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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Trade-offs in response to environmental constraints among strains of *Scenedesmus dimorphus*

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Kathleen Chun-I Kwok

Committee in charge:

Professor Jonathan Shurin, Chair
Professor Lin Chao
Professor Brian Palenik

2014

The Thesis of Kathleen Chun-I Kwok is approved and it is acceptable
in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2014

DEDICATION

This Thesis is dedicated to my family, especially I Lin
Tang, Shirley Kwok, Leo Kwok, Eugene Kwok, and
Jonathan Tzeng.

They have sacrificed much for me, pushed me to achieve,
and picked me up when I was down. I would not be who I
am today without them, and I will eternally be grateful
for their love and support.

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This Thesis contains research that is being prepared for submission for publication. Kwok, Kathleen; Shurin, Jonathan. "Trade-offs in response to environmental constraints among strains of *Scenedesmus dimorphus*". The Thesis author is the primary investigator and author of this paper.

ABSTRACT OF THE THESIS

Trade-offs in response to environmental constraints among strains of *Scenedesmus dimorphus*

by

Kathleen Chun-I Kwok

Master of Science in Biology

University of California, San Diego, 2014

Professor Jonathan Shurin, Chair

Trade-offs, or negative functional associations among traits that affect fitness, are fundamental to ecological and evolutionary processes and are the basis for non-neutral species diversity through niche differentiation. Recent studies indicate that trade-offs occur in multiple dimensions involving population limitation by alternative resources or abiotic constraints and may drive species interactions and structure ecological communities. I tested for multivariate trade-offs among 32 natural

populations of the freshwater algae *Scenedesmus dimorphus* in response to light, phosphorus, salinity, nitrogen source (nitrate vs. ammonium) and the presence of pathogenic fungi. Surprisingly, no evidence of trade-offs was found and positive or neutral correlations were observed among strains in response to the treatments. Strains that grew the fastest and reached the highest density at steady state tended to be least affected by light limitation, phosphorus limitation, increased salinity, and switching to ammonium as the nitrogen source. This may suggest an absence of functional constraints in *S. dimorphus* that limit intraspecific variation in response to several important environmental factors, nutrients and energy availability. Trade-offs may occur with other potential limiting factors such as grazers or temperature. Further research is needed to identify important axes of differentiation that constrain diversification and coexistence among *Scenedesmus* genotypes.

Introduction

Hutchinson (1959) asked a simple question that became one of the major conceptual dilemmas of ecology: How do so many species coexist in the same habitat? Classical models predict that an environment with few potentially limiting resources and limited opportunities for niche differentiation should be dominated by one or a few species. Hutchinson called the apparent contrast between classical predictions from theory and the great species diversity observed in natural ecosystems the “Paradox of the Plankton” (1961). Any body of water, such as a lake, may have only a few limiting nutrients and constraining physical factors, yet contain hundreds of species of phytoplankton. Hutchinson proposed a number of violations of the many simplifying assumptions of these classical models that might explain the disparity. For instance, spatial or temporal habitat heterogeneity, or the inability of systems to reach equilibrium due to extrinsic or intrinsic population variation, might allow numerous species to coexist. Tilman and Pacala (1993) reviewed models with more physical and biological complexities that predict the stable or unstable persistence of a potentially unlimited number of species. These models generally conclude that niche differentiation is not just controlled by competition for limiting resources, but also by spatial and temporal heterogeneity of resource availability, top-down/bottom-up control, environmental gradients, and, ultimately, trade-offs in organisms’ abilities to respond to these constraints (Tilman and Pacala 1993). Ptacnik et al. (2010) argue that pelagic systems are temporally and spatially variable in so many aspects of the physical environment that the high diversity they contain should be expected.

However, the processes that promote niche differentiation among phytoplankton remain unknown.

Trade-offs are fundamental to ecological and evolutionary processes, and they are assumed to underlie the maintenance of non-neutral species diversity through niche differentiation. Trade-offs are exhibited as a negative functional interaction between traits that affect fitness (Kneitel and Chase 2004), for instance, between the ability to compete for alternative resources, survive under different conditions, or resist the effects of predators or diseases. Trade-offs may arise from limited ability to allocate resources to alternative functions such as, for example, among growth, reproduction or defense. In a community context, trade-offs represent niche differentiation among species, which emerge from individual-level constraints imposed by the environment (Chase and Leibold 2003). Within a species, trade-offs can also affect intraspecific variation and evolutionary potential, as they may constrain the trajectory of phenotypic evolution (Blows and Hoffmann 2005).

Little is known about which trade-offs structure phenotypic diversity in different groups of organisms (Edwards et al. 2011). Recent reviews have emphasized that trade-offs can be detected and linked to changes in species composition along environmental gradients by quantifying correlations among important functional traits across species (McGill et al. 2006, Litchman and Klausmeier 2008). Plants show a negative association between ability to compete for nutrients vs. tolerate herbivory, indicative of a trade-off between susceptibility of top-down vs. bottom-up control (Lind et al. 2013). Three-way trade-offs among plants in the ability to compete for resources, tolerate environmental stress and reproduce quickly in disturbed

environments are the basis for Grime's Triangle (Grime 1979). Coral species have also recently been shown to exhibit similar associations along the same three niche axes (Darling et al. 2012). Recently, Edwards et al. (2011) showed the importance of multivariate approaches to functional traits related to acquisition of nitrogen and phosphorus in phytoplankton. These studies indicate that trade-offs may occur in multiple dimensions, and such trade-offs may drive species interactions and structure ecological communities.

Although phytoplankton were the inspiration for Hutchinson's Paradox of the Plankton, the trade-offs that govern coexistence in phytoplankton remain largely unknown. Microscopic phytoplankton are a diverse, polyphyletic group of photosynthetic eukaryotes and cyanobacteria that produce about half of global primary production, and affect the biogeochemical cycling of many elements, including carbon, nitrogen, and phosphorus (Falkowski et al. 2004). Litchman and Klausmeier (2008) argue that phytoplankton provide an ideal system to test trait-based approaches because of their relative simplicity and the well-defined traits that determine their ecological niche (i.e., cell size and shape, nutrient uptake and requirements).

Several interspecific trade-offs have been demonstrated in phytoplankton. A trade-off between maximum growth rate and competitive ability, termed by Grover (1997) as the gleaner-opportunist trade-off, has been postulated to be caused by differential investment in growth machinery (P-rich ribosomes) vs resource acquisition machinery (N-rich proteins; Klausmeier 2004). However, evidence for this trade-off is not apparent in some groups of species, such as marine diatoms (Litchman 2007). Trade-offs between competitive abilities for different resources, including nutrients

and light, have also been hypothesized as important in structuring phytoplankton communities (Passarge et al. 2006, Tilman 1982, Leibold et al. 1997). Edwards et al showed a three-way trade-off between nitrogen and phosphorus competitive abilities and cell size (2011). Smaller cells are better competitors for both N and P, consistent with surface-area constraints, however, after controlling for cell size, N and P competitive abilities were negatively correlated across taxa. A higher minimum quota for nitrogen under low irradiance has also been demonstrated, and is hypothesized to be due to an increase in nitrogen requirements when light-harvesting pigments are increased (Rhee and Gotham 1981). Competitive abilities for light and phosphorus have been shown to be positively correlated and are unlikely to explain biodiversity in phytoplankton communities (Passarge et al. 2006). However, a trade-off between susceptibility to grazing and competitive abilities for phosphorus and light may indicate that predators can maintain diversity in phytoplankton (Leibold 1996, Yoshida et al. 2004). Grazer resistance in phytoplankton is often achieved by increasing cell size or by colony formation, which both decrease nutrient competitive abilities due to less efficient surface area to volume ratios (Reynolds 1988).

I explored the existence of trade-offs along multiple trait axes among different strains of the freshwater phytoplankton *Scenedesmus dimorphus*. *S. dimorphus* (Class: Chlorophyceae) is a widespread member of freshwater plankton communities as well as a prime candidate for algal biofuel use due to its rapid growth, low nutrient requirements, and high lipid content. However, its nutrient and water requirements are still high compared to other potential biofuel feedstock, such as switchgrass and corn (Clarens 2010). To analyze the existence of multivariate trade-offs in natural

populations of *S. dimorphus*, I measured maximum growth rate and carrying capacity of up to 32 different strains of *S. dimorphus* under contrasting environmental conditions. The conditions studied included light limitation, phosphorus limitation, use of nitrate vs. ammonium as a nitrogen source, increased salinity, and addition of *Amoebophilidium protococcarum*, chytrid-like fungus known to parasitize *S. dimorphus* (Letcher et al. 2013). All of these treatment constraints represent different forms of environmental variability that exist in nature and may affect the fitness of *S. dimorphus* under varying conditions. Negative correlations among treatment effects on growth would suggest trade-offs in the abilities of *S. dimorphus* strains to respond to them. Positive correlations between growth under benign conditions and treatment effects would indicate a trade-off in the abilities of *S. dimorphus* strains to maximize growth and compete for resources or deal with abiotic stress.

I hypothesized that each treatment condition would affect growth negatively and that trade-offs would be found between a *S. dimorphus* strain's ability to grow well under ideal (control) conditions and its ability to respond to treatment conditions. I also predicted that we would find trade-offs in *S. dimorphus* strain abilities to respond to treatment constraints. That is, if the response to one form of population limitation (e.g., light limitation) is negatively correlated among strains with the response to another experimental factor (e.g., nitrogen source), then this indicates a trade-off in sensitivity to the two environmental constraints. Two sets of experiments were used to test for potential trade-offs by examining growth in both semi-continuous and batch cultures.

Materials and Methods

We tested for potential trade-offs in *S. dimorphus* strains by comparing growth under different environmental constraints in both semi-continuous and batch cultures. We initially used batch culture, but we repeated the measurements in semi-continuous culture because of concern that we could not measure the effect on cell densities over time of the chytrid-like fungus *A. protococcarum* in our batch culture measurements.

Semi-continuous culture

Five replicate populations of each of 28 *S. dimorphus* strains (UTEX 1237 plus 27 strains isolated from natural environments by researchers from Sapphire Energy) were grown under six separate experimental treatments—control, NH₄, salt, low phosphorus, low light, and chytrid—in a semi-continuous culture for 12-14 days (Table 1). Experiments were done in groups over a period of several months, with up to 14 different strains grown with up to 3 treatments at a time. Strains were inoculated into sterilized 50 mL flasks with cotton plugs at an *in vivo* chlorophyll-*a* (*chl**a*) density of approximately 200 µg/L. Each flask contained 20 mL of WC media (Guillard 1975), adjusted for the treatment conditions as necessary. For the chytrid treatment, spores of the chytrid-like fungus *A. protococcarum* (strain FD01) were inoculated from a culture grown in WC and fed with *S. dimorphus* (UTEX 1237), after examination of the culture under microscopy showed no live *S. dimorphus* cells. All replicates were grown under 24 hour photoperiod at 28°C. Media was diluted at 10 percent per day, every other day (*i.e.*, 20% every two days). Samples were taken before each dilution and *chl**a* was measured at 750 nm using a Turner fluorometer (Model 7200-000) to approximate population biomass.

Batch Culture

Five replicates of each of 32 *S. dimorphus* strains (the same strains grown in semi-continuous culture, with 3 additional strains received from Sapphire Energy) were grown under five separate experimental treatments—control, NH₄, salt, low phosphorus, and low light—in a batch culture for 19-20 days (Table 2). Strains were inoculated into sterile 96-well plates at an optical density (OD) of approximately 0.025 at 680 nm. Each flask contained 300 μL of WC media (Guillard 1975), adjusted for the treatment condition as necessary. All replicates were grown on orbital shakers at 120 rpm and under 24 hour photoperiod at 23°C. OD at 680 nm was measured every 2 days with a Thermo Scientific Multiskan FC plate reader to approximate population biomass.

Statistical Analysis

Maximum exponential growth rate and carrying capacity were used as the parameters to describe the growth of each strain by treatment. The maximum exponential growth rate of was defined as the maximum observed growth between two sampling intervals during the experiment:

$$\mu = \max \left(\frac{\ln(N_x/N_{x-1})}{t_x - t_{x-1}} \right)$$

where N_x is the density at time t_x and N_{x-1} is the density at time t_{x-1} . The carrying capacity, K , was defined as the maximum density (expressed in OD or *chl**a*) reached over the course of the experiment. The parameters μ and K were calculated for each replicate and averaged for each strain by treatment. The effect of a treatment condition on each strain, $E_{treatment}$, was estimated as the difference between the growth

parameters under treatment and the growth parameters of the control group (*i.e.*, Salt effect on $K = E_{Salt}$ on $K = K_{control} - K_{Salt}$, for each strain). To test for trade-offs between strain responses to treatments, we calculated the Pearson's product-moment correlation (r) between treatment effects on growth and carrying capacity across all strains. To discover whether a trade-off exists between strains' performance under ideal (control) conditions and under treatment conditions, we also calculated r between growth under control conditions and each treatment effect. We used principal component analyses (PCAs) on the treatment effects to illustrate the correlations between treatment effects. Treatment effects, correlations, and principal components were all estimated for μ and K in both the semi-continuous culture and batch culture experiments. All statistical analyses were conducted using the statistical programming language R, version 3.0.2 (R Core Development Team, 2013).

Results

Figure 1 shows the growth curves for each *S. dimorphus* strain under control and treatment conditions for the semi-continuous cultures, and Figure 2 shows growth curves in batch cultures. Figure 3 shows the treatment effects (treatment minus control) on μ and K for the semi-continuous cultures, and Fig. 4 for the batch cultures. Treatments effects ($E_{Treatment}$) were considered negative for a parameter if the lower bound of the 95% confidence interval for the difference between treatment and control levels was positive. In the semi-continuous cultures, E_{Salt} , $E_{Low\ Light}$, and $E_{chytrid}$ on μ was negative for most strains, while E_{NH4} was negative on μ for 13 strains and $E_{Low\ Phosphorus}$ for only 6 of the 28 strains (Fig. 3a-e). E_{NH4} and E_{Salt} were negative on K for all strains, and $E_{Low\ Phosphorus}$, $E_{Low\ Light}$, and $E_{chytrid}$ were negative on K for most strains (Fig. 3f-j). In the batch cultures, E_{NH4} and E_{Salt} on μ were negative for most strains, but $E_{Low\ Phosphorus}$ was negative on μ for only 6 and $E_{Low\ Light}$ for only 5 of the 32 strains (Fig. 4a-d). E_{NH4} was negative on K for all strains, while E_{Salt} and $E_{Low\ Phosphorus}$ on K were negative for most strains, and $E_{Low\ Light}$ on K was negative for 12 of the 32 strains (Fig. 4e-h).

Significant correlations were observed between many of the treatments effects in both semi-continuous and batch cultures (Fig.s 5 and 6, respectively). In the semi-continuous cultures, positive correlations were found between E_{NH4} and $E_{Low\ Light}$, between E_{Salt} and $E_{Low\ Phosphorus}$, and between $E_{Low\ Phosphorus}$ and $E_{Low\ Light}$ for both μ and K . Additionally, significant positive correlations were found between E_{NH4} and $E_{Low\ Phosphorus}$ and between E_{Salt} and $E_{chytrid}$, on μ . A negative correlation was found between μ 's of the control group ($\mu_{control}$) and E_{Salt} on μ . There was also a negative correlation

between K 's of the control group ($K_{control}$) and every treatment effect on K , except for the chytrid treatment (Fig. 5). In the batch cultures, E_{Salt} and $E_{Low Phosphorus}$ were positively correlated for both μ and K , as well as between $E_{Low Phosphorus}$ and $E_{Low Light}$ for K . $\mu_{control}$ was negatively correlated to every treatment effect on μ . $K_{control}$ was negatively correlated to every treatment effect on K , except for the low light treatment effect (Fig. 6).

PCA's illustrate the multivariate correlations of treatment effects on μ and the treatment effects on K . In the semi-continuous cultures, the first two principle components (PC1 and PC2) extracted from the treatment effects on μ explained 72% of the variation in treatment effects among strains. PC1 and PC2 from the treatment effects on K explained 78% of the variation in strain responses (Fig. 7). In the batch cultures, PC1 and PC2 of the treatment effects on μ explained 72% of the variation between strain responses. PC1 and PC2 of the treatment effects on K explained 76% of the variation between each strain's responses to the treatments (Fig. 8). The PC analyses illustrate that all of the treatment effects were either positively or neutrally correlated (*i.e.*, treatment effect loadings are pointing in the same direction or in perpendicular directions, respectively), indicating the absence of any apparent trade-offs among strains in response to the treatments imposed.

Discussion

Our experiments found no evidence of trade-offs among genotypes of *S. dimorphus* in response to nitrogen source, high salinity, phosphorus limitation, light limitation, or the presence of pathogenic fungi in the environment. Rather, the strains that showed the highest growth and densities under benign conditions (in the control) tended to maintain the highest growth under treatment conditions. In general, strains that were most strongly affected by one form of physiological stress (e.g., salinity) in the environment were also susceptible to others (e.g., low phosphorus). Since all of the strains except UTEX 1237 were recently isolated from the natural environment as part of an industrial search for viable bioenergy strains, these results raise the question of how the strains that performed poorly under ideal conditions and in response to environmental stress manage to persist in the face of competition from the more robust and resilient strains. A number of possible explanations remain unexamined (e.g., abilities to disperse among habitats, resist the effects of grazing zooplankton, or grow at different temperatures, just to name a few). However, our results indicate that some strains performed well under all experimental and control conditions while others were susceptible to multiple forms of environmental stress.

Most of the treatment conditions had a significant negative effect on the carrying capacity, K , for the majority of strains, in both semi-continuous cultures and batch cultures, indicating that the treatments imposed limitations on cell densities (Fig.s 3f-j and 4e-h). The only exception was the low light effect, $E_{Low\ Light}$, on K in batch culture, which exhibited a significant negative effect on only 6 of the 32 strains studied (Fig. 4h). This may have been a result of the difference in culture conditions

between the batch and semi-continuous cultures: for the strains grown in batch culture (in 96-well plates), it's possible that the much smaller culture volume (300 μ L vs. 20 mL) and use of shakers eliminated the self-shading that would be present in flasks, and the control light intensity actually caused photo-inhibition while the low light treatment relieved it.

For μ , most treatments had a significant negative effect on the majority of *S. dimorphus* strains in both types of culture, suggesting that the treatments limited cell growth rates (Fig.s 3a-e and 4a-d). In batch culture, $E_{Low\ Light}$ on μ was only negative on 5 of 32 strains, similar to $E_{Low\ Light}$ on K in batch culture and probably caused by the photo-inhibitive light levels of the control treatment described above (Fig. 4d and 4h). Additionally, $E_{Low\ Phosphorus}$ was only significantly negative for 6 of 32 strains a few strains on μ but was negative for most strains on K , in semi-continuous and batch cultures. This suggested that the phosphate concentration in the low phosphorus treatment media was not limiting to cell growth during the beginning exponential growth phase, but it was limiting at the end when cell densities were higher. This explanation is supported by Passarge et al. (2006), who found that external phosphorus concentrations were rapidly depleted in chemostat and batch cultures, and population densities continued to increase on a slower time scale.

We did not find evidence for the gleaner-opportunist trade-off between maximum growth rate and competitive ability proposed by Grover (1997) and supported mechanistically by Klausmeier et al. (2004). In figures 5 and 6, this trade-off would be represented by a positive correlation between a strain's maximum growth rate under ideal conditions, $\mu_{control}$ and its response to resource limitation (e.g., the

effects of nitrogen source, low phosphorus, or low light). Surprisingly, we only found negative correlations for μ and a few treatment effects when comparing *S. dimorphus* strains. Negative correlations were also found for K under ideal conditions, $K_{control}$, and all treatment effects on K , except for the chytrid effect in semi-continuous culture. This implies that strains that have a higher μ and K under ideal conditions also tend to be more tolerant of environmental constraints. However, a strain's $\mu_{control}$ when grown in semi-continuous culture was only significantly correlated with the salt effect on μ , whereas $\mu_{control}$ in batch culture and $K_{control}$ in both kinds of culture were much better predictors. This is also reflected in the treatment effect PCAs (Figs 7 and 8). Klausmeier et al.'s (2004) mechanistic support for the gleaner-opportunist trade-off was based on N:P stoichiometry, which was not measured in this experiment. In their analysis of literature on major functional traits in phytoplankton, Litchman et al. (2007) also did not find support for interspecific trade-offs between maximum growth rate and competitive ability for resources among major groups of phytoplankton.

Although investigators have previously demonstrated trade-offs between phytoplankton species in their competitive abilities for different resources (*i.e.*, Tilman 1982, Leibold et al. 1997), we found none of these trade-offs between *S. dimorphus* strains. Trade-offs in competition for alternative resources is the basis for the Resource Ratio Hypothesis (Tilman 1982), one of the most often invoked explanations for coexistence in phytoplankton (Interlandi and Kilham 2001) and plant (Harpole and Tilman 2007) communities. A trade-off between competitive abilities for different resources would be represented by a negative correlation between treatment effects on strains in figures 5 and 6, but all significant correlations found were positive. E_{Salt} was

consistently positively correlated with $E_{Low\ Phosphorus}$ on both μ and K , in both semi-continuous and batch cultures. I did not find any evidence in the literature that would support a positive correlation between the ability of freshwater phytoplankton to respond to high salinity and the ability to compete for phosphorus. On the contrary, a negative correlation would be expected since phosphorus is shown to be more readily available for absorption in seawater than freshwater, as well as along salinity gradients in estuaries, although this is attributed to higher sulfate concentrations in seawater (Hartzell and Jordan 2012, Blomqvist et al. 2004).

$E_{Low\ Light}$ and $E_{Low\ Phosphorus}$ were positively correlated among *S. dimorphus* strains on K for both types of culture and on μ for batch culture. This agrees with the findings of Passarge et al. (2006) that phytoplankton species with strong competitive abilities for light were also strong competitors for phosphorus. This positive correlation did not, however, coincide with a negative correlation with susceptibility to chytrid grazing, as Yoshida et al. (2004) would have expected to find in support of keystone predation. E_{NH4} and $E_{Low\ Light}$ were positively correlated on μ and K for strains grown in semi-continuous cultures. This may be a result of some strains adapting to eutrophic conditions, where high cell densities that limit light penetration of the water and higher ammonium concentrations coincide (MacIsaac and Dugdale 1972).

The correlations between E_{NH4} and $E_{Low\ Phosphorus}$ and between E_{Salt} and $E_{chytrid}$ were significantly positive only on μ in semi-continuous cultures. It has been shown that *Scenedesmus* sp. cells do not differentiate between nitrate and ammonium as a nitrogen source (Park et al. 2010), but cause of the observed positive relationship of

ammonium response to phosphorus limitation response is unknown. The chytrid-like fungus *A. protocoecarum* was only recently isolated, and there is little data on what factors influence its effect on algal growth (Letcher et al. 2013). *A. protocoecarum* is a pest capable of significantly crashing *S. dimorphus* biofuel crop ponds, and further research is needed to determine if phytoplankton resistance to salt is indeed correlated to resistance to this parasite and the mechanism behind it.

The lack of any evidence for trade-offs among *S. dimorphus* strain abilities to respond to treatment effects is surprising. We also did not anticipate that a strain's $\mu_{control}$ and $K_{control}$ would be negatively correlated with treatment effects, or that multiple treatment effects would be positively correlated. All of these findings indicate an absence, in *S. dimorphus*, of functional constraints that would limit intraspecific variation or hinder adaptation to the environmental limitations we imposed.

However, further research is needed to ascertain if we would find different trait relationships by measuring other biological parameters, as there could be many weak trade-offs in multiple dimensions. Edwards et al. (2011) found that competitive abilities for nitrogen and phosphorus were positively correlated in major groups of phytoplankton, but both traits decreased as cell size increased, and a negative correlation or trade-off between the traits was only evident when cell size was controlled for. Since we used strains of the same species that are similar in size, it is unlikely that controlling for cell size would change our results, but it is possible that different trait correlations would arise if we controlled for average *S. dimorphus* colony size, which varies between strains. Garland (2014) also asserted that organisms comprise an almost infinite number of measurable traits, and trade-offs may only be

apparent when we include more traits in an analysis. For example, measuring parameters related to N:P stoichiometry would be another way we could tease out trade-offs, and it would be interesting to see if we could find the same evidence Klausmeier et al. (2004) did for a trade-off between maximum growth rate and competitive abilities.

Examples of other traits that could control trade-offs are the ability to compete for nitrate, compete for urea, and susceptibility to zooplankton grazing, all of which would have impacts on *S. dimorphus*'s suitability as a bioenergy crop. In particular, there is very little literature that explores the relationship between algal responses to different nitrogen sources and competitive ability for other resources, and this knowledge would facilitate researcher and industry decision-making on whether or not *S. dimorphus* is fit for use in wastewater management (Park et al. 2010). Another way to test for the existence of trade-offs and whether genetic constraints on adaptation exist is an experimental evolution experiment, in which we could test *S. dimorphus*'s abilities to adapt to the one or a combination of the treatment conditions we used. There is evidence that *S. dimorphus* can adapt to higher salinities without a significant sacrifice in growth rates or lipid content (Gigante 2013). If *S. dimorphus* could also simultaneously adapt to the other treatments in this study, this would have important implications for the cost projection on algal biofuels. For example, adaptation to low phosphorous levels would lower fertilizer costs, and adaptation to lower light levels would decrease energy consumption. These would all help *S. dimorphus* improve its life cycle assessment in terms of biofuel production.

This Thesis contains research that is being prepared for submission for publication. Kwok, Kathleen; Shurin, Jonathan. "Trade-offs in response to environmental constraints among strains of *Scenedesmus dimorphus*". The Thesis author is the primary investigator and author of this paper.

Figures and Tables

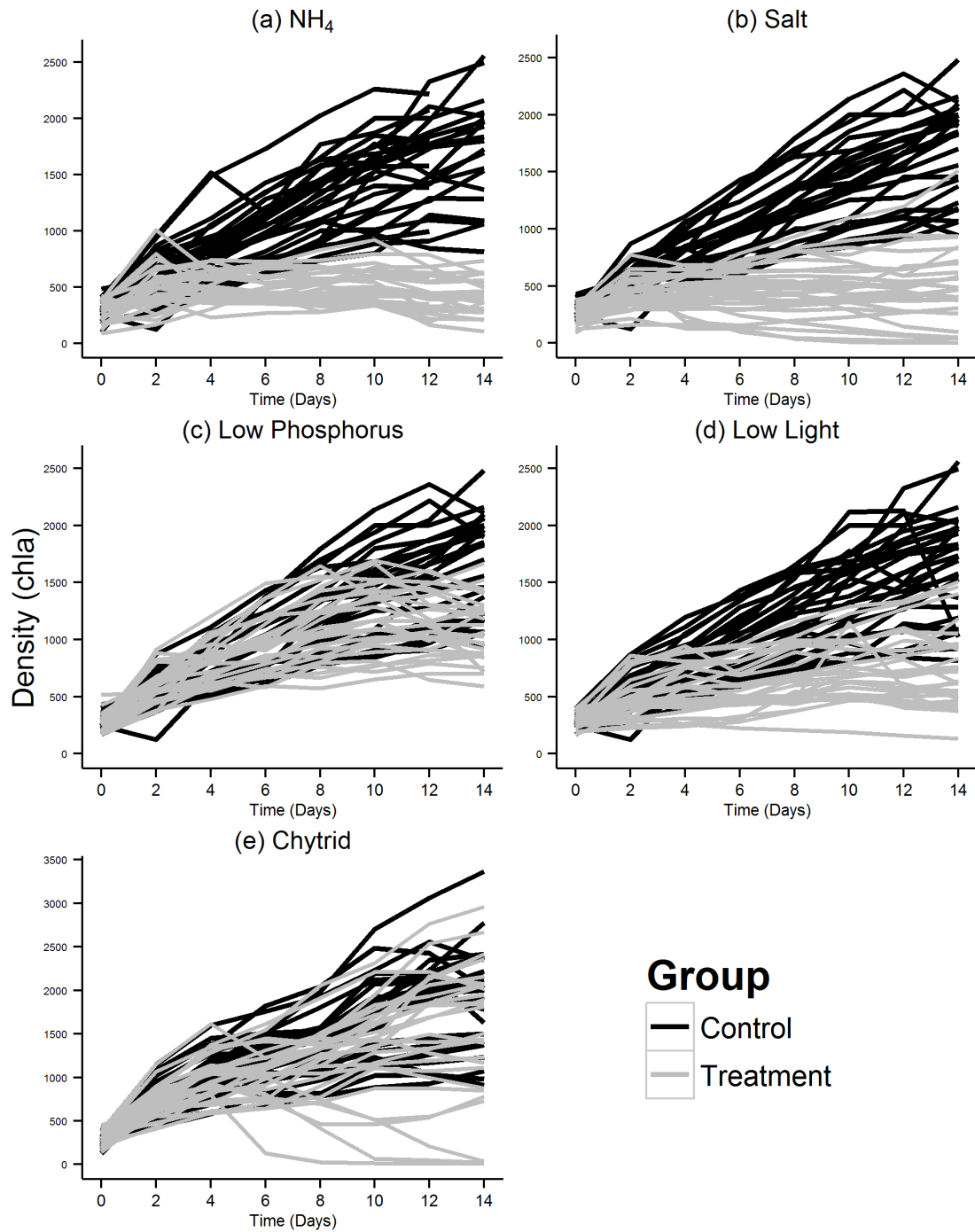


Figure 1: Growth curves of strains grown in semi-continuous culture, by treatment. Density values are averaged across replicates.

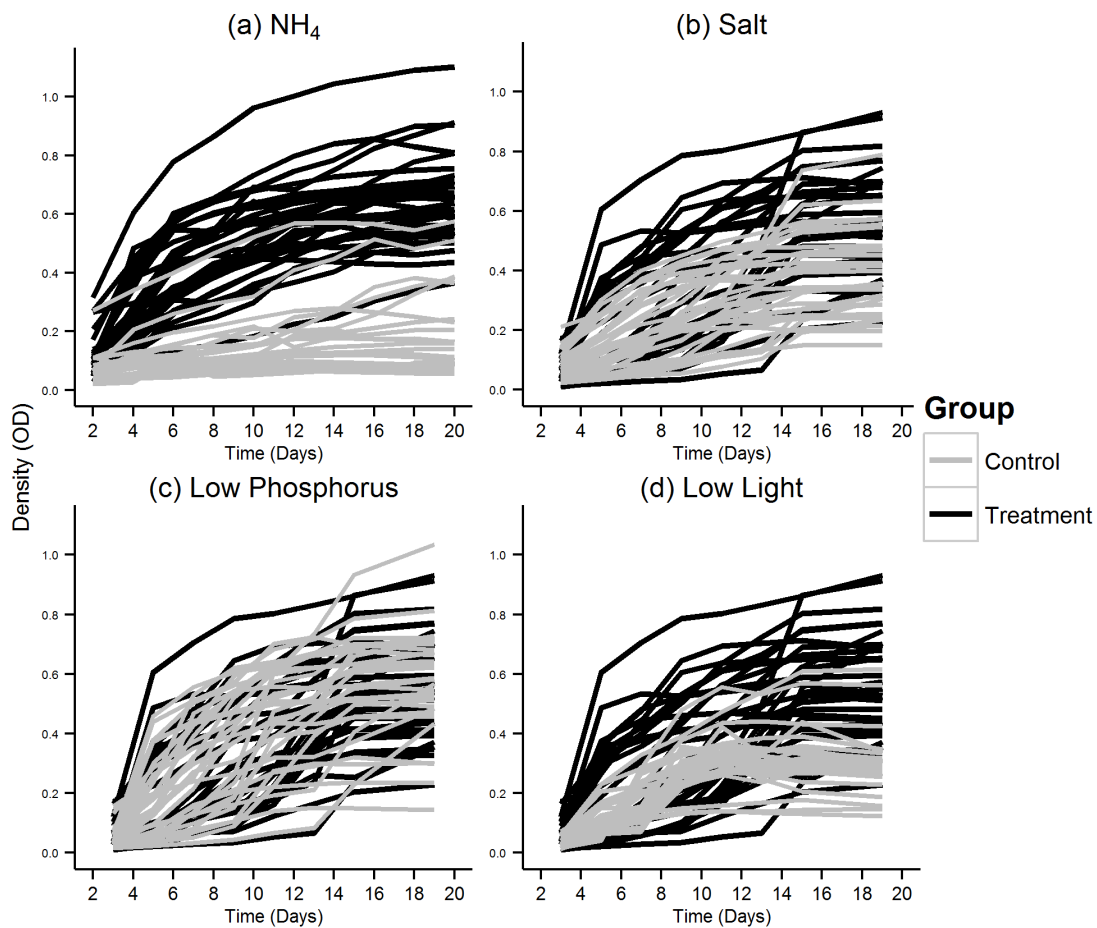


Figure 2: Growth curves of strains grown in batch culture, by treatment. Density values are averaged across replicates.

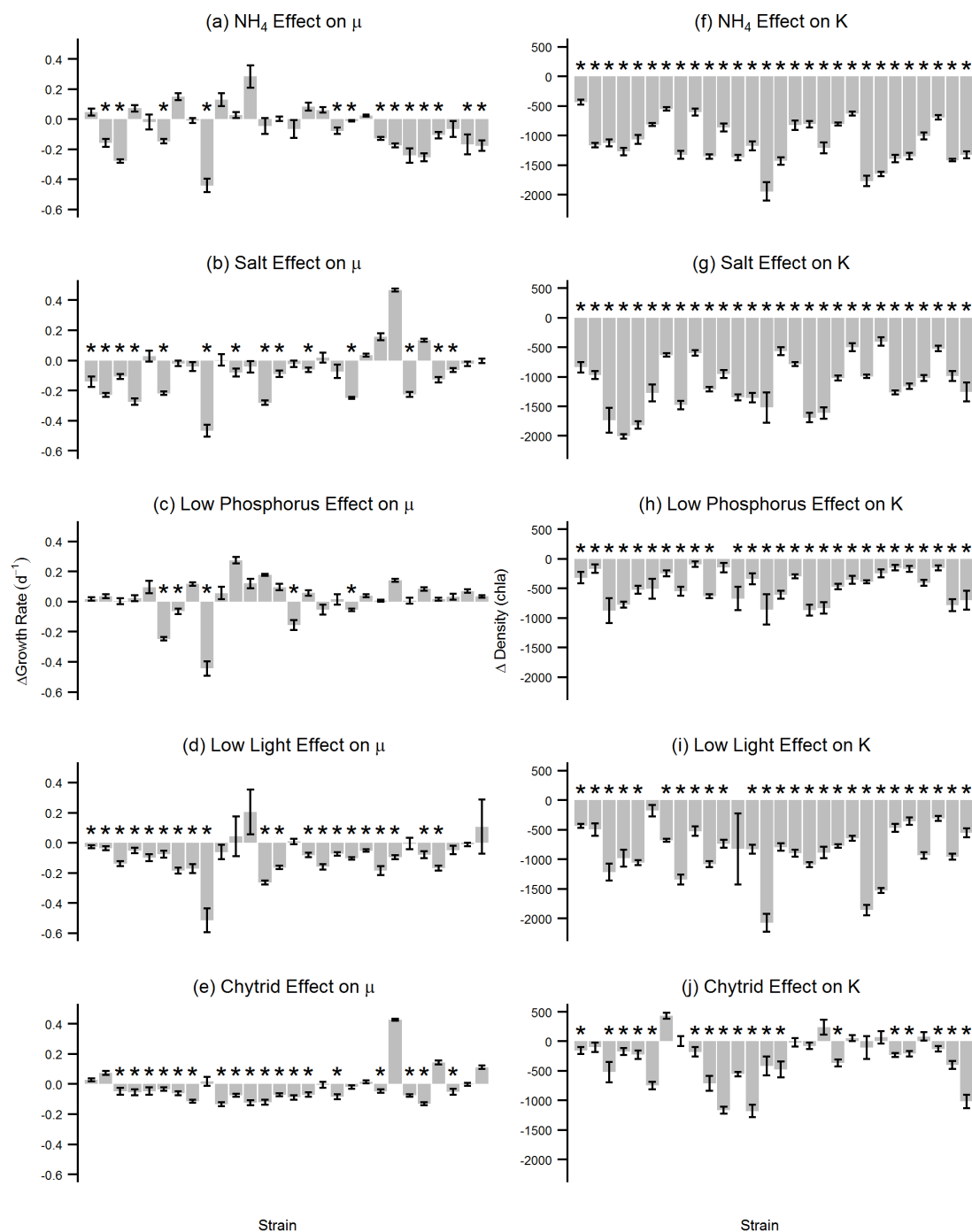


Figure 3: Effect of each treatment (control minus treatment) on average maximum growth rate μ (a-e) and average maximum density K (f-j) for each strain in semi-continuous cultures, with standard error bars and significance stars for negative effects ($p < 0.5$).

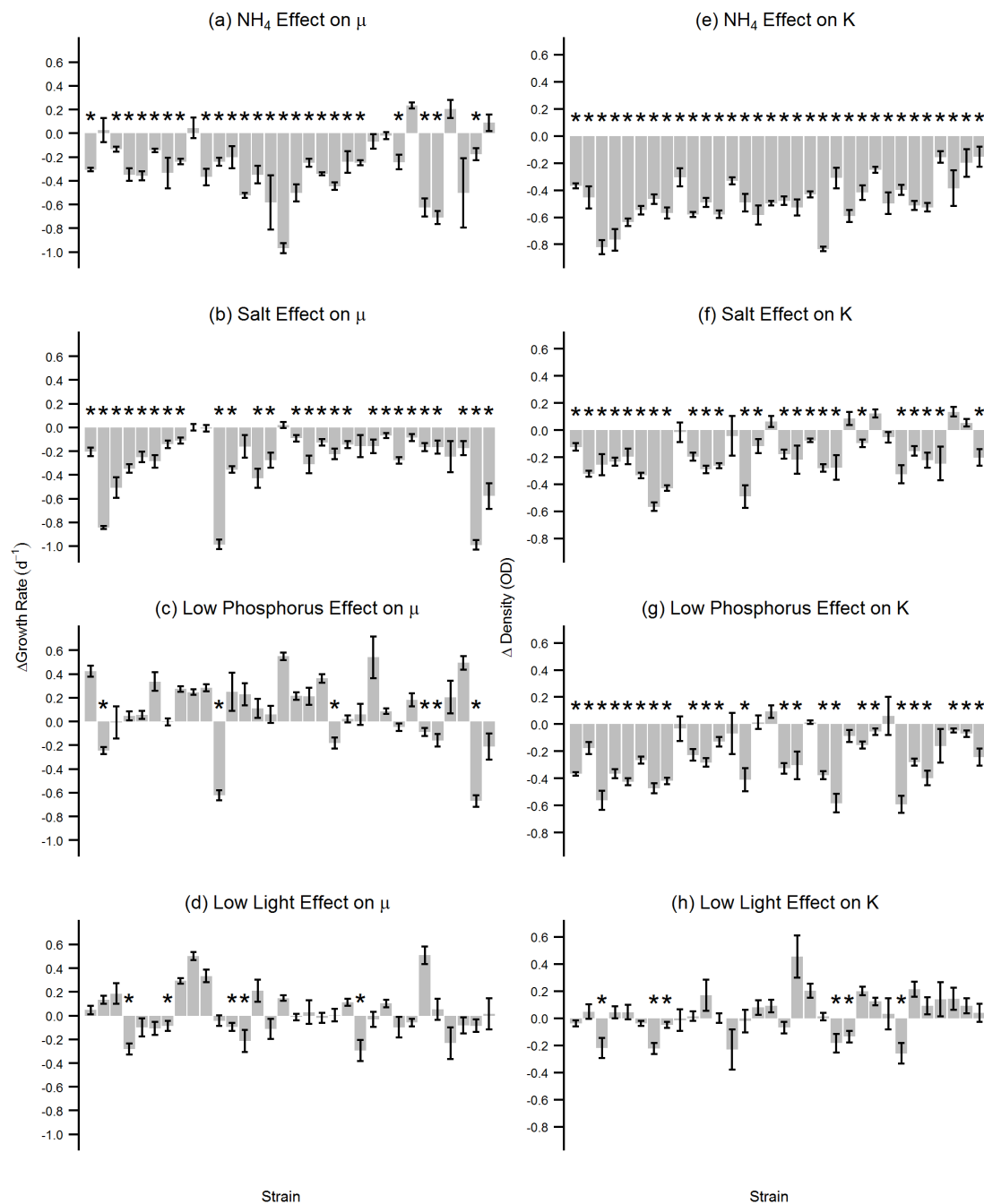


Figure 4: Effect of each treatment (control minus treatment) on average maximum growth rate μ (a-d) and average maximum density K (e-h) for each strain in batch culture, with standard error bars and significance stars for negative effects ($p < 0.05$).

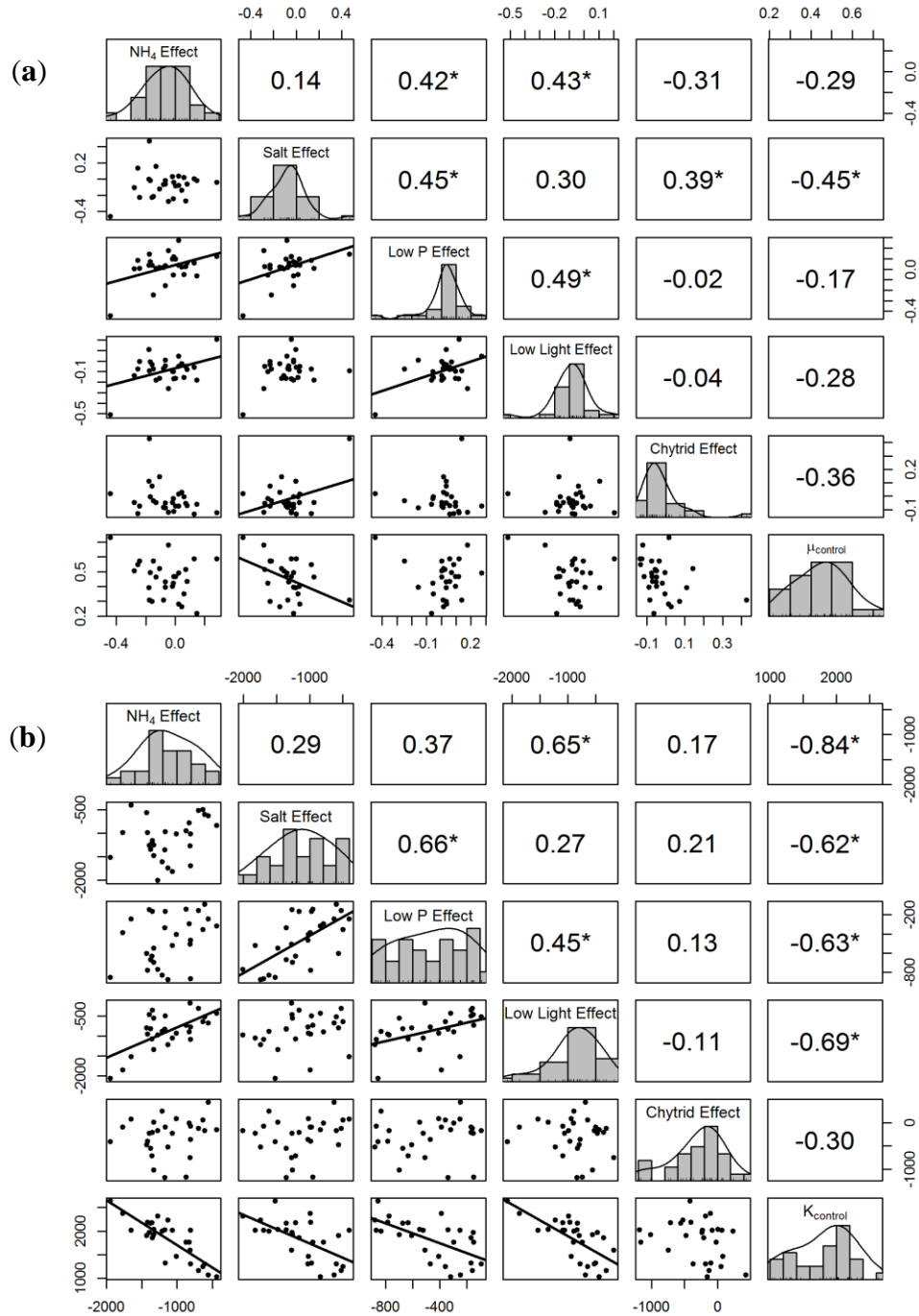


Figure 5: Correlation matrix for (a) treatment effects on μ and μ_{control} , and between (b) treatment effects on K and K_{control} , for each strain in semi-continuous culture. The diagonal shows the histogram of each treatment effect or growth parameter, as well as the axes labels for the scatterplots below the diagonal (x axis is in the histogram above each scatterplot, and y-axis is in the histogram to the right of each plot). The boxes above the diagonal contain treatment effect correlation coefficients, labelled with a ‘*’ if significant at $p < 0.05$. Regression lines are also plotted in the scatterplots with significant correlations.

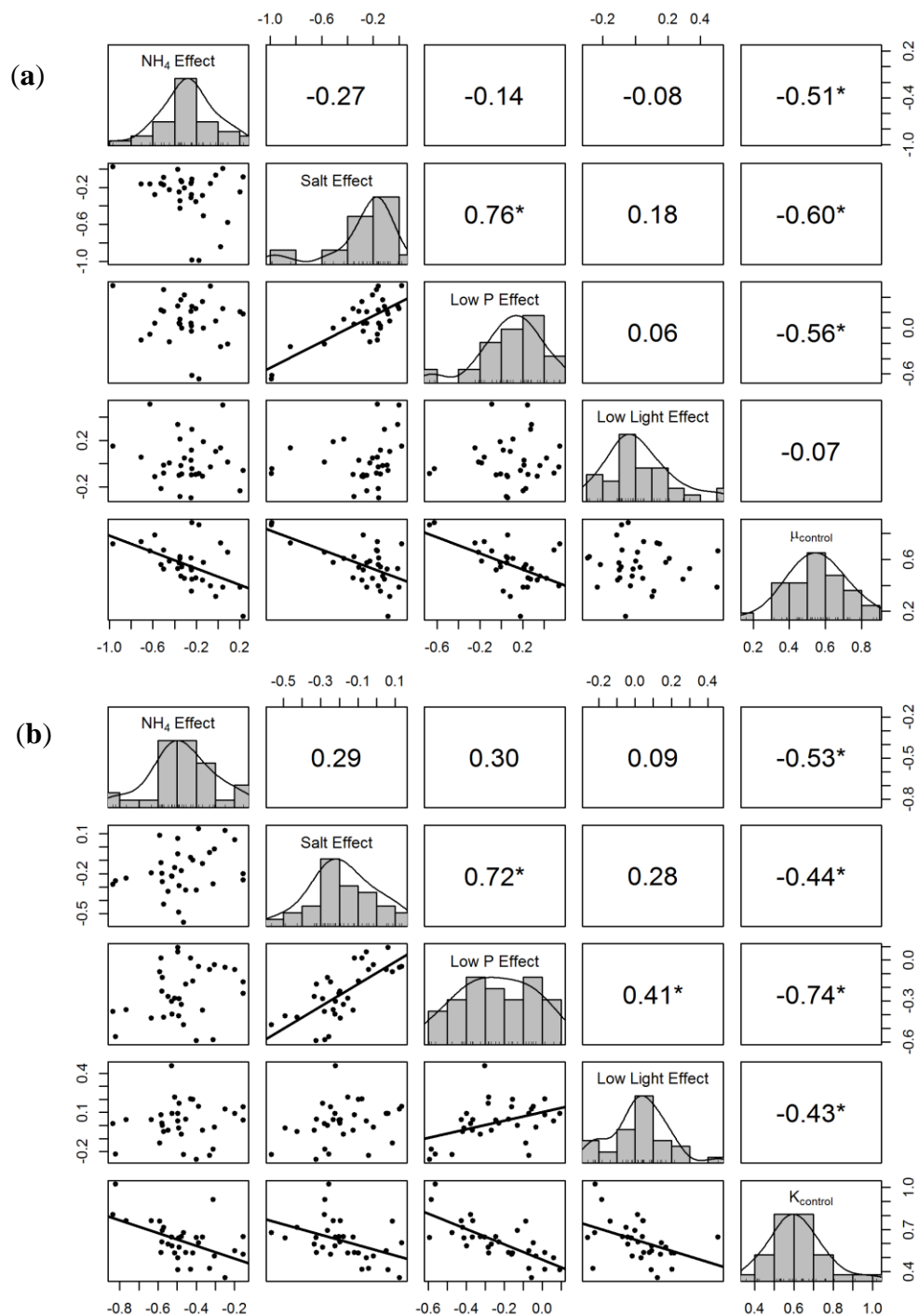


Figure 6: Correlation matrix for (a) treatment effects on μ and μ_{control} , and between (b) treatment effects on K and K_{control} , for each strain in batch culture. The diagonal shows the histogram of each treatment effect or growth parameter as well as the axes labels for the scatterplots below the diagonal (x axis is in the histogram above each scatterplot, and y-axis is in the histogram to the right of each plot). The boxes above the diagonal contain correlation coefficients, labelled with a ‘*’ if significant at $p < 0.05$. Regression lines are also plotted in the scatterplots with significant correlations.

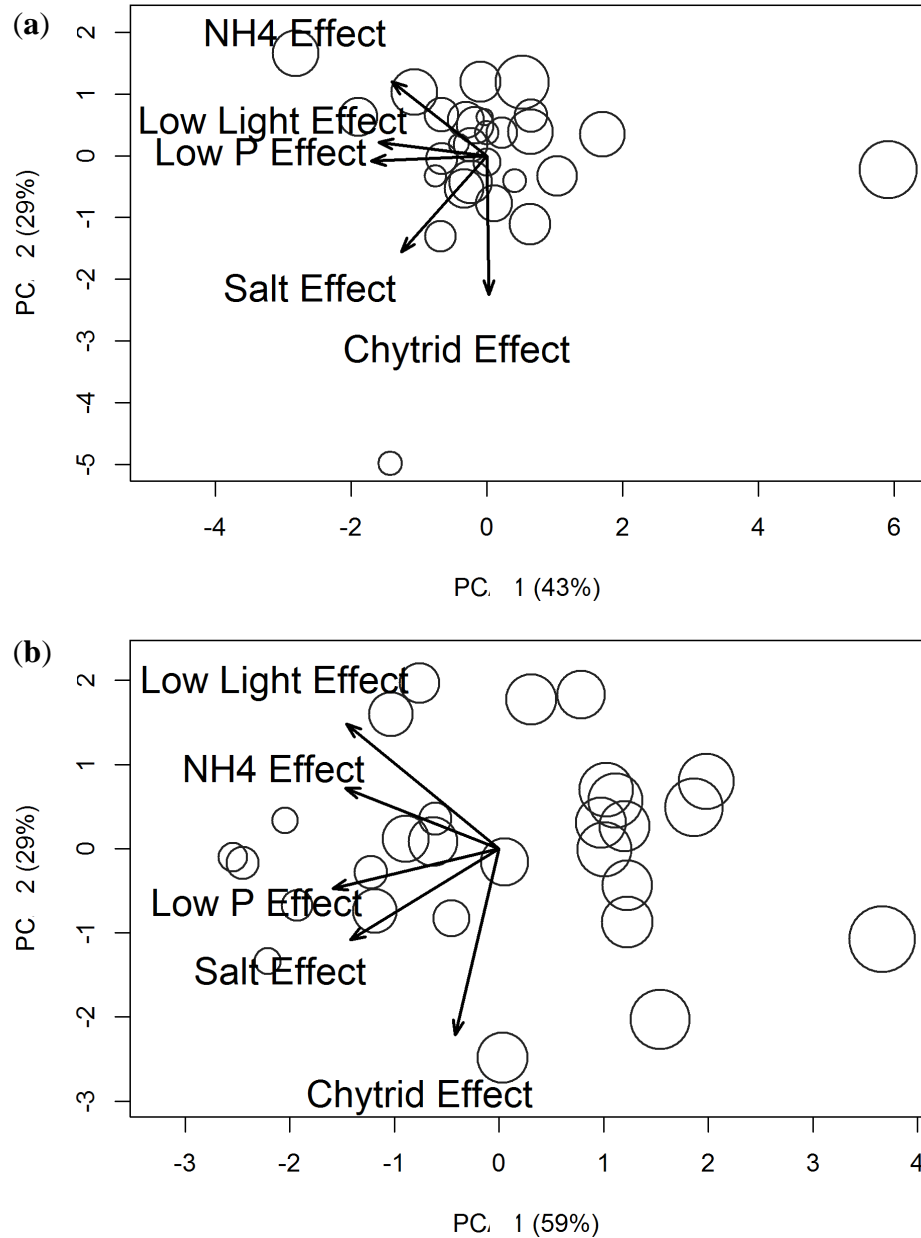


Figure 7: (a) Loading of treatment effects on μ in semicontinuous culture onto the first two principle component axes of a PCA using these 5 variables. Respective loading coefficients of E_{NH4} , E_{Salt} , $E_{Low\ Phosphorus}$, $E_{Low\ Light}$, and $E_{chytrid}$ on μ are $(-0.47, -0.42, -0.57, -0.53, 0.01)$ on PC1 and $(0.40, -0.52, -0.03, 0.07, -0.75)$ on PC2. (b) Loading of treatment effects on K in semicontinuous culture onto the first two principle component axes of a PCA using these 5 variables. Respective loading coefficients of E_{NH4} , E_{Salt} , $E_{Low\ Phosphorus}$, $E_{Low\ Light}$, and $E_{chytrid}$ on K are $(-0.49, -0.47, -0.53, -0.49, -0.14)$ on PC1 and $(0.24, -0.36, -0.16, 0.49, -0.74)$ on PC2. All loadings are multiplied by 3 for clarity. Percentages in the axis labels are the percentage of variation explained by each axis. Each circle represents a strain, with the size of the diameter corresponding to $\mu_{control}$ in (a) and $K_{control}$ in (b).

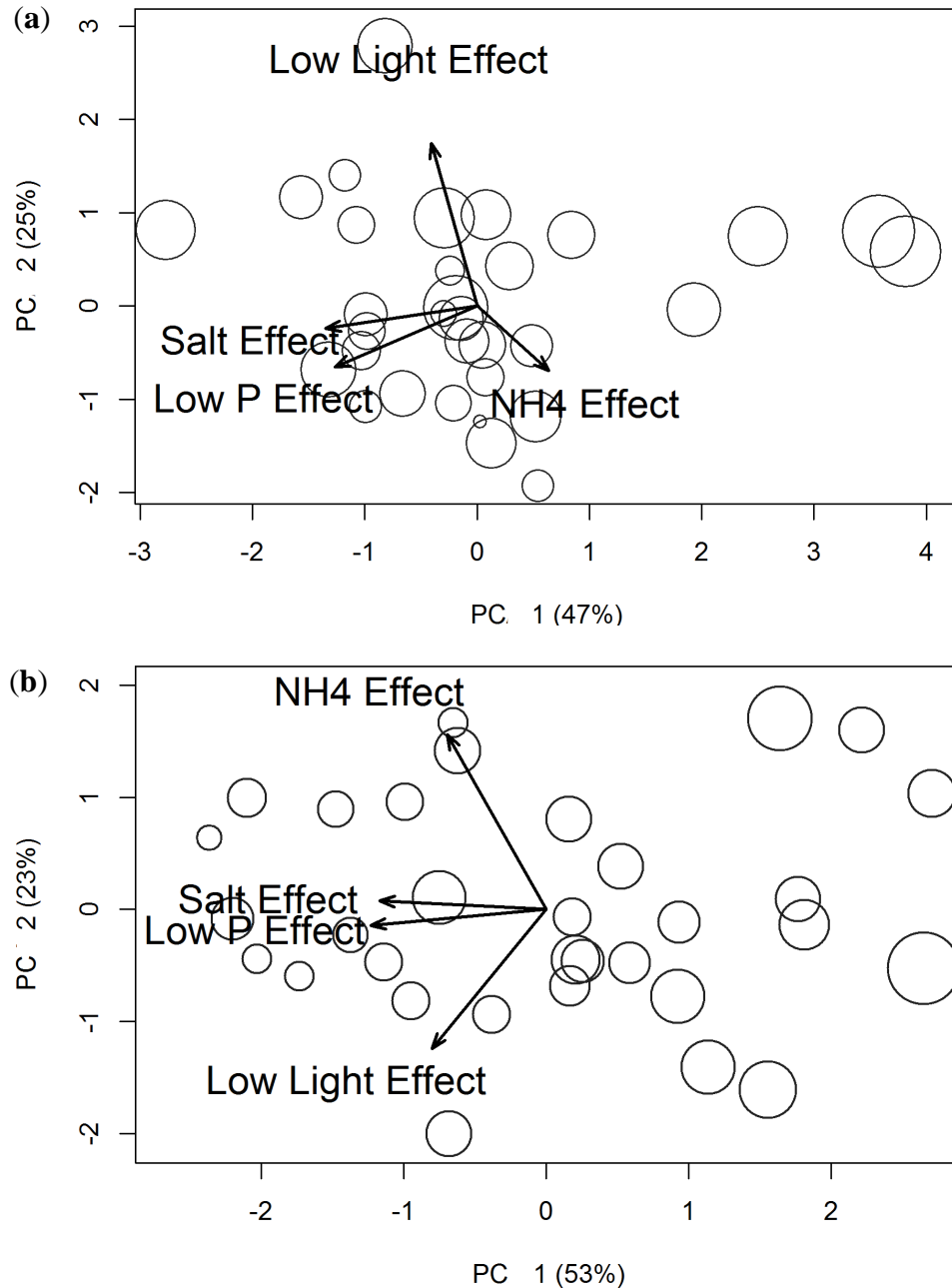


Figure 8: (a) Loading of treatment effects on μ in batch culture onto the first two principle component axes of a PCA using these 4 variables. Respective loading coefficients of E_{NH_4} , E_{Salt} , $E_{Low\ Phosphorus}$, and $E_{Low\ Light}$ on μ are (0.32, -0.67, -0.63, -0.20) on PC1 and (-0.31, -0.11, -0.32, 0.89) on PC2. (b) Loading of treatment effects on K in batch culture onto the first two principle component axes of a PCA using these 4 variables. Respective loading coefficients of E_{NH_4} , E_{Salt} , $E_{Low\ Phosphorus}$, and $E_{Low\ Light}$ on K are (-0.35, -0.58, -0.61, -0.40) on PC1 and (0.78, 0.04, -0.07, -0.62) on PC2. All loadings are multiplied by 2 for clarity. Percentages in the axis labels are the percentage of variation explained by each axis. Each circle represents a strain, with the size of the diameter corresponding to $\mu_{control}$ in (a) and $K_{control}$ in (b).

Table 1: Treatment conditions for strains grown in semi-continuous culture.

Treatment Condition	NH₄	Salt	Low Phosphorus	Low Light	Chytrid
Control levels	1 mM NO ₃ ⁻ as nitrogen source	0 M NaCl	50 μM PO ₄ ³⁻	25-30 μM s ⁻¹ m ⁻²	No chytrid
Treatment levels	1 mM NH ₄ ⁺ as nitrogen source	0.25 M NaCl	5 μM PO ₄ ³⁻	5-10 μM s ⁻¹ m ⁻²	3 mL/L chytrid fungus

Table 2: Treatment conditions for strains grown in batch culture.

Treatment Condition	NH₄	Salt	Low Phosphorus	Low Light
Control levels	1 mM NO ₃ ⁻ as nitrogen source	0 M NaCl	50 μM PO ₄ ³⁻	25-30 μM s ⁻¹ m ⁻²
Treatment levels	1 mM NH ₄ ⁺ as nitrogen source	0.1 M NaCl	5 μM PO ₄ ³⁻	5-10 μM s ⁻¹ m ⁻²

References

- Blomqvist, S., Gunnars, A., & Elmgren, R. (2004). Why the limiting nutrient differs between temperate coastal seas and freshwater lakes: A matter of salt. *Limnology and Oceanography*, 49(6), 2236-2241.
- Blows, M. W., & Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology*, 86(6), 1371-1384.
- Chase, J. M., & Leibold, M. A. (2003). *Ecological niches: linking classical and contemporary approaches*. University of Chicago Press.
- Clarens, A. F., Resurreccion, E. P., White, M. A., & Colosi, L. M. (2010). Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science & Technology*, 44(5), 1813-1819.
- Darling, E. S., Alvarez - Filip, L., Oliver, T. A., McClanahan, T. R., & Côté, I. M. (2012). Evaluating life - history strategies of reef corals from species traits. *Ecology Letters*, 15(12), 1378-1386.
- Edwards, K. F., Klausmeier, C. A., & Litchman, E. (2011). Evidence for a three-way trade-off between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. *Ecology*, 92(11), 2085-2095.
- Falkowski, P. G., Katz, M. E., Knoll, A. H., Quigg, A., Raven, J. A., Schofield, O., & Taylor, F. J. R. (2004). The evolution of modern eukaryotic phytoplankton. *Science*, 305(5682), 354-360.
- Garland Jr, T. (2014). Trade-offs. *Current biology: CB*, 24(2), R60-1.
- Gigante, Bethany. "SALINE ADAPTATION OF THE MICROALGA *Scenedesmus dimorphus* FROM FRESH WATER TO BRACKISH WATER." Electronic Thesis or Dissertation. Cleveland State University, 2013. OhioLINK Electronic Theses and Dissertations Center. 09 Feb 2014.
- Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American naturalist*, 1169-1194.
- Grover, J. P. (1997). *Resource competition* (Vol. 19). Springer.
- Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In *Culture of marine invertebrate animals* (pp. 29-60). Springer US.
- Harpole, W. S., & Tilman, D. (2007). Grassland species loss resulting from reduced niche dimension. *Nature*, 446(7137), 791-793.

Hartzell, J. L., & Jordan, T. E. (2012). Shifts in the relative availability of phosphorus and nitrogen along estuarine salinity gradients. *Biogeochemistry*, 107(1-3), 489-500.

Hutchinson, G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals?. *The American Naturalist*, 93(870), 145-159.

Hutchinson, G. E. (1961). The paradox of the plankton. *The American Naturalist*, 95(882), 137-145.

Interlandi, S. J., & Kilham, S. S. (2001). Limiting resources and the regulation of diversity in phytoplankton communities. *Ecology*, 82(5), 1270-1282.

Kneitel, J. M., & Chase, J. M. (2004). Trade - offs in community ecology: linking spatial scales and species coexistence. *Ecology Letters*, 7(1), 69-80.

Klausmeier, C. A., Litchman, E., Daufresne, T., & Levin, S. A. (2004). Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature*, 429(6988), 171-174.

Leibold, M. A. (1996). A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. *American Naturalist*, 784-812.

Leibold, M. A., Chase, J. M., Shurin, J. B., & Downing, A. L. (1997). Species turnover and the regulation of trophic structure. *Annual review of ecology and systematics*, 467-494.

Letcher, P. M., Lopez, S., Schmieder, R., Lee, P. A., Behnke, C., Powell, M. J., & McBride, R. C. (2013). Characterization of *Amoebophilum protocoecum*, an algal parasite new to the Cryptomycota isolated from an outdoor algal pond used for the production of biofuel. *PloS one*, 8(2), e56232.

Lind, E.M., Borer, E., Seabloom, E., Adler, P., Bakker, J.D., Blumenthal, ... Wragg, P.D. (2013). Life-history constraints in grassland plant species: a growth-defense trade-off is the norm. *Ecology Letters*, 16(4), 513-521.

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade - offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecology letters*, 10(12), 1170-1181.

Litchman, E., & Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, 39, 615-639.

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21(4), 178-185.

Park, J., Jin, H. F., Lim, B. R., Park, K. Y., & Lee, K. (2010). Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. *Bioresource technology*, 101(22), 8649-8657.

Passarge, J., Hol, S., Escher, M., & Huisman, J. (2006). Competition for nutrients and light: stable coexistence, alternative stable states, or competitive exclusion?. *Ecological Monographs*, 76(1), 57-72.

Ptácnik, R., Andersen, T., Brettum, P., Lepistö, L., & Willén, E. (2010). Regional species pools control community saturation in lake phytoplankton. *Proceedings of the Royal Society B: Biological Sciences*, 277(1701), 3755-3764.

R Core Team (2013). R: A language and environment for statistical computing (Version 3.0.2) [Software]. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.R-project.org/>.

Reynolds, C. S. (1988). Functional morphology and the adaptive strategies of freshwater phytoplankton. Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, 388-433.

Rhee, G. Y., & Gotham, I. J. (1981). The effect of environmental factors on phytoplankton growth: light and the interactions of light with nitrate limitation. *Limnology and Oceanography*, 26.

Tilman, D. (1982). Resource Competition and Community Structure.(MPB-17) (No. 17). Princeton University Press.

Tilman, D., & Pacala, S. (1993). The maintenance of species richness in plant communities. *Species diversity in ecological communities*, 13-25.

Yoshida, T., Hairston, N. G., & Ellner, S. P. (2004). Evolutionary trade-off between defence against grazing and competitive ability in a simple unicellular alga, *Chlorella vulgaris*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1551), 1947-1953.