TRAIT-BASED APPROACHES IN AQUATIC ECOLOGY

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Lay Abstract

Predicting what will happen to a habitat after a disturbance is critical for conservation and management. Species specific information is useful for building a mechanistic understanding of ecology. Predictions that include underlying processes (mechanisms) may be more robust to a changing environment than predictions based on correlations. Eutrophication, the addition of excess nutrients, is a common problem in freshwater habitats. Being able to predict the effects of nutrient addition is critical for ensuring the health of freshwater ecosystems. By using species-specific life history and morphological information and a simple lab system, I test different methods of predicting and understanding the consequences of eutrophication. I find that the ramifications of eutrophication are not easily predicted by species' categorizations or with a more detailed mechanistic model.

Abstract

Ecologists try to understand how changing habitats alter the populations of organisms living within them, and how, in turn, these changing populations alter the environment. By linking individual or cellular (physiological) processes to system level responses, mechanistic models can help describe the feedback loops between organisms and the environment. Aquatic systems have long used mechanistic models, but increasing model complexity over the last 50 years has led to difficulty in parameterization. In fact, it is often unclear how researchers are choosing parameters at all, even though small changes in parameters can change qualitative predictions. I explore the challenges in parameter estimation present in even an ideal situation. Specifically, I conduct individual experiments for all of the needed parameters to describe a simple lab-based, aquatic system; estimate those parameters using the results from these experiments supplemented with literature data; and run a large experiment designed to test how well the lab-estimated parameters predict actual zooplankton populations and nutrient changes over time. I document best practices for finding and reporting parameter choices and show whole ecosystem level consequences of a variety of decisions. To get the best predictions I find that a mix of parameter estimation methods are necessary. Trait-based approaches are another method to understand species-environment interactions. Trait-based methods aggregate species into functional traits, perhaps making qualitative predictions easier. Theory suggests that more functionally diverse systems will be more resilient. I test this prediction in a simple aquatic system but am unable to find consistent support for this hypothesis, and instead finding that results are highly dependent on what measures of ecosystem recovery are used. Overall, more species-specific information is critical to building better models for both mechanistic and trait-based approaches. I expand species-specific data by providing new information,

and collating information from literature on a small, tropical Cladocera.

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Table of Context

List of Figures and Tables

Chapter 2

Chapter 3

Chapter 4

Appendix A

Appendix B

Appendix C

List of Abbreviations and Symbols

NPZ Nutrient-phytoplankton-zooplankton

ODE Ordinary Differential Equation

PCoA Principal Coordinates Analysis

PERMANOVA Permutational multivariate analysis of variance

Declaration of Academic Achievement

This standard thesis contains an introduction (Chapter 1), a manuscript in revision (Chapter 3), two drafts of a manuscript in the early stages of preparation for publication (Chapter 2, 4), and a conclusion (Chapter 5). Chapters 1 and 5 were wholly written by me. Drs. Ben Bolker and Jurek Kolasa are co-authors for chapter 2. We all conceived of the idea. Dr. Bolker and I did the analyses. I ran the experiments and wrote the manuscript with edits from Dr. Kolasa and Dr. Bolker. Chapter 3 is a collaborative project with four undergraduate students. Dr. Kolasa and I conceived of the experiment. Alexander Phong, Lakhdeep Brar, Acacia Frempong, Ofure Oware and I ran the experiments. Mr. Phong and Ms. Frempong identified all of the algal communities, and Mr. Phong ran preliminary analysis on community structure. Mr. Brar ran preliminary analyses for *Daphnia magna* populations. I ran all final analyses. I wrote the manuscript with edits from all co-authors. Chapter 4 was co-written by myself and Dr. Kolasa. I ran the lab experiments and gathered the literature data. Dr. Kolasa collected the field data. I wrote the manuscript with edits from Dr. Kolasa.

Chapter 1:   Introduction

Functional trait-based approaches help answer fundamental ecological questions about species distributions and ecosystem functioning (e.g. Bremner, 2008; Cadotte et al., 2011; Cardinale et al., 2000) by focusing on mechanisms of species-environment interactions. Functional traits are a measurable aspect of a species that directly impact a particular piece of the ecosystem. This approach places less emphasis on species-species interactions and steps away from metrics like species richness, instead focusing on functional diversity. Functional diversity is a part of biodiversity that specifically relates to what traits, rather than what species, are present. Thus, functional trait-based approaches examine how species' functional traits interact with that species' niche. In doing so, these approaches are well suited for describing how species impact ecosystem function (e.g. Srivastava and Vellend 2005; biogemochemical cycles (Srivastava, 2002); productivity (Cardinale et al., 2000); and resilience (Peterson et al., 1998)).

   While not new, trait-based approaches have increased in usage since 2006, partially because they help describe species-environment interactions across gradients (McGill et al., 2006). For example, Kunstler et al. (2012) used traits to determine tree species distribution and found that differences in trait values (e.g. leaf mass area) were the best determinants of species presence. Similarly, following large scale fire events, Spasojevic et al. (2016) found that which sites were able to recover depended on the traits (e.g. seed type) of trees nearby and in the plots pre-fire. Beyond predicting what type of species will be present after a disturbance or across an environmental gradient, using functional traits instead of taxonomy has led to better predictions of ecosystem functioning over space and time (Mouillot et al. 2013; Mori et al. 2013, but

see Cadotte et al. 2009). Importantly, trait-based approaches suggest that either a single trait or a group of traits affect a specific part of the ecosystem and that this effect will be clear and predictable.

Methods that incorporate environmental gradients, like trait-based approaches, are increasingly important given the accelerating rate of anthropogenic disturbance. A changing environment leads to shifts in species' populations and distributions, which in turn leads to further changes in the environment. However, most trait-based studies emphasize the explanatory power of a focal trait on a specific ecosystem service (McGill et al., 2006). Thus, when ecologists want to ask questions about feedback loops between habitat change and shifting species demographics, a different approach than classic statistical regression or variance partitioning between traits and environmental factors is necessary.

A way to approach questions about the feedback loops between habitat and species demographics is through mechanistic models that incorporate species traits. Mechanistic models are mathematical models that link individual or cellular (physiological) level processes or interactions to whole system dynamics. Mechanistic models are used throughout ecology for a variety of purposes (e.g. to study disease transmission (Grassly and Fraser, 2008); to calculate sustainable harvests (Olmsted and Alvarez-Buylla, 1995; Kokko and Lindström, 1998; Beissinger and Bucher, 1992); and to gain insight to understand population dynamics such as periodicity (McCauley et al., 2008a) or predator-prey cycles (Shertzer et al., 2002)). Like classical trait-based approaches, mechanistic models are suited to asking questions about the impact of individual species on their environment. However, unlike trait-based methods, mechanistic models have the capacity to explicitly explore feedback loops and make projections into the future and into new conditions. Population and ecosystem mechanistic models in aquatic systems have led to insights about when predictions are most likely to be accurate

(e.g. when physical rather than chemical or ecological factors drive observed patterns (Robson, 2014b), the effects of climate change on population dynamics (Hart and Gotelli, 2011), and nutrient-consumer feedback loops (Nisbet et al., 1991)).

While trait-based approaches can answer broad questions about habitat change (e.g. what types of traits do we expect to see post disturbance?), mechanistic models can answer specific questions such as: "how long until species X is extinct?"; or, "how will changes in the population of species Y affect species X or ecosystem function Z?". Answers to both types of questions are critical for moving toward a broader understanding of ecology.

In this thesis I combine trait-based ecology with mechanistic approaches to address questions about aquatic systems post-perturbation. While most work in trait-based ecology has been done in plants (e.g. Ackerly, 2003; Lavorel and Garnier, 2002; Funk and Wolf, 2016; Funk et al., 2017) recent studies have gathered species-specific information in order to increase the use of trait-based methods in aquatic systems (e.g Litchman et al., 2010; Hébert et al., 2016). Connecting effect traits,that is, traits that directly effect the ecosystem, such as nutrient recycling, to ecosystem functioning is the next step in trait-based frameworks.

One of the major perturbations in aquatic systems is eutrophication. Therefor, I use a eutrophication event to explore the utility of trait-based approaches and mechanistic models in predicting responses to changes in aquatic systems. Cultural eutrophication occurs when run-off from farms, factories, or other anthropogenic sources add nutrients to a water body leading to algal blooms and hypoxic conditions. The most effective way of stopping cultural eutrophication is by stopping phosphorus inputs. However, many phosphorus inputs are from non-point sources and are therefore difficult to control (e.g. Scavia et al., 2014). Studying eutrophication is difficult because some of the consistent results found from field studies, for example, that phosphorous reductions

alone can reverse eutrophication, cannot be replicated in small scale nutrient addition experiments (Smith and Schindler, 2009; Schindler et al., 2016). Therefore, interactions between nutrients, algae and primary consumers remain poorly understood (Schindler et al., 2016). I determine if a mechanistic model can make reasonable predictions about nutrient, algal, and primary consumer concentrations after a eutrophication event in a simple system in Chapter 2. Additionally, functional diversity is thought to help stabilize systems and increase resilience after a perturbation. In Chapter 3 I ask if trait diversity can lessen the impact of eutrophication.

Resilience is an important measure of an ecosystem in regards to disturbance. Resilience was originally defined by Holling (1973) as the amount of disturbance a system can absorb without changing states. Understanding resilience and predicting when a system may be resilient is an important because as the world changes deciding which areas to preserve, how to increase the natural world's capacity to "bounce back", or resist change is an important conservation mandate. However, in practice, resilience has been defined in several different ways: the amount of perturbation a system can sustain (Gunderson, 2000; Ludwig et al., 1997), a measure of time until return to a state pre-disturbance (elasticity e.g. sensu Hodgson et al., 2015), or a combination of both (Hodgson et al., 2015; Yeung and Richardson, 2016; Hodgson et al., 2016; Côté and Darling, 2010). Despite the relatively simple idea, the variety of definitions of resilience and the difficulty in measuring resilience has led to inconclusive and often contradictory findings about what makes a system more likely to be resilient (Todman et al., 2016; Ingrisch and Bahn, 2018). And since resilience is often a conservation goal, contradictory evidence and vague definitions leave policy and planning difficult (Newton, 2016). Resilience is often thought to increase with functional diversity due to insurance; that is, multiple species may perform the same function, so that if one is extirpated another can take its place. However, this may not be accurate: resilience may

increase with biodiversity itself or may be mediated through more complex pathways (Downing and Leibold, 2010). In Chapter 3, I aim to distinguish between functional diversity and species diversity itself by setting up treatments that either increased in both richness and in trait diversity, or only in richness. Unfortunately, one of our filter feeding species was unable to survive and as such I was unable to perform the experiment as planned. I do however, find that resilience is not simply a function of diversity and is highly dependent on what ecosystem measures are tracked. These results further lend support to the idea that for policy in particular, goals and outcomes need to be specific.

To continue to explore the relationship, and particularly feedback loops, between species' traits and resilience in an aquatic system I built a mechanistic model that includes species-specific information for the three non-microbial components of a simple aquatic system: algae, zooplankton and nitrogen. The foundation for the mathematical part of this model is well understood, as aquatic systems have a long history of mechanistic modeling. Some of the earliest aquatic mechanistic are Nutrient-Zooplankton-Phytoplankton (NPZ) models that have been used since the 1930s (Robson, 2014a; Fleming, 1939). There has been extensive work on understanding model behavior and the effects of various functional forms (e.g. Fulton et al., 2003). Simple versions have been used to explore population dynamics and periodicity (e.g. McCauley et al., 1999, 2008b). However, aquatic mechanistic models have greatly increased in complexity over the past twenty years. As a result these models require a large number of parameters, but there has not been a concurrent increase in new data (Silberstein, 2006). Thus, these models' many assumptions are rarely well explored, nor are their predictions routinely checked. Due to the high dimensionality of ecosystem mechanistic models they are often either highly specific to a location (e.g. parameters are meaningless outside of the very specific system (Franks, 2009)), difficult to explore mathematically,

hard to verify, or it is difficult to gain any insight and results in being more descriptive than actually mechanistic (Franks, 2009).

Simple NPZ models have been extended to full ecosystem models including multiple functional groups of phytoplankton and zooplankton as well as microbes and biogeochemical processes. Comprehensive reviews of aquatic mechanistic models were published in Franks (2002), Franks (2009) and Robson (2014b). The primary takeaways from these reviews are: 1) use of mechanistic models requires better justification, because there currently is no evidence that they do a better job at predictions than statistical models; 2) mechanistic models should be used primarily to test specific hypotheses or when there are clear feedback loops; 3) most papers do not give quantitative measures of goodness of fit of their models; 4) better parameterization is necessary to improve these models and 5) uncertainty in parameters and model results needs to be thought through more carefully and reported. The bulk of my thesis attempts to address the final two problems highlighted in these reviews. My thesis largely agrees with point one, in that predictions I find from simple mechanistic models fail to match experimental data well. Parameterization is a particular problem because, frighteningly often, a parameter's source is not reported or single values are used or perpetuated from study to study without rigorous experiments. Even if a parameter is reported confidently, there is often variation in both the units in which a parameter is measured and in the actual reported value of the parameter (Robson et al., 2018). In large, multi-species or multi-functional group aquatic models (Blackford et al., 2004) this problem is exacerbated by the sheer number of unknowns, creating enough uncertainty in each step that making predictions outside of the original conditions (one of the goals of mechanistic models) problematic.

One promising avenue for improving parameter estimates is the use of Bayesian approaches. Since 2014 there has been a rise in the use of Bayesian methods in ecology

(e.g. Obenour et al., 2014). Bayesian approaches can account for different types of uncertainty (Schartau et al., 2017) and incorporate data from multiple different sources, hopefully making parameter estimates more accurate, or at the very least, making the source of variation more clear (Robson et al., 2018). Statistical advances (largely through improved technology) and the creation of large data amalgamations (e.g. Hébert et al., 2016; Robson et al., 2018) make it possible to make better parameter estimates and thus better predictive mechanistic models. In Chapter 2 I use a mix of approaches, including Bayesian approaches, to make parameter estimates and determine parameter uncertainty. This chapter highlights best practices for parameter estimation and documentation that need to become commonplace if aquatic mechanistic models are to be useful moving forward. While the resulting mechanistic model fails make good predictions post-perturbation, the methods I lay out show what needs to happen for mechanistic models to be more robust.

Finally, in Chapter 4, I provide life-history data from laboratory experiments and distributions from a literature search for a widespread, tropical zooplankton species, *Ceriodaphnia rigaudi*, in an effort to expand the available data sources on species-specific traits in zooplankton (as captured by the growing literature of zooplankton traits (Hébert et al., 2016; Barnett et al., 2007)).

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Chapter 2: Links between data and models: cautionary notes and limits

2.1 Abstract

Mechanistic models represent biological systems with mathematical equations that link individual level processes to whole system dynamics. Aquatic systems have a long history of mechanistic modelling for the purpose of prediction. However, these models have steadily increased in complexity, outpacing data capacity and model verification. One of the ongoing issues in aquatic modelling is the propagation of unverified parameters and lack of incorporation of parameter uncertainty. In this chapter we demonstrate ways to incorporate lab and literature data into parameter estimates and how to include uncertainty. We evaluate different possible decisions for each process and how each decision affects the final mechanistic predictions. We find that for best results a mixed approach for obtaining and estimating parameters is necessary.

2.2   Introduction

Mechanistic models are mathematical descriptions of biological processes that link interactions at the individual or cellular (physiological) level to whole-system dynamics (population growth, disease transmission, energy flows, and other features of interest) (Flynn et al., 2014; Robson, 2014b). Mechanistic models can be used to describe, for example, predator-prey dynamics or disease transmission (Keeling and Rohani, 2011), or used to make land management decisions (e.g. harvest rates: Merganičová et al., 2005; Johnston et al., 2015; Xie et al., 1999), using parameters such as the rates of cellular uptake of nitrogen, individual contact or organism death rates. Mechanistic modeling is sometimes used as a tool to reveal unknown properties of systems, narrow the plausible range of some process, or study emergent proprieties of complex biological systems resulting from feedback loops and non-linearities (e.g. McCauley et al., 2008). However, because mechanistic models are built upon fundamental biological interactions, in ecology they are often used to predict system-level processes in new environments (e.g. population growth (Scherrer et al., 2019) or habitat shifts with climate change (Thomas et al., 2016)).

In this context, the usefulness of a mechanistic model depends on how well the underlying biological processes are known. For example, mechanistic models can be poorly suited to describing systems where individual behavior does not translate to ecosystem and community level processes cleanly (e.g. Peckarsky et al., 1997), or where many of the interactions underlying observed dynamics are unknown (e.g. communities are made up of many understudied groups (Fulton et al., 2003)). When the goal is to predict system-level processes with a mechanistic model but underlying processes are unknown, it is inadvisable to rely on mechanism; if used, results from mechanistic models could be misleading and must be approached with skepticism (Anderson, 2005).

In complex aquatic systems, for example, there is no evidence that mechanistic models are better at making predictions than other methods (e.g. using statistical models to estimate correlations from observational data (Pennekamp et al., 2017)).

Aquatic systems models have forged ahead into increasingly complex models without careful attention to what is known of underlying processes (Flynn, 2005). The aquatic system literature is full of mechanistic models, with nutrient-phytoplankton-zooplankton (NPZ) models appearing as early as the 1930s. Over time aquatic models have steadily increased in complexity (with many recent models having over 100 parameters (see Fulton et al., 2003)). Yet, it remains unclear if these models are able to describe aquatic systems in the present, predict responses in new locations, or forecast into the future (Anderson, 2005). These outcomes remain unclear due to a widespread lack of validating models or reporting any goodness of fit metrics (Robson, 2014a). When validation or goodness of fit metrics do exist, models are do not predict population cycles of zooplankton and phytoplankton populations (Robson, 2014a; Arhonditsis and Brett, 2004; Arhonditsis et al., 2006). Therefore, when making predictions for large systems (whole lakes or oceans) our confidence in those predictions should not be high; yet they are often treated as "true" (e.g. highly cited with parameter estimates propagated through the literature and time (Robson, 2014a)).

One reason that these models fall short is due to the large number of parameters and the volume of data needed to parameterize. This quantity of parameters introduces ambiguity because different parameters can lead to different predictions (e.g Jiang et al., 2018); it is difficult to determine where models fail if parameters are unknown and it is unclear what contributes uncertainty in models is coming from (Robson, 2014a). Even with qualitative matching of ordinary differential equations (ODE) predictions and reality, large systems can notoriously give the same dynamics with different parameter combinations, which can lead to erroneous conclusions about a

biological system, possibly resulting in mismanagement or inappropriate interventions (Kao and Eisenberg, 2018).

Here we create a simple system where we know all of the parameters in order to determine if a simple NPZ model can predict ecosystem level process (ammonium cycling). We use this simple system to demonstrate that parameterization and fitting decisions at every level changes an ODEs predictions. We systematically document best-practices for picking and fitting parameters, and show how each step involves many researcher degrees of freedom (e.g. a garden of forking paths Gelman and Loken, 2014) that will ultimately affect final model predictions.

Choosing functional forms and parameter units is difficult; ideally the functions would be based on cellular activity (Bonachela et al., 2011; Allen and Polimene, 2011), but these processes are unknown. This difficulty is especially true if current mechanistic forms assume biological processes that are not the reality (e.g. trade-offs that *don't do not* exist Fiksen et al., 2013); since most researchers are not parameterize their models themselves and instead take data from the literature, often only old functional forms, like michaelis-menten, that are biologically less accurate, are the only parameters that are available (Fiksen et al., 2013). But getting the right parameters and functional form can greatly increase the accuracy of a model (e.g. Hararuk et al., 2015; Lignell et al., 2013).

Part of getting better parameter estimates is being able to use as much data as possible in order to reduce the number of true free parameters. Unfortunately, many obstacles get in the way of acquiring parameter values. Robson et al. (2018) outline several key issues: finding appropriate literature values is difficult, and often, even if there is literature, it is difficult to get the correct units or the magnitude varies hugely between studies; the process of parameterization is often poorly documented; and parameter uncertainty is not well understood. This type of uncertainty makes

it difficult to figure out where the model is wrong; a model with a large number of parameters can lead to over-fitting, where the model appears to perform well but be biologically incorrect (e.g. Mitra et al., 2007). Bayesian approaches are able to address some of these problems (Arhonditsis et al., 2008) because these approaches allow incorporation of multiple data sources (e.g. Obenour et al., 2014) and allow parameters to be constrained to biologically probable ranges (Zhang and Arhonditsis, 2009; Schartau et al., 2017). Despite difficulties with methods (e.g. sensitivity to prior choice), hierarchical Bayesian models can produce improved parameter estimates and predictions (Norros et al., 2017).

By carefully documenting our parameterization methods and using hierarchical Bayesian methods we hope to highlight options for researchers building their own models and demonstrate how decisions around parameterization should be documented so as to be able to critically evaluate the accuracy and implications of a given model. Furthermore, we are able to give uncertainty on each parameter itself. In this chapter we determine if a simple mechanistic model can predict a simple lab system.

2.3   Methods

2.3.1   Nutrient-Phytoplankton-Zooplankton (NPZ) models

Early NPZ models had three state variables (Franks, 2002), but have since been extended to include multiple species or functional groups at both the zooplankton (e.g. Hinckley et al., 2009) and phytoplankton levels (e.g Banas, 2011). Classically these models are constructed with respect to biomass, as each state variable can contain multiple species. Recent models often include microbial or detritus state variables and geographical structure as well (e.g. Schartau and Oschlies, 2003). For a comprehensive

review of how NPZ models are built see Franks (2002).

Using these models as a foundation, we write our NPZ model with two size classes of *Daphnia magna*, but follow *Daphnia* by individual and not biomass. Our full model is as follows:

$$\frac{dN}{dt} = -\frac{(aN)}{(k+N)}A + d_1lA - cN + x_ah_aD_a + x_jh_jD_j \tag{2.1}$$

$$\frac{dA}{dt} = \frac{(aN)}{(k+N)}fA - d_1A - h_aAD_a - h_jAD_j \tag{2.2}$$

$$\frac{dD_a}{dt} = gD_j - d_2D_a \tag{2.3}$$

$$\frac{dD_j}{dt} = \frac{b_1A}{(b_2A)}D_a - d_3D_j \tag{2.4}$$

where, $\frac{dN}{dt}$ describes the change in ammonium per unit time, and dA, dD_a, and dD_j referring to algae, individual adult Daphnia, and individual juvenile Daphnia respectively. All parameters are described in Table 2.1.

Table 2.1: NPZ model parameters.

Parameter	Description	Unit
a	algal maximum uptake of NH_4	mg N/(μg chl a*day)
k	algal half-saturation point for uptake of NH4	mg N
l	amount of NH_4 released upon algal death	mg N/ μg chl a
d_1	proportional death of algae	1/day
c	proportion ammonium lost to air or to other forms of N	1/day
x_a	adult daphnia excretion	mgN/μg chl a
x_j	juvenile daphnia excretion	mg N/ μg chl a
f	proportional algal growth given uptake	μg chl a/mgN
h_a	feeding rate of adult daphnia	μg chl a/(daphnia$_a$*day)
h_j	feeding rate of juvenile daphnia	μg chl a/(daphnia$_j$*day)
g	growth	1/day
d_2	adult daphnia death	1/day
b_1	maximum birth rate	daphnia$_j$/(daphnia$_a$*day)
b_2	food concentration at which half-maximum daily birth is achieved	μg chl a
d_3	juvenile death rate	1/day

2.3.2   Lab experiments for parameter estimation

We sought to experimentally estimate all of the parameters in our NPZ model (Table 2.1) so that it was fully calibrated to our lab conditions and organism lineages.

Experiments for *Daphnia magna*-associated parameters

Feeding and ammonium recycling rates: To determine the uptake rate of *Haematococcus* sp. by *Daphnia magna* (h_a), as well as the rate at which *Daphnia magna* recycled ammonium (x_a), we ran an experiment with seven treatments or algal concentrations (Table A.1) in which we measured starting algae and ammonium and then measured both again after six hours. Each treatment was replicated five

times. Each replicate contained approximately 15 individual *Daphnia magna* (counted at the end to avoid stressing the individuals prior to the experiment and potentially biasing estimates); changes in algae and ammonium were scaled to *per capita* estimates. We included three replicates for each treatment that did not contain any *D. magna* to account for any growth or death of *Haematococcus* sp. unrelated to grazing. This experiment was repeated for both *D. magna* size classes (<1mm or >1mm).

$$N_a = \frac{aTNP}{1 + aT_hN} \qquad (2.5)$$

Equation 2.5: where N is prey (here algae) density, a is attack rate, T is total search time,P is predator, here *D.magna*, density, and T_h is handling time.

While there is general support for a Holling type II functional response (Equation 2.5 of zooplankton consumption of phytoplankton (Mccauley et al. 1990; Paloheimo et al. 1982; but see Morozov 2010), this relationship varies for different resources (e.g. Gentleman et al., 2003; Jeschke et al., 2004, support a type I response). In our experiment *D.magna* food consumption remained linear over the range of algal concentrations we used; thus we assumed a linear relationship.

Birth, growth and death rates: From a lab maintained stable population of *Daphnia magna*, we haphazardly sub-sampled 73 individuals. Each individual was transferred into her own container and provided a constant food environment (Table A.2); replicate numbers varied because some adults from the lab-maintained population died prior to giving birth. We followed each individual until she had her first clutch, and then removed the adult and all but one randomly chosen juvenile. We

then followed the single juvenile *D. magna*, maintaining the constant food environment, until it grew to the second size class (>1mm), and then until death. All offspring were removed and counted over the lifespan of all tracked individuals.

Algae-associated parameters

Nitrogen uptake and population growth: To determine the growth rate of phytoplankton (*Haematococcus* sp.) at different nitrogen concentrations we used six treatments with different starting ammonium concentrations (Table A.3). Each treatment was replicated five times. We used a Miracle-Gro ® mixture that had an N:P ratio of 20:8, well above the required ratio needed for *Haematococcus* sp. (Fábregas et al., 2000). We therefore assume that nitrogen is the only limiting nutrient. Each replicate began at 46.19 ± 0.569 μg/L of chlorophyll-a. We measured chlorophyll-a, as a proxy for cell density and ammonium daily for twelve days. We assumed a saturating relationship between phytoplankton growth and nitrogen (Franks, 2002, see equation 4).

2.3.3   Estimating Parameters

In many ODE models a single value from the literature is used as a point estimate for parameter value, without accounting for variation in the parameter either in the actual biological response or from measurement error. This lack of variation is a problem because parameters can vary widely over space and time; even in a single instance there is uncertainty in a single parameter because of the estimation process (Robson, 2014a). Models can also be parameterized with local experimental data, but just using local data is still using only one measurement; incorporating previous knowledge about the system or species may be desirable. Depending on the choices a researcher makes,

predictions may differ substantially. Here we show strategies for fitting each of these techniques and implications for final outcomes. All models were fit in `R` (R Core Team, 2019) using the package `stan` (Carpenter et al., 2017; Stan Development Team, 2020). All Bayesian models were run using four chains; mixing was tested for by ensuring the Gelman-Rubin $\hat{R}$ statistic was < 1.01, that there were no divergent transitions in the accepted chains, and that Effective Sample Size (ESS) was greater than 10% of the total sample size.

For each parameter we outline each method we used to fit that parameter. We start with methods that only use our lab data, then expand into methods that incorporate literature data. The parameter estimates for each method are available in Table A.4 and fits with each method are found in the graphs listed in the description of the parameter. All code and data can be found on github at https://github.com/jwerba14/Species-Traits.

Fecundity: We model the relationship between daily fecundity and food availability (Chlorophyll-a: Chl) using a saturating relationship, represented with a Michaelis-Menten equation (Equation 2.6)

$$\frac{b_1 \cdot Chl}{b_2 + Chl} \tag{2.6}$$

We fit b_1 and b_2 using the following methods:

1. First, we used only our lab data and fit the Michealis-Menten equation using non-linear least squares. This fit is shown in Figure 2.1A.

2. For our second fit we used a Bayesian framework with wide priors. We assumed a half-normal prior, to restrict values to positive numbers, for b_1, b_2, and daily

fecundity, all with mean 0 and standard deviation 100 (Figure 2.1B.). Based on literature values daily birth rate is between 1.5 and 6.5, so this prior distribution is sufficiently wide to be relatively uninformative.

3. We incorporated literature values in mixed model framework fit in `stan`. We treat each study, including our own, as a replicate, allowing parameters b_1 and b_2 to vary by study (a random effect of study). We had five studies that reported food environment. Unfortunately, the food environment in the studies we found were measured by cell count. It is difficult to translate between cell count and chlorophyll-a because the relationship is species- specific and depends on the stage of the growth cycle. We found a possible conversion equation from (Ferreira et al., 2016). However, it is unclear how accurate this conversion is. Using this conversion puts most of the literature's algal data in the far low end of our experiments. Priors on b_1 and b_2 were relatively wide (half-normal(0,10)). (Figure 2.1C).

4. Since we were not confident of the conversion our food environments, for our fourth fit we used a prior on daily fecundity itself based on the distribution of daily fecundity from 11 papers (lognormal(1.4,0.6)); priors on b_1 and b_2 were half-normal(0,100). We constructed the prior distribution using all literature values with the package `fitdist` (Delignette-Muller and Dutang, 2015). Using this type of prior constrains the mean fecundity equally across all food types. (Figure 2.1D).

5. As another method to adjust our estimation process for the mismatched units of algal abundance we incorporated the literature only as a means to restrict maximum birthrate (b_1). To set a maximum birth rate we use the highest four fecundity numbers and assumed a fixed high food environment. We then

incorporated these studies in the same way as the mixed model framework above.
Priors on b_1 and b_2 were half-normal(0,10). (Figure 2.1E).

6. Finally, we parameterized fecundity using only literature data. We fit these data
   in `stan` with a lognormal prior on b_2 to restrict it to being positive. (Figure 2.1F).
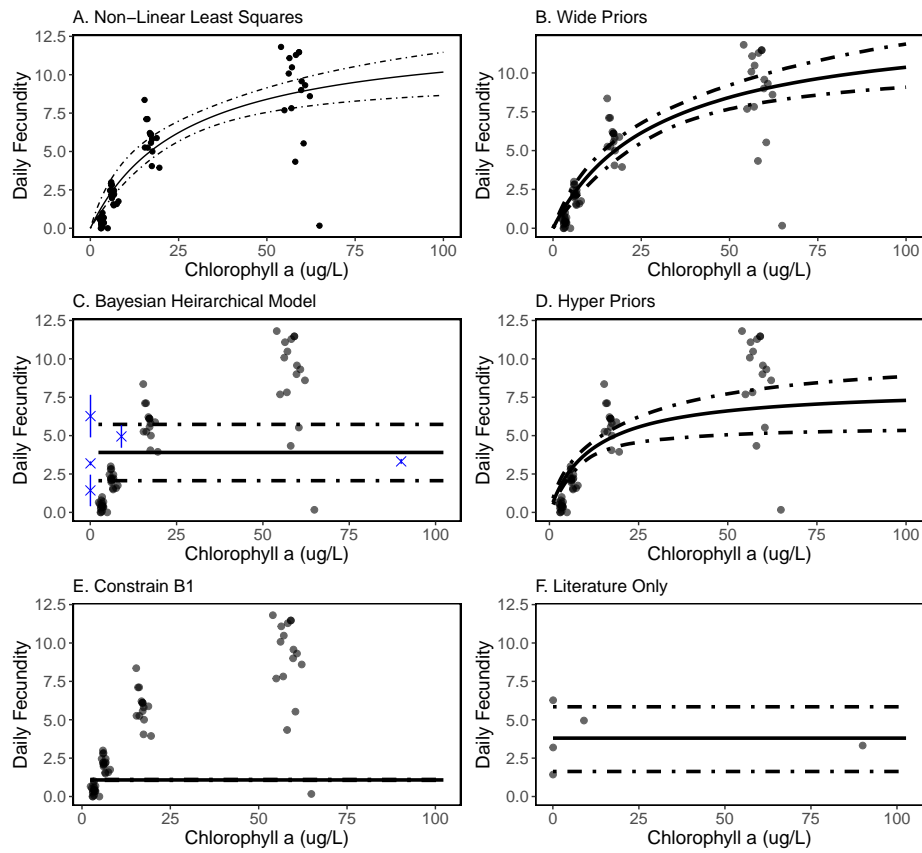

Figure 2.1: Fecundity predictions plotted with our lab collected data.
Dotted lines and envelope represent 95% confidence intervals. In
panel C. the blue "Xes" are literature values and error bars are
reported standard deviations.

Feeding and Excretion: We modelled adult feeding as a linear relationship to food
available based on our lab generated data:

$$h_a \cdot Chl_0 \tag{2.7}$$

Because the literature used a per hour scale we also fit on the hour scale and adjusted to a daily rate for the final model. As with fecundity, most literature reports algal concentrations in cell counts, while our data is in units of chlorophyll-a. So again, the literature values fall only in the lower end of our experimental data.

We only modelled excretion with our lab data. Excretion is a function of uptake. The relationship between excretion and uptake is often accomplished, in theory, by a parameter that represents how much nitrogen assimilation occurs; however, this is not measurable in our lab. Instead we fit a strict proportion to what was eaten:

$$x_a \cdot h_a \cdot Chl \tag{2.8}$$

Additionally, none of the literature values we found for excretion reported anything about food availability, uptake or assimilation so we could not incorporate literature values to constrain our estimates. Because excretion is directly dependent on feeding we fit both parameters simultaneously, in five different ways:

1. We used a Bayesian models with wide priors (prior on $x_a \sim$ normal(0,10), $h_a \sim$ normal(0,10)). See Figure 2.2A.

2. We ran a mixed model in `stan`. Only three studies reported error on their estimates of feeding rate, so only those were included here. The three literature values and our data are used to estimate a distribution of feeding rate, where feeding rate is allowed to vary by study (random effect of study). Due to the paucity of data, priors on error had to be fairly tight in order for this to meet our mixing guidelines outlines above. When there is little data, or the data are not very informative, determining the posterior is difficult and the answer depends strongly on the prior. (Figure 2.2B).

3. Alternatively, we used all the feeding literature data but estimated the standard deviations of all literature values where standard deviations were not reported. We estimated the un-reported standard deviations based on the distribution of reported standard deviations. (Figure 2.2C).

   We then estimated the feeding rate with only literature values using either:

4. including estimates of standard deviations for each study (i.e. accounting for measurement error) but assuming the parameters were the same for every study (i.e. no random effect of study) (Figure 2.2D) or,

5. without measurement error (did not include standard deviation) but allowing estimates to vary by study (kept a random effect of study). (Figure 2.2E).
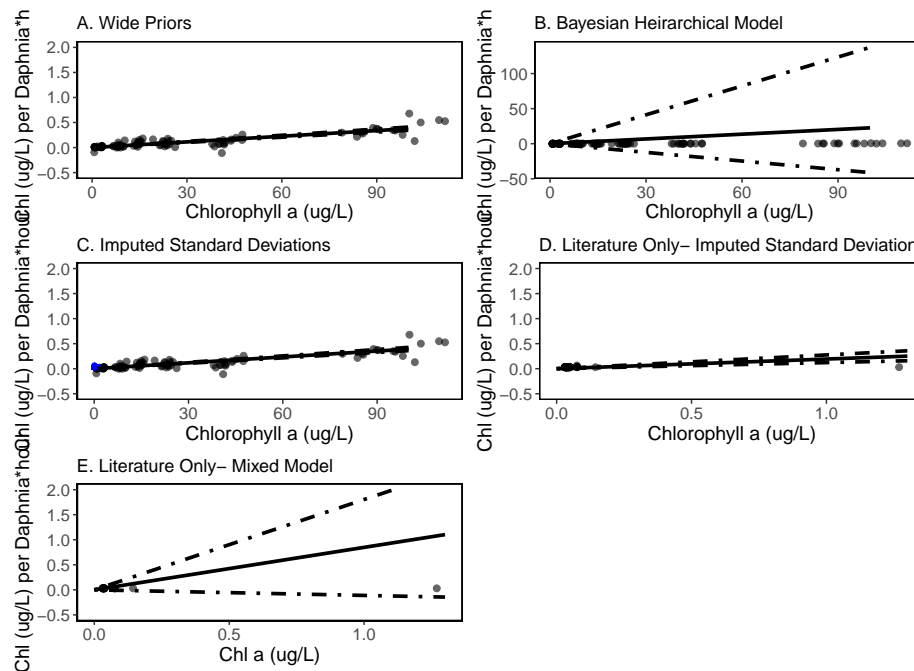

Figure 2.2: Feeding predictions plotted with our lab collected data. Dotted lines represent 95% confidence intervals. In panel C. the blue dots are literature values

The estimates for excretion were consistent across models (Figure 2.3).
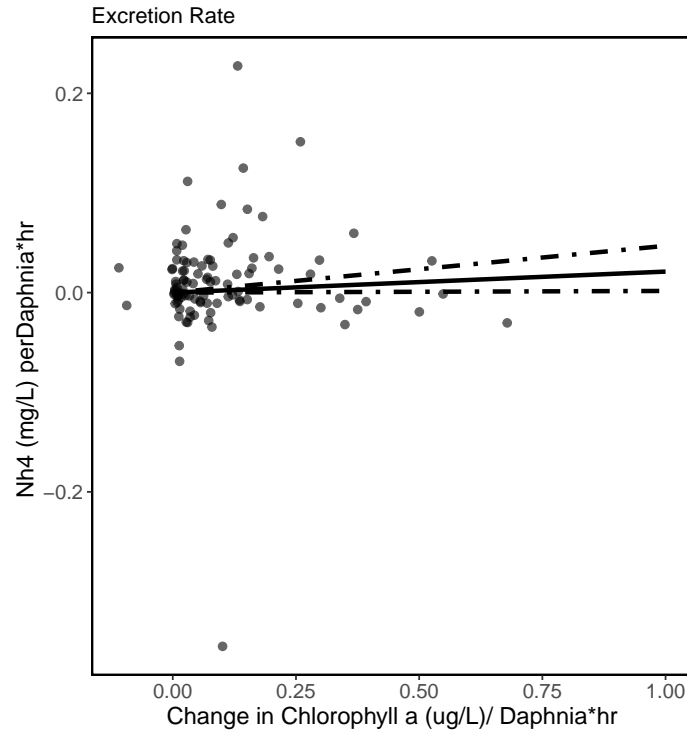
Excretion Rate

Figure 2.3: Predicted excretion per *D.magna* per hour predictions plotted with our experimental data. Dotted lines are 95% CI

Adult Death: We assumed exponential decay for death:

$$e^{-\frac{days}{d_2}} \tag{2.9}$$

Where $\frac{1}{d_2}$ is the rate and d_2 is the *e-folding time*, or the number of days until a fraction $\frac{1}{e} \approx 37\%$ of the original population is left. We didn't include food environment as a predictor for time until death, even though food certainly has an effect on longevity. In particular we see (and expect) that in low food environments individuals live longer (up until a point of starvation). We do not include food environment for simplicity and because the effect doesn't appear large in our data and often doesn't correspond to many, if any, new births in the extra days of life.

We modelled death in `stan` using just our data with a wide prior on death rate, (normal(0,100)) (Figure 2.4A). The data for this parameter was the most straightforward since most papers actually report the parameter itself. To incorporate the literature values we used a prior distribution based on the distribution of the literature values, fit using the `R` package `fitdistplus` (Delignette-Muller and Dutang, 2015) (normal(55,22)) (Figure 2.4B). For the literature only estimates we took the mean of the literature values in three different ways: weighted by 1/standard deviation, weighted by sample size, or unweighted (Figure 2.4C). Weighting by sample size assumes that the underlying variance is equivalent across studies, therefore, the inverse variance of the parameter is proportional to n.
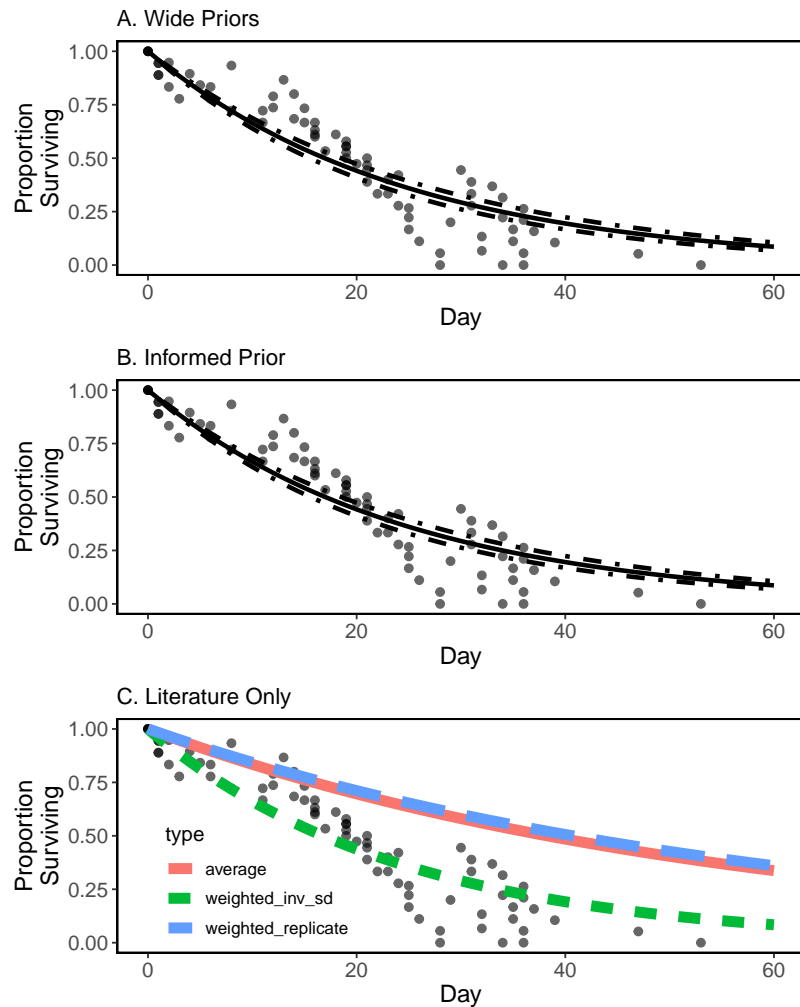
Figure 2.4: Proportion of *D.magna* surviving over time. Dotted lines in panels A and B are 95% CI. Panel C shows the three estimates from using literature values only.

Juvenile parameters: Because our measurement for our size classes was based on a filter (i.e. a size criterion) and not on molting stage it was difficult to find literature that matched our data well. Therefore, for all juvenile parameter estimates we only used our lab data.

Juvenile time to maturation: We fit juvenile maturation as an exponential function of time (the same model as time to death). This could likely be improved by

including different feeding environments, though there wasn't a clear difference between feeding groups in maturation time except for at the very lowest feeding conditions (Figure 2.5).
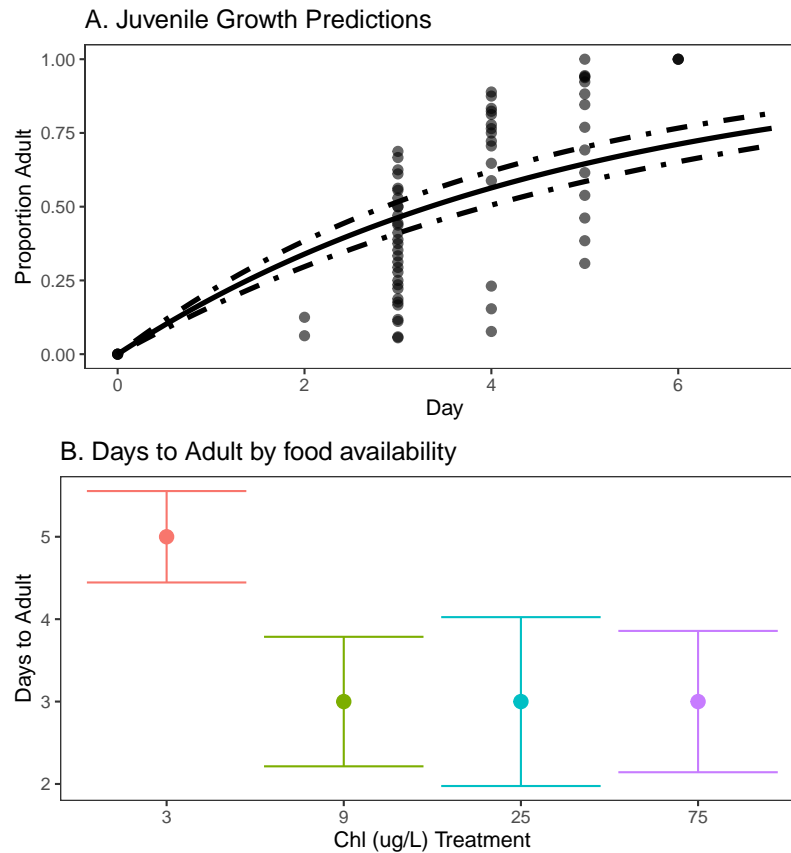
Figure 2.5: Predicted proportion of juveniles that move size classes by a given day plotted with our lab collected data, dotted lines represent 95% CI (A) and median days ± sd of days until death by treatment (B.)

Juvenile Feeding and Excretion rates: We fit juvenile feeding and excretion simultaneously with the same model we used for the adults (with wide priors, no literature values were incorporated)(Figure 2.6). We force juvenile excretion to be greater than zero by placing a lognormal prior on the slope parameter (x_j). We have to do this because our data is not sufficient to distinguish x_j from zero, which it must

be biologically.
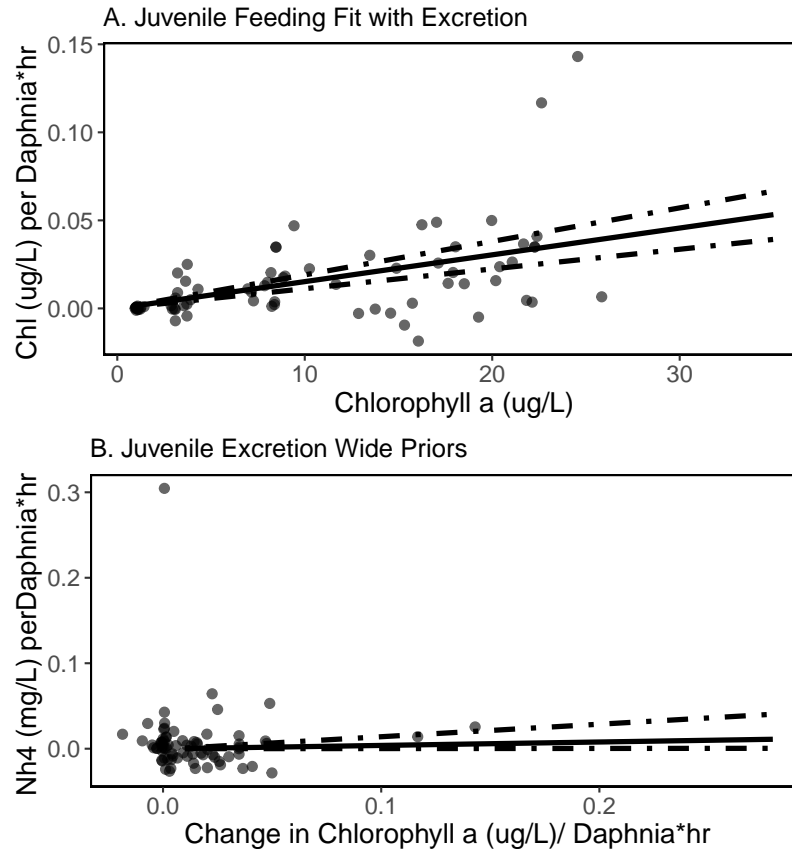
Figure 2.6: Predicted feeding rate (A) and excretion rate (B) for juvenile *D.magna* per hour. Dots are our lab data. Dotted lines are 95% CI.

Ammonium and algal growth and uptake: Because algal growth and nutrient uptake are occurring concurrently and depend on each other we fit these parameters simultaneously. We created a 500000 entry Sobol sequence (a sampling algorithm known to cover sampling space more efficiently than Latin Hypercube (Burhenne et al., 2011)) using a function from the R package pomp (King et al., 2020). We built the Sobol sequence using a range of parameters based on the range of literature values we found. We looked for the parameter combination with the highest negative log likelihood for each treatment. To find confidence intervals for the parameters we used a weighted

quantile function in the package `hmisc` (Frank E Harrell Jr, 2020), where weights were set as exponential decay of the likelihoods (Figure 2.7). Fits for parameters can be seen in Figure 2.8.
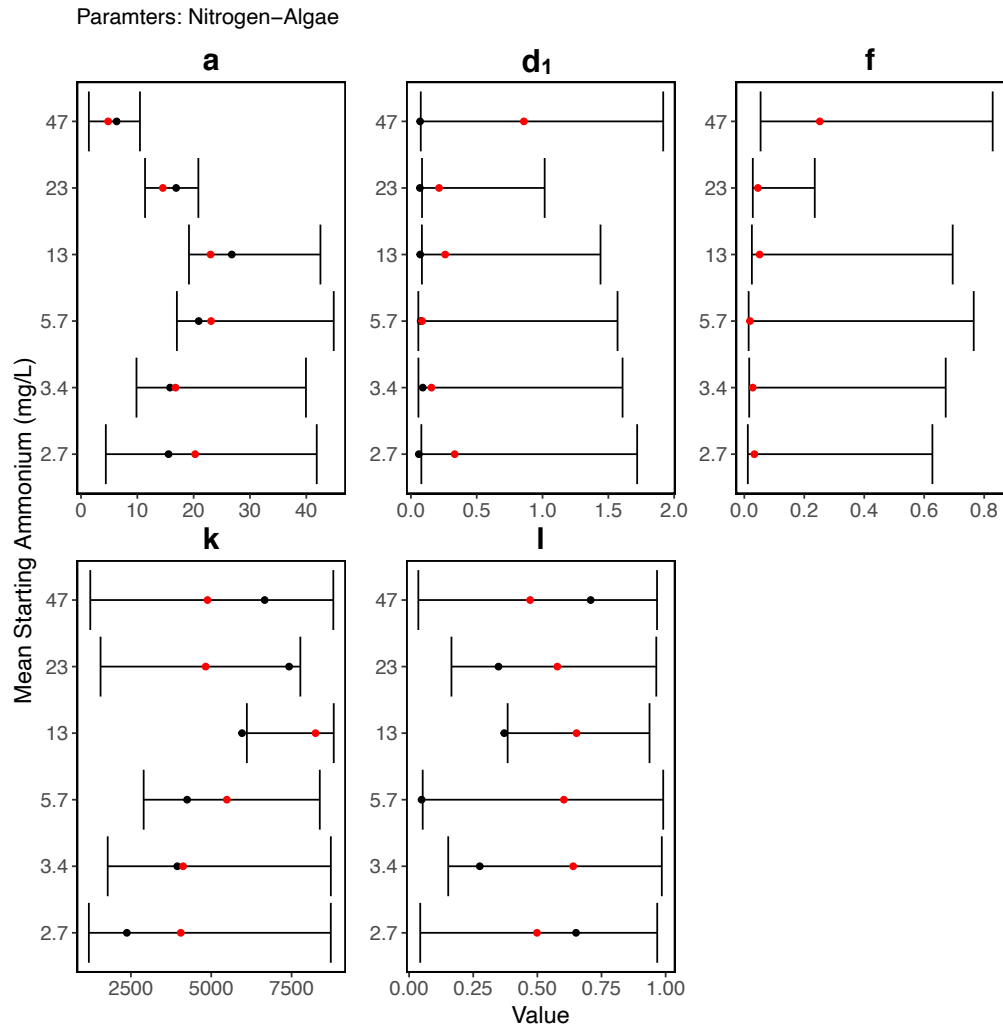
Figure 2.7: Parameter estimates and CI for all nitrogen-algae parameters. These parameters were fit simultaneously. The black dot is the best log likelihood estimate and the red dot is the median estimate based on our method of calculating quantiles.
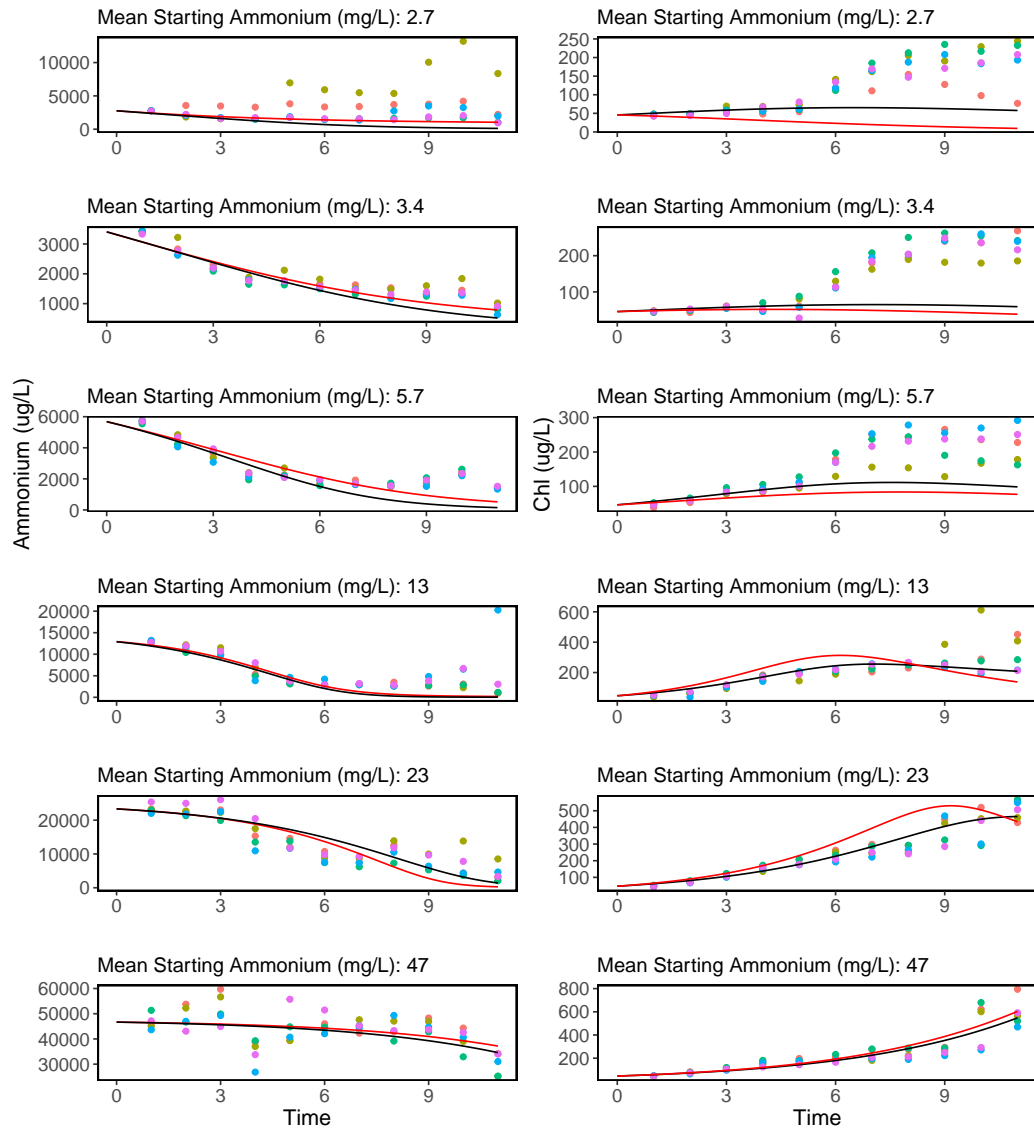
Figure 2.8: Best fit parameter estimates and CI for all nitrogen-algae parameters for each starting ammonium treatment. These parameters were fit simultaneously. The black line is the best log likelihood prediction and the red line is the median predictions based on our method of calculating quantiles.

2.3.4   Literature search for parameters

We paired our lab-estimated parameters with parameters obtained from published literature to build appropriate priors for each parameter. We were not trying to

complete a comprehensive literature review for each parameter. We were trying instead to gather enough information to construct informative priors.

For *Daphnia magna* parameters, we searched on July 1, 2019 using Google Scholar for: `"daphnia magna" and "growth" and "survival" and "reproduction"`, which resulted in 19600 hits. For this and all other searches we looked at the first 50 papers.

We only downloaded the paper if it was clearly about *Daphnia magna*, which resulted in 31 downloads of which 16 had data we could use. We then searched Google Scholar again on September 10, 2019 for: `"ammonium excretion" and "daphnia magna"` which resulted in 5,310 results. We downloaded 29 articles that were clearly about *Daphnia magna*, five of which had appropriate data. For our final Google Scholar search we used the terms `"daphnia magna" and "grazing rates"` (on September 12, 2019), which resulted in 7790 hits. We downloaded any papers about *Daphnia magna* that hadn't previously been found, which resulted in 38 hits. We downloaded these, and one additional paper that was cited in one of the others, for a total of 39; 15 of these had appropriate data.

For algal parameters we searched Google Scholar on May 29, 2019 for: `"algae death rates"` which resulted in 190,000 hits. We removed any papers about blue-green algae, any saline/marine species, or if the paper was about another organisms' reaction to different algal types. We only took articles that were looking at actual algal populations and not humans/cancer etc. or otherwise obviously not about freshwater algae. We excluded books. We looked at the first 200 papers of which we downloaded 55. From those 55 we found nine additional papers. From the total of 64 papers 21 had data we could use. We also used the same search strategy for `"algal growth rate"` for which we downloaded 56 papers and took data from 18; we also searched `"algal nitrogen uptake"` for which we downloaded 63 and took data from 16 papers.

Many papers had information on more than one parameter; for the actual number of data points per parameter see each particular model. A full list of downloaded papers and notes about data that we did or did not extract (including extracted data) can be found in the github repository. When data was presented in a graph we used Data Thief (Tummers, 2006) to extract the data.

2.3.5   Full lab experiment

We designed an experimental lab system to conform to the NPZ equation presented previously. Then, using the *a priori* estimated parameters we compare model results to experimental results.

Experimental Design: The experiment ran for six weeks. All tanks began with algal concentrations of 14.5 ± sd 2.3 μg Chlorophyll-a/L. We filled tanks with 1 L of water and either with (20 tanks) or without (20 tanks) 20 adult (>1 mm) *Daphnia magna*. We measured chlorophyll-a daily (using an AquaFlor flourimeter) and measured ammonium (mg/L) twice a week (using a YSI Pro Plus). We counted *Daphnia magna* populations twice a week by using four 10 mL sub-samples. *D. magna* were counted in two separate size classes: < 1 mm and > 1 mm. Tanks were kept at a median temperature of 19°C. After three weeks, half of the tanks received a one-time influx of nutrients (100 mL of 1g/L concentration of Miracle Gro®) as a single perturbation event. We then continued data collection for an additional three weeks.

Experiment and Model Evaluation: We ran the full ODE over all 375 parameter combinations and evaluated fit using log likelihood. We did this separately for model/treatments with and without a perturbation and for the treatments with and without *Daphnia magna*. Because we have no data for juvenile death we ran the best

fit sets over a range of possible juvenile death parameters.

Additionally, we created a 500000 entry Sobol sequence over a biologically reasonable range of all parameters to find the best parameter fit and then compared those parameters to our data.

To calculate confidence intervals on the full model we used the posterior distributions for the parameters that were estimated in `stan`. For the algal-ammonium parameters (a, k, l, f, d_1) we created a weighted distribution by likelihood from the original fits. Then we ran the ODE across the created 4000 parameter sets and selected the 95% quantities for each state variable.

2.4   Results

We found the best fit parameter set (largest negative log likelihood) uses a mix of parameter estimation methods (Table 2.2), but even the best set does not predict the experimental data particularly well (Figure 2.9).

Table 2.2: Set of parameter values that produced the largest negative log-likelihood

Parameter	Method	Value
b_1	mixed model	3.9
b_2	mixed model	6.9
h_a	wide priors	0.09
x_a	mixed model, varying slopes	53.9
d_2	literature only- weighted by replicate	58.8

Figure 2.9: Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) for the best parameter set (black line). Dotted lines are 95% CI.

When we predict with literature only parameters (where available) we get a worse match (Figure 2.10) than either our best set or a parameter set (Figure 2.9, Δ log-likelihood = 61) and from our lab experiments (Figure 2.11 Δ log-likelihood = 35).

Figure 2.10: Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) using only literature values (black line). Dotted lines are 95% CI.

Figure 2.11: Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) using only lab values (black line). Dotted lines are 95% CI.

This is consistent in the disturbed treatments (Figure 2.12, Figure 2.13 Δ log-likelihood 12 from greatest negative log-likelihood, and Figure 2.14 Δ log-likelihood = 24 from greatest negative log-likelihood).
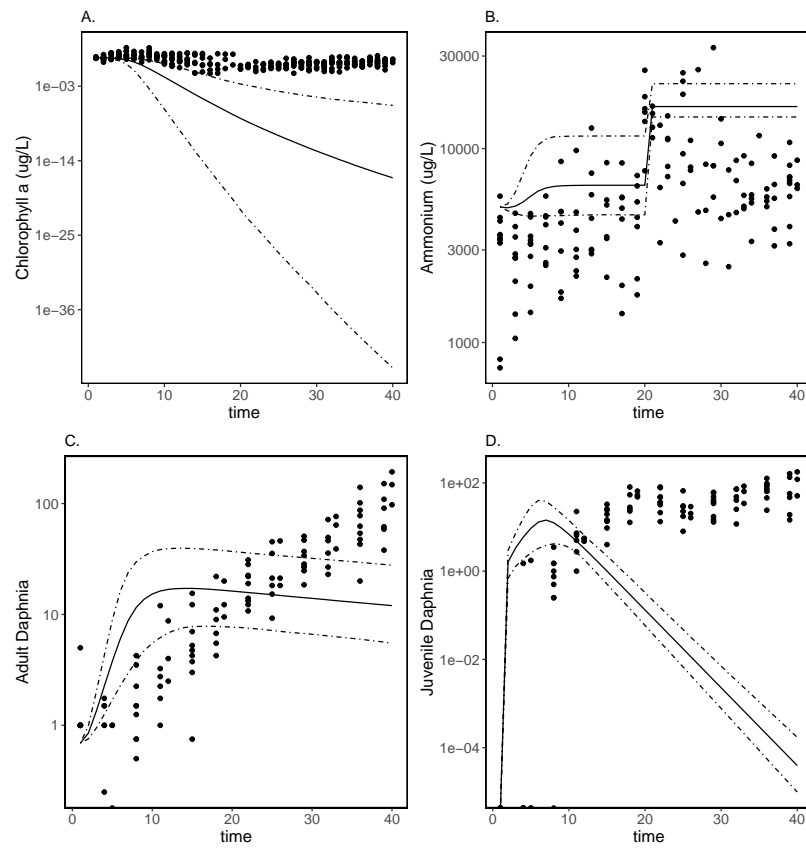
Figure 2.12: This figure shows our best parameter set when there is a single eutrophication event. Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) (black line). Dotted lines are 95% CI.
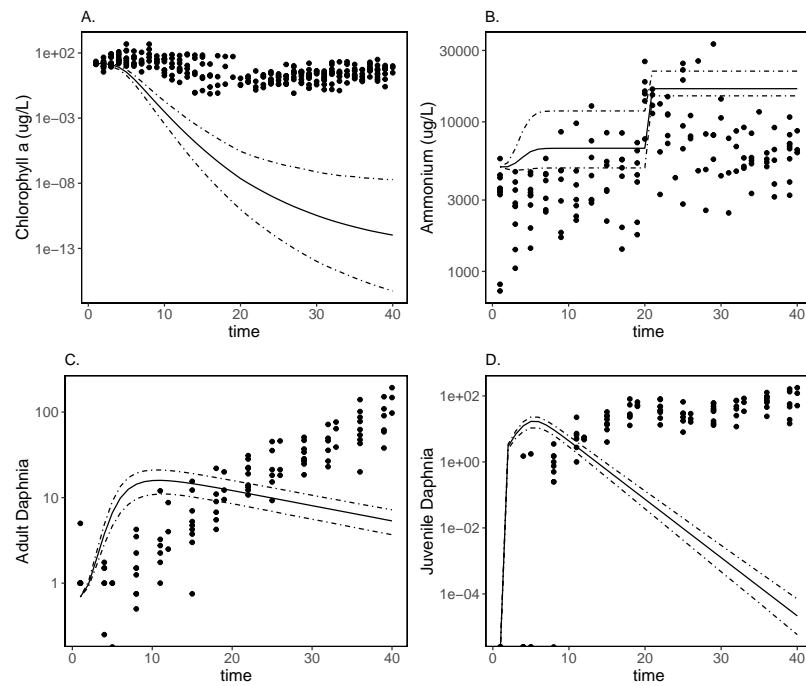
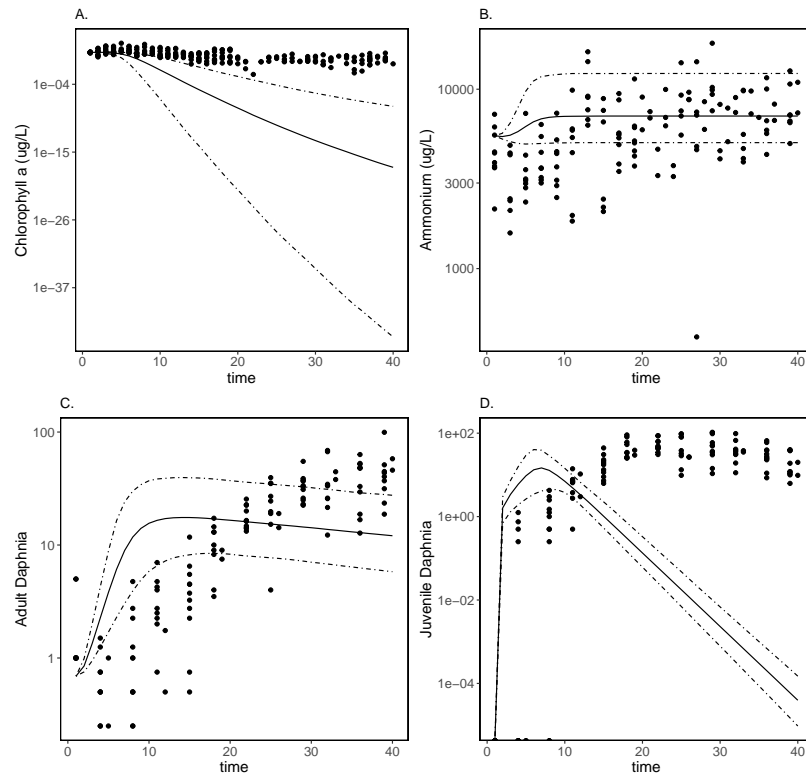Figure 2.13: Predictions with a eutrophication event using only lab values when available. Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) are represented by the black line. Dotted lines are 95% CI.

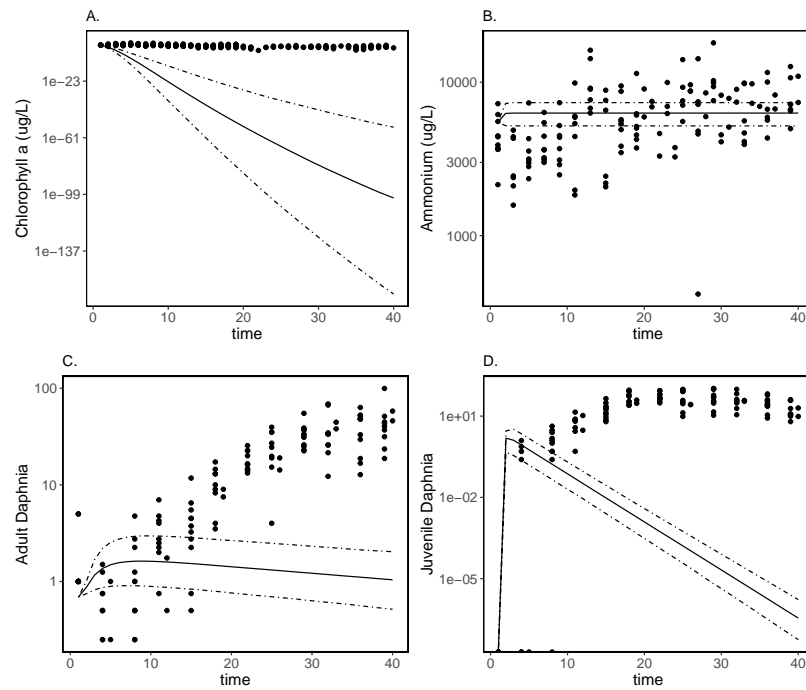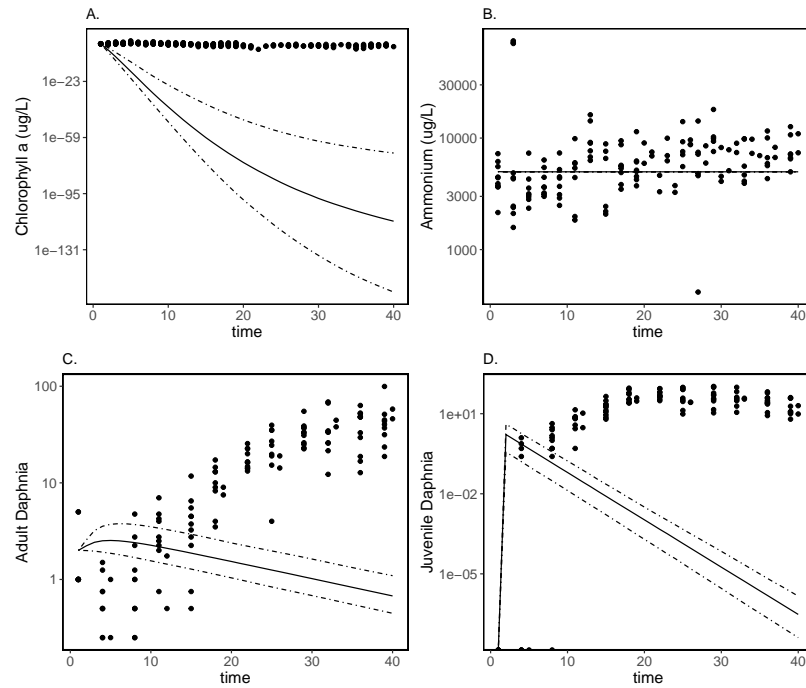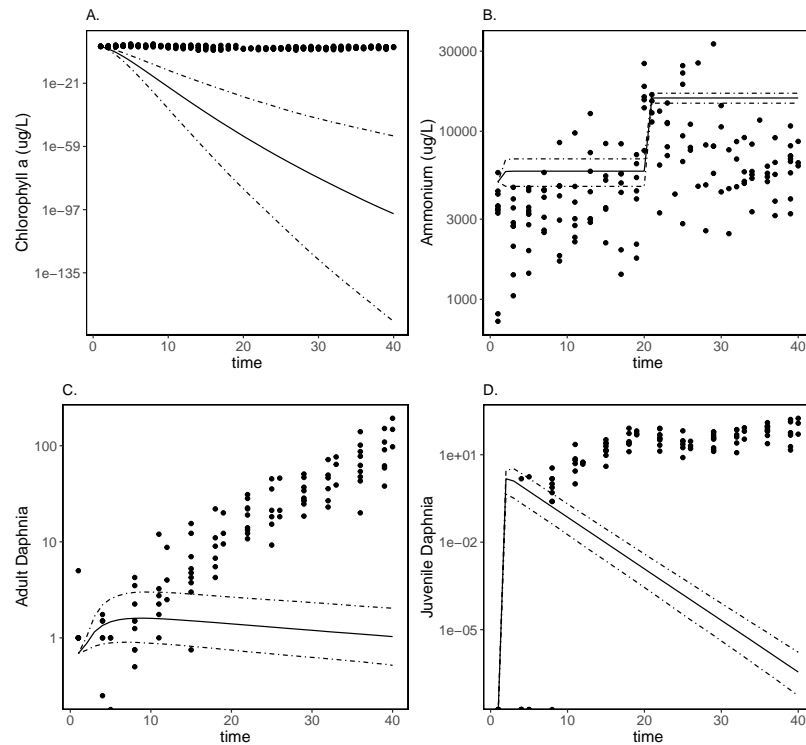Figure 2.14: Predictions with a eutrophication event using only literature values when available. Predicted outcomes for each of state variable, algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* (D.) are represented by the black line. Dotted lines are 95% CI.

2.5   Discussion

Simple mechanistic models are a cornerstone of research in biology. They have advanced our understanding of many biological processes including, for example, disease spread (Heesterbeek and Roberts, 2015; Keeling and Rohani, 2011), population dynamics (e.g. Scherrer et al., 2019), and nutrient cycling (e.g. McMurtrie, 1985). Mechanistic models are particularly well suited for making predictions in situations where there are strong feedback loops (Robson, 2014b) or when the environment will be different from when the initial measurements were made (i.e. out of sample predictions, (e.g. with

different climate scenarios: Montalto et al., 2016)). In aquatic sciences, management plans require accurate predictions in the face of large scale environmental problems (Pace, 2001). However, aquatic mechanistic models have become increasingly more complex. These complex mechanistic models often make less accurate out-of-sample predictions than either simpler mechanistic models or statistical models (Robson, 2014b). In aquatic ecology, a recent trend towards more complex models with unvetted predictions is, at best, reducing the impact of these studies, and at worst, leading to unsupported management plans or constructing a fragile foundation upon which other work builds.

In theory, a better understanding of a system should lead to better models and predictions (Pace, 2001). At the very least, a deeper mechanistic understanding of a system helps reduce bias in predictions (Schuwirth et al., 2019). However, obtaining a well-parameterized mechanistic model of a complex aquatic system is difficult, even if the mechanisms operating in that system are well described, because of the sheer number of parameters needed to describe the known biological interactions operating in that system. Finding or estimating each parameter value is difficult: often literature values for parameters are irrelevant (e.g. only from related species, or from a different location), in units that are not easily convertible, or the variation in parameter values found in the literature is very large (Robson et al., 2018). Because of the difficulties in model parameterization, most mechanistic models often use point estimates, ignoring uncertainty in these values (Ramin et al., 2011; Arhonditsis et al., 2007). However, poor parameterization or ignoring uncertainty can lead to biased and overly confident answers, poor predictions or an inability to accurately account for what mechanisms are actually driving dynamics. Ultimately, this can result in a deep misunderstanding of a system (e.g. for disease predictions Kao and Eisenberg, 2018). While this is problematic for all research, it is especially detrimental for applied research designed

to make management decisions, as these require risk assessment in order to develop realistic expectations for different management strategies (Reichert, 2020; Schuwirth et al., 2019; Ramin et al., 2011).

In this chapter we showed multiple ways to find, fit, and report parameter values, and illustrated the implications of these choices for predictions in a simple aquatic system.

Despite careful parameter estimation, not a single state variable's data from our full lab experiment was well described by our system of ODEs. In particular, algal populations were poorly represented across all parameter sets; in all cases model predictions show algal populations declining to approximately 0 rapidly which causes *D. magna* populations to crash, and neither algal nor *D.magna* populations are able to recover. Similarly, dynamics of juvenile *D.magna* were poorly explained by our model across all parameter sets; in all parameter sets the peak population of juvenile *D.magna* occurs almost two weeks before the actual peak and is smaller than what we observed in the experiment.

There are a number of defensible choices we *could* have made but didn't (e.g. change functional forms such as allowing *D. magna* feeding with a Hollings type II curve (Mccauley et al., 1990; Paloheimo et al., 1982), or using a Droop equation for algal nutrient uptake (Droop, 1974)); this non-exhaustive lists highlight the wide range of possible dynamics that could be predicted in even a simple system. Importantly, many alternative decisions would lead to different outcomes. For example, changing the form of transfer function of feeding by *D. magna* can cause populations to change from stable to oscillating (Gentleman and Neuheimer, 2008). In systems where many pieces are unknown, the number of different possible decisions greatly increases, leading to a compounding number of possible outcomes. Given the plethora of possible decisions, it is critical that all research using mechanistic models clarify how parameters are chosen

(which sadly is rarer than would be expected (Robson, 2014a; Robson et al., 2018)), incorporate uncertainty, and use as much biologically appropriate data as possible (e.g. parameter values from the appropriate environment).

There are several other potential reasons why even our best parameter set poorly described our experimental results. First, the base structure of NPZ models may not fit our system well. This was unanticipated, because our lab system was designed to conform to the simplest version of an NPZ model (Franks, 2002). The fact that our NPZ model was unable to describe the experimental system well raises many questions. Is leaving out, for example, microbial cycling so detrimental to predictions of zooplankton and algae populations that standard NPZ models are not worth fitting? Even in nitrogen limited systems (e.g. oceans) does phosphorus need to be included in the model (Nelson et al., 2020)? Perhaps algal populations need to be measured in biomass instead of chlorophyll-a (Nelson et al., 2020) or split into morphological groups (Kruk et al., 2011)? While it is possible that such a simple model misses some nuance in the data, the fact that it misrepresents the data this poorly is surprising. Even if we allow all parameters to vary we are unable to replicate the dynamics of the lab system particularly well (Figure 2.15). If this were a "real" system (i.e. not a controlled lab system), we would have successfully shown that our hypothesis, the NPZ model, did not model the dynamics of our system (Franks, 2002).
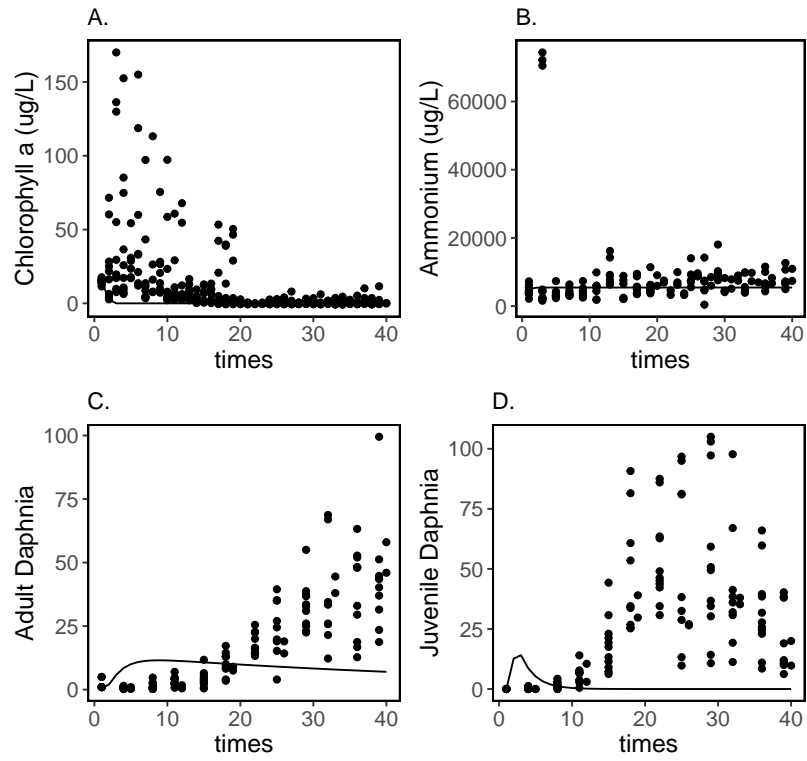
Figure 2.15: Model predictions for the best parameter from all combinations from our Sobol sequence. Dots are experimental data. Dark line is mean prediction and dotted lines are 95 % CI on the prediction.

Second, we made several assumptions about *D. magna's* interaction with algae that may have resulted in poor predictions of *D. magna* populations. We assumed that *D. magna* birth rate is only related to algal supply; however, because *D. magna* eat bacteria—and we had no way of tracking bacterial populations— perhaps *D. magna* won't stop reproducing as algal concentrations reach low numbers. In our current model *D. magna* populations crash earlier than expected and fail to reach expected population levels. We are able to improve model predictions of *D. magna* by adding a single additional food source (Figure 2.16).

Figure 2.16: Model predictions for each algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* across 0.01 to 0.4 for a new food source for *D.magna* once chlorophyll-a drops below a specific thresh-hold (0.01 μ g/L).

The type of food may have also caused problems in our predictions. *D. magna* interaction with food (algae) from our initial experiments potentially did not translate well to our full experiment because in our initial lab algal stock species was predominately *Haemetococcus* sp. and *Scenedesmus* sp. and in the main experiment we had mostly *Chlorella* sp. and *Scenedesmus* sp. (see Chapter 3, Figure B.6). These species composition differences likely led to different interactions with both nitrogen and *D. magna*, though all these species would likely be grouped together in most phytoplankton models as a green algae group (Litchman et al., 2007). Adding either microbial cycles or more algal groups (Kruk et al., 2011) would be important to accurately model *D. magna* or algal populations. However, this would add more parameters to estimate and more functional forms to consider. With so many caveats and unknowns even in

a controlled lab system, we expect this problem to be exacerbated in more complex systems.

A third possible problem is that we could not find nor estimate a juvenile *D.magna* death rate. We could not estimate juvenile death rate because in our initial life history experiments only three juveniles out of over fifty died. This rate is far lower than what we would expect in an actual population. We did not however, find major differences in fits across a range of juvenile death parameters (Figure 2.17).
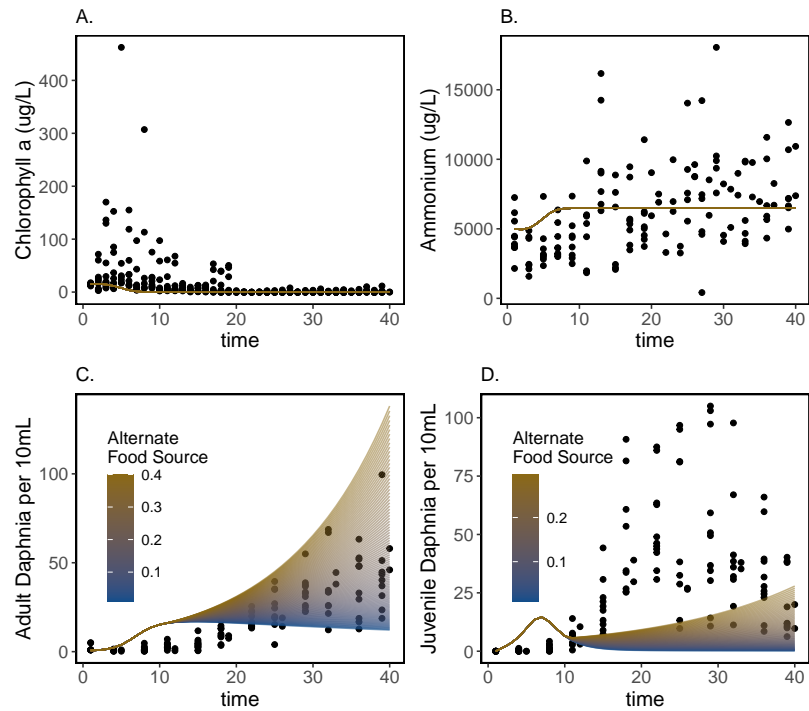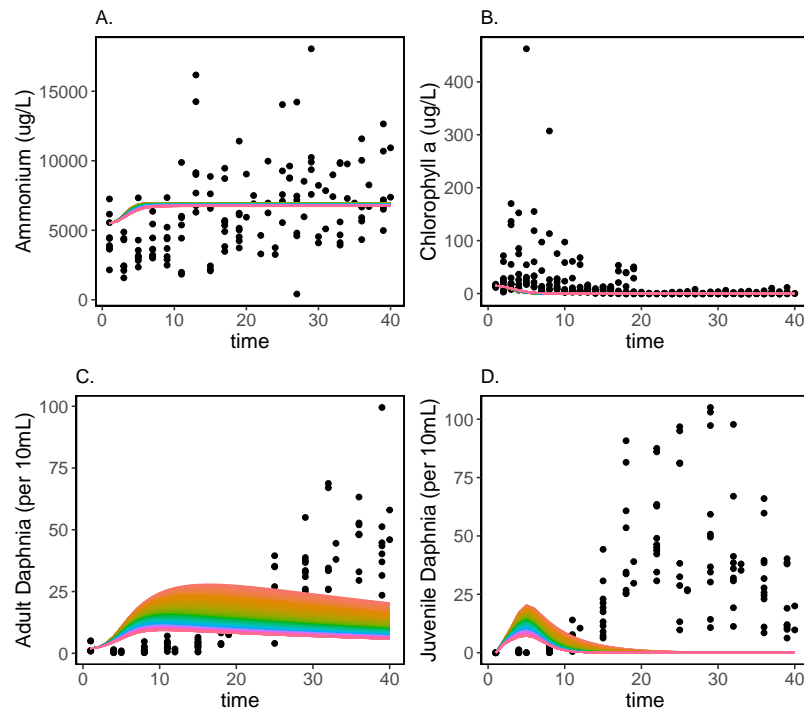

Figure 2.17: Model predictions for each algae (A.), ammonium (B.), adult (C.) and juvenile *D.magna* across 0.001 to 0.9 for juvenile death. Pink to orange gradient is small(0.001) to large (.9) values.

Overall, our models would be greatly improved by ensuring all of our priors are biologically informed. For example, in situations where we couldn't find species-specific parameters in the literature (e.g. juvenile excretion) we could use allometric equations to determine an expected range for the parameter (e.g. Lignell et al., 2013). While we

carefully estimated parameters, and calculated parameters based on food environment, parameters could also change over other environmental gradients (e.g. temperature Mordecai et al., 2013). Therefor, in our system, particularly the perturbed system, where nutrients levels changed drastically, it is possible we did not accurately model species behavior because parameter. More complete simulations of our model's behavior would help highlight if changing parameter estimates could account for differences between model predictions and experimental data (Norros et al., 2017).

Even though our best parameter set did not fit the experimental data well, when we used only literature values for parameters all state variable median estimates were worse and confidence intervals were too small (Figure 2.10). Literature values were regularly not in an appropriate scale (e.g. hours vs days) or in difficult to convert units (e.g. cell count to chlorophyll-a). We naively assumed that hourly rates linearly translated to day, but actual diel cycles of *Daphnia* sp. are varied (Haney and Hall, 1975; Haney, 1985). This assumption could lead to faster resource depletion. Overall, the differences in the way physiologists measure life history traits versus ecosystem ecologists leads to a disconnect between lab experiments and fitting to large scales. For example, *Daphnia* sp. physiologists generally measure food particles in cell count over the course of single hour, in starved *Daphnia* sp.; but, in a large lake this might not translate to the way *Daphnia* sp. eat or excrete over the course of a week (McMahon and Rigler, 1963). Even though many ecosystem models measure algae with chlorophyll-a, physiology experiments almost never do. The conversion between cell count and chlorophyll-a, is complex and possibly impossible (see methods). These mismatches (Robson et al., 2018) make it difficult to trust parameters for *your* specific model from the literature, but using only your own lab generated data is not only not practical but likely impossible for most models. Even in a well-studied system (Daphnia-algae), with a huge amount of literature, and in which we had the capacity to run multiple

experiments, it was still very difficult to find the "right" transfer functions and "right" parameters, and we ended up with poor fits.

Instead of building increasingly complex aquatic mechanistic models, our results suggest that more information is needed at the species (i.e. physiological) level in order to increase the accuracy of predictions of basic biological interactions. However, the mechanistic models with the best predictive results generally focus on only key pieces of the system (Pace, 2001; Robson et al., 2018; Chang et al., 2019). Given this we could deliberately change small pieces one at a time to understand what describes our system (Franks, 2009), in order to find those "key" pieces for our system (e.g. Chang et al., 2019). In fact, in systems that are mostly driven by physical forces we already are able to get reasonably good predictions (Robson, 2014b) so determining if a system needs biological aspects as part of the model could be a potential first step. But for systems that aren't well explained by physical parameters our project suggests that our current knowledge is likely insufficient to make predictions at large scales based on species specific information.

2.6   Acknowledgemnts

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Chapter 3: Increasing functional diversity increases some, but not all, measures of resilience in an aquatic system

3.1 Abstract

Biodiversity is valued due to its potential to stabilize the function of natural ecosystems. Theory suggests that increasing functional rather than taxonomic diversity should have a greater effect on system-level resilience (recovery following a perturbation). We test this idea experimentally in a lab system consisting of algae consumed by zooplankton, snails, or both, using an eutrophication event as a perturbation. We examined sediment load, algal and ammonium concentration as gauges of resilience. We find that *Daphnia magna* increased our measures of resilience. But this effect is inconsistent across ecosystem measures; in fact *D.magna* increased the difference between disturbed and undisturbed treatments in sediment loads. We have some evidence of shifting reproductive strategy in response to perturbation in *D.magna* and in the presence of the pond snail, *Physa* sp. These shifts correspond with altered population levels in *D.magna*, suggesting feedback loops between the herbivore species. While these results suggest only an ambiguous connection between diversity of species and to ecosystem resilience, they point to the difficulties in establishing such a link: indirect effects of one species on reproduction of another and different scales of response among components of the system, are just two examples of dimensions that can compromise the power of simple predictions.

3.2   Introduction

Biodiversity enhances resilience after a perturbation (Oliver et al., 2015). However, the mechanisms by which biodiversity enhances resilience are poorly understood. For example, while differences in species response (response diversity- *sensu* Elmqvist et al., 2003; Hébert et al., 2016) to a perturbation can cushion its impact on the aggregate performance of the whole ecosystem, it is unclear what mechanisms help with the speed of recovery. Beyond the number of species, the diversity of redundant and complementary functions performed by species (Yachi and Loreau, 1999) also matters. As a larger number of species implies an increase of complementary functions, it may be hard to disentangle which aspect of diversity — the number of species or the number of complementary functions — drives resistance and recovery of a living community of organisms.

Several empirical studies support the idea that resilience of a system will increase with increased richness (e.g. Allan et al., 2011; Tilman and Downing, 1994). However, these findings are not universal. For example, Guelzow et al. (2017) found an increase in recovery in phytoplankton communities with increased heterogeneity among patches and with increased connectivity, but did not detect increased recovery with increased diversity. It could be that different functional groups, not just specific species, are themselves more resilient to disturbance (e.g. Karp et al., 2011). Functional groups are likely to differ in their responses, which could be important in nutrient cycling, for example, because nutrients can be recycled at different rates and in different parts of the nutrient cycle (e.g. decomposition, herbivory) (Hulot et al., 2014).

Our goal is to gain insights into the role of functional traits in the recovery of a simple ecosystem from perturbation. We approach this empirically by contrasting a simple experimental community of primary producers (algae) alone with communities

that also include one or two consumers with distinct feeding modes. Our working hypothesis is that treatments with both functional groups will recover more completely (i.e. be more similar to an undisturbed system) than treatments with only one functional group or only primary producers.

Because eutrophication of freshwaters is a common threat to biodiversity and functioning of aquatic communities (Saunders et al., 2002; Carpenter et al., 1999), we chose it as our experimental perturbation. Eutrophication generally occurs from run-off from agricultural or mining lands. This run-off leads to algal blooms that can be toxic, or so excessive as to drastically deplete oxygen in the water body (Smith, 1998), and can even change evolutionary trajectories of species (Brede et al., 2009). Eutrophication can be difficult to reverse, as the removal of nutrients alone is sometimes insufficient to reverse all effects (Carpenter et al., 1999; Brede et al., 2009). Thus, understanding which aspects of a system will increase resilience to eutrophication is useful.

Our two functional groups of interest are grazers, represented by the snail *Physa* sp., and filter feeders represented by the Cladoceran, *Daphnia magna*. We chose these species because of their well-known effects on aquatic systems. For example, snails alter multiple pieces of a freshwater ecosystem including nutrient loads, fish and even bird communities (Gilioli et al., 2017). *Physa acuta* tolerates polluted systems with high nutrient loads and low dissolved oxygen (Kalyoncu et al., 2009) and thus are able to survive in highly impacted systems. Snails are important for energy inputs in aquatic systems because they are major decomposers (Brady and Turner, 2010). Snails also alter periphyton abundance and diversity (Swamikannu and Hoagland, 1989). While snails are likely important for nutrient cycling (even contributing up to 2/3 of all ammonium in a system) this may be due to their high biomass in many natural systems rather than high individual efficiency (Hall Jr et al., 2003).

Cladoceran filter feeders, such as *Daphnia magna*, are important for several functions in aquatic systems. *Daphnia* spp. increase pH and available oxygen (Wojtal-Frankiewicz and Frankiewicz, 2011), alter disease risk (e.g. Kagami et al., 2004), and increase water clarity (Walsh et al., 2016). They are important in nutrient cycling, specifically increasing N or NH_4 in the water column and reducing P (Paterson et al., 2002; Mackay and Elser, 1998; Wojtal-Frankiewicz and Frankiewicz, 2011). These impacts alter cyanobacterial competitive advantage (Mackay and Elser, 1998) (but see Paterson et al., 2002). However, changes in nutrient loads can in turn change the population dynamics of *Daphnia magna* (Kleiven et al., 1992; Sterner and Hessen, 1994). These feedback loops suggests that *Daphnia* spp. response to a nutrient perturbation is subtle and thus, greater understanding of these feedback loops could lead to a better overall understanding of system level recovery.

As recovery from perturbation is only indirectly accessible via signature variables, we look at several different endpoints: sediment load, ammonium concentration, algal concentrations, filter feeder *(Daphnia magna)* populations, grazer *(Physa* sp.) survival and reproduction, and algal community composition. We examine sediment loads because they are important to freshwater health as they block light for macrophyte growth and sequester nutrients. Additionally, increased sediment loads decreases gastropod and benthic invertebrate abundance and diversity (e.g. Donohue et al., 2003) which in turn alters sediment accumulation (James et al., 2000).

In this experiment we thus focus on the differential effects of *Physa* sp. and *Daphnia magna* on ecosystem recovery that follows a eutrophication event. We expect more complete recovery if the species have differential responses to the perturbation. Additionally, we expect population cycles of *Daphnia magna* to be more stable with two species. We acknowledge that a new stable state is also possible (e.g. colonization by cyanobacteria) in which case, regardless of diversity, the system will not be able to

rebound after a disturbance (Standish et al., 2014).

3.3 Methods

3.3.1 Experimental Set-Up

Experiments were run in the greenhouse at McMaster University between January and March 2019. The herbivores we used, *Daphnia magna* and *Physa* sp. and the mixed algae, predominantly *Chlorella* spp. (Figure B.6, panel A.), were all from lab maintained populations.

Our experiment consisted of four herbivore treatments: no herbivores, *Daphnia magna* only, *Physa* sp. only, and both. Tanks were either perturbed with single eutrophication event or were not disturbed. Each treatment had ten replicates, for a total of forty tanks.

General Set-Up: Tanks were filled with 1 L of water from the lab cultures of *Daphnia magna*. Next, the algal mix was added until starting concentrations of 14.5 ± sd 2.3 chlorophyll-a μg/L were reached. Tanks began with ammonium concentrations (mg/L) of 3.86 ± 0.564. Each tank with *Daphnia magna* received twenty individuals larger than 1 mm. Each tank with *Physa* sp. received four individuals with an average size of 4.6mm ± sd 1 mm. Following the introduction of the organisms, tanks were left undisturbed for three weeks (at a median temperature of 19 °C during the day, no shade) and monitored. After three weeks, we added 100 mL of 1g/L concentration of a nutrient mixture (Miracle-Gro ®) to tanks selected for the disturbance treatment.

Data Collection: We measured chlorophyll-a (AquaFlor flourimeter), temperature, and pH daily (both with HACH Pocket Pro) daily as well as NH_4 (YSI Pro Plus) twice a week. *Daphnia magna* populations were estimated twice a week by counting four 10

mL samples of tank water from each tank. We kept track of two separate *D. magna* size classes: < 1 mm and > 1 mm. We also recorded snail egg masses and juveniles when they appeared and at the end of the experiment. We measured snail length at day 19 or 20 and at the end of the experiment, 39 or 40 days. We also collected 5 mL water samples for algal identification on the same sampling days as snail measurements. All algal samples were placed in a cooler for five to seven days before being taken to the lab to be analyzed. From each five mL sample we extracted a ten μl sub-sample, which was placed on a hematocytometer for counting. Algae were identified using a Zeiss Primo Star compound microscope. The program Zen was used to capture and process each algal image at 10x magnification. We took four pictures of each sample. Algae captured on each photo were manually counted and identified using Manaaki Whenua Landcare Research algae guide (2014).

3.3.2 Analysis

All analyses were completed in the `R` statistical programming environment (R Core Team (2016) version 3.6.1). Data and code are publicly available at: https://github.com/jwerba14/Disturbance.

All of the following generalized linear mixed models were run using the `lme4` package (Bates et al., 2015) and included a random effect of start date since our experiment started over the course of three weeks.

For both chlorophyll-a and ammonium models we ran log transformed linear models with an interaction term between herbivore treatment and disturbance. We used the `emmeans` or `multcomp` package to compare our *a priori* contrasts (Lenth, 2020; Hothorn et al., 2008). Specifically our interest is in the difference between disturbed and undisturbed treatments across herbivore treatments. For example, is

the difference between *D. magna* disturbed and undisturbed treatments greater than or less than the difference between the difference between the *Physa* sp. only disturbed and undisturbed treatments? We ran all six possible contrasts. Bonferonni adjustment for multiple contrasts was used to calculate p-values.

For final chlorophyll-a concentrations we used the final four days of the experiment. The maximum post-disturbance chlorophyll-a data used for analysis was a mean of values recorded in a three day window starting one day after the disturbance. For ammonium we ran the same model as for chlorophyll-a but took a single day maximum and the final day value because of the lower measurement frequency.

We examined the effect of disturbance on final populations of *Daphnia magna* using a generalized linear model, with a negative binomial error perturbation. We used disturbed (y/n) and herbivore treatment as predictors. Maximum populations were defined as the mean of all four sub-samples. We log transformed organisms' max populations and used a weighting factor of 1/variance of the sub-samples used to measure their abundance for the linear model.

We ran exploratory analyses to find out if the presence or absence of resting eggs, ephippia, in *Daphnia magna* at the end of the experiment were affected by disturbance and grazer presence (binomial generalized linear mixed model).

Physa sp. survival was modeled as the proportion surviving given day and treatment, using a generalized linear mixed model with a binomial error perturbation and individual tank as a random effect. The probability of snails laying eggs by the end of the experiment was also modeled with a binomial generalized linear model with herbivore and disturbance treatment as fixed effects.

Algal community turnover between treatments and time were explored using a PCoA with a Bray-Curtis dissimilarity matrix of algal species abundance. Variation explained by grazer treatment and time were analyzed using a permutational multivariate analysis

63

of variance (PERMANOVA). These analyses were done in the `Vegan 2.3.3` package (Oksanen et al., 2016).

3.4 Results

3.4.1 *Chlorophyll-a* concentrations

After an eutrophication event, algal spikes are of concern. Thus, the maximum levels and long term levels of chlorophyll-a are important endpoints to evaluate. We directly test how herbivore treatments (none, *Physa* sp. alone, *Daphnia magna* alone, or *Physa* sp. and *Daphnia magna* in combination) affect the difference between disturbed systems and undisturbed systems. In particular, does a more diverse herbivore community treatment reduce the difference between the undisturbed and disturbed treatments? We find, for both final chlorophyll-a concentration and maximum chlorophyll-a concentration, that in the presence of *Daphnia magna* either alone or in combination with *Physa* sp., the difference between the undisturbed and disturbed treatments are smaller than when no herbivore is present (Figures 3.1 and 3.2, for model estimates Table B.1, for raw data Figures B.1 and B.2). These results suggest that a filter feeder presence is critical for limiting algal blooms after an eutrophication event.

Figure 3.1: Log10 fold change in final chlorophyll-a between undisturbed and disturbed treatments by herbivore combination. Distance from 0 (the dashed vertical line) indicates the magnitude to the change. This scale is maintained for Figures 3.1 through 3.5. Both treatments containing *Daphnia magna* reduced the proportional change between disturbed and undisturbed treatments when compared to *Physa* alone or to no herbivore. Points are means and error bars are 95% CI.
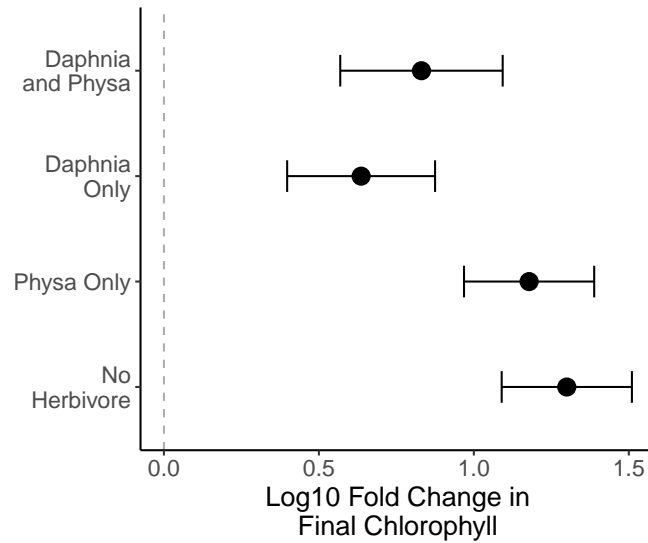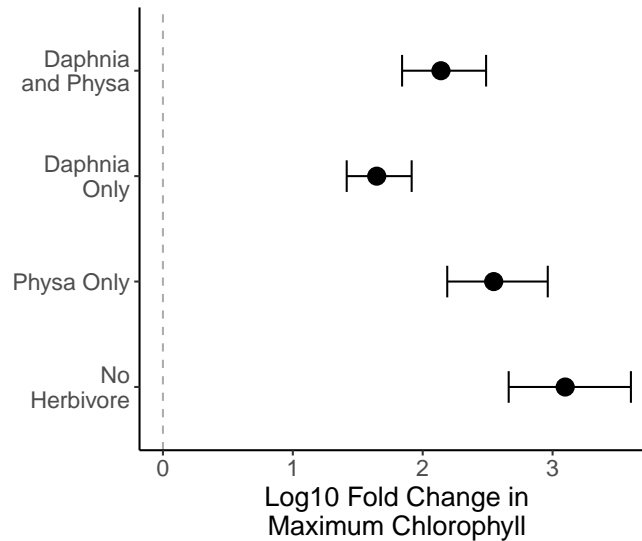
Figure 3.2: Log10 fold change in maximum chlorophyll-a between undisturbed and disturbed treatments by herbivore combination. Both treatments containing *Daphnia magna* reduced the proportional change between disturbed and undisturbed treatments when compared to *Physa* alone or to no herbivore. Points are means and error bars are 95% CI.

3.4.2   Ammonium concentrations

We are unable to detect any effect in the size of the difference between disturbed and undisturbed treatments for final (Figure 3.3) or maximum (Figure 3.4) ammonium concentrations across herbivore treatments. (Model estimates can be found in Table B.1 and raw data can be found in Figures B.3 and B.4).

Figure 3.3: Log10 fold change in final ammonium concentrations between undisturbed and disturbed treatments. None of our herbivore treatments were statistically different from the others. Points are means and error bars are 95% CI.

Sediment

We find that *Daphnia magna* alone increases the difference in sediment between disturbed and undisturbed treatments when compared to either no herbivore or only *Physa* are present (Figure 3.5). But *D. magna* appear to lower the raw sediment substantially in disturbed treatments (Figure B.5, Table B.1 for model estimates).
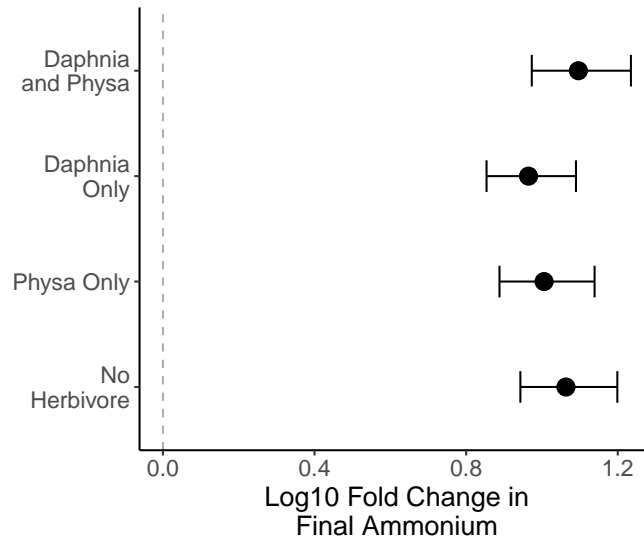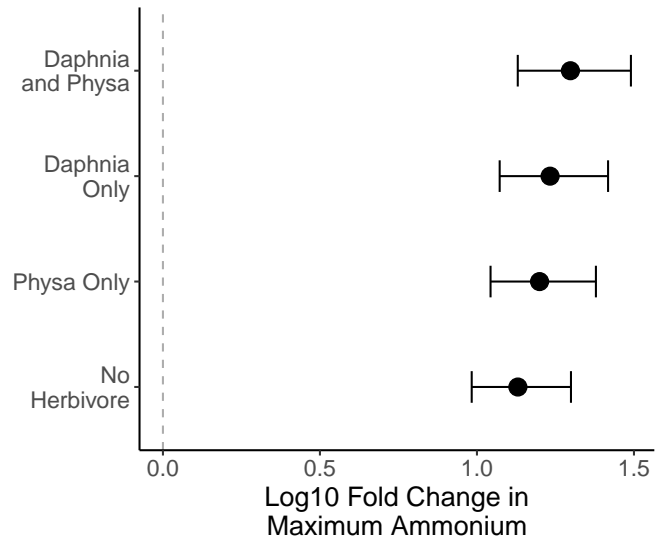
Figure 3.4: Log10 fold change in maximum ammonium concentrations between undisturbed and disturbed treatments. None of our herbivore treatments were statistically different from the others. Points are means and error bars are 95% CI.
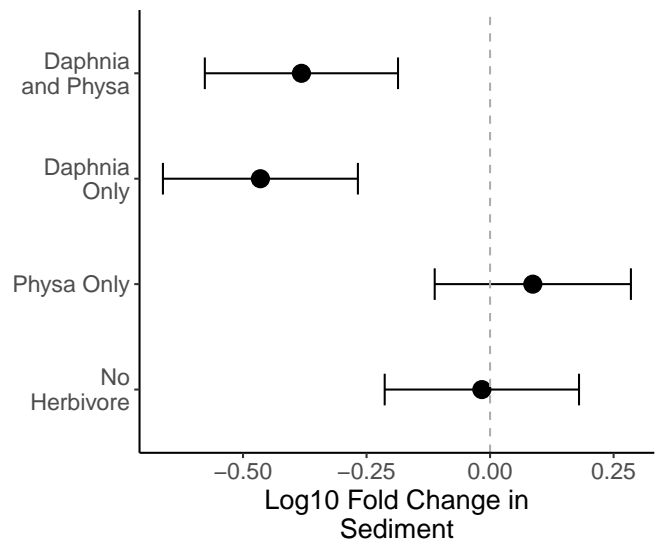
Figure 3.5: Log10 fold change in sediment between undisturbed and disturbed treatments. *Daphnia magna* only treatments had a larger change between disturbed and undistured treatments than either no herbivore or compared to *Physa sp* only treatments. *Physa sp.* only treatments had a smaller change in sediment than the treatment with both herbivores. Points are means and error bars are 95% CI.

Daphnia magna **reproduction and population**

Ephippia production represents a different mode of reproduction for *Daphnia magna.* We detected a clear change in reproductive strategy towards ephippia production in disturbed treatments (Figure 3.6), likely at least partially due to the higher maximum and final populations of *D. magna* in disturbed treatments (Figure 3.7).
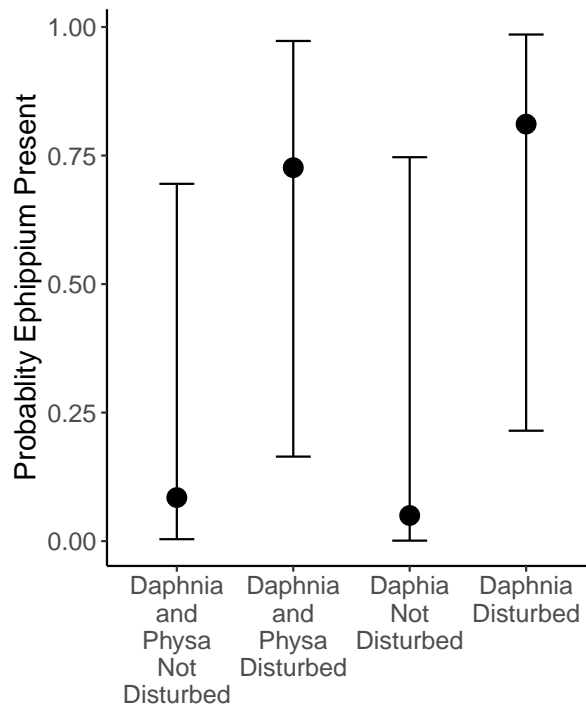
Figure 3.6: *Daphnia magna* were more likely to produce ephippia by the end of the experiment (three weeks post disturbance) in disturbed treatments. Points are means and error bars are 95% CI.
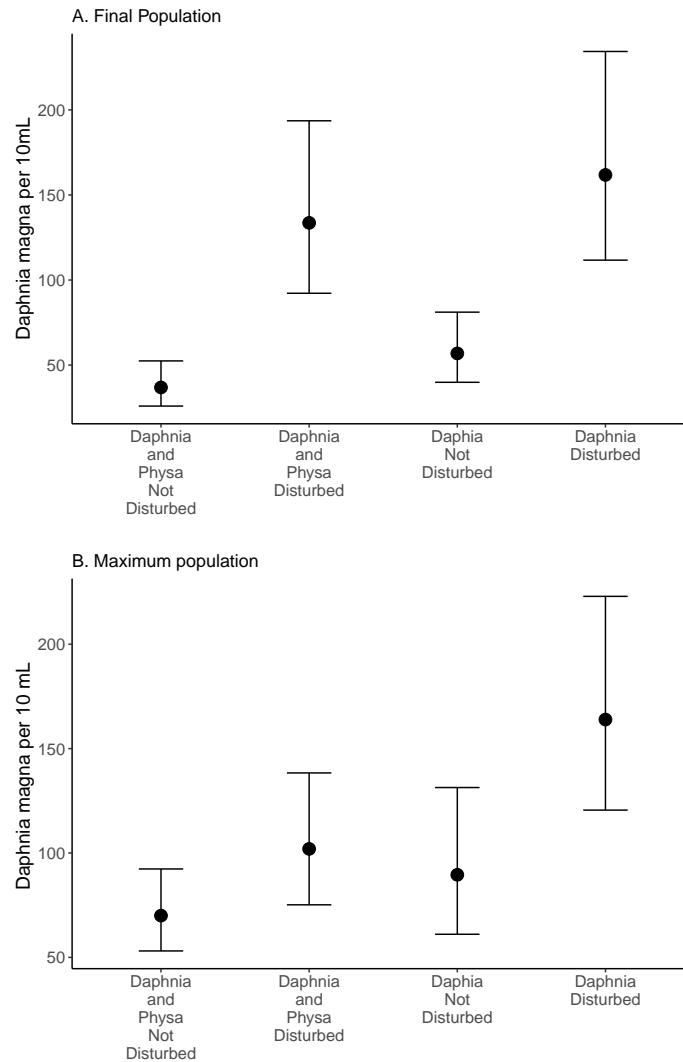
Figure 3.7: Final (A.) and Maximum (B.) *Daphnia magna* populations were higher in disturbed treatments. Final populations were also higher when *Daphnia magna* was the only herbivore present. Points are means and error bars are 95% CI.

And while we did not observe a downstream effect on ephippia production we do detect a reduction in the final populations of *Daphnia* populations when *Physa* are present (Figure 3.7, panel A.). We cannot, however detect a difference in the change between undisturbed and disturbed treatments in either final (Figure 3.8) or maximum (Figure 3.9) *D. magna* populations when *Physa* are or are not present. All model

estimates can be found in Table B.2.
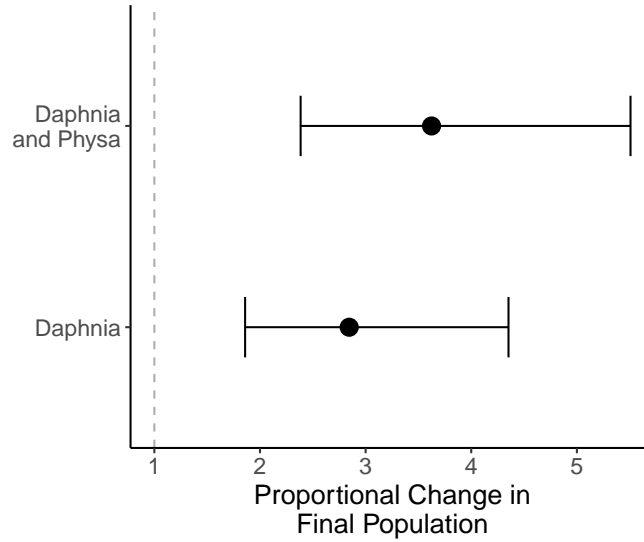
Figure 3.8: Proportional change in final *Daphnia magna* populations between undisturbed and disturbed treatments. Distance from 1 (dashed lined) represents the magnitude of the difference between treatments. Points are means and error bars are 95% CI.
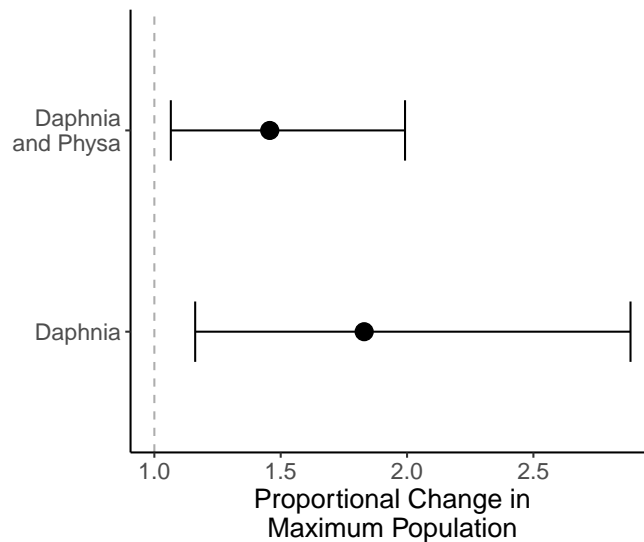
Figure 3.9: Proportional change in maximum *Daphnia magna* population between undisturbed and disturbed treatments. Distance from 1 (dashed lined) represents the magnitude of the difference between treatments. Points are means and error bars are 95% CI.

Physa sp.

We found no significant differences in final snail survival among treatments, though *Physa* sp. in tanks without *D. magna* died more quickly than in tanks with *D. magna* (Figure 3.10).
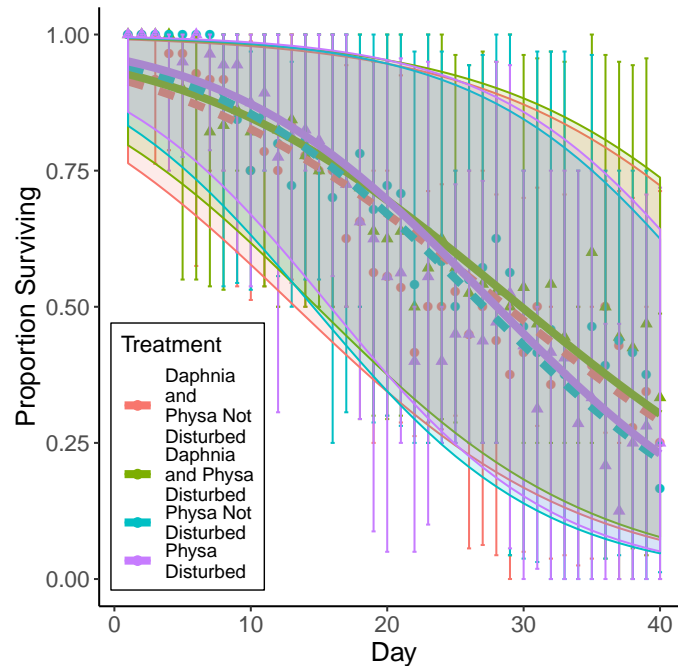
Figure 3.10: *Physa* sp. survival over the course of the experiment. *Physa* sp. alone (blue and purple lines) died more quickly than when *Daphnia magna* were also present (red and green lines). We don't detect a difference in survival between disturbed treatments (solid lines) and undisturbed treatments (dashed lines). Points are means and error bars and envelope are 95% CI.

Physa sp. egg production also increased when *Daphnia* were present (Figure 3.11).
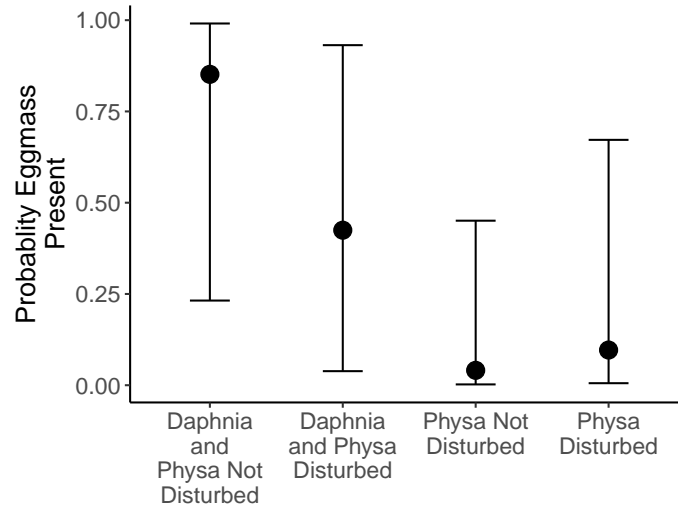
Figure 3.11: *Physa* sp. were more likely to lay eggs when *Daphnia magna* were present. Points are means and error bars are 95% CI.

Additionally, when *Daphnia magna* are present there is a larger difference in egg production between the disturbed and undisturbed treatments (Figure 3.12). All model estimates can by found in Table B.3.
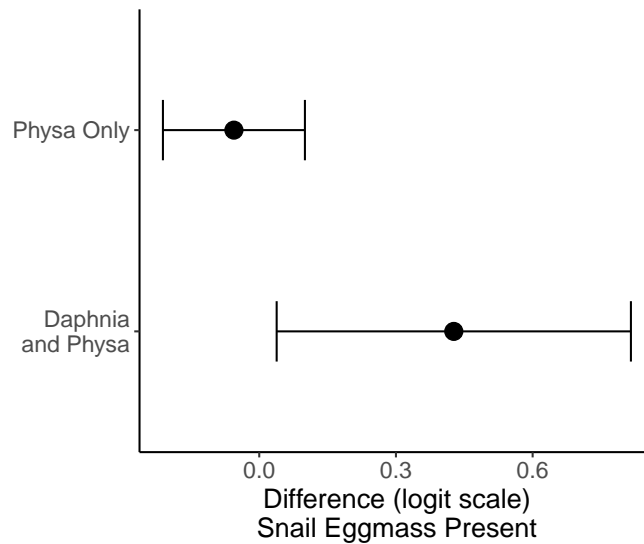
Figure 3.12: The difference between disturbed and undisturbed treatments in the likelihood of snails laying eggs increased when *Daphnia magna* were present. Points are means and error bars are 95% CI.

Algal communities

At the beginning of the experiment algal communities were indistinguishable between treatments (Figure 3.13, panel A). Directly before the disturbance there was some separation in algal community along the primary PCoA axis between treatments with or without *Daphnia magna* (Figure 3.13, panel B). Three weeks post-disturbance the treatments were clearly delineated into two groups, those with *Daphnia magna* and those without. There is minor separation along the secondary PCoA axis between the disturbed and undisturbed treatments when *Daphnia magna* is absent (Figure 3.13, panel C). PERMANOVA results suggest that herbivore treatment explained the most marginal variance in the algal community ($R^2 = 0.1, p < 0.05$), followed by time (which was modelled as a continuous variable) ($R^2 = 0.06, p < 0.05$). We are unable to distinguish between disturbance treatments, and the majority of variance in the community structure was not explained by any of our fixed effects (Residual Variance $= 0.8$).
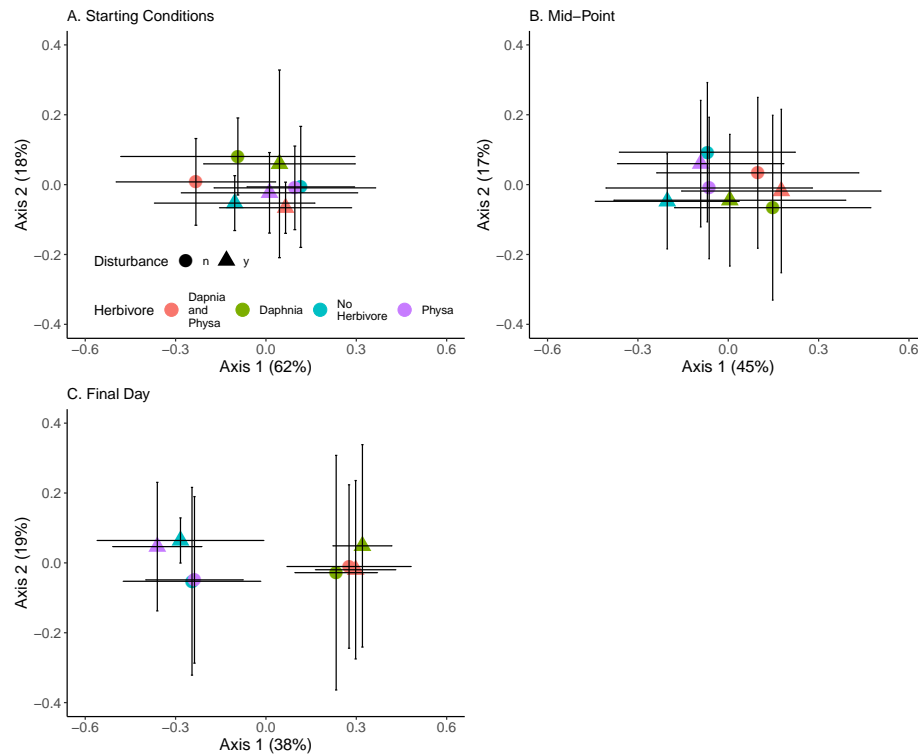
Figure 3.13: Points represent the centroid of the algal communities. Error bars show standard deviation. Panels represent different time points: A) starting structure, B) Mid-point, and C) Final day. Shapes indicate disturbance treatment: circles show no disturbance, triangles show disturbed treatments. Colors represent herbivore combination. Axis are PCoA 1 (x-axis) and PCoA 2 (y-axis).

Only three species made up the majority of the algal community at the start of the experiment (Figure B.6 panel A.). Shortly before disturbance one species was replaced by another but still the communities were made primarily of three species (Figure B.6 panel B.). Three weeks post-disturbance the disturbed treatments were slightly more diverse, with up to five species with > 5 percent of abundance.

3.5   Discussion

By definition a more resilient system will return to the pre-disturbed state more fully than a less resilient system. Across most ecosystem level metrics, presence of *Daphnia magna* was the only driver of increased resilience, although this was not universal; in some metrics (snail egg production, sediment) our resilience measure was worsened by the presence of *Daphnia magna* (Figures 3.5 and 3.12).

Resilience is expected to be higher in more diverse systems. However, we find in contrast to our hypothesis, that having two different herbivore functional groups did not detectably reduce the effects of eutrophication. While the herbivore treatments, with or without disturbance, had clear and often strong effects on chlorophyll-a, the effect of ammonium and sediment levels (Figures B.1, B.2, B.3, B.4, and B.5) on resilience was less clear.

Sediment loads are a problem following eutrophication events. Snails are generally understood to reduce suspended sediment particle concentrations. As sediment reduction allows light to penetrate further in the water column and helps support macrophytes instead of algae, this is one of the primary top-level ecosystem functions snails perform. The fact that we could not detect the effect of *Physa* sp. on sediment loads is likely due to the high snail mortality (Figure 3.10) and low birth rates across treatments. Indeed, it was not until the end of our experiment that we began to see newborn snails and egg clutches. *D. magna* presence decreased the resilience of sediment as shown by the increased difference between perturbed and recovered system (Figure 3.5), but the disturbed system when *D. magna* were present had reduced sediment compared to undisturbed systems (Figure B.5). This suggests that having a filter feeder may be critical to reducing the downstream effects of increased sediment. This reduction could be due to decreased carbonate precipitation through controlling

algal biomass and decreasing both N and P sedimentation overall (Sarnelle, 1993) but why this occurs more in a disturbed system is unclear, possibly tied to the increased population of *D. magna* in disturbed systems (Figure 3.7).

Daphnia magna are understood to be important in nutrient cycling particularly by increasing dissolved NH_4 (e.g. Paterson et al., 2002). Additionally, it seems that snail presence increased maximum NH_4 concentration in the disturbed treatments (Figure B.4), which is surprising because our snail populations were small and the effect of snails on nutrients is expected to be at least partially based on their large populations (Hall Jr et al., 2003). Evidence suggests that snails can raise NO_2 (Mulholland et al., 1991) and can be an important source of ammonium (Griffiths and Hill, 2014). Additionally, snails can alter nitrogen availability via selective grazing (Arango et al., 2009; Liess and Kahlert, 2009), though we do not see evidence of this in the treatments (Figure B.6). We do see evidence of *Daphnia magna* altering community composition (Figure 3.13), which could indirectly alter NH_4 concentration. For this ecosystem-level response, presence of more herbivore functional groups appears to worsen, at least initially, the effect of eutrophication. The longer term consequences of herbivores are unclear and are likely to have complex interactions with primary producers. Additionally, no combination of herbivore species detectably altered the difference between disturbed and undisturbed treatments (Figures 3.3 and 3.4).

Daphnia magna produce ephippia when daylight is less than 12 hrs, at low food, when populations exceed 0.4 individuals per mL (Carvalho and Hughes, 1983), and when stressed by pollution (Ringot et al., 2018). We observe this shift in reproductive behavior far more frequently in disturbed treatments than in undisturbed treatments (Figure 3.6). We can rule out light as the cause because tanks shared a uniformly illuminated space. However, *D. magna* densities were much higher in perturbed treatments (Figures 3.7) so this likely contributed to switching reproductive tactics,

77

though Booksmythe et al. (2018) did not find increased density leading clearly to increased ephippia production. *Daphnia magna* can switch to ephippia production in new trophic states because the ephippia are resilient to changes in trophic state (Isanta Navarro et al., 2019). This change in reproductive strategy could signal a transition to a different state of the system. Alternative stable states often result from eutrophication (Carpenter et al., 1999).

Physa sp. were more likely to lay eggmasses within the 6 weeks of our experiment if *D. magna* were present (Figure 3.11). *Lymnea* sp. has also been shown to increase fecundity in the presence of congeneric species, though other snails, though the mechanism remains unclear (Hershey, 1990). The combination of reduced *D. magna* population when *Physa* sp. is present but increased *Physa* sp. eggs (and likely future increased population) when *D. magna* are present suggests a possible feedback loop: increase in *D. magna* → increase in *Physa* sp. reproduction (eggs) → eventually increase in *Physa* sp. and, likely, some impact of higher *Physa* sp. populations on *D. magna*. In nature, this coincides with other factors contributing to *D. magna* reduction over the course of summer. We mention this to highlight that, although, some experimental results are informative, long-term consequences of interactions among the components, even in the simple experimental system we used, may be confounded by processes unfolding at different time scales. This applies to dynamics observed under lab conditions, and even more to natural situations.

The differences in the algal community were mostly driven by herbivore treatments rather than perturbation. This first effect is not surprising as herbivores influence algal communities through direct and indirect routes (Abrantes et al., 2006; Sterner, 1989). However, an undetectable signature of perturbation is a surprise because algal species are known to perform differentially under different nutrient regimes (e.g. Tilman, 1977). Herbivores alter algal communities directly by grazing on edible, high quality

algae increasing the mortality rate of some species and increasing the survival of other inedible algae (Agrawal, 1998; Lampert et al., 1986; Sterner et al., 1993). Indirect affects on algal community occur due to shifting nutrient ratios (Abrantes et al., 2006; Pinowska, 2002; Sommer, 1988; Sterner, 1989) which can create advantageous and disadvantageous environments for different species of algae (Schlesinger et al., 1981). We do indeed observe *Daphnia magna* shifting the algal community. However, we expected snails to modify algal communities as well (Mulholland et al., 1994; Arango et al., 2009; Liess and Kahlert, 2009) but we don't observe this, likely due to the low total *Physa* sp.) populations. *Scendesmus* spp. made up close to a quarter of the algal community over time, but is greatly reduced in *D. magna* only treatments by the end of the experiment (Figure B.6). Disturbed treatments had higher percentage of some species that were not well represented in non-disturbed treatments(Figure B.6, panel C.). In sum, it is possible that the combined and opposing effects of *D. magna* grazing and nutrient augmentation introduced noise that masked nutrient treatment. We thus reiterate that herbivore presence is far more important to algal community structure than a single eutrophication event. This observation has some management implications for fish stocking: a stocking decision that cascades down to reduce grazer numbers would have a potentially multiplicative effect on magnitude of an eutrophication event.

3.6   Conclusions

Overall, we have some evidence that multiple functional groups have combined, and sometimes, synergistic effects on the system but we do not see that translate to resilience across ecosystem measures. Additionally, we are unable to say much about long-term complex interactions such as long-term ammonium availability and shifts in

algal communities and edibility. Our study further suggests that resilience may not be predictable by a simplified approach. Instead, it involves complex interactions that require knowledge of adaptive species responses and indirect effects.

3.7   Acknowledgments

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Chapter 4: The challenge of life history traits - a small cladoceran, Ceriodaphnia rigaudi

4.1 Abstract

Using accurate and precise species-specific parameters in mechanistic models can lead to better predictions of population dynamics and ecosystem function (e.g. nutrient cycling) across a range of environmental conditions. Zooplankton are important in the aquatic food web and for nutrient cycling but are highly diverse, but there is only limited information on specific species. Knowledge of species-specific attributes is patchy. In particular tropical species are underrepresented in this regard. Here we gather all the known information about a wide-spread tropical zooplankton member, *Ceriodaphnia rigaudi*, and add new information from lab and field experiments. We determine feeding rate across a range of food concentrations and food-dependent population growth rate of *C. rigaudi*. Additionally, we use 16 years of occurrence data from rock pools in Jamaica to explore environmental characteristics of the habitat in which *C. rigaudi* live. We compare our data to world wide records of the species attributes and create a reference map of its occurrence.

4.2   Introduction

Functional traits are morphological or physiological aspects of a species that impact fitness (Violle et al., 2007); often functional traits are also traits of the organisms that directly interact with the environment (*sensu* effect traits Hébert et al., 2017; Lavorel and Garnier, 2002). Functional traits are important for two reasons: 1) making model predictions involving a specific species of interest (e.g. van der Meer, 2006); 2) enabling classifications of species into functional groups for classic trait-based models (e.g. Vogt et al., 2013). Trait-based models are particularly important in aquatic systems due to high species richness (e.g. phytoplankton) and the desire for species-specific models (e.g. fisheries). Aquatic systems are well suited to trait-based methods due to a long history of modelling (Robson, 2014) and combining of high species richness into subgroups (e.g. phytoplankton: Litchman et al. 2010; zooplankton: Litchman et al. 2013). Yet, despite advances in data amalgamation for aquatic species (e.g. Hébert et al., 2016; Robson et al., 2018), more information is always needed for additional species to expand the usefulness of trait-based methods.

Zooplankton represent a large and important part of aquatic systems and yet species-specific information is limited. Zooplankton classifications are usually based on size and feeding strategy. However, size may not be a good predictor of traits of interest (e.g. grazing) outside of temperate regions (Pinheiro-Silva et al., 2020) which may be a larger problem than currently realized because tropical species tend to be underrepresented (e.g. Hébert et al., 2016; Rizo et al., 2017). One challenge is the limited scope of studies on life history that makes it impossible to tell if species traits are consistent throughout their range. With more resolved life history information more informed and nuanced decisions for trait based classifications should be easier. Here we provide additional information for a small, tropical cladoceran, *Ceriodaphnia*

rigaudi.

Cladocerans are a crucial link between lower trophic levels (microbial) and upper trophic levels (fish) of the freshwater food web (Burns and Schallenberg, 2001). They alter algal production and community structure (Sommer et al., 2001), and impact nutrient cycling (e.g. Migal, 2011). However, most cladoceran species have little life history information; a meta-analysis by Hébert et al. (2016) found that the genus Daphnia comprises half of all known cladoceran information. Despite a known 620 unique cladoceran species (Karuthapandi and Rao, 2016), a meta-analysis found species trait information on less than 70 (Hébert et al., 2016) which is concerning for the ubiquitous use of species traits in aquatic modelling. Gathering more information on smaller cladocerans will help identify when size is or is not a good proxy for traits of interest.

Ceriodaphnia rigaudi are a small cladoceran commonly found throughout tropical freshwater ponds. Originally thought to be distinguished from *Ceriodaphnia cornuta* by the absence of head, shoulder and tail spikes, this classification turns out to potentially be the result of differential predation and not a true physiological characteristic (Rietzler et al., 2008). However, recent genetic tests do in fact place *C. rigaudi* as a separate species from *C. cornuta* (Sharma and Kotov, 2013). *C. rigaudi* have been considered for use as a water quality indicator species and as such have been used in some toxicology experiments (e.g. Raymundo et al., 2019; Mohammed, 2007), and may be important community indicators (Márquez et al., 2016). Because it is also widespread and may play a dominant role in some systems, understanding its traits and distribution could be important for nutrient cycling and food web models in specific locations. However, little is known about their life history traits. Here we amalgamate all data on the life history and geographical spread of the species and add information from our own lab experiments and field observations. Life-history information can

help inform future trait-based studies,parameterize mechanistic models, or determine if this species is a good indicator species for tropical systems.

4.3 Methods

All analyses were completed in `R` (R Core Team, 2019). Data and code are available at https://github.com/jwerba14/Species-Traits/tree/master/Ceriodaphnia.

4.3.1 Lab Conditions

Ceriodaphnia rigaudi has been maintained in our lab continuously for several years. All individuals used in our experiments came from our lab population. The colony originated from Jamaican rock pools. Water temperature of the stock cultures varied from 17-22°C.

4.3.2 Feeding rates

To determine *Ceriodaphnia rigaudi* uptake rate of green algae (predominantly, *Haematococcus* sp.) we used five algal concentration treatments (mean $\pm$ sd: $0.778 \pm 0.0985\mu$g - Chl-a/L, $3.11 \pm 0.321\mu$g - Chl-a/L, 9.43 ± 1.01 μg - Chl-a/L, 16.7 ± 1.24 μg - Chl-a/L, 20.9 ± 1.27 μg - Chl-a/L) with five replicates each. Each replicate had an average of 75 individuals. We measured the chlorophyll-a after six hours. We did not expect discernible growth in the *Haematococcus* sp. population over six hours, but we did include three replicates without any *C. rigaudi* for each chlorophyll-a treatment to account for any growth or death of algae unrelated to grazing to be used as a reference. We subtracted the average change of the controls, by treatment, from the experimental replicates. We ran a linear model across food treatment to determine average uptake

rate.

4.3.3   Population growth

For population growth we began colonies in 1.1L of water with 30 individuals of *Ceriodaphnia rigaudi* each at four food treatments (mean $\pm$ sd: $2.93 \pm 0.905\mu$g - Chl-a/L, $9.39 \pm 22.5\mu$g - Chl-a/L, $22.3 \pm 6.32\mu$g - Chl-a/L, 65.5 ± 10.1 μg - Chl-a/L) with six replicates each. Every day either algae or distilled water was added to bring the tanks back to the food treatment level. *C. rigaudi* were counted weekly in eight fifty-mL sub-samples. The experiment lasted eight weeks. We fit a logistic growth curve (4.1) to each replicate separately using the `nlmrt` package (Nash, 2016) and then ran a linear regression of each parameter (r,k) against the food treatment.

$$N_t = \frac{k}{1 + \frac{k - N_0}{N_0}e^{(-rt)}} \tag{4.1}$$

where k is the population asymptote, r is the growth rate and N_0 is initial population size

4.3.4   Literature search

A Google Scholar search on April 23, 2020 for "Ceriodaphnia rigaudi" resulted in 523 hits. We downloaded those papers that had survey data or had life history information for a total of 196 papers. Of those we found 98 with either geographical information (96) or life history information (2 papers). After confirming geographical coordinates we accepted 137 records as sufficiently reliable.

4.3.5   Distribution

We mapped *C. riguadi* with the `maps` package (code by Richard A. Becker et al., 2018). Our map includes 138 locations (one is our own data). Whenever possible we mapped the exact sampling locations (considered exact if the coordinates were reported in the manuscript or we found the body of water mentioned). We did not consider any river an exact location (unless coordinates were given). All coordinates are the closest we could establish based on the location description found in the paper.

4.3.6   Jamaican data and Habitat

Rock pools are located on the west coast of Discovery Bay, Jamaica. 50 out of over 200 pools were sampled annually from 1989-2006, (except 2004). We also obtained salinity and oxygen concentrations as well as pH and temperature from each pool. To determine which if any environmental factors were associated with the presence or absence, or level of abundance of *C. rigaudi* we ran a hurdle model with a negative binomial error distribution in `glmmTMB` (Brooks et al., 2017).

4.4   Results

Feeding rate: Individual *C. rigaudi* took up marginally more chlorophyll-a per hour as chlorophyll-a availability increased (Estimate:7.42e-10 μg chl-a per hour, p = 0.009, Figure 4.1).
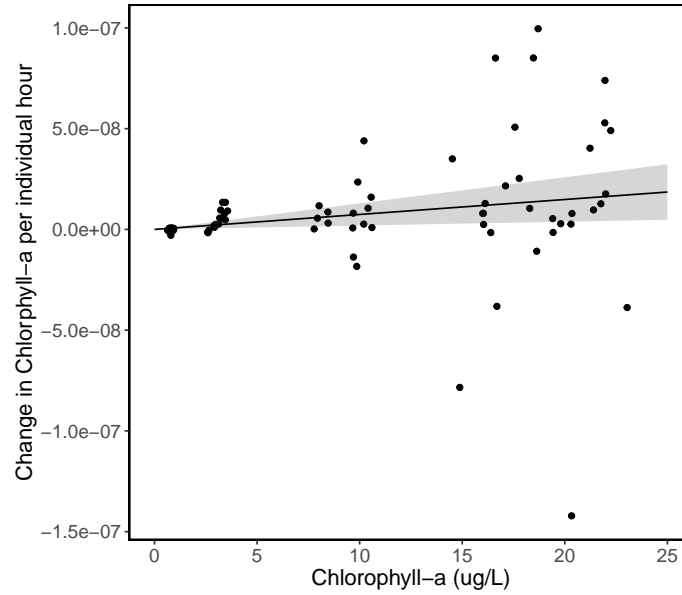
Figure 4.1: Feeding rate of each individual per hour across Chl-a concentrations

Growth rate: We were unable to estimate a non-zero slope across feeding treatments for either r or k ($p > 0.05$). Food treatment did not explain much of the variance in either parameter ($R^2 < 0.1$). The range of r and k estimates for each treatment was very variable by replicate (Figure 4.2).
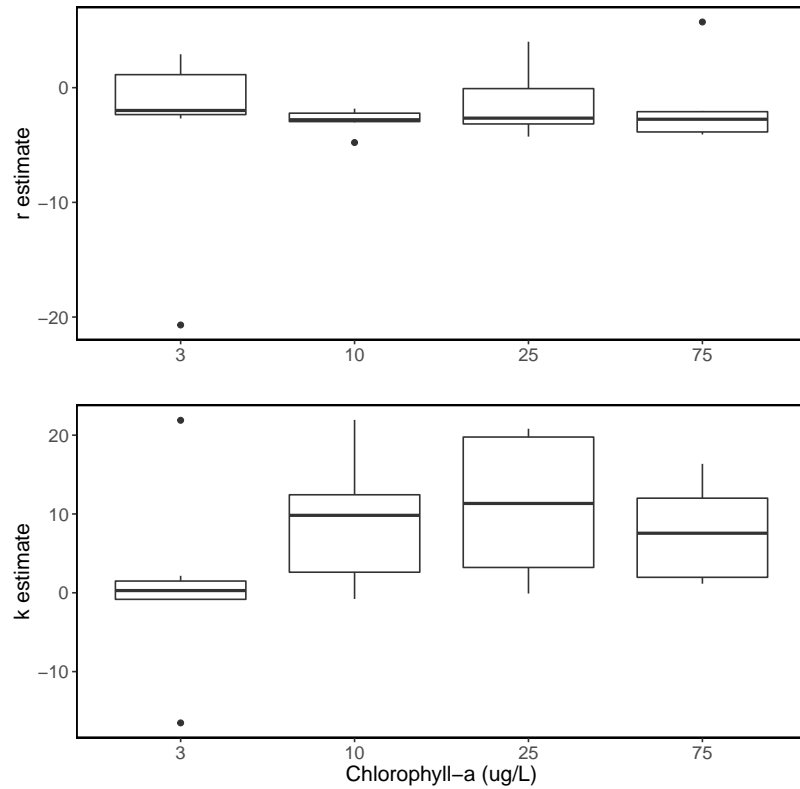
Figure 4.2: Estimates for r and k for C. rigaudi populations at different constant food treatments. r and k estimates are on the log scale

Environmental correlates: We find that as pH, oxygen and salinity increase, the probability of *C. riguadi* being present declines ($p < 0.05$, Figure 4.3). Decreases in pH and salinity increase the abundance of *C.riguadi* ($p < 0.05$, Figure 4.3).
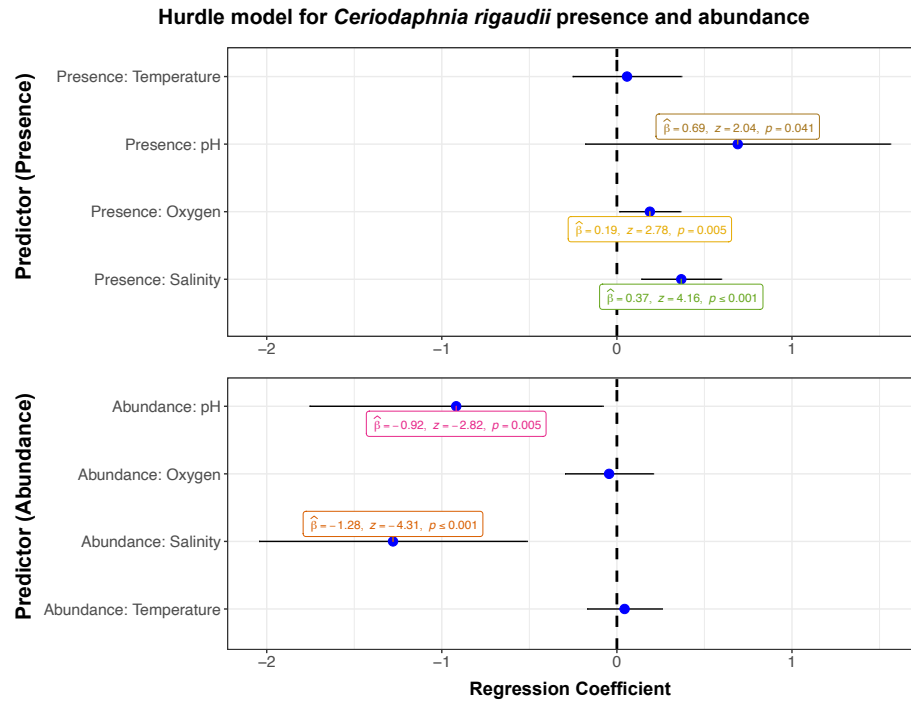
Figure 4.3: Environmental factors contributing to *C. rigaudi* presence in Jamaican rock pools

Map of species distribution: We were able to find 138 records of *C. rigaudi* in survey data. We see clusters in southern Brazil. Otherwise records spread throughout Africa, south Central Asia and parts of central America (Figure 4.4). While *C. rigaudi* is generally thought of as a tropical species we found records outside the tropics or subtropics, in the United States and southern Europe.
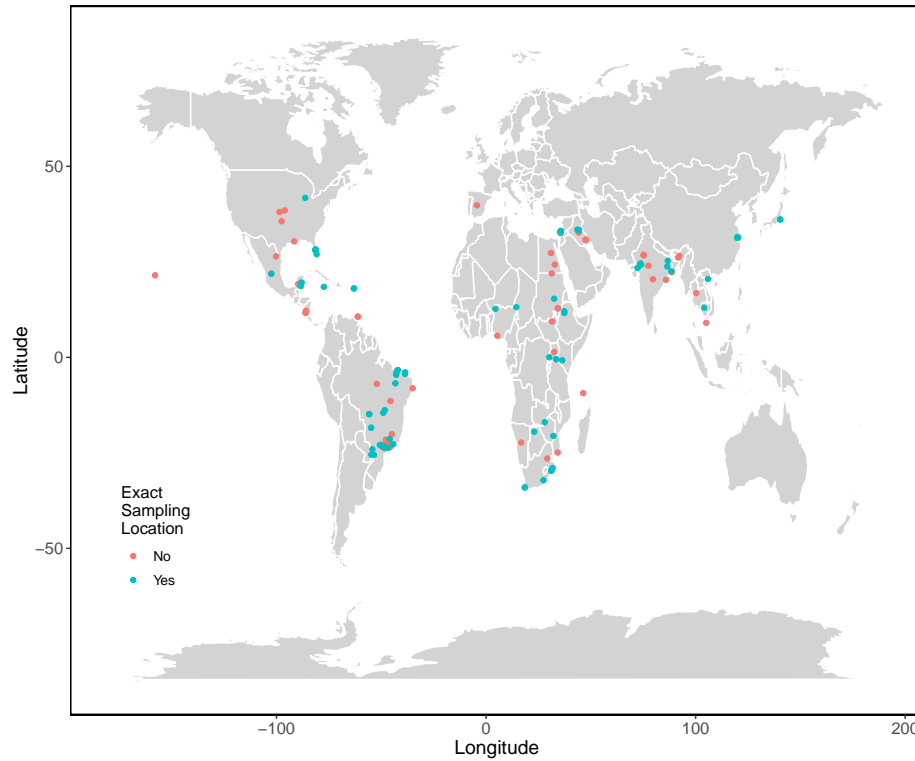
Figure 4.4: All of the locations where we found reported sightings of *C. rigaudi.* Blue dots indicate exact sampling locations, red dots indicate general location

4.5   Discussion

We find that feeding rate increases very little as food concentration increases. As far as we are aware this is the first study of *C. rigaudi* feeding rate. Estimating population growth rate was challenging and our fits gave wide possible ranges for k and r. Due to the high variation among replicates we could not identify a pattern across food concentration for either parameter. Interestingly, higher food concentrations did not give reliably higher populations at the end of eight weeks, suggesting that either our algal species (*Scendesmus* sp.) were not favored food or that other factors were limiting their growth (Figure C.1). Previous studies have shown clutch size, survival and day to first reproduction to vary with different food types and concentrations (all extracted

data can be found in our metafile (Burgis, 1973; Mohammed, 2007; Martinez-Jeronimo and Ventura-Lopez, 2011)). Excretion and uptake rates of nutrients for *C. riguadi* would be helpful additional traits to measure.

While we find *C. rigaudi* throughout the tropic and subtropic regions (Figure 4.4), some of the records are quite old and may no longer be accurate. For example we didn't include a record from Garças Reservoir, Sao Paulo, Brazil (Di Genaro et al., 2015) because the most recent study did not find any *C. rigaudi* and it appears to have disappeared since 1997. However, it is unclear if this was seasonal and the species will return or if it is in fact extirpated. Many of our records are quite old (e.g. Jones, 1958; Egborge, 1987) or single sightings (e.g. Hart and Boane, 2004) and thus make any extrapolation about continued presence or changes over time impossible.

Tropical freshwater species are understudied. *C. rigaudi* is a widespread species that could be useful as a water quality indicator species, but more information about the species is critical. Here we provide some of the needed information. It is important to continue to learn about tropical species for water quality and toxicity because their responses can be quite different than temperate species (Mohammed, 2007).

Trait-based approaches are powerful tools for understanding community assembly (Cornwell and Ackerly, 2009), species distributions (McGill et al., 2006), and the impact of particular species on the environment (Lavorel and Garnier, 2002). However, trait-based methods require a large database of information about a wide array of species. Furthermore, our experiments indicate that the estimates of crucial parameters prerequisite for the quantitative evaluation of *C. rigaudi* role in aquatic systems are difficult to obtain even under controlled conditions and considerable time investment. Superimposed on the possible regional variation, this difficulty limits the range of predictions that trait-based models involving zooplankton species will be able to make. At the same time, our observations highlight a need for further research into the

variation of r and k and conditions explaining such variation.

Recent meta-analyses have made tremendous progress gathering data of zooplankton (Hébert et al., 2016; Robson et al., 2018) traits. These data include sizes, excretion rates, and nutrient ratios. These types of data can be used for easier parameterization of aquatic models or as a basis for understanding trait-environment interactions in zooplankton. Community and ecosystem ecology greatly benefits from these databases because they allow for more accurate tests of community assembly hypothesis, particularly trait-based approaches (McGill et al., 2006). The only way to build foundational databases is to have data; therefor we think it is imperative for more, particularly tropical species, to be used in lab experiments for life history information.

4.6 Acknowledgments

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Chapter 5:   Concluding Remarks

In this thesis I explored various methods for using species-specific traits to understand broad ecological questions.

In Chapter 2 I built and tested a simple nutrient-zooplankton-phytoplankton model (Franks, 2002) using relationships among nitrogen, algae and *Daphnia magna* in a lab-contained system. My goal was to determine how well a simple nutrient-phytoplankton-zooplankton (NPZ) model could describe a lab system and predict a response to perturbation, using only parameters and functional forms in the model fitted *a priori* to the same organisms and laboratory conditions.

When I started this journey I thought that building and verifying the "correctness" of a mechanistic model in a simple, lab system would be straightforward and produce expected results (i.e. good fits). I planned to explore how different configurations of species in functional groups improved or worsened the ability of mechanistic models to make good out of sample predictions. Instead, I discovered that even in a simple system where most of the interactions between groups has been well studied, a simple model failed to capture most of the dynamics. Part of this was driven by difficulties in obtaining appropriate parameter estimates from lab experiments; even using lab experiments designed to estimate specific parameters was not straightforward. Many studies in aquatic systems have shown that complex mechanistic models make poor predictions, and that most successful predictions have been from models where only a few key model components are the main drivers of the system (e.g. physical dynamics (Robson, 2014), lake depth; residence time and nutrient load (Chang et al., 2019)). Moving forward, predictive aquatic mechanistic models require a system-specific focus — that is, models should seek to understand particular systems without simply adding

complexity. However, I also believe that knowledge in aquatic ecology has developed enough that these system-specific models can be built upon well tested mechanistic foundations. For example, Chang et al. (2019) provides a mechanistic model for lake managers to understand and plan for eutrophication in their management area. More general models like this can be used in a first round of analysis in aquatic systems to identify key components of that system. As a follow-up, more detailed models can be built and parameterized to understand that system. At the very least, the recent trend towards more complicated models (Arhonditsis et al., 2007; Robson et al., 2018) without this foundation is setting the field on a dangerous path.

The Chang et al. (2019) model specifically focuses on lakes and eutrophication; future work could explore either other aquatic habitats or different or combined perturbation types. This strategy, of finding only a couple of easily measurable parameters, is particularly useful for management where larger models are unlikely to be parameterizable and a primary goal is to make relatively fast decisions and update those decisions as more data becomes available (Schuwirth et al., 2019; Arhonditsis et al., 2008).

In Chapter 3 I examined the utility of using functional groups to determine resilience after a eutrophication event. Theory suggests that increasing diversity should increase resilience (e.g. Allen and Polimene, 2011; Tilman and Downing, 1994). However, results remain mixed (e.g. Guelzow et al., 2017); perhaps diverse functional groups instead of taxonomy are more important for resilience (Karp et al., 2011; Hulot et al., 2014). The goal of this chapter was to try to separate the effects of functional diversity and taxonomic diversity on resilience. I was unable to complete as robust a test of this theory as I initially planned because one of the functional groups, a filter-feeder,(*Ceriodaphnia rigaudi*), was unable to live in the greenhouse; thus, I was unable to separate functional group increase from increases in diversity. However, I think

that this type of experimental set-up (multiple species in the same functional group compared to multiple species in different functional groups) is a critical experiment for furthering understanding of the role of diversity in resilience and as a robust test of ecological theory. Overall, I found that the relationship between diversity and resilience is not consistent and instead is based on which ecosystem metrics are used.

One unexpected result from these experiments is that the *Physa* sp. laid more eggs when *D.magna* were present than when they were not. Since snails and Cladocera are both important to nutrient cycling, from an ecosystem perspective understanding more about their interactions could be beneficial. From a community ecology perspective, these two groups are not generally studied together. Most community ecology studies about species interactions (e.g. not neutrality) focus on within-group interactions (e.g. direct competition), interactions through the food web (e.g. predator-prey, or trophic cascades), or direct interactions between disparate species (e.g parasites and hosts, plants and microbial symbionts) with less work that incorporates indirect interactions in distantly related genera. The interaction here between *Physa* sp. and *D. magna* falls into the last category, though the mechanism driving this finding remains unclear. The first step would be to verify that this interaction is actually occurring, and not just a by-product of our experiment since we didn't directly set out to test this interaction.

A replicable interaction would raise questions that could lead to insights about freshwater systems. For example: are *D.magna* directly interacting with *Physa* sp. in some way? Is there an interaction through water chemistry? Why was this interaction exacerbated by nutrient enrichment? Is the interaction density-dependent? Do more snail eggs translate into more snail adults? This relationship could lead to new insights about community development and population dynamics.

In Chapter 4 I add life history information for a ubiquitous tropical Cladocera, *Ceriodaphnia rigaudi*, from literature, lab and field experiments. Potential future work

could include building a species distribution model. In order to use traits to either build mechanistic models or to make statistical inference larger databases that include species from a wide range of habitats must be collected. Currently, zooplankton data is skewed toward temperate, saline species. This can cause biased expectations because even relationships that are regarded as true (e.g. size to grazing effort) may be different in tropical species (Mohammed, 2007). Chapter 4 contributes life history information in order to build a better repository of zooplankton data.

If trait-based approaches are the way forward in uniting ecological theory (McGill et al., 2006; Litchman et al., 2007) more robust tests of theory must occur. In addition, more species-specific information must be available across a wide range of habitats and taxa. Altogether, I demonstrate different ways of incorporating species-specific trait data to explore both theory and practical applications in aquatic systems.

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Appendix : Additional tables and figures for Chapter 2

Authors: Jo A. Werba, Ben Bolker and Jurek Kolasa

Treatment	Chl (μg/L)	SD Chl	NH4 (mg/L)	SD NH4
1	0.903	0.205	10.9	2.31
2	2.91	0.217	11.5	2.29
3	8.18	1.04	12.3	2.30
4	14.5	1.36	13.4	2.33
5	22.6	1.71	14.7	2.67
6	41.8	3.16	18.1	1.72
7	93.6	8.27	17.2	1.55

Table A.1: Starting food conditions for the feeding and nitrogen recycling rate experiment.

Treatment	Chl (μg/L)	SD Chl	Replicates
1	3.36	0.806	17
2	6.76	2.06	19
3	18.2	4.43	19
4	61.6	11.6	18

Table A.2: Starting food conditions for the birth and maturation rate.

Treatment	NH4 (mg/L)	SD NH4
1	2.76	0.033
2	3.40	0.045
3	5.68	0.09
4	12.91	0.209
5	23.32	1.23
6	46	2.92

Table A.3: Starting nitrogen concentrations for the nitrogen uptake and population growth experiment.

param	method	median	lower	upper
b_1	wide	13.46	10.93	17.67
b_2	wide	29.38	18.66	51.24
b_1	literature only	3.80	1.63	5.85
b_2	literature only	1.00	0.14	6.90
b_1	mixed model	3.90	2.06	5.73
b_2	mixed model	6.92	0.45	22.70
b_1	hyper	8.13	5.65	10.45
b_2	hyper	11.39	5.43	18.64
b_1	constrained b_1	6.33	4.38	8.23
b_2	constrained b_1	0.25	-19.55	19.59
d_2	unweighted	50.41	22.24	84.00
d_2	weight: 1/sd	67.55	65.17	69.92
d_2	weight: replicate	58.75	55.22	62.28
d_2	wide	24.43	22.33	26.70
d_2	informed	24.51	22.46	26.72
h_a	wide	0.09	0.08	0.10
x_a	wide	52.02	-18.20	124.39
h_a	mixed model	5.43	-9.88	32.96
x_a	mixed model	53.97	-16.29	121.64
h_a	literature only: impute sd	4.63	2.92	6.60
h_a	literature only: vary slope	20.36	-2.67	43.29
h_a	impute sd	0.09	0.09	0.10
x_a	impute sd	51.08	-13.04	118.71
h_j	wide	0.04	0.03	0.05
g	wide	4.82	4.13	5.68
x_j	wide	155.55	6.91	549.13

Table A.4: Model parameter estimates and 95% confidence intervals for each estimation method for all *D. magna* parameters.

Appendix : Additional tables and figures for Chapter 3

Authors: Jo A. Werba, Alexander C. Phong, Lakhdeep Brar, Acacia Frempong-Manso, Ofure Vanessa Oware and Jurek Kolasa

Model	Comparison	Estimate (log10)	SE	p
Chlorophyll Final	None - Physa	9.2	8.4	0.3
	None-Daphnia	33	12.3	0.02
	None - both	33	12.3	0.02
	Physa - Daphnia	24	9.19	0.02
	Physa - both	24	9.18	0.02
	Daphnia - both	-0.02	0.99	0.98
Chlorophyll Maximum	None-Physa	10.8	11.9	0.4
	None-Daphnia	60	12.1	0.0001
	None - both	61	12.2	0.0001
	Physa - Daphnia	49	10.6	0.0001
	Physa - both	50	10.7	0.0001
	Daphnia - both	0.97	1.7	0.58
Ammonium Final	None - Physa	1.05	1.5	0.5
	None-Daphnia	1.7	1.5	0.25
	None - both	-0.31	1.5	0.8
	Physa - Daphnia	0.64	1.4	0.65
	Physa - both	-1.36	1.4	0.35
	Daphnia - both	-2	1.3	0.14
Ammonium Maximum	None-Physa	-1.7	2.6	0.5
	None-Daphnia	-2.35	2.6	0.4
	None - both	-4.6	2.85	0.12
	Physa - Daphnia	-0.66	2.7	0.8
	Physa - both	-2.9	2.95	0.3
	Daphnia - both	-2.2	2.9	0.4
Sediment	None - Physa	-0.24	0.3	0.5
	None-Daphnia	0.96	0.35	0.008
	None - both	0.52	0.3	0.08
	Physa - Daphnia	1.2	0.35	0.001
	Physa - both	0.765	0.3	0.01
	Daphnia - both	-0.44	0.3	0.56

Table B.1: Model estimates for each of our measures of resilience. The estimate is difference between log10(disturbed)-log10(undisturbed) between herbivore treatments.

Model	Comparison	Estimate	StDev	p
Final Populations	Disturbed-Undisturbed	1.04	0.2	< 0.0001
	Daphnia-Both	-0.19	0.2	0.4
	Disturbed:Treatment	0.24	0.3	0.4
Maximum Populations	Disturbed-Undisturbed	0.6	0.2	0.005
	Daphnia-Both	-0.25	0.18	0.18
	Disturbed:Treatment	0.23	0.23	0.3
Probability Ephippia Present	Disturbed-Undisturbed	-4.4	2.2	0.04
	Daphnia-Both	-0.48	1.4	0.7
	Disturbed:Treatment	1.04	2.3	0.65

Table B.2: Model estimates for *Daphnia magna* populations

Model	Comparison	Estimate	StDev	p
Survival	Day	-0.09	0.006	< 0.0001
	Snail-Both	0.45	0.4	0.2
	Disturbed-Undisturbed	0.19	0.4	0.6
	Day:Herbivore Treatment	-0.02	0.008	0.004
	Day:Disturbance	-0.003	0.007	0.7
Probability Eggmass Present	Disturbed-Undisturbed	-2.05	0.55	0.0002
	Snail-Both	-4.9	0.7	< 0.001
	Disturbed:Treatment	2.97	0.8	0.0002

Table B.3: Model estimates for *Physa* sp. populations
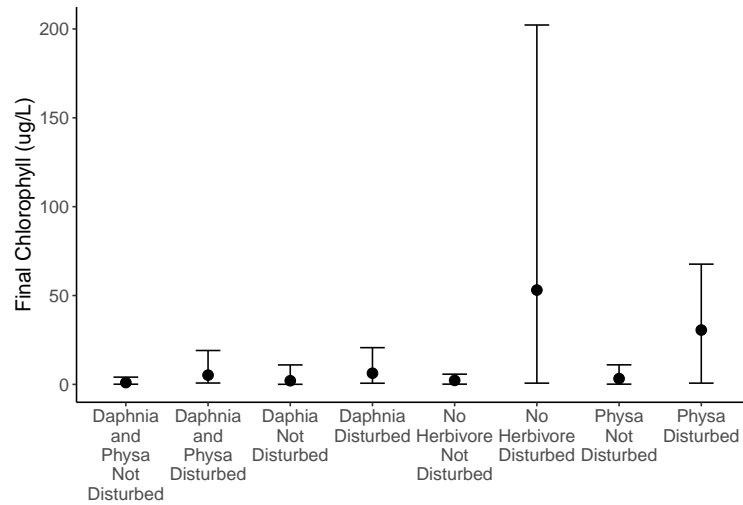
Figure B.1: Chlorophyll-a (μg/L) final concentrations by treatment. Points are means and error bars are 95% CI
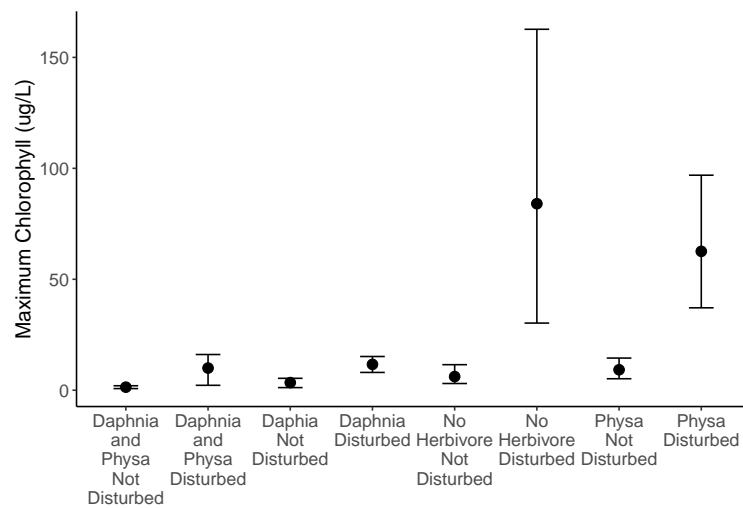

Figure B.2: Chlorophyll-a (μg/L) maximum concentrations by treatment. Points are means and error bars are 95% CI
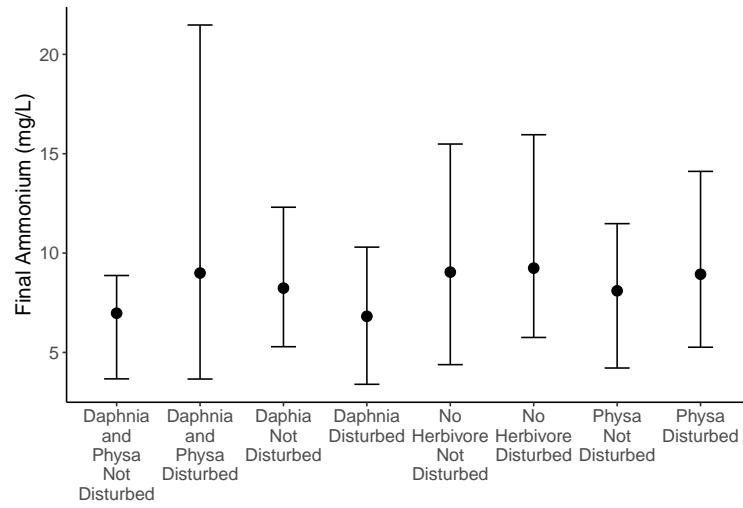
Figure B.3: Ammonium (mg/L) final concentrations by treatment. Points are means and error bars are 95% CI
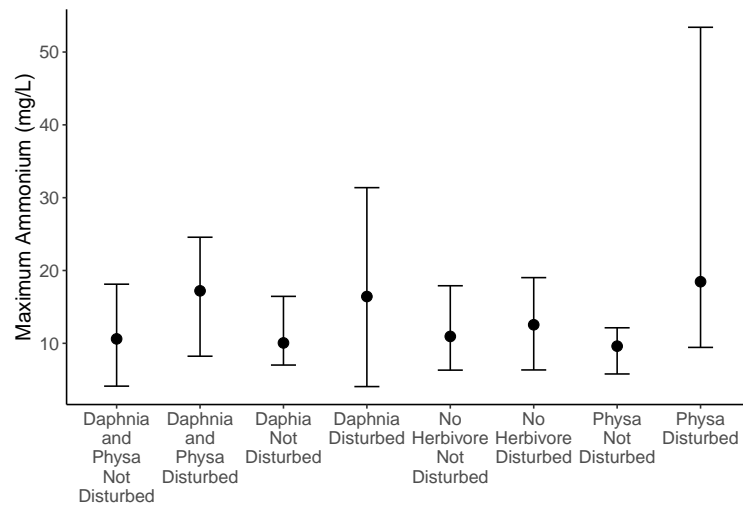

Figure B.4: Ammonium (mg/L) maximum concentrations by treatment. Points are means and error bars are 95% CI
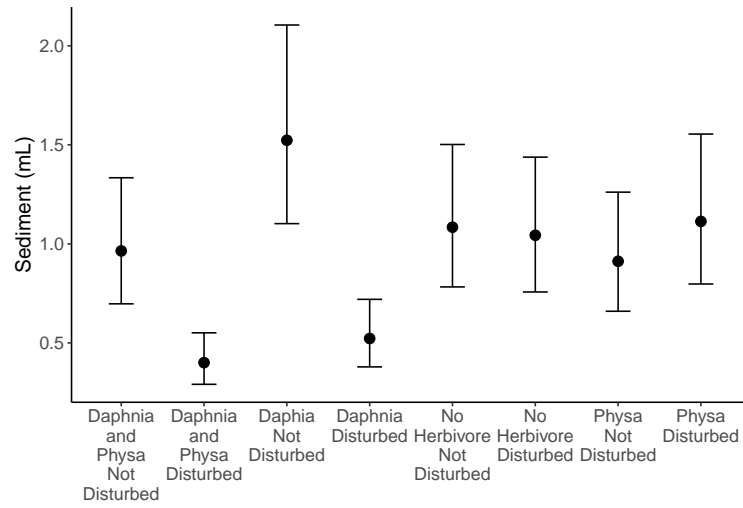
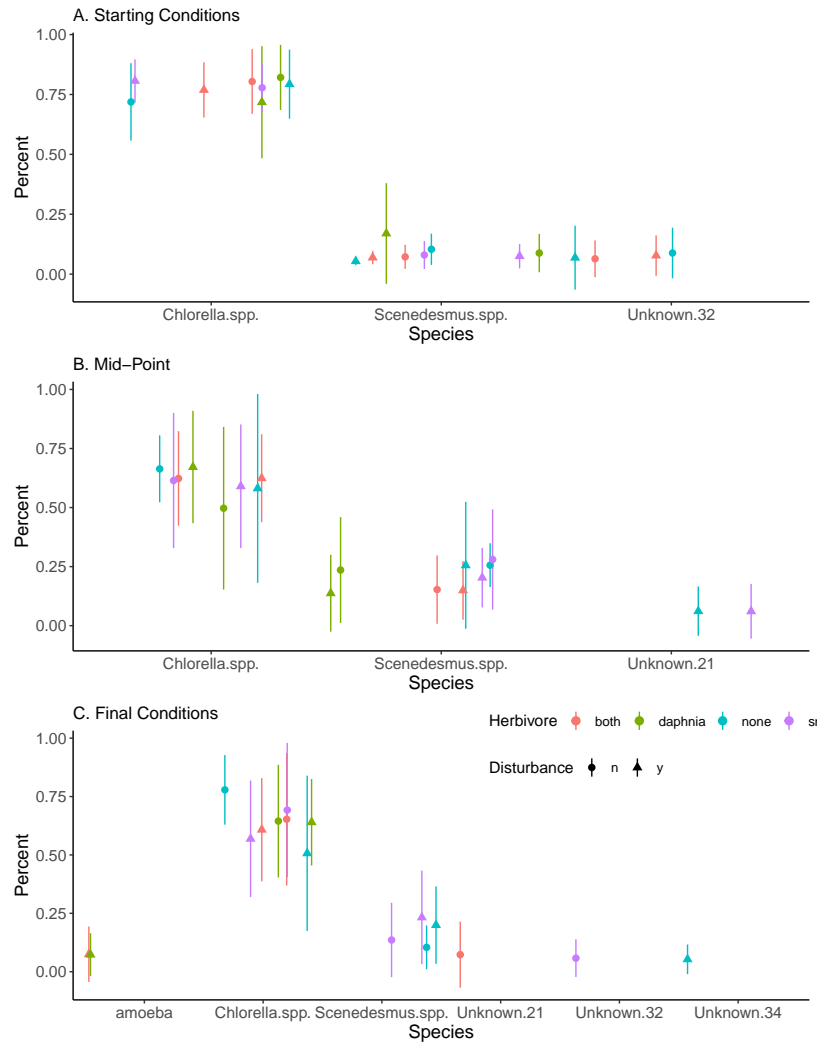Figure B.5: Sediment (mL) final amount by treatment. Points are means and error bars are 95% CI

Figure B.6: Algal species that represent greater than 0.5% of the community for the starting (A.), mid-point (B.) and end of the experiment (C.). Point color is herbivore combination. Point shape is disturbance treatment. Points are mean and error bars are standard deviation.

Appendix : Additional tables and figures for Chapter 4
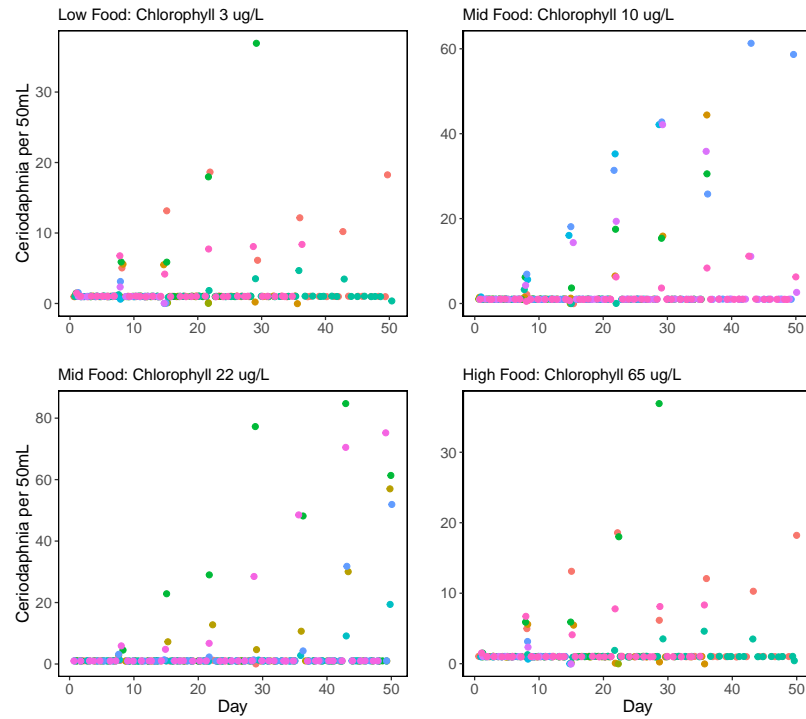
Authors: Jo A. Werba and Jurek Kolasa

Figure C.1: Daily *C.rigaudi* population per day. Color represents individual populations. The dots are a mean of eight sub-samples.