatoms Asterionella Formosa and Chylotella meneghiniana in batch and semicontinous culture," *Journal of Phycology*, vol. 12, pp. 375–383, 1976.

[14] D. Tilman, "Resource competition between plankton algae: an experimental and theoretical approach," *Ecology*, vol. 58, pp. 338–348, 1977.

[15] R. P. Niedz and T. J. Evens, "A solution to the problem of ion confounding in experimental biology," *Nature Methods*, vol. 3, no. 6, p. 417, 2006.

[16] R. H. Myers and D. C. Montgomery, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, John Wiley & Sons, New York, NY, USA, 2nd edition, 2002.

[17] J. A. Cornell, *Experiments with Mixtures: Designs, Models and the Analysis of Mixture Data*, John Wiley & Sons, New York, NY, USA, 3rd edition, 2002.

[18] G. E. P. Box and N. R. Draper, "Factorial designs, the X'X criterion, and some related matters," *Technometrics*, vol. 13, pp. 731–741, 1971.

[19] G. F. Piepel, "Programs for generating extreme vertices and centroids of linearly constrained experimental regions," *Journal of Quality Technology*, vol. 20, pp. 125–139, 1988.

[20] S. Weisberg, *Applied Linear Regression*, John Wiley & Sons, New York, NY, USA, 2nd edition, 1985.

[21] T. J. Evens, R. P. Niedz, and G. J. Kirkpatrick, "Temperature and irradiance impacts on the growth, pigmentation and photosystem II quantum yields of *Haematococcus pluvialis* (Chlorophyceae)," *Journal of Applied Phycology*, vol. 20, no. 4, pp. 411–422, 2008.

[22] M. J. Anderson, *RSM Simplified: Optimizing Processes Using Response Surface Methods for Design of Experiments*, Productivity Press, New York, NY, USA, 2005.

[23] G. E. P. Box and D. R. Cox, "An analysis of transformations (with discussion)," *Journal of the Royal Statistical Society: Series B*, vol. 26, pp. 211–246, 1964.

[24] D. A. Belsley, E. Kuh, and R. E. Welsch, *Regression Diagnostics: Identifying Influential Data and Sources of Collinearity*, John Wiley & Sons, New York, NY, USA, 1980.

[25] R. H. Myers, *Classical and Modern Regression with Applications*, PWS-KENT Publishing Co., Boston, Mass, USA, 2nd edition, 1990.

[26] R. D. Cook and S. Weisberg, *Residuals and Influence in Regression*, Chapman and Hall, New York, NY, USA, 1982.

[27] J. A. Nelder and R. Mead, "A simplex method for function minimization," *Computer Journal*, vol. 7, pp. 308–313, 1965.

[28] G. Derringer and R. Suich, "Simultaneous optimization of several response variables," *Journal of Quality Technology*, vol. 12, pp. 214–219, 1980.

[29] G. J. Hahn and W. Q. Meeker, *Statistical Intervals: A Guide for Practitioners*, John Wiley & Sons, New York, NY, USA, 1991.

[30] W. D. Schecher and D. C. McAvoy, *MINEQL+ a Chemical Equilibrium Modeling System: Version 4.0 for Windows User's Manual*, Environmental Research Software, Hallowell, Me, USA, 1998.

[31] J. A. Nelder, "The selection of terms in response-surface models—how strong is the weak-heredity principle?" *American Statistician*, vol. 52, no. 4, pp. 315–318, 1998.

[32] J. Peixoto, "A property of well-formulated polynomial regression models," *American Statistician*, vol. 44, pp. 26–30, 1990.

[33] J. A. Raven, "Nutrient transport in microalgae," *Advances in Microbial Physiology*, vol. 21, pp. 47–226, 1981.

[34] M. R. Droop, "Some thoughts on nutrient limitation in algae," *Journal of Phycology*, vol. 9, pp. 264–272, 1973.

[35] D. B. Johnson, "Biodiversity and ecology of acidophilic microorganisms," *FEMS Microbiology Ecology*, vol. 27, no. 4, pp. 307–317, 1998.

[36] B. C. Redfield, "The biological control of chemical factors in the environment," *American Scientist*, vol. 46, pp. 205–221, 1958.

[37] T. J. Smayda, "Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea," *Limnology and Oceanography*, vol. 42, no. 5, pp. 1137–1153, 1997.

[38] T. J. Evens and R. P. Niedz, "Are Hofmeister series relevant to modern ion-specific effects research?" *Scholarly Research Exchange*, vol. 2008, Article ID 818461, 9 pages, 2008.

[39] T. J. Evens and R. P. Niedz, "Quantification of nutrient-replete growth rates in five-ion hyperspace for *Chlorella vulgaris* (Trebouxiophyceae) and *Peridinium cinctum* (Dinophyceae)," *European Journal of Phycology*, vol. 45, no. 3, pp. 247–257, 2010.

[40] R. W. Sterner and J. J. Elser, *Ecological Stoichiometry: The Biology of the Elements from Molecules to the Biosphere*, Princeton Univesity Press, 2002.