Factors influencing the coexistence of bromeliad-dwelling chironomids on Ilha do Cardoso, Brazil

by

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Abstract

Species interactions can influence the spatial distribution of organisms and the composition of local communities. To investigate how interactions influence the coexistence of invertebrates living in bromeliad phytotelmata, I combined methodological development and empirical exploration with the aim of understanding: 1) Which species in a community show signs of strong interactions, 2) Whether predators influence the outcome of competitive interactions and 3) Whether equalizing or stabilizing interactions between species change depending on context. To detect interactions between species given observational field data, I designed a method of finding negative co-occurrence patterns (using checkerboard units) between species based on their abundances in nature. Using this method, I found that three chironomid (Diptera: Chironomidae) species showed very strong negative co-occurrence patterns, suggesting that they experience net negative interactions (e.g. competition) or habitat filtering. Next, I performed a predator-addition experiment to assess the importance of predators in mediating the coexistence of the three chironomid species. Three predator species were added to bromeliads containing the three chironomid species. Although field observations suggested that at least one chironomid species should improve performance in the absence of predators, there was only a slight differential response to predators. Furthermore, one species of chironomid was competitively superior to the others in both the presence and absence of predators. We suspect that differing habitat preferences and the presence of other prey may be more important to coexistence than the presence or absence of predators alone. Finally, I performed an experiment to assess how habitat and ontogeny affect the outcome of competition between

the two most common chironomid species. When reared at the same body size, the two chironomids exhibited a stable relationship that we term here asymmetric equivalence: in one species experiences the world neutrally but the other does not. However, when species differed in their ontogenetic stage, the asymmetric equivalence disappeared. Taking all three studies together, I found that competition, but not predation, is an important factor in chironomid coexistence, but that differences in context lead to different coexistence outcomes.

Preface

All chapters in this thesis are the original work of A. D. Letaw. The ideas for all chapters were developed by A. D. L. with supervisor D. S. Srivastava. All chapters were written by A.D.L. in the form of manuscripts and edited by the co-authors. Chapter 2 is co-authored with D. S. S., who also collected the data used in that chapter. Chapter 3 is co-authored with D. S. S. and G. Q. Romero, who contributed to the ideas and manuscript. Demographic models used in this chapter were developed with A. Andrew M. MacDonald. Chapter 4 is co-authored with D. S. S. Experimental design for all chapters was developed by A. D. L. with D. S. S. Empirical work was completed by A. D. L. with field assistants Robert Fisette and Aline Nishi. All programming and analysis for all chapters was completed by A. D. L.

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For my family

CHAPTER 1

Introduction

"In that murky zone girdled by
Where have we come from
and Where are we bound
We exist.

Sometimes science can shine a light
into this dark region
betwixt Whence and Whither
Sometimes not."

–Marcia E Letaw

The most basic questions in community ecology concern the distribution of organisms across the biosphere. Why are species where they are? Answering this question could help us predict how environmental changes might lead to changes in species distributions over time. At the global scale, it is not difficult to understand why species are where they are: Organisms are the product of the environment in which they have evolved and therefore cannot persist under any arbitrary set of conditions. However, as we consider ever finer geographical scales, it becomes more difficult to understand which forces lead to the particular composition of a local community.

At a local level, species interactions may be one of the most important determinants of species distributions. In fact, according to early theory, very similar species that have highly overlapping resource use should not be able

to coexist at all (Gause, 1934; Grinnell, 1904; Hardin, 1960; Hutchinson, 1957; MacArthur and Levins, 1967) because one will always be better on average at obtaining or assimilating resources. Under this premise, it is then difficult to understand why similar species can be found in the same community together and how they can coexist. Fortunately, there are multiple explanations for how various aspects of a community and environment can maintain similar species at the metacommunity scale. These include explanations that utilize niche-based models, such as: habitat/spatial heterogeneity (Amarasekare, 2003; Chesson, 2000b; Loreau, 2004), phenology/temporal heterogeneity (Chesson and Warner, 1981; Godoy and Levine, 2014), multi-species interactions (Holt *et al.*, 1994; Spiesman and Inouye, 2015) and species traits (Bassett, 1995; Miner *et al.*, 2005); as well as explanations that propose alternatives to niche based models, such as neutral models (Connor and Simberloff, 1979; Hubbell, 1997, 2001); and combinations of niche and neutral (Cadotte, 2007; Gravel *et al.*, 2006). While niche models employ species differences to understand coexistence, neutral models consider species to be ecologically equivalent (equivalent in terms of competition and fitness), and explain coexistence as a result of random processes. Importantly, niche models usually focus on stable coexistence, in which species could theoretically cooccur together indefinitely, whereas neutral models accept an unstable version of coexistence, in which species may go extinct over time (Chesson, 2000b; Hubbell, 1979, 1997).

Modeling coexistence is difficult, both because community composition is often the result of multiple processes, and because the identity of those processes often vary between systems. Having a library of well understood systems could help us find larger scale ecological patterns. Some research programs have attempted to get at this deeper understanding. For example: studies of interactions between *Tribolium* spp. beetles investigated multiple

factors contributing to the outcome of competitive coexistence, including temperature, humidity, and relative abundance (Leslie *et al.*, 1968; Park, 1948, 1954, 1957); interspecific interactions such as competition and predation have been found to limit the distribution of barnacles in the intertidal zone in both Scotland and the San Juan islands (Connell, 1961a,b, 1970); competition, predation and dispersal are all factors affecting the distribution of zooplankton in lakes (Shurin, 2001; Shurin and Allen, 2001). Not only are these studies useful for providing a foundation from which to seek out patterns that cross geographic and ecosystem boundaries, they can also lead to the development of new ideas. For example, the unpredictable outcome of coexistence between *Tribolium* beetles under some environmental conditions is a good example of stochasticity in ecology; Connell's intertidal zone studies provide a textbook example of fundamental and realized niches.

In the following body of research, I study the factors that lead to coexistence (both stable and unstable) in a bromeliad invertebrate mesocosm. Bromeliads are a family of neotropical plants that provide habitat for aquatic invertebrates, primarily insect larvae. The bromeliad system consists of a detritus-fed food web maintained by fallen leaves from the surrounding canopy. In some cases, especially in open-canopy areas, algae replace detritus as the primary basal energy source (Brouard *et al.*, 2011, 2012; Frank and Lounibos, 2009). Allochthonous material is reduced from its whole form to fine particulate organic matter via an invertebrate-facilitated processing chain (Starzomski *et al.*, 2010). In turn, bromeliads obtain increased nitrogen input, especially when predators are present (Ngai and Srivastava, 2008), or terrestrial fauna (Gonçalves *et al.*, 2014; Romero *et al.*, 2006, 2010). Many species of invertebrates are supported by this system, most of which are larval Dipterans, including several species of mosquitoes (Culicidae) and chironomids (Chironomidae). The top predator is usually a damselfly, though leeches, corethrellids, ta-

banids and tanypodine chironomids are often present as well (Frank and Lounibos, 2009). Bromeliads are excellent systems for community ecology research because the invertebrate communities that live among their leaves are possible to delineate and easy to manipulate (Srivastava *et al.*, 2004). This ease of manipulation makes it possible to modify community structure in order to study the effects of species interactions and habitat characteristics on populations or the community as a whole.

The body of research concerning bromeliad-invertebrate community ecology has been growing rapidly over the past several years. We are starting to formulate ideas about which large-scale processes are the main factors influencing food-web structure. Macroinvertebrate diversity often increases with bromeliad size or water volume (Armbruster *et al.*, 2002; Dézerald *et al.*, 2014; Jabiol *et al.*, 2009; Jocque and Field, 2014); community composition may change with canopy cover, with predators favoring more open areas (Brouard *et al.*, 2012; Dézerald *et al.*, 2013). Furthermore, biotic interactions, especially predation (Dézerald *et al.*, 2014; Hammill *et al.*, 2015a,b; Starzomski *et al.*, 2010), but also competition (Lounibos *et al.*, 2003) are known to influence community composition, and sometimes ecosystem function as well. Now that the importance of these factors has been highlighted, more work is needed to understand how they work in combination to influence species coexistence at the local level.

In order to answer the question of how similar species can coexist in bromeliad food webs, I manipulated invertebrate communities in the state park of Ilha do Cardoso, Brazil (see more about the study site below). I started with the development and application of a new methodological approach to predict which species exhibit strong negative interactions based on observational data. I followed this with two empirical methods to determine whether species interactions and environmental variables led to coexistence of

the species indicated in the first portion of the research. I sought the answers to three main questions:

1. Which species exhibit signs of competition?
2. Is competition between the target species mediated by predators?
3. Do habitat and species-level traits change the outcome of competition between the target species?

To answer the first question, I modified a method of finding negative co-occurrence patterns called checkerboard analysis (Gotelli, 2000; Stone and Roberts, 1990). In checkerboard analysis, observational field data are used to identify cases of mutual exclusion between species in a community. Existing forms of the analysis use species incidence data, a practice that can compromise the biological relevance of the analysis. In Chapter 2, I modify the existing method to use species abundances and to differentially weight observed patterns based on those abundances. Then I use diagnostic testing to compare the abundance-based method with the original incidence-based version of checkerboard analysis. Questions two and three are answered by applying empirical methods to the species pairs identified in Chapter 2. Chapter 3 involves the addition of predators to whole bromeliads to determine whether or not they mediate the coexistence of competing detritivores. Following the predator-addition experiment, I compare total emergences between species and use a demographic model to compare their demographic (emergence and death) rates as well. The analysis of demographic rates allows us to get more information about how predators impact the three species differently than we can obtain from final measures. The development of this three-fate model also represents a methodological contribution to ecological analyses. In Chapter 4, I manipulate bromeliad size, ontogeny (body size) and relative abundance of two species to determine whether habitat type and phenotypic differences

change the outcome of competition. Manipulations of relative abundance allow us to answer questions about local coexistence, while manipulations of habitat and ontogeny lead to conclusions about regional coexistence.

1.1 THE STUDY SITE: ILHA DO CARDOSO, BRAZIL

My research was carried out in the state park of Ilha do Cardoso in São Paulo, Brazil (Figure 1.1). The habitat at the field site is comprised of *restinga* forest, a type of low canopy forest with sandy soil. Bromeliads are ubiquitous in the region, making it an ideal research site for bromeliad food web ecology. All experiments, were performed using the terrestrial bromeliad *Quesnelia arvensis* - the most common bromeliad species in the area. This species has serrated leaf margins and sharp spines at each leaf tip. The richness of bromeliad-dwelling invertebrate species is high in this area compared to other regions (Bromeliad Working Group, unpubl. data), with at least 166 species now documented (Romero and Srivastava 2010; P. M. Omena and G. C. Piccoli, pers. comm.). The high abundance of species makes Cardoso a great place to study species coexistence, as many taxonomically similar species cooccur in the region.

FIGURE 1.1: *Quesnelia arvensis* in the *restinga* of Ilha do Cardoso, Brazil.

CHAPTER 2

Assessing species associations using abundance weighted checkerboard patterns and null model analysis

2.1 INTRODUCTION

Ecologists have long been interested in understanding patterns of community structure. Earlier research often assumed that habitat filtering and biotic interactions are the most important determinants of species distributions (Vandermeer, 1972; Whittaker and Levin, 1975). More recently, greater consideration has been given to random events, such as dispersal and drift, when explaining species distributions (Hubbell, 1997, 2001; Vellend, 2010). Early efforts in disentangling these drivers of community structure were limited by the restrictions of empirical inference. However, modern computing power has made it feasible to perform large-scale simulations in order to understand the impact of random events on community assembly.

Checkerboard analysis was developed with the intention of distinguishing between communities structured primarily by competitive interactions and randomly assembled communities (Diamond, 1975; Gotelli, 2000; Stone and Roberts, 1990), and is still used to make inferences about community structure (e.g. Barberán *et al*. 2012; Bik *et al*. 2010; Horner-Devine *et al*. 2007; Presley *et al*. 2010). Checkerboard analysis works by measuring numbers of checkerboard units (CU) between species pairs. A checkerboard unit is a 2 x 2, species-by-site sub-matrix in which one species is present in the first site only, and the

second species is present in the second site only. In other words, the two species are mutually exclusive at the two sites. When repeated over multiple species pairs, this generates a checkerboard-like pattern to occurrence, thereby giving the name checkerboard unit. Usually, researchers are interested not in the CUs between species pairs, but in the average number of CUs per species pair for a community, known as the checkerboard score (C-score). Using a null model to shuffle the original data matrix, the C-score can be compared to C-scores generated from shuffled matrices. The null model is intended to simulate random processes and thus produce a community without the structuring effects of deterministic processes, such as competition (Gotelli and Ulrich, 2012). Thus, if the original C-score is higher than 95% of shuffled matrices, we can say that the community is likely structured more by those forces that lead to checkerboard patterns than by random events.

Although checkerboard analysis was designed to highlight the structuring effects of competitive interactions (Diamond, 1975), checkerboard patterns can also arise when species have a predator-prey relationship (Englund *et al.*, 2009; Jackson *et al.*, 1992), or when they are adapted to different sub-habitats. In the case of a predator-prey relationship, we note that this interaction should lead to elimination of the prey unless there is some other factor allowing the prey to coexist with or avoid predators. Therefore, it is likely that even in the case of predation-driven checkerboarding, environmental factors are also present. These alternative interpretations of checkerboard patterns add potential for discovering not only which species pairs are driving community-level patterns, but also which underlying mechanisms are responsible for mutual exclusion.

Historically, checkerboard analysis has been performed on presence-absence matrices. In these matrices, columns represent sites and rows represent species. The matrix is then filled with 1s and 0s to represent the presence or absence

of a particular species at a particular site. A checkerboard unit (CU) is then defined as a sub-matrix with the form:

$$\begin{array}{lccc} & \textit{Site } x & & \textit{Site } y \\ \textit{Species } A & 1 & \cdots & 0 \\ & \vdots & & \vdots \\ \textit{Species } B & 0 & \cdots & 1 \end{array}$$

where Species A is found in Site x but not y, and Species B is found in Site y but not x. (The dots above illustrate the fact that the two sites and species need not be found next to each other in the data matrix; other numbers may occur between them.) Full details of the methodology can be found in Stone and Roberts (1990).

Unfortunately, there are two problems with using presence-absence, rather than abundance data. First, sub-matrices that nearly comprise a CU are not counted under the strict presence-absence regime. For example, consider the following abundance-based sub-matrix:

$$A_1 = \begin{bmatrix} 100 & 0 \\ 1 & 98 \end{bmatrix}$$

Ecologically speaking, sub-matrix A_1 shows strong evidence for competitive exclusion or habitat filtering. However, the presence of a single individual outside of the checkerboard pattern means that this sub-matrix will not count as a CU. This is a major fault with standard checkerboard analysis for three reasons: First, species that experience strong competition will not necessarily compete to the point of exclusion of one or the other. For example, species that change habitat at different life stages (e.g. aquatic larvae vs. terrestrial adults) experience a limited period of competition before moving on to their new environment. Second, species distribution data are merely a snapshot of

the state of a community. A species that is in the process of extinction (e.g. as the result of competition) at a site could still have a few lingering individuals at the time of data collection. Third, single individuals are associated with high error because they could have been misidentified or recorded incorrectly during data collection.

A second problem with using presence-absence data over abundance data is that sub-matrices that qualify as CUs may actually provide only weak evidence of competitive exclusion because of overall low abundances of individuals; these sub-matrices still contribute equally to the overall checkerboard score. Low abundances correspond with high error rates, reducing our confidence in the pattern. For instance, consider sub-matrix A_2 below:

$$A_2 = \begin{bmatrix} 1 & 0 \\ 0 & 2 \end{bmatrix}$$

A_2 is considered to be a CU following the traditional definition, but the probability that this is a real checkerboard pattern as opposed to a random distribution of three individuals is quite low. The three individuals present in sub-matrix A_2 may be in the process of going extinct from the site, may have been misidentified, or may be in the process of moving to another site. Therefore, the low abundances reduce confidence in the ecological relevance of the pattern. In contrast, A_1 has high abundances and the relative difference between high abundance and low abundance cells is much larger in absolute terms. From a purely biological perspective A_1 provides much stronger evidence of a biologically important relationship than A_2. Thus, the strength of checkerboard patterns varies depending on observed abundances, but patterns of varying strength are given the same weight, and some strong patterns are not included at all.

To summarize, two problems exist in the current use of checkerboard analysis: 1) weak patterns are incorporated with equal weight to strong ones, and 2) strong patterns are eliminated completely due to the presence of a single or a few individuals. A recent method by Ulrich and Gotelli (2010) began to address the problems with checkerboard analysis by presenting an abundance checkerboard unit (ACU). Using these ACUs ensures that submatrix A_2 above would be included in the analysis. However, the more important problem of strong and weak ACUs both receiving equal weights still remains.

As mentioned above, checkerboard analysis is normally paired with a null model to test whether the score for the data matrix differs significantly from what is expected if random processes are responsible for species distributions. The null model takes the original data matrix and shuffles the values in a manner that is supposed to "randomize" the data, removing the mechanism of interest (Gotelli and Ulrich, 2012). However, there are a variety of possible algorithms that could be used to shuffle a data matrix and the best one depends on what mechanism is being studied. In incidence-based checkerboard analysis, the preferred algorithm shuffles presences and absences by preserving row and column totals (Gotelli, 2000; Stone and Roberts, 1990). Since columns represent sites and rows represent species, this is the same as forcing species richness per site and the number of sites per species to be constant. Although this method works well for incidence-based checkerboard analysis, the same is not true when abundances are introduced; Checkerboard analysis on abundances has favoured a probabilistic model, where row and column sums are not fixed but the probability of placement in rows and columns is based on those sums (Ulrich and Gotelli, 2010). Because it is difficult to know a priori which method may be best for a particular analysis, it is essential to

test a range of null model algorithms on sample matrices to calculate the Type I and Type II error rates associated with each (Gotelli and Ulrich, 2012).

In this paper, we present and test a new method for measuring species segregation using abundance data, solving existing problems with checkerboard analysis. With this method, we define an abundance checkerboard unit (ACU) and apply a weight and strength to this unit to generate an abundance-weighted checkerboard unit (AWCU). Finally, we generate an abundance-weighted checkerboard score, or AWC-score to replace the traditional C-score as a metric of species segregation in a community. To determine the best null model for use with our new metric, we tested nineteen null model algorithms with sample matrices to find the Type I and Type II error rates associated with each one.

As a follow-up to developing this new method of checkerboard analysis, we were interested in comparing our method with the incidence-based (Stone and Roberts, 1990) and abundance-based (Ulrich and Gotelli, 2010) methods that have preceded it. To this end, we conclude by analysing one of our own datasets using all three methods.

2.2 METHODS

2.2.1 *Abundance Weighted Checkerboards*

An abundance checkerboard unit (ACU) is defined as follows. Given any 4-cell sub-matrix $ABxy$, where A and B are species, x and y are sites, and Ax, Ay, Bx, and By are abundances of the respective species at the respective sites:

$$ABxy = \begin{bmatrix} Ax & \cdots & Ay \\ \vdots & & \vdots \\ Bx & \cdots & By \end{bmatrix}$$

$ABxy$ comprises an abundance checkerboard unit if:

[1] $Ax > Bx$ and $By > Ay$

OR

[2] $Ax < Bx$ and $By < Ay$

To distinguish between high and low abundance ACUs, we created a weighting method. Abundance weighting is performed in two steps. First, we defined a weight, W, for each ACU:

$$W = \left| \frac{Ax}{At} - \frac{Bx}{Bt} \right| + \left| \frac{Ay}{At} - \frac{By}{Bt} \right| \tag{2.1}$$

where At and Bt are equal to the total abundance of species A and B, respectively. This generates a value between 0 and 2. The differences calculated give a measure of the amount of overlap between species at the given sites. If the difference is small, then species show a near absence of exclusion, which will generate a small weight. Dividing by species abundance totals standardizes the abundance measures, accounting for species that naturally occur at widely different abundances. For example, in bromeliad invertebrate communities in Ilha do Cardoso, Brazil, damselfly nymphs *Leptagrion* spp. are comparatively massive and the average observed abundance is three per bromeliad (D. S. Srivastava and G. Q. Romero unpubl. data). At the same site, ostracods *Elpidium bromeliarum* are barely visible to the eye and have been observed at abundances into the thousands (D. S. Srivastava and G. Q. Romero unpubl. data). Thus, using ratios avoids skewing the data in favour of species combinations that involve naturally abundant species paired with naturally rare species.

The inclusion of the weight is still not sufficient to solve all problems, however. Using the above calculation for W, we generate some ACUs with equivalent values of W but different strengths corresponding with different overall abundances. For example, suppose we have three ACUs as follows:

$$ACU_1 = \begin{bmatrix} 200 & 100 \\ 100 & 200 \end{bmatrix}$$

with $At = 300$ and $Bt = 300$

$$ACU_2 = \begin{bmatrix} 20 & 10 \\ 10 & 20 \end{bmatrix}$$

with $At = 30$ and $Bt = 30$

$$ACU_3 = \begin{bmatrix} 2 & 1 \\ 1 & 2 \end{bmatrix}$$

with $At = 3$ and $Bt = 3$. Because the ratios Ax/At, Ay/At, Bx/Bt and By/Bt are equal in all three sub-matrices, all three ACUs have weight, $W = 0.667$. However, ACU_1 represents a stronger checkerboard than ACU_2 and ACU_3 because of higher overall abundances. We therefore create a measure of strength based on abundances only, in order to increase the value of the abundance-weighted CU when abundances are high but ratios are equivalent. Strength, S, is calculated as:

$$S = \log_{Nmax}(A_{max}) + \log_{Nmax}(B_{max}) \tag{2.2}$$

where N_{max} is the maximum abundance value in the matrix, and A_{max} and B_{max} are the maximum values of species A and B within the sub-matrix. This

generates a value between 0 and 2 (the same range as W). Using log base N_{max} scales the values such that the cell with highest abundance will give a value of 1. This means that S is scaled to the abundance distribution of each specific matrix. Assuming the above three sub-matrices come from a matrix with $N_{max} = 200$, the strengths are as follows:

$$S(ACU_1) = 2$$

$$S(ACU_2) = 1.131$$

$$S(ACU_3) = 0.262$$

Finally, these values are added to W, to get a set of abundance-weighted checkerboard units (AWCU):

$$AWCU_1 = S_1 + W_1 = 2.667$$

$$AWCU_2 = S_2 + W_2 = 1.798$$

$$AWCU_3 = S_3 + W_3 = 0.929$$

To calculate an abundance weighted checkerboard score for an entire matrix, AWCUs are calculated for each unique 2 x 2 sub-matrix in the data set (i.e. every pair of species at every pair of sites). The abundance weighted checkerboard score is the mean of all AWCUs for the matrix.

2.2.2 *Null Models*

When converting from an incidence-based to an abundance-based matrix, the number of possible null model algorithms increases. We evaluated nineteen null model algorithms for shuffling matrices. Nine of these were previously

employed by Ulrich and Gotelli (2010) and the remaining ten were of our own design.

Null models for shuffling abundance data can be categorized in two main ways. First, zero cells in the original data can either be retained or ignored in the null matrices. Fixed zero null models retain the placement of zero cells and floating zero models allow species to be placed in sites where they did not exist in the original matrix (Ulrich and Gotelli, 2010). Second, null models can be categorized based on whether populations, or individuals are rearranged between the cells. In population-based models, the entire population of one species at one site is shuffled into another cell. In individual-based models, species are placed one-by-one into new cells (Ulrich and Gotelli, 2010). Biologically, a population-based model assumes that dispersal is clustered. The bromeliad-dwelling ostracod *Elpidium bromeliarum*, for example, is largely unable to migrate between bromeliads, so offspring remain together after reproduction. Individual-based models, on the other hand, assume that dispersal is individualized, as in bromeliad-dwelling frogs of the *Scinax perpusillus* group, which oviposit one egg at a time, splitting their clutch between bromeliads (AlvesSilva and da Silva, 2009).

We constructed nineteen null models and named them based on the following conventions: 1) As the first letter, **I** = individual-based, **P** = population-based; 2) As the second letter, **X** = fixed-zero, **L** = floating-zero. **D** is used as a stand-in in the individual-based models to represent "dropped" zeros. In other words, the zeros are neither preserved by "fixing" them, nor by "floating" them; they are merely dropped; 3) As the third, and possibly fourth letter(s), **R** means row sums are preserved, **C** means column sums are preserved, **RC** means both row and column sums are preserved, and **M** means the sum of the entire matrix is preserved (but row and column sums may fluctuate); 4) As the final letter(s), **U** means there is a uniform probability of

being placed in any cell, (though this is not strictly true if row and/or column sums are preserved), **R** or **C** means placement probabilities are proportional to row or column sums, and **RC** means probabilities are proportional to row AND column sums. Following is the complete list of null models that were tested:

- **PXRU**, **PXCU**: Population-based models with fixed zeros and row or column sums, respectively, preserved.
- **PXMU**: A population-based model with fixed zeros and populations placed in any cell with equal probability.
- **PLRU**, **PLCU**, **PLMU**: Population based models with preserved row, column, or matrix sums, respectively. Same as PXRU, PXCU, and PXMU above, but with floating zeros.
- **IDRU**, **IDCU**: Individual-based models with row or column sums, respectively, preserved.
- **IDRCU**: An individual-based model with preserved row and column sums.
- **IXRU**, **IXCU**: Same as IDRU and IDCU above, but with fixed zeros.
- **IDMR**, **IDMC**: Individual-based models with placement probabilities proportional to, respectively, row or column sums.
- **IDMRC**: An individual-based model with placement probabilities are proportional to both row and column sums.
- **IXMR**, **IXMC**, **IXMRC**: Same as IDMR, IDMC and IDMRC above, but with fixed zeros.
- **IDMU**, **IXMU**: Individual-based models with, respectively, fixed or dropped zeros where placement in any cell is equiprobable.

2.2.3 *Diagnostic Testing*

We ran diagnostic tests to determine the ability of each null model to correctly distinguish random from structured (checkerboarded) data. We estimated Type I and Type II error rates of each null model by creating 400 each of random and structured test matrices and performing the null model analysis on each one. For each test matrix, we ran each null model 1000 times, generating 1000 shuffled matrices per test matrix.

Diagnostic testing was performed first on the random test matrices in order to obtain a Type I error rate. Random matrices were created in two different ways (designated as M_R and M_S) by sampling from a log-normal distribution. A full description and defence of the diagnostic testing can be found in Ulrich and Gotelli (2010; see also Gotelli and Ulrich, 2012). Type I error was calculated as the number of test matrices showing significantly high or low ($\alpha = 0.05$) amounts of structure compared to shuffled versions of the matrix (either $k \leq 0.025$ or $k \geq 0.975$ where k is the proportion of shuffled matrices with a lower AWC score than the test matrix). Only models that generated $p \approx 0.05$ were tested for Type II error. This corresponded to 10 out of 400 test matrices with $k \leq 0.025$ and 10 with $k \geq 0.975$.

Type II error rates were calculated by adding structure to random matrices to find the point at which structure was detected. Rows of structure were added one at a time and then the new matrix was run through the null model again. Each row was selected randomly from a uniform distribution. Structure was generated by taking the maximum value, r_{max} in the selected row, and alternating that value with 0s. Depending on whether the row and column index were even or odd, we switched between alternating in a 0, r_{max}, ... pattern or a r_{max}, 0, ... pattern. This ensured that 0s would be offset and ACUs generated. Once the matrix showed more structure than 95% ($k \geq 0.95$) of the shuffled matrices, we stopped adding structure and the proportion of

structured rows was recorded. After all 400 test matrices were analysed, we generated a mean proportion of structure needed for the model to generate $k \geq 0.95$.

2.2.4 *Analysis of Community Data*

To compare the results of using incidence-based, abundance-based and abundance-weighted checkerboard analysis, we performed all three methods on a data set of bromeliad-invertebrate communities (D. S. Srivastava and G. Q. Romero unpubl. data) using the null model algorithm best suited to each one. The Bromeliaceae is a neotropical plant family that collect water in their leaf axils, providing habitat for aquatic insect larvae and other small fauna. Our data were collected from Ilha do Cardoso, an island off the coast of São Paulo province in Brazil. This community is home to over 100 species of invertebrates (Romero and Srivastava 2010; Srivastava 2015 pers. comm.), many of which may have strong interactions. In particular, three species of Chironmidae (*Chironomus detriticola*, *Polypedilum kaingang* and *Polypedilum marcondesi*) are observed to occur in different mean bromeliad sizes (Letaw, 2015), but also co-occur frequently. We therefore expected that these might display strong checkerboard patterns in the abundance-weighted metric, but not the incidence- or abundance-based ones.

For each analysis, we ran the best suited null model 5000 times. For the incidence-based analysis, we used null model IDRCU, with fixed row and column sums (Gotelli, 2000). For the abundance-based analysis we used null model IDMRC, with probabilistic placement based on row and column sums (Ulrich and Gotelli, 2010). For our own abundance-weighted analysis, we used the optimal null model as determined by this research.

Each of the aforementioned analyses generates a community-level score and a p-value describing whether the community differs significantly from

the null expectation of random species segregation. In the event of significant checkerboarding, we were interested in determining which species pairs were driving the pattern. We did this by analysing the distribution of units (CU, ACU, or AWCU) for every species pair. From this distribution, we found the mean number of units and the standard deviation. Any species pairs that had more than the mean + 2 SD were designated as the highly segregated species pairs driving the checkerboard pattern.

2.3 RESULTS

As a result of the diagnostic testing, we found that most null models had very high Type I error rates, with test matrices exhibiting lower AWCU scores than their corresponding shuffled matrices (Table 2.1). This pattern held across both M_R and M_S type matrices. Models that had some constraint on both row and column values gave the lowest error rates, although this did not hold when zeros were fixed. The model with lowest Type I error was IDRCU, a model that fixes both row and column sums, which generated an error rate of 0.025 for M_R matrices and 0.05 for M_S matrices.

Because all other models had unacceptably high error rates, we only assessed Type II error for model IDRCU. In the M_R type matrices, the mean fraction of structured rows needed to generate $k \geq 0.95$ was 0.221 ± 0.223. 11 of the 200 matrices never generated $k \geq 0.95$. For the M_S type matrices, the mean fraction of structured rows was 0.132 ± 0.161. 8 of the 200 matrices never generated $k \geq 0.95$. Those matrices that never generated $k \geq 0.95$ correspond to a Type II error rate of 0.04 to 0.055.

Null Model	M_R		M_S	
	$k < 0.025$	$k > 0.975$	$k < 0.025$	$k > 0.975$
PXCU	172 [0.860]	0 [0.000]	193 [0.965]	0 [0.000]
PXRU	115 [0.575]	0 [0.000]	193 [0.965]	0 [0.000]
PXMU	174 [0.870]	0 [0.000]	194 [0.970]	0 [0.000]
PLCU	176 [0.880]	0 [0.000]	197 [0.985]	0 [0.000]
PLRU	191 [0.955]	0 [0.000]	196 [0.980]	0 [0.000]
PLMU	190 [0.950]	0 [0.000]	199 [0.995]	0 [0.000]
IDCU	147 [0.735]	0 [0.000]	196 [0.980]	0 [0.000]
IDRU	125 [0.625]	2 [0.010]	192 [0.960]	2 [0.010]
IDRCU	2 [0.010]	3 [0.015]	4 [0.020]	6 [0.030]
IDMU	175 [0.875]	0 [0.000]	199 [0.950]	0 [0.000]
IXCU	169 [0.845]	0 [0.000]	195 [0.975]	0 [0.000]
IXRU	177 [0.885]	1 [0.005]	193 [0.965]	0 [0.000]
IXMU	158 [0.790]	5 [0.025]	197 [0.985]	0 [0.000]
IDMC	133 [0.665]	2 [0.010]	191 [0.955]	0 [0.000]
IDMR	70 [0.350]	4 [0.020]	185 [0.925]	2 [0.010]
IDMRC	0 [0.000]	33 [0.165]	1 [0.005]	11 [0.055]
IXMC	125 [0.625]	19 [0.095]	168 [0.840]	1 [0.005]
IXMR	0 [0.000]	167 [0.835]	130 [0.650]	5 [0.025]
IXMRC	0 [0.000]	196 [0.980]	0 [0.000]	193 [0.965]

TABLE 2.1: Number of matrices with higher AWCU than at least 95% of shuffled matrices. A model with a standard Type I error rate of $\alpha = 0.05$ should generate around 5 matrices with $k > 0.975$ and 5 with $k < 0.025$ where k is the proportion of shuffled matrices with a lower AWC score than the test matrix. Error rates are calculated in square brackets.

2.3.1 *Analysis of an Example Community Dataset*

We now consider the performance of the AWC approach, as compared to C and AC approaches, in terms of analysing a real ecological dataset: the bromeliad macroinvertebrate communities of Ilha do Cardoso, Brazil. All three checkerboard metrics generated significant checkerboard scores when

tested against the relevant null model: incidence-based ($CS = 7.46$, $p = 0.0056$), abundance-based ($ACS = 9.98$, $p = 0.0002$), abundance-weighted ($AWCS = 10.14$, $p = 0.0002$). However, each model generated a very different set of highly segregated species pairs (Table 2.2), with C and AC approaches sharing 22% of species, AC and AWC sharing 37% of species, and C and AWC sharing only 2.6% of species; only a single species pair was preserved across all three methods.

2.4 DISCUSSION

Here we developed a novel method of performing checkerboard analysis on abundance data and weighting checkerboard scores based on these abundances. Further, we found that a null model that preserves matrix column and row sums was the best choice for using with AWC analysis.

Evaluating new analyses with diagnostic testing is an essential part of ensuring the analysis is robust (Gotelli and Ulrich, 2012). In the case of null model shuffling algorithms, each one should be tested for Type I and Type II error rates to confirm that the model is creating random matrices as expected (Gotelli, 2001; Gotelli and Ulrich, 2012). In other words, a shuffling algorithm should neither reject the null hypothesis that a data matrix is random too often ($\alpha = 0.05$), nor should it falsely reject the alternate hypothesis that the data matrix is structured too often (β). For our AWC analysis, we found that most null model algorithms had error rates well above the desired α level, and were therefore unsuitable for use (Table 2.1). In fact, this is not unusual; previous analyses using similar diagnostic tests have also shown that many null model algorithms are prone to high Type I error (Gotelli, 2000; Ulrich and Gotelli, 2010). In our case, the only model with low susceptibility to Type I error was IDRCU, the "fixed-fixed" model that fixes the sum of row and column

abundances. This result is not surprising because the fixed-fixed model is also considered to be the best null model for incidence-based checkerboard analysis (Gotelli, 2000). A good null model algorithm will attempt to remove the mechanism of interest, and in all checkerboard analyses that is the structuring caused by net negative species interactions or habitat filtering.

Because model IDRCU was the only model with reasonable Type I error rates, this model was the only one tested for Type II error. When rows of structure were added to the model, 13% to 20% of an otherwise random matrix had to be filled with structured rows in order to generate a $p < 0.05$. This value is similar to the abundance-based metric, which found significant structure when checkerboarding was increased by 1-10% (Ulrich and Gotelli, 2010). Compared to the incidence-based metric, however, these values are somewhat lower (Gotelli, 2000). In that analysis, Type II error was measured by adding randomness to structured matrices, the reverse process to that employed here and by Ulrich and Gotelli (2010). Structure was still detectable when up to $\sim$50% of the matrix had been randomized. This difference between incidence and abundance-based methods is likely related to the probability of generating checkerboard units at random in each method. If CUs appear frequently by chance, a large percentage of structure should be required to generate a significantly high level of checkerboarding because the average number of checkerboards in shuffled matrices will be high. In contrast, if CUs appear rarely by chance, a smaller percentage of structure will be required to push the matrix to the alpha level of significance. In fact, logic tells us that the probability of generating CUs by chance must be lower when abundances are used. Because the random matrices are generated using a log-normal distribution (see Methods), some species are much more abundant than others and it is unlikely for very abundant species to form checkerboard patterns with rare species; rare species will frequently not have high enough

abundances to be more abundant at a given site than the common species. However, if abundances are converted to incidences, the effect of high and low abundances is removed and any species can randomly checkerboard with any other species.

The relative ease of producing a CU by chance under different types of checkerboard analysis can inform us about the differences between the methods. Because ACUs are more difficult to generate by chance than incidence CUs, and because it takes a smaller fraction of structure in the matrix to generate a significant AC- or AWC-Score, abundance methods should give higher significance levels to matrices with equal or lower amounts of checkerboarding. In practice, we found this to be true. Our analysis of an empirical data matrix showed significant amounts of checkerboard structure in all three analyses. However, the level of significance was much higher in both abundance-based methods than in the standard incidence analysis.

Checkerboard analysis has primarily been used to assess the level of competition and species segregation within a whole community. An extension of this is to consider which species pairs are driving the pattern of segregation. This information can lead to fruitful empirical examinations of species in situ. We analysed our empirical data to find out which highly segregated species pairs were driving the patterns of checkerboarding in the community. To illustrate the usefulness of analysing segregated pairs, we draw the reader's attention to response of midge larvae in the family Chironomidae. Three species of Chironomidae – *Polypedilum kaingang*, *Polypedilum marcondesi*, *Chironomus detriticola* – are known competitors with different preferences for plant size (Chapter 4). On average, *P. kaingang* are found in small plants ($\sim$ 250 mL), *P. marcondesi* are found in medium plants ($\sim$ 525 mL) and *C. detriticola* are found in large plants ($\sim$ 875 mL). However, the three species also experience some overlap and do not show complete mutual exclusion,

suggesting that abundance and incidence measures of co-occurrence will not be identical. Under incidence-based checkerboard analysis, no pairwise combination of the three species showed high levels of segregation (Table 2.2). Once abundances were used by either the AC or AWC method, *P. marcondesi* and *P. kaingang* had ACUs in the high end of the distribution. Furthermore, using our abundance-weighted metric, *C. detriticola* and *P. marcondesi* also appeared as highly segregated. The findings from our AWC analysis pair well with our own knowledge and experimental results on the interaction between the three species. In particular, *C. detriticola* and *P. marcondesi* have been found to have an interesting competitive relationship when reared together in bromeliads (Chapter 4). However, using incidence-based or abundance-based CA, we would not have detected this species pair as a duo of interest.

Interestingly, there was little overlap between the highly segregated species according to incidence-based checkerboard analysis and those found with the two abundance-based analyses. This suggests that the incidence-based patterns we found in our data matrix were often weak or rare when 1s and 0s were converted to abundances. Switching from incidence-based to abundance-based, if there are many more ACUs than CUs, the species pairs with high CUs will be moved to the middle or lower part of the distribution and no longer appear as highly segregated. Furthermore, converting to AWCUs, the actual value of abundances becomes important. If pairs with high CUs or ACUs are comprised of low total abundances, they may be moved even lower in the distribution and disappear from the list of highly segregated species pairs when the abundance-weighted analysis is used. It is instructive to compare species abundances underlying a species pair from Table 2.2 with high CU (Corethrellidae sp.1, *Wyeomyia* sp.) and one with high AWCU (*P. marcondesi*, *Scirtes* sp.). In Table 2.3, abundances are shown for these two species pairs. The high CU pair show a high degree of apparent mutual exclusion, but

overall abundances are relatively low. Interestingly, this is a predator-prey combination (Corethrellids consume mosquito larvae, including *Wyeomyia*). *Wyeomyia* mosquito larvae are known to avoid their predators by occurring in smaller bromeliads than most other bromeliad-dwelling insects, (Hammill *et al*., 2015a). In comparison, the high AWCU pair have a lot of species overlap at sites, but high abundances and apparent mutual suppression have led to a high number of AWC units. Once again, this highlights the utility of AWC analysis in detecting structured patterns between species pairs; when putative pairs of interacting species co-occur, standard incidence-based CA is unable to detect them.

In spite of the advantages of using AWC analysis, incidence-based CA may still be a useful method for some purposes. We have seen that the incidence-based method tends to be more conservative in its estimates of the amount of structure in a community. The fact that approximately 50% of the community must be structured to generate a significant C-score (Gotelli, 2000) means that the incidence-based method gives a score more representative of the community as a whole than do the abundance-based and abundance-weighted methods. Combined with its strict adhesion to complete mutual exclusion, these qualities suggest that standard CA would be useful for detecting a strong environmental filter within a community. If the area of interest is actually species-level interactions, then we recommend the AWC analysis described here. Because our analysis is more sensitive to the presence of checkerboarding and better at detecting interactions between species, it is a good way to find strong interactions between species that may actually be co-occurring within sites. Furthermore, following the AWC analysis with a post-hoc test to find highly segregated species pairs, as we did here, can lead to the identities of competing species and open the door to further empirical study.

Species 1	Species 2	C	AC	AWC
Orthocladiinae sp.	*Tanytarsus* sp.	49	67	–
Ephydridae sp.	*Monopelopia caraguata*	50	–	–
Bezzia sp.	*Polypedilum kaingang*	51	–	–
Corethrellidae sp. 2	*Polypedilum kaingang*	51	–	–
Dasyhelea sp.	*Polypedilum marcondesi*	51	–	–
Polypedilum kaingang	Psychodidae sp.	52	72	–
Corethrellidae sp. 1	*Dasyhelea* sp.	52	–	–
Tubificidae sp.	*Wyeomyia* sp.	52	–	–
Elpidium bromeliarum	Ephydridae sp.	56	72	–
Ephydridae sp.	*Trentepohlia* sp.	56	72	–
Dasyhelea sp.	Tubificidae sp.	56	–	–
Leptagrion andromache	*Wyeomyia* sp.	56	–	–
Elpidium bromeliarum	*Wyeomyia* sp.	60	73	–
Trentepohlia sp.	*Wyeomyia* sp.	60	–	–
Scirtes sp.	*Wyeomyia* sp.	64	68	64.7
Diptera sp.	*Elpidium bromeliarum*	64	–	–
Diptera sp.	*Trentepohlia* sp.	64	–	–
Corethrellidae sp. 1	*Wyeomyia* sp.	65	–	–
Polypedilum kaingang	Trichoptera sp.	–	68	87.7
Culex sp.	*Elpidium bromeliarum*	–	72	91.2
Polypedilum kaingang	Tubificidae sp.	–	75	67.1
Polypedilum kaingang	*Polypedilum marcondesi*	–	76	92.8
Corethrellidae sp. 1	*Polypedilum kaingang*	–	81	80.5
Polypedilum kaingang	*Scirtes* sp.	–	82	99.1
Polypedilum kaingang	*Tanytarsus* sp.	–	87	73.5
Polypedilum kaingang	*Trentepohlia* sp.	–	87	81.9

Species 1	Species 2	C	AC	AWC
Elpidium bromeliarum	*Polypedilum kaingang*	–	118	125.8
Culex sp.	*Trentepohlia* sp.	–	–	64.7
Elpidium bromeliarum	*Trentepohlia* sp.	–	–	65.8
Elpidium bromeliarum	*Monopelopia caraguata*	–	–	65.8
Polypedilum marcondesi	*Wyeomyia* sp.	–	–	67.0
Chironomus detriticola	*Polypedilum kaingang*	–	–	73.6
Elpidium bromeliarum	Tubificidae sp.	–	–	77.8
Elpidium bromeliarum	Trichoptera sp.	–	–	78.4
Culex sp.	*Polypedilum kaingang*	–	–	78.9
Chironomus detriticola	*Elpidium bromeliarum*	–	–	84.8
Polypedilum marcondesi	*Scirtes* sp.	–	–	100.5
Elpidium bromeliarum	*Scirtes* sp.	–	–	107.8
Elpidium bromeliarum	*Polypedilum marcondesi*	–	–	117.3

TABLE 2.2: Checkerboard (C), abundance checkerboard (AC) and abundance-weighted checkerboard (AWC) units for the most highly segregated species pairs under each analysis. "Highly segregated" species are those from a community with a significant checkerboard score (incidence-based, abundance-based, or abundance-weighted) and whose number of units exceeds the mean + 2 SD for the dataset.

Bromeliad	High CU pair		High AWCU pair	
	Corethrellidae sp. 1	*Wyeomyia* sp.	*P. marcondesi*	*Scirtes* sp.
B1	23	0	76	90
B2	18	0	89	65
B3	10	0	82	69
B4	10	0	19	0
B5	8	0	57	63
B6	8	0	11	39
B7	6	0	31	13
B8	5	0	29	55
B9	4	0	21	22
B10	2	0	0	17
B11	1	0	12	0
B12	1	0	7	37
B13	1	0	7	1
B14	0	0	14	4
B15	0	0	11	9
B16	0	0	4	7
B17	0	0	2	4
B18	0	0	0	1
B19	0	0	0	0
B20	0	0	0	0
B21	0	1	33	6
B22	0	2	0	0
B23	0	6	0	0
B24	0	11	0	0
B25	0	15	14	0

TABLE 2.3: Abundances of four species in 25 bromeliads from Brazil. The first two columns show a species pair with high numbers of checkerboard units, while the second pair has high abundance-weighted checkerboard units. The high-CU pair has many instances of mutual exclusion, whereas the high-AWCU pair has higher overall abundances and a lot of species overlap.

CHAPTER 3

Predator-mediated competition does not facilitate coexistence of bromeliad-dwelling Chironomidae in Brazil

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3.1 INTRODUCTION

According to classical niche theory, the coexistence of identical species is impossible (Hutchinson, 1957) because one species will ultimately exploit shared resources more efficiently than the other. In ecosystems where similar species cooccur, a conceptual challenge therefore arises in understanding how species avoid local extinction. Fortunately, several possible solutions to this puzzle have already been formulated - including neutrality, and niche differentiation through trade-offs in species performance. Under neutral theory, species coexistence is unstable and stochastic events (e.g. drift, dispersal) determine which species coexist (Chave, 2004; Hubbell, 1997, 2001); Species may coexist for multiple generations, but the ultimate fate is extinction. Further, species in the model are assumed to be equivalent, so species similarity in reality is not a problem. Niche differentiation leads to coexistence of similar species by reducing overlap in their use of resources or microhabitats, as is the case for mosquitoes that divide space vertically within bromeliad tanks (Gilbert *et al.*, 2008). This occurs because of trade-offs in performance under different conditions. Differential exploitation of multiple resources (Tilman, 1977, 1990) and differences between species in their competitive and colonization abilities (Turnbull *et al.*, 1999; Levine and Rees, 2002) are classic examples of trade-offs

that have been used to explain coexistence of similar species. We focus on the role of trade-offs in coexistence for the remainder of this work.

There are numerous types of trade-offs that could lead to coexistence, a number of which involve the effects of predators. Predators can have large impacts on the abundance of prey populations, and species may trade-off sensitivity to predation with sensitivity to other biotic or abiotic stressors. For example, trade-offs may occur between predator resistance and drought resistance, as in bromeliad-dwelling mosquito larvae (Hammill *et al*., 2015a) or between predator resistance and competitive ability, as in larval anurans (Werner and Anholt, 1996; Werner and McPeek, 1994). The trade-off between predator resistance and competitive ability in particular can result in coexistence of similar species in the same habitat under predator-mediated coexistence (PMC). PMC has been shown to be important in several systems, including insect larvae in container habitats (Bradshaw and Holzapfel, 1983; Kesavaraju *et al*., 2008), as well as amphibians (Peacor and Werner, 2000; Reich *et al*., 2000), marine invertebrates (Wulff, 2005), fish (Persson, 1993), mites (Karban *et al*., 1994), and birds (McKinnon *et al*., 2013). The classic test for PMC is a difference in competitive outcomes following the exclusion (or inclusion) of the predator.

In this study we examine whether trade-offs in predator resistance lead to PMC in aquatic invertebrate communities within the water-filled tanks of bromeliads. In bromeliads, multiple invertebrate species of the same family, or even genus, are often found co-occurring within a single plant, suggesting that strong competitive interactions may be present. Further, predation has been shown on multiple occasions to be an important factor in invertebrate species distributions among bromeliads (Gilbert *et al*., 2008; Hammill *et al*., 2015a,b).

We conducted our research on Ilha do Cardoso in São Paulo, Brazil (see Chapter 1). In this area, there are many species in closely related taxonomic

groups, suggesting that these species may have a high degree of niche overlap and may be involved in strong interspecific interactions. In particular, there are at least 4 species of chironomid midge in Cardoso, two of which are congeneric (G. Q. Romero, unpubl. data). We studied the relationship between the three most common of these chironomids: *Chironomus detriticola* Correia & Trivinho-Strixino 2007, *Polypedilum marcondesi* Pinho & Mendes 2010, and *Polypedilum kaingang* Pinho, Mendes & Andersen 2013.

In addition to being closely related, the three study chironomid species have previously been shown to have negative co-occurrence patterns according to abundance-weighted checkerboard analysis (AWCA; see Chapter 2). AWCA detects species segregation using relative differences in abundances between species at overlapping sites. Negative co-occurrence patterns suggest that the species either have differential responses to an abiotic aspect of the habitat, or are undergoing net negative interactions. If the latter, competition could be driving their negative co-occurrence, though we cannot tell with AWCA alone whether predators are involved in mediating coexistence.

Here we experimentally test whether the metacommunity-scale co-occurrence of three chironomid species, including two congenerics, is due to PMC. If so, we predict that:

1. Chironomid species differ in their vulnerability to predation.
2. Chironomid species show different competitive outcomes within bromeliads, depending on the presence or absence of predators.
3. The distribution of chironomid species among bromeliads is primarily determined by the distribution of predators, rather than any other habitat attribute of bromeliads (e.g. size).

We conducted a predator-addition experiment to determine whether or not expectations (1) and (2) were met. We followed this with an analysis of

observed co-occurrence patterns of predators and chironomids in differently-sized bromeliads to determine whether expectation (3) was met.

3.2 METHODS

3.2.1 *Predator-addition experiment (Expectations 1 and 2)*

We conducted a predator-addition experiment to examine the effects of a community of three different predators on the three focal chironomid species. If PMC was important, we would expect chironomid species to differ in their susceptibility to predators, and for predators to alter the outcome of competition between chironomids.

Our experiment was carried out between January and April of 2011. Three species of predators were used: *Leptagrion andromache* (a damselfly; Odonata: Coenagrionidae), *Monopelopia caraguata* Mendes, Marcondes & Pinho 2003 (a predatory chironomid; Diptera: Chironomidae), and Hirudinea sp. (a green leech). These species were chosen due to their relative ubiquity as well as their phenotypic differences; We used three unrelated but common predator species in order to sample the multiple types of predator behaviour that chironomids normally experience in bromeliads. In this study site, bromeliads generally contain 5 to 11 (mean $\pm$ SD) species of predators, so chironomids usually co-occur with a multi-species predator community. *L. andromache* is the most common of a few odonate species found in Cardoso. Odonates are the top predators in bromeliad food webs when present (Frank and Lounibos, 2009). They are sit-and-wait predators that lurk in the bottom of the leaf well and grab approaching prey with their extensible labium. *M. caraguata* is a small Tanypodine chironomid with piercing mouth-parts. In spite of their small size, Tanypodine chironomids have been observed to consume prey as large as, or larger than, themselves (A. A. M. MacDonald and D. S. Srivastava, unpubl.

results). The green leech, Hirudinea sp., feeds by draining blood from its prey. These leeches were observed to feed on many invertebrates in the bromeliad food web, including chironomids (A. A. M. MacDonald and D. S. Srivastava, unpubl. results).

All invertebrates were collected from naturally occurring bromeliads by removing the contained water with a large pipette. Bromeliad water was sieved sequentially through sieves of two mesh sizes (850 and 150 μm) to separate organisms from fine particulate organic matter. Once the invertebrates of interest were obtained, remaining organisms were returned to bromeliads in the field.

Two treatments were used to determine the effects of predators on different chironomid species: a predator-present treatment and a predator-absent control. In the predator treatment, the three species of chironomids and the three predator species were added to cleaned (the cleaning process is described below) bromeliads. In the predator-absent control, chironomids were added to cleaned bromeliads without predators. In all treatments, every bromeliad received 10 individuals of each chironomid species. In the predator treatment, we also added one individual each of *L. andromache* and Hirudinea sp., and two individuals of *M. caraguata*. Densities of chironomids and predators were chosen based on the range of densities found in a 2008 field survey of bromeliads (described below; D. S. Srivastava and G. Q. Romero, unpubl. data). Chironomids are known to benefit from detrital shredding, which can create the small detrital and fecal particles that they collect (Starzomski *et al.*, 2010). Therefore, in both treatments, a nymph of Trichoptera sp. (a caddisfly) was added to promote shredding of the coarse detritus. Each treatment was repeated in 15 replicate bromeliads.

Each replicate was run within an entire bromeliad, which was returned to the forest after cleaning, for the duration of the experiment. Bromeliad

sizes ranged from 100 to 200 mL. Bromeliads were prepared by first removing them from the soil and pipetting out all water. They were then washed out thoroughly with a hose and submerged, upside down, in a tank of water for 24 hours to promote the exodus of any remaining organisms. Finally, bromeliads were hung upside down to dry for 48 hours so that any remaining organisms would desiccate. Three bromeliads were cleaned to test the cleaning method. In the three test bromeliads, only two living organisms were found: a partially desiccated chironomid larva, and an individual of the ostracod species *Elpidium bromeliarum*. Washing *Q. arvensis* bromeliads has previously been estimated to remove 94% of existing fauna (Romero and Srivastava, 2010).

Chironomid communities were added to bromeliads along with fine detritus (to provide food and materials for the chironomid cases) and dried leaves (to provide the habitat complexity found naturally). Fine detritus was collected from bromeliads using a 150 μm sieve. Detritus was then boiled to ensure that no living organisms remained, and concentrated by allowing the detrital material to settle overnight and pipetting off the remaining water. We inoculated all bromeliads with 44.4 mL of this same batch of detritus solution, ensuring that the water to detritus ratio remained constant. Whole leaves were also collected from bromeliads and dried in an oven at the lowest possible temperature. After measuring out 7.9 g of dried leaves per bromeliad, leaves were soaked in water overnight to avoid eutrophication of the bromeliad caused by the initial influx of nutrients, and then distributed evenly between the axils of each bromeliad. Chironomids were placed in bromeliads 24 hours prior to predators to allow dispersal through the bromeliad.

Bromeliads were placed in three different sections of the forest. In each section, the experiment was initiated two weeks subsequent to the previous section, creating three temporal-spatial blocks, all run for the same length of time (six weeks). The temporal staggering allowed us to perform an experi-

ment with many replicates while reducing mortality in the captive chironomid larvae, which tend to have poor survivorship in captivity. Furthermore, the incorporation of time and location as random variables allows us to test the generality of the experimental treatments, ensuring that results are not determined by the date or physical placement of the bromeliads.

Bromeliads were covered with mesh cages to prevent migration into and out of the experimental units. Attached to the top of each cage were collection traps for corralling adult chironomids (Figure 3.1). Traps were constructed from inverted, 2-liter, clear beverage bottles that had been thoroughly cleaned prior to the start of the experiment. The dispensing-end of the bottle was removed, turned upside-down, and attached to the interior of the bottle creating a funnel type trap. Traps were checked daily for emerged insects, which were identified and released. If any predators emerged as adults, they were replaced with another individual of the same species as soon as possible.

The experiment was carried out for six weeks, which should have been a sufficient amount of time for all chironomids to emerge under normal conditions (Canteiro and Albertoni, 2011; Oliver, 1971), after which the bromeliads were removed and dissected, and the contained communities censused.

3.2.2 *Analysis of survey data (Expectation 3)*

We analysed an observational data set from our field site in Ilha do Cardoso, Brazil, collected in 2008 (D. S. Srivastava and G. Q. Romero, unpubl. data) to determine whether chironomid species differed in their relationship with either predator biomass or bromeliad size. If predator-mediated coexistence (PMC) is important, we would expect predators rather than plant size to determine differences between chironomid species in their distribution. Plant size is examined in particular here as, of all bromeliad attributes, it is the most common correlate of invertebrate composition in bromeliads (e.g. Amundrud

and Srivastava 2015; Gilbert *et al*. 2008; Srivastava *et al*. 2008). We used linear models to predict how chironomid abundance was related to total predator biomass, bromeliad size and their interaction. Models were fit separately for each chironomid species (*Chironomus detriticola*, *Polypedilum kaingang* and *Polypedilum marcondesi*). Non-significant terms were removed and the models were compared with AIC (Akaike's Information Criterion) and ANOVA to select the best one. We assessed model residuals and QQ-plots to confirm that the selected models fit well.

3.2.3 *Statistical Analysis of Experiment Data*

All analyses were performed using the statistical computing language, R (R Core Team, 2014). Experimental data were analysed using a two-way analysis of variance (ANOVA) to compare the survival and emergence of the three species at the end of the experiment. The response variable (either survival or emergence) was transformed using a $log10$ transformation.

To get an idea of how emergence rates were affected by the treatments, we analysed the number of days until first emergence for each species within each treatment using two-way ANOVA.

Because our data were right-censored (the experiment was ended on a fixed date regardless of the fate of chironomids), we needed to model emergences over time to determine whether chironomids might have emerged after the end of the experiment. Survival analysis is able to deal with data with binary fates (e.g. dead, alive), but our data had three fates (dead, alive but not emerged, alive and emerged). Therefore, we created a demographic model to predict the final values of survival and emergence for each species in each treatment. We used a density independent model of population growth (A. D. Letaw and A. A. M. MacDonald, unpubl. results) to predict the adult

population size based on the larval population size, emergence rate, and death rate. The model construction is as follows:

First, larval population decline over time (t) is modelled as a function of number of larvae (L), larval death rate (d) and adult emergence rate (a):

$$\frac{dL}{dt} = -(a+d)L \tag{3.1}$$

Solving the differential equation gives:

$$L(t) = L_0 e^{-(a+d)t} \tag{3.2}$$

where Lo is the number of immature individuals at the start of the experiment. Next, we model adult population growth as:

$$\frac{dA}{dt} = aL \tag{3.3}$$

where A is the adult population size. Since we have already solved the equation for larvae, we can substitute that into the formula above, getting:

$$\frac{dA}{dt} = aL_0 e^{-(a+d)t} \tag{3.4}$$

Now, solving the equation for the adult population gives:

$$A(t) = \frac{aL_0 e^{-(a+d)t}(e^{(a+d)t} - 1)}{a+d} \tag{3.5}$$

which can be simplified, giving:

$$A(t) = \frac{a}{a+d} L_0 (1 - e^{-(a+d)t}) \tag{3.6}$$

To estimate the parameters of our demographic model, we first calculated the cumulative emergence over time, within treatments and replicates, for each chironomid species in our data set. Next, we fit our model to the cumulative

data using two rounds of non-linear least squares (NLS) estimation. In the first round, we used a "brute-force" technique that generates 1000 random parameter values within a given starting grid. The starting grid was created by establishing upper and lower bounds for each parameter (e.g. death rate could not be higher than 10 as there were only 10 individuals of each species per replicate). This process was completed using the R package *nls2* (Grothendieck, 2013). Using the estimates generated in the first round of fitting as new start values, we performed NLS a second time, this time with the Levenberg-Marquardt algorithm (Marquardt, 1963) to generate a better fit. We used the R package *minpack.LM* (Elzhov *et al.*, 2015) for the second fit. Error in model parameter estimates was calculated using bootstrapping to resample the data 100 times and perform the two rounds of NLS on resampled data. Percentile-based 95% confidence intervals were calculated based on the bootstrap residuals and used to compare parameter estimates.

3.3 RESULTS

3.3.1 *Predator-addition experiment*

Chironomids took longer to start emerging in the presence of predators (treatment: $F_{1,82} = 9.440$, $p = 0.00289$; Figure 3.2), but this effect of predators on days to first emergence did not differ between species (species x treatment interaction: $F_{2,82} = 0.993$, $p = 0.375$). In fact, there was no overall difference in days to first emergence between species (species: $F_{2,82} = 1.365$, $p = 0.261$), though *P. kaingang* tended to have a higher median in days to first emergence than the other species (Figure 3.2). There was a significant block effect ($F_{1,82} = 8.302$, $p = 5.21 \times 10^{-4}$).

Overall, predators reduced the survival and emergence of chironomids (survival: $F_{1,82} = 24.836$, $p < 0.0001$; emergence: $F_{1,82} = 18.983$, $p < 0.0001$

FIGURE 3.1: Set up of the predator-addition experiment. Bromeliads were enclosed in mesh cages to prevent organisms from entering or exiting the experiment. Cages were topped with traps to capture emerging adult chironomids.

; Table 3.1). However, predators had proportionally similar effects on all chironomid species, both in terms of survival (species x predation: $F_{2,82} = 0.375$, $p = 0.688$) and emergence (species x predation: $F_{2,82} = 0.239$, $p = 0.788$). In fact, regardless of treatment, there was little difference between chironomid species in their survival (species: $F_{2,82} = 0.427$, $p = 0.654$) and emergence (species: $F_{2,82} = 0.205$, $p = 0.815$). Both survival ($F_{2,82} = 10.280$, $p = 1.04 \times 10^{-4}$) and emergence ($F_{2,82} = 11.195$, $p < 0.0001$) showed block effects. We caution that, in addition to being performed on censored data, these ANOVAs consider only the total number of emergences or surviving larvae by the end of the experiment. By considering how the number of emergences change over time we can gain additional insight into the underlying rates of these processes.

Our models of adult population growth were a reasonable fit to adult emergence considering the amount of noise in the data (Figure 3.3). Predicted estimates of overall emergence from these models were often similar to the observed values at the end of the experiment, but in four cases, the model predicted further emergences after the end of the experiment (Table 3.1) suggesting that some information was lost by using censored data for the ANOVA. According to the models, all chironomid species had significant decreases (around 2 to 3-fold) in emergence rates in the predator treatment (Figure 3.4) suggesting the species tend to delay emergence under threat of predation (also supporting the analysis of days until first emergence, above). All three species had similar emergence rates in the control treatment. However, when predators were present, *P. kaingang* emergence was lower than the other two species. Predators also led to non-significant increases in death rate for *P. marcondesi* (2-fold) and *C. detriticola* (3-fold), but did not change the death rate for *P. kaingang* (Figure 3.4). Both *P. marcondesi and C. detriticola* had similar death rates in both the control and predator treatments. However, while *P.*

		Control		with Predators	
		Emergence	Survival	Emergence	Survival
C. detriticola	Obs.	2.67 ± 0.61	3.33 ± 0.72	1.13 ± 0.33	1.27 ± 0.36
	Pred.	4.90	NA	1.50	NA
P. kaingang	Obs.	3.27 ± 0.73	4.20 ± 1.00	1.27 ± 0.45	1.40 ± 0.51
	Pred.	9.10	NA	7.52	NA
P. marcondesi	Obs.	2.33 ± 0.37	2.40 ± 0.38	1.00 ± 0.24	1.07 ± 0.27
	Pred.	4.00	NA	1.34	NA

TABLE 3.1: Mean $\pm$ SE emergence and survival (Obs), and predicted (Pred) emergence (out of 10 individuals per replicate), for each species x treatment combination. Gray cells represent those treatments in which further emergence is predicted after the end of the experiment (emergences greater than μ + 2 SE). *P. kaingang* was predicted to have the most emergences in both control and predator treatments.

kaingang overlapped with *C. detriticola* in the control treatment, *P. kaingang* death rate was lower than the other two species in the predator treatment. These differences suggest a species-by-treatment interaction in which species rates are the same or nearly so in the control treatment, but *P. kaingang* is distinguished by having lower emergence and death rates in the predator treatment.

3.3.2 *Analysis of survey data*

Chironomid species differed in which factors (predator biomass or bromeliad size) best predicted their abundance (Figure 3.5, 3.6). *Polypedilum marcondesi* abundance increased with predator biomass ($t_{16} = 3.683$, $p = 0.00201$; Figure 3.5A); *P. kaingang* abundance was best fit by a model including a negative effect of predator biomass, but the slope of the relationship did not differ from zero ($t_{16} = -0.799$, $p = 0.436$; Figure 3.5B); *C. detriticola* abundance increased with plant size ($t_4 = 5.759$, $p = 0.00451$) but decreased with predator biomass ($t_4 = -2.839$, $p = 0.04692$; Figure 3.6).

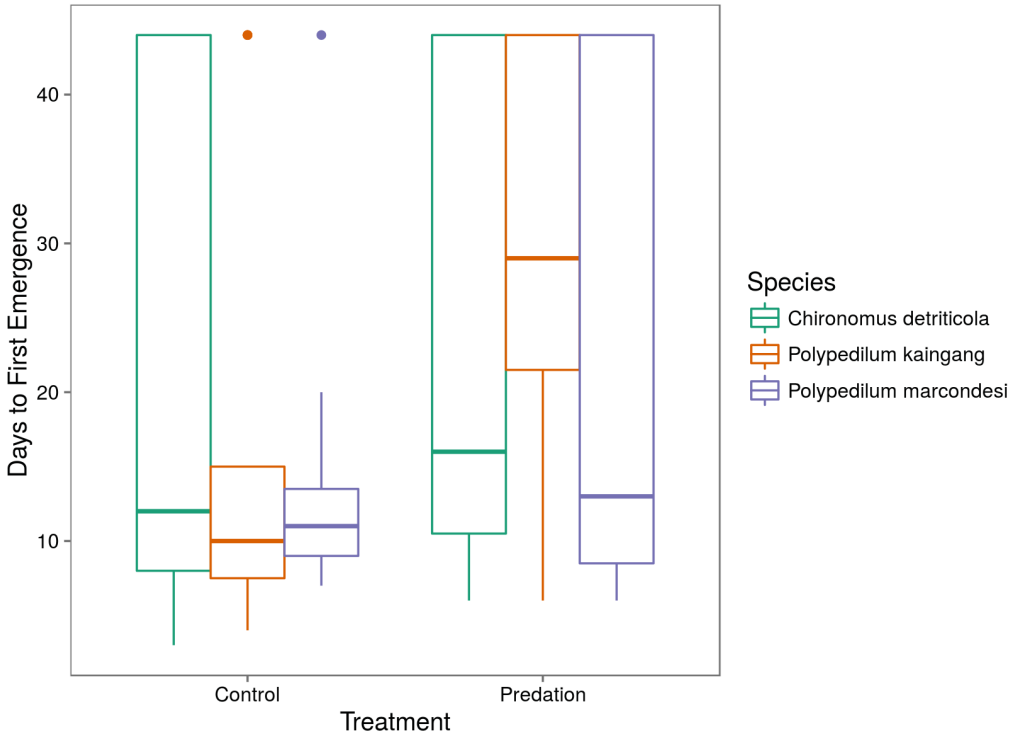

FIGURE 3.2: Days to first emergence increased ($F_{1,82} = 9.440$, $p = 0.00289$) from the control to predator treatments, suggesting that chironomids delayed emergence in the presence of predators. However, although predation increased time to first emergence in all species, there were no differences between species (species: $F_{2,82} = 1.365$, $p = 0.261$; species x treatment interaction: $F_{2,82} = 0.993$, $p = 0.375$)

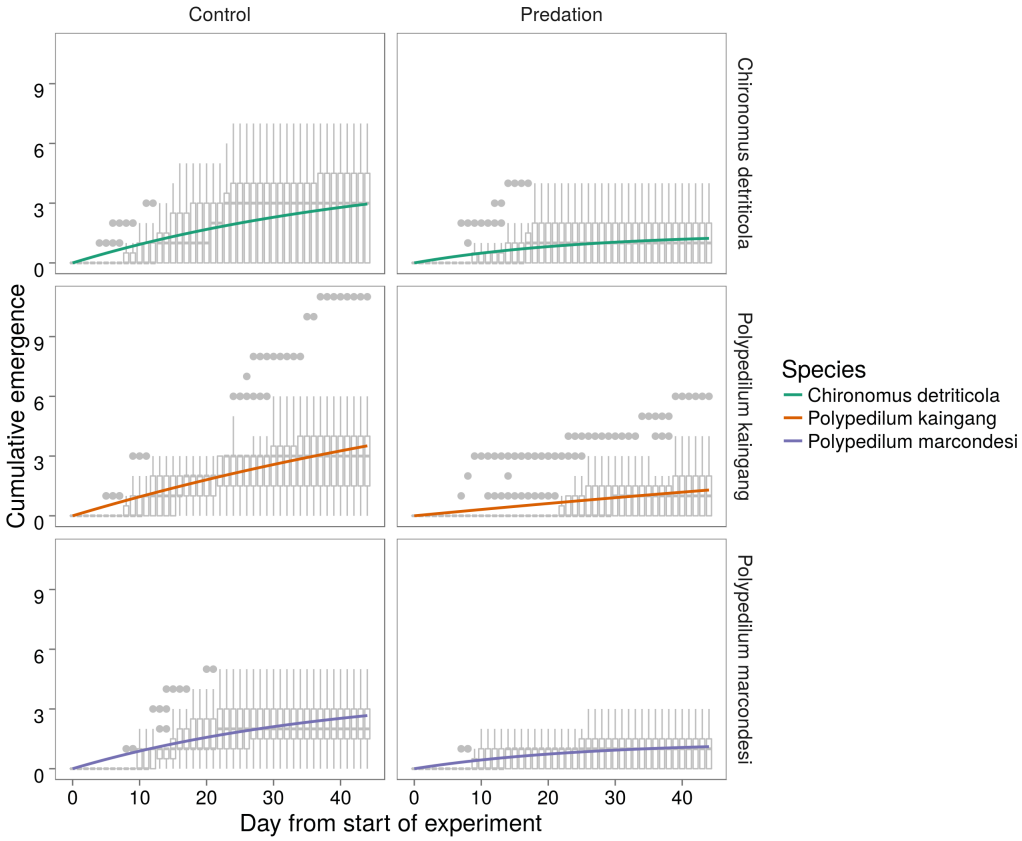

FIGURE 3.3: Demographic model fits to the emergence data for both treatments. The coloured prediction lines are backed with the actual cumulative emergence data displayed as boxplots. Emergence was slowed and reduced in the predator treatment. Model fits suggest that *P. kaingang* would have had the highest emergence values if the experiment had been run for a longer period of time.

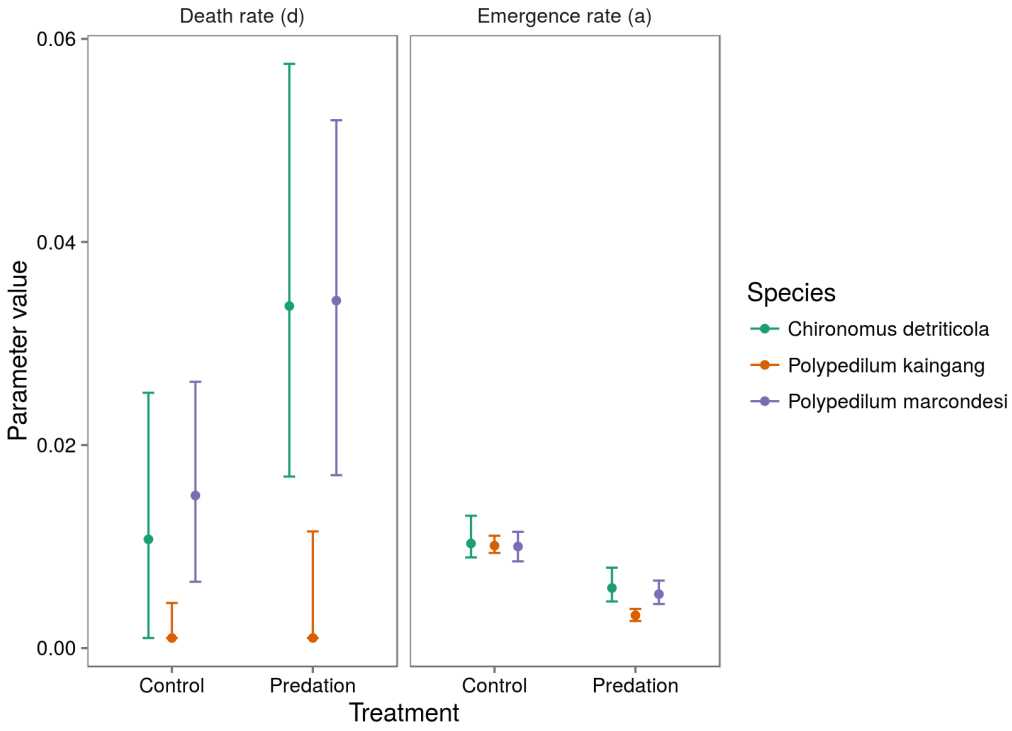

FIGURE 3.4: Comparison of the parameter estimates from the demographic model for each species by treatment combination. Points show means and confidence intervals. All species showed significant decreases in emergence rate (a) when predators were present. Compared to *C. detriticola* and *P. marcondesi*, *P. kaingang* had lower death rates (d), suggesting higher fitness in the conditions of the experiment.

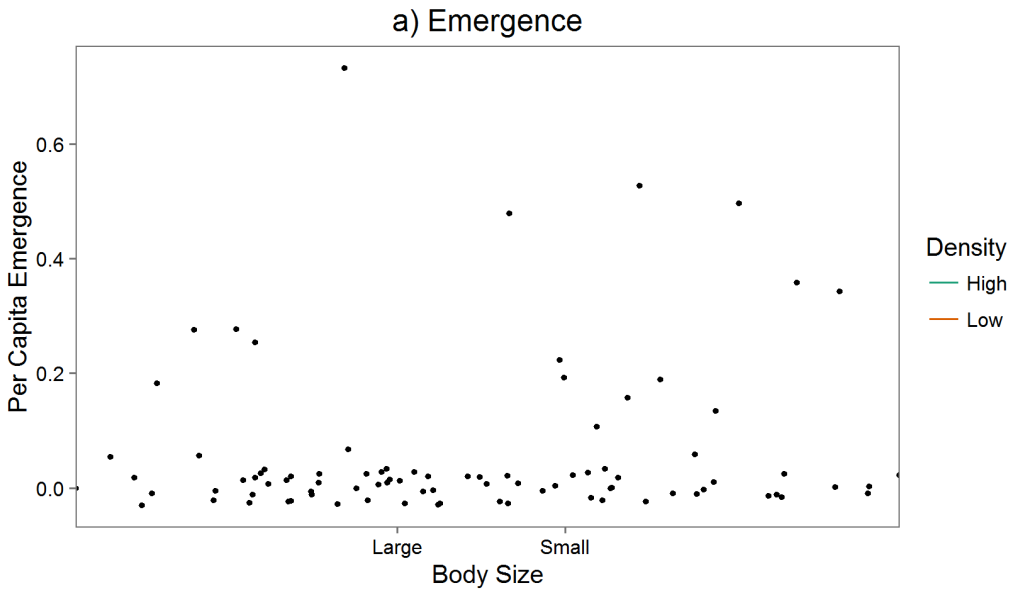

FIGURE 3.5: Survey data showing the relationship between the two *Polypedilum* species and predator biomass. Points show the actual data while lines are the predicted values generated by linear models. A: *P. marcondesi* abundance increased with predator biomass ($p = 0.00201$). B: *P. kaingang* was best fit by a model containing only predator biomass, but the slope of this relationship was not different from zero ($p = 0.436$).

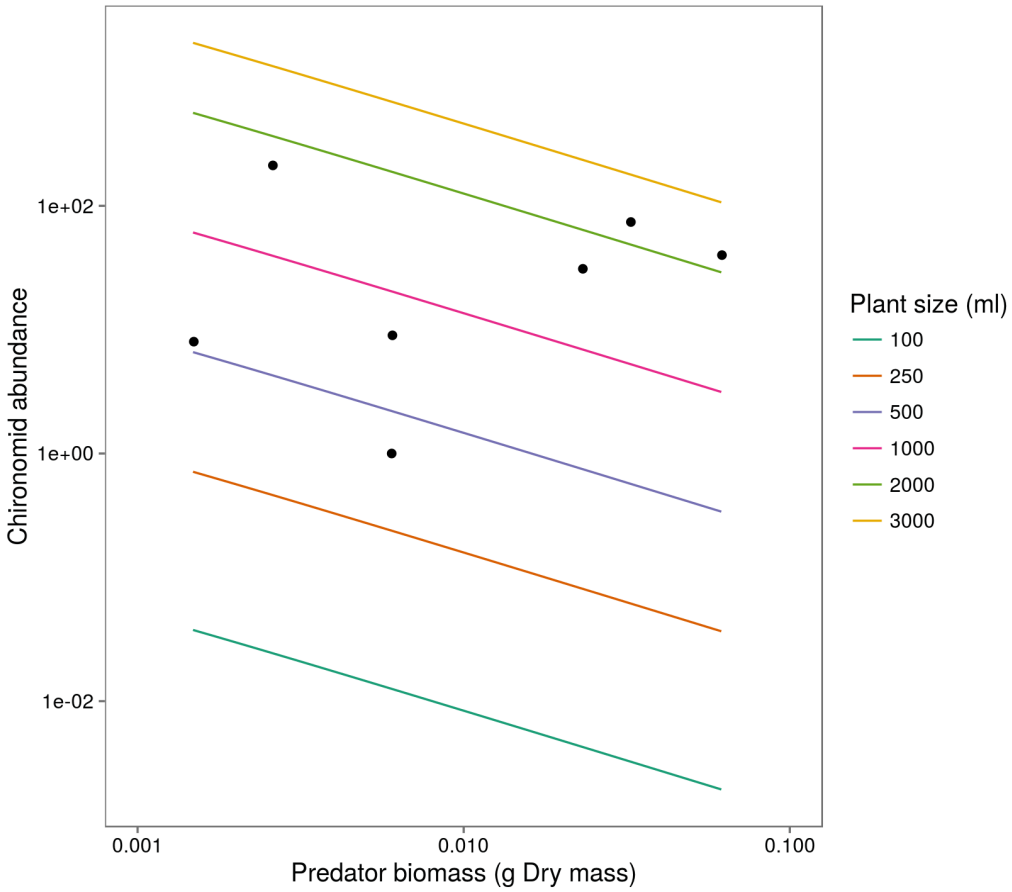

FIGURE 3.6: Survey data showing the relationship between *C. detriticola* and plant size and predator biomass (log scale). Points show the actual data while isocline lines depict the predicted values generated by the linear model. *C. detriticola* abundance increased with plant size ($p = 0.00451$) but decreased with predator biomass ($p = 0.04692$). The biological significance of the relationship with predator biomass was lower at small plant sizes in the size range we used in our experiment (due to the log scale).

3.4 DISCUSSION

Here, we combine observational and experimental data to test whether the coexistence of three chironomid species in bromeliad tanks is mediated by the presence of predators. Our predator-addition experiment suggests that there may be differential responses to predator-presence between the three species. This was supported by the survey data, in which each species responded differently to increased predator biomass. We conclude that predators have different effects on different species, but that this does not result in predator-mediated coexistence because the identity of the highest performing species remains constant across treatments.

Predators had strong negative effects on all chironomid species, realized more through reductions in the emergence rate than increases in the death rate (changes in the death rate were not significant). Consequently predators increased the time to first emergence in all species (Figure 3.2). Delayed development in response to predators has been found in many other insect communities as well (e.g. McKie and Pearson 2006; Stoks 2001; van Uitregt *et al*. 2012). The importance of non-consumptive effects such as this is increasingly being highlighted (Davenport *et al*., 2014; Preisser *et al*., 2005; Werner and Peacor, 2003). In the bromeliad system, predators have previously exhibited non-consumptive effects on the community, leading to differences in community composition and ecosystem function (Hammill *et al*., 2015a; Marino *et al*., 2015). Though non-consumptive effects have yet to be studied for the species used in our experiment, other species of chironomids change their behaviour in the presence of predators, increasing burrow depth (Hölker and Stief, 2005) and reducing foraging (Ball and Baker, 1996; Hölker and Stief, 2005). Reduced foraging can increase development time, which would naturally lead to decreased emergence rates as we saw here.

Although predators affected all three species of chironomids, the strength of this effect differed between species. Results of the predator-addition experiment suggest a difference between *P. marcondesi* and *C. detriticola* on the one hand and *P. kaingang* on the other. Specifically, demographic rates were nearly equivalent between species in the control, with only *P. kaingang* and *P. marcondesi* differing slightly in death rate (Figure 3.4). When predators were added, however, the death rates of *P. marcondesi* and *C. detriticola* increased such that *P. kaingang* then had a lower death rate than both of the other two species (Expectation 1; Figure 3.4). Emergence rates were also depressed in the presence of predators for all three species, with *P. kaingang* standing out again as having significantly lower emergence rate than the other two species (Figure 3.4). Even though *P. kaingang* larvae had low emergence rates in the presence of predators, so many larvae survived to pupation that the total number of predicted emergences was higher for this species than the other two species (Table 3.1). The results from our demographic model demonstrate that chironomid species differ in the extent to which predation depresses their demographic rates and that this led to differences in predicted numbers of emergences (Table 3.1). Although our ANOVA of cumulative emergences does not show such a species-by-predation interaction, this likely is an artefact of censoring the data set for that analysis.

In terms of the natural distribution of the three chironomids, all three had different responses to predator biomass. Distributions of *P. marcondesi* and *C. detriticola* were both predicted by the biomass of predators (Expectation 3; Figures 3.5A, 3.6) but *P. kaingang*, differed, having no relationship with either predator-biomass or plant size (Figure 3.5B). Furthermore, while *C. detriticola* was negatively affected by predator biomass, *P. marcondesi* was actually positively affected. Although both the survey and the experiments show that *P. kaingang* is the least affected by predators, we were expecting - based on the

experimental results - for both *P. marcondesi* and *C. detriticola* abundance to be negatively (not positively) affected by predator biomass. The only way that this apparent contradiction in the response of *P. marcondesi* could be resolved is if predation risk is actually lower in bromeliads with high predator biomass. This could occur if predators interfere with each other (Bruno and O'Connor, 2005; Griffin *et al*., 2008), or if predators have alternate prey. Both of these seem plausible based on previous data from this system. Two studies have now shown that predators in bromeliads have strong antagonistic effects, reducing their net predation rate (Atwood *et al*. 2014; A. A. M. MacDonald, unpubl. data). Thus, chironomids may obtain an advantage by occurring in bromeliads with diverse predator assemblages (the number of predator species increases with predator biomass in our survey data: $r = 0.891, p < 0.0001$). Alternatively, high predator biomass in bromeliads may reflect high biomasses of alternate prey, including tipulids, scirtids and mosquitoes. Odonate predation on these alternate prey species has been shown, at our field site, to be greater than that on chironomids (LeCraw, 2014). Chironomids may escape predation in such situations.

Notably, we found block effects showing differences in response when the location and time of experiment start was changed. Possibly there are differences in the robustness of individuals that are the result of earlier colonization compared to those that develop later. There was no way to control for such differences as our reason for blocking was to avoid high larval mortality in captivity. However, these block effects may point to the importance of colonization timing, also suggested by Chapter 4. Further investigation is needed into the role of time on chironomid survival, emergence and coexistence.

Is there PMC in this system? Although predators had differential effects on the three chironomid species, the species that appeared to be the best at resisting predation, *P. kaingang*, was not competitively inferior in the absence

of predators; In fact it had slightly lower larval mortality than the other species even in the absence of predators suggesting if anything it was the competitive dominant in all situations (a "Darwinian demon"). There are many possible alternatives to PMC as the mechanism allowing these chironomid species to inhabit the same region. One is that the species do not compete, but our other research does not support that conclusion (see Chapter 4). More likely, there are other habitat variables mediating the coexistence of chironomids. One of these is likely plant size. Other studies of the system suggest that plant size is an important factor in determining community composition (Gilbert *et al.*, 2008; Hammill *et al.*, 2015a). Field survey data showed that at least *C. detriticola* seems to have a positive relationship with plant size, meaning this species may also be competitively dominant in larger plants. Any interaction between predation and plant size, could not be captured in our experiments, which were conducted in a single plant size.

We therefore conclude that predation has little role in mediating coexistence between bromeliad-dwelling chironomid species. Importantly, this result was not predicted by our analysis of observational data, which suggested that predator presence would benefit at least one species of chironomid. The mismatch between experimental and observational results suggests fertile ground for future research. For example, manipulating predator effects in conjunction with bromeliad size could determine whether effects are context-dependent; Examining the effects of alternate prey and predator antagonism on chironomid survival could answer questions about the positive relationship between *C. detriticola* and predator biomass. Finally, future behavioural or caged-predator studies could give more information about the non-consumptive effects we suspect are driving chironomid response here.

CHAPTER 4

Asymmetric ecological equivalence and context-dependent competition between chironomids in bromeliads

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4.1 INTRODUCTION

Species coexistence can be explained by both deterministic, niche processes and stochastic, neutral processes (Chesson, 2000a,b; Vellend, 2010). Niche models explain coexistence by employing species differences whereas neutral models (Hubbell, 1997, 2001) explain coexistence through random events, such as drift and dispersal. Furthermore, neutral models assume species are ecologically equivalent. Ecologically equivalent species interact as if they are the same species, so the neutral processes of drift and dispersal determine long-term dynamics. More specifically, ecologically equivalent species are equivalent in terms of both fitness (fitness equivalence; $\lambda_i = \lambda_j$, where λ is the species average fitness) and competition (competitive equivalence; $\alpha_{ii} = \alpha_{jj} = \alpha_{ij} = \alpha ji$, where α_{xy} is the effect of species y on species x).

In a neutral model, ecological (fitness and competitive) equivalence of species is sufficient for coexistence, albeit one that is vulnerable to drift or perturbation (unstable coexistence). In niche models, however, fitness or competitive differences among species do not guarantee coexistence. Instead, competitive differences must be such that they stabilize the multispecies system (stable coexistence) by increasing the strength of intraspecific (as compared to interspecific) competition when a species is represented by an increasing

proportion of individuals in a community (Chesson, 2000b). Conversely, competitive differences can destabilize the system if intraspecific competition diminishes in importance as a species increases its relative proportion, generally leading to local extinction of one species. Unlike competitive differences, fitness differences between species never promote coexistence at a local scale (although local fitness differences can promote coexistence at a metacommunity scale, as we discuss shortly). Fitness differences lead to competitive exclusion when they cannot be offset by competitive stabilizing mechanisms (Chesson, 2000b).

Between the extremes of ecologically equivalent species and niche-differentiated species, lies a suite of other possible relationships. By mathematically manipulating the relative importance of intra- and interspecific competition, it is possible to uncover alternative relationships. As previously shown (Adler *et al.*, 2007; Chesson, 2000b; Godoy and Levine, 2014), when species exhibit fitness differences, coexistence occurs if the species with higher fitness also has stronger intraspecific competition (competitive stabilization; Appendix A.1). However, under fitness equivalence, the strengths of competition for both species determine whether or not this criterion is satisfied, and a few different scenarios allow for coexistence (Table 4.1A; Appendix A.1). For example, one species may be equally affected by intra- and interspecific competition even if the other is not, a phenomenon we call *asymmetric ecological equivalence*. In these scenarios, one species responds to the world neutrally while the response of the second species is decidedly non-neutral, being more limited by either conspecifics or heterospecifics. The dynamics influencing the non-neutral species (i.e. competitive stabilization vs. destabilization) determine whether or not the two species can coexist.

Even if species do not coexist at the local scale, context-dependent fitness differences can promote coexistence at the metacommunity scale (Table 4.1B).

For example, environmental differences in local patches can lead to habitat partitioning when each species performs better under a different environmental context. Many experiments on insects have shown differences in the outcome of competition to be dependent on environmental variables such as habitat size (Juliano, 2009), temperature (Park, 1954), humidity (Costanzo *et al.*, 2005; Park, 1954), and resource distribution (Atkinson and Shorrocks, 1981). Stochastic differences in colonization order can also lead to coexistence by producing ontogenetic differences in body size; when both species have an advantage at one body size (e.g. the larger species always has higher fitness), variation in colonization timing can lead to variation in the identity of the superior competitor in a given patch - a priority effect (Rasmussen *et al.*, 2014; Shorrocks and Bingley, 1994). Larger individuals often prey on their smaller counterparts (Fox, 1975; Polis, 1981; Schröder *et al.*, 2009) while smaller individuals may be more adept at obtaining or utilizing resources (Claessen *et al.*, 2000; Werner, 1994). Thus, even if species are identical in their competitive abilities in general, if one arrives earlier and is therefore larger at the time they interact, there may be some fitness or competitive difference driven by colonization order and ontogeny alone (Gilbert *et al.*, 2008).

This research investigates local and regional coexistence by manipulating relative abundance, habitat type and body size in a bromeliad invertebrate system using two species of chironomid larvae (Diptera: Chironomidae). One of the most important habitat variables for bromeliad insects is bromeliad size (Jocque and Field, 2014; Marino *et al.*, 2011; Petermann *et al.*, 2015). In surveys of bromeliad contents, many species appear to exhibit some preference for bromeliad size (Amundrud and Srivastava, 2015). Smaller bromeliads generally dry out faster (Schmidt and Zotz, 2001; Srivastava *et al.*, 2008) while larger bromeliads generally contain more predators (Amundrud and Srivastava, 2015; Srivastava, 2006; Srivastava *et al.*, 2008). For example, coexistence

in bromeliad mosquitoes may depend on a trade-off between the ability to resist desiccation and the ability to resist predation (e.g. Hammill *et al*. 2015b). Bromeliad size may also influence larval fitness by influencing resource availability, such as detritus density and algal productivity (Marino *et al*., 2011).

We performed two experiments with two chironomid species that are often found coexisting within a single plant. We aimed to answer three questions:

1. What type of competitive relationship, if any, do the two species have (e.g. competitive equivalence) and which mechanisms drive the relationship (e.g. stabilizing mechanisms)?

2. Does context (habitat or ontogeny) alter the outcome of competition?

3. Are the two species expected to coexist locally? Regionally?

In the first "equivalence" experiment we tested the importance of intra- and interspecific competition by manipulating the relative abundance of the two species (Question 1). Recent research with congeneric damselfly nymphs used a similar experimental design and demonstrated ecological equivalence (Siepielski *et al*., 2010). In the second, "ontogeny" experiment, we tested the effect of manipulating ontogeny by altering the relative body size of the chironomid species (Question 2). In both experiments, we performed manipulations in two plant sizes to measure the effect of changing habitat conditions on local coexistence (Question 2). We crossed all treatments with a manipulation of absolute density. Species average fitness is related, in part, to their ability to perform despite increased density of competitors, and differences between species in this component of fitness help predict whether species would coexist at the local scale (Question 3). We followed the experiments with an analysis of survey data to determine whether any habitat context-dependence seen in the experiment matched habitat-dependent patterns observed in natural bromeliad communities (Question 2).

In the equivalence experiment, we expected to find a combination of equalizing and stabilizing processes consistent with local coexistence (Table 4.1A) as the study species are naturally found together within a patch. Two possible outcomes are consistent with local coexistence (Figure 4.1): 1) If the community was neutrally structured with ecological equivalence between species, the relative abundances of the two species are expected to have no systemic trend from the beginning to the end of the experiment (Figure 4.1), indicating that species identity is unimportant in determining performance; 2) By contrast, if the community was structured by stabilizing mechanisms, intraspecific competition is expected to increase as the relative abundance of that species increases. Therefore, when a species begins with high relative abundance, intraspecific competition should be strong leading to a lower per capita response (Figure 4.1). A third possible outcome is that species interactions could lead to exclusion of one or the other species. This could happen either if the species with higher initial abundance also has higher final abundance (competitive destabilization; Figure 4.1), or if one species always has higher final abundance (fitness inequality is not compensated by competitive stabilization). As described previously (Table 4.1A), the presence of competitive equivalence, stabilization, and destabilization can be asymmetric between the species, with different combinations leading to different coexistence outcomes.

Because we assumed density, that is, the ratio of organisms to food resources, not abundance, was the important factor in competition, our design crossing habitat size and density necessarily resulted in a range of total abundances or organisms even within density treatments. However, it is conceivable that organisms instead respond to abundance, for example if organisms do not compete for food resources but instead for a scale-invariant resource. We therefore tested whether there was any relationship between the species response and total abundance. If total abundance were important, we

would expect that equalizing mechanisms would show a decline in response with total abundance (Figure 4.1a), and stabilizing mechanisms would show the steepest decline in response with total abundance of conspecifics only (rather than with heterospecifics, or both) (Figure 4.1b).

In the ontogeny experiment and in the habitat manipulations, we expected to find different responses to habitat and body size, especially if the results of the equivalence experiment led to local extinction of one species. Specifically, species could still persist at the metacommunity scale, even with local extinction, if each species exhibited higher performance than the other species in a particular context (habitat or relative body size; Table 4.1B). However, if species had the same performance in each context, then coexistence is expected to be either unstable (neutral dynamics at local and metacommunity scales) or one species will go extinct in the metacommunity, in the absence of any other stabilizing factors. Scenarios suggesting regional extinction of one species would be surprising because the two species have been observed in the same region over multiple years (G. Q. Romero unpubl. data, A. D. Letaw pers. obs.). If our results indicated such an outcome, we would postulate that there must be another mechanism besides those we investigate here, allowing coexistence at the metacommunity scale.

4.2 METHODS

We performed two experiments, manipulating relative abundance (ecological equivalence) and relative body size (ontogeny) in two chironomid species. To test if performance in different plant sizes in experiments matched observed plant size preferences, we also analysed survey data for the two species from a previous year (D.S. Srivastava & G. Q. Romero, unpubl. data). Finally, we

A: Outcome of local coexistence ($\lambda_i = \lambda_j$)			
	$\alpha_{ij} = \alpha_{ii}$	$\alpha_{ij} > \alpha_{ii}$	$\alpha_{ij} < \alpha_{ii}$
$\alpha_{ji} = \alpha_{jj}$	Unstable coexistence ($\rho_{ij} = \lambda_j/\lambda_i$)	No coexistence (asymmetric destabilization)	Coexistence (asymmetric stabilization)
$\alpha_{ji} > \alpha_{jj}$	No coexistence (asymmetric destabilization)	No coexistence (destabilization)	Contingent coexistence: Species coexist if $(\alpha_{ii} \times \alpha_{jj}) > (\alpha_{ij} \times \alpha_{ji})$
$\alpha_{ji} < \alpha_{jj}$	Coexistence (asymmetric stabilization)	Contingent coexistence: Species coexist if $(\alpha_{ii} \times \alpha_{jj}) > (\alpha_{ij} \times \alpha_{ji})$	Coexistence (stabilization)
B: Outcome of regional coexistence			
	$\lambda_{i,x} = \lambda_{j,x}$	$\lambda_{i,x} > \lambda_{j,x}$	$\lambda_{i,x} < \lambda_{j,x}$
$\lambda_{i,y} = \lambda_{j,y}$	Unstable coexistence	Species j goes extinct	Species i goes extinct
$\lambda_{i,y} > \lambda_{j,y}$	Species j goes extinct	Species j goes extinct	Coexistence via habitat partitioning or priority effects
$\lambda_{i,y} < \lambda_{j,y}$	Species i goes extinct	Coexistence via habitat partitioning or priority effects	Species i goes extinct

TABLE 4.1: Outcomes of local and regional coexistence predicted for our experiments. A: Lighter grey cells exhibit asymmetric ecological equivalence while darker cells exhibit symmetric ecological equivalence (neutrality). Assuming fitness equivalence, local coexistence, depends on relative values of competition coefficients (α_{xy} = the effect of species y on species x). Species coexistence is stable when $\rho_{ij} < \lambda_j/\lambda_i$ (ρ_{ij} is a measure of niche overlap; Appendix A.1). The outcome of competition is driven by the non-equivalent species. If species do not have equal fitness, coexistence occurs through competitive stabilization only. B: Dark cells are compatible with a hypothesis of neutrality at the metacommunity scale. Species can coexist via habitat partitioning or priority effects if each species has higher fitness in one context (e.g. small plants vs. large plants) than the other species, where different contexts are indicated by x and y.

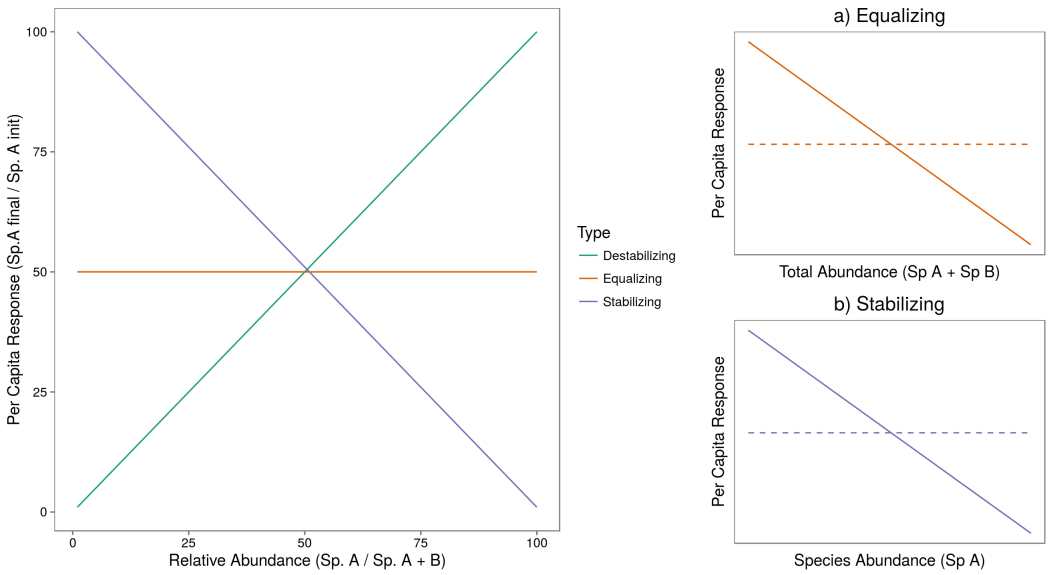

FIGURE 4.1: Possible results for the ecological equivalence experiment. The relationship between initial and final proportions of a species reveals the type of mechanisms affecting the species interaction: A flat line suggests equalizing mechanisms because the per capita response is not affected by initial relative abundance; Negative slopes suggest a stabilizing relationship in which species with high initial relative abundance limit their own population sizes; Positive slopes suggest a destabilizing relationship in which one species excludes the other. Side plots show expected response to absolute density in a) Equalizing or b) Stabilizing scenarios. Species will either respond to the total abundance of both species (a), to the abundance of conspecifics only (b), or have no relationship with abundance (a and b, dashed lines).

estimated average fitness of species in order to predict whether coexistence would occur (Table 4.1) in each treatment.

Experiments were run between late February and early June of 2013. Two species of aquatic bromeliad-dwelling chironomid larvae were used: *Chironomus detriticola* Correia & Trivinho-Strixino, 2007 and *Polypedilum marcondesi* Pinho & Mendes, 2010. These were chosen as focal species based on their high relative abundance compared to other bromeliad invertebrates, including other chironomid species.

4.2.1 *Equivalence Experiment*

To determine the relative importance of intra- and interspecific competition, we set up a 2 x 2 x 3 factorial experiment (Figure 4.2a), manipulating absolute chironomid density (low vs. high, described below), plant size (large vs. small, described below), and relative abundance. Relative abundance treatments followed a substitutive design, with each chironomid species making up either 25, 50, or 75% of the total larval population. The substitutive design is necessary for detecting equivalence and frequency-dependence of species responses; an additive design would confound the results because of the increased absolute density.

4.2.2 *Ontogeny Experiment*

The ontogeny experiment used a 2 x 2 x 2 factorial design (Figure 4.2a), manipulating absolute chironomid density (low vs. high, described below), relative body size (large vs. small), and plant size (large vs. small, described below). Relative body size was manipulated in order to represent differences in colonization order (Hernandez and Chalcraft, 2012; Rasmussen *et al.*, 2014), though we acknowledge that this fails to capture advantages in access to resources provided to early colonizers. This indirect manipulation

of colonization order allowed us to ensure that all chironomids experienced the same length of the experiment; had we added one species before the other, the effects of the size difference would be confounded with differences in the number of exposure days. Larger bodied organisms were also put into the experiment 24 hours prior to the smaller organisms.

4.2.3 *All Experiments*

In all experiments, invertebrates were collected from bromeliads by removing dead leaves from the bromeliad tanks with large forceps and pipetting all impounded water into a bucket using a large pipette. Bromeliad contents were sorted into coarse and fine organic matter, using an 850 μm sieve followed by a 150 μm sieve. Materials retained on sieves were then searched for larvae of the relevant species.

Two plant size classes and two absolute densities of chironomids were used. Plants were designated either as large or small, where small plants had volumes of 500 ml or less and large plants had volumes of 1500 ml or more. These volumes were determined based on data from bromeliads in natural conditions (A. A. M. MacDonald and D. S. Srivastava 2010, unpubl. data); *P. marcondesi* tend to be found in small plants whereas *C. detriticola* are more often found in large plants. Two levels of absolute density were used to examine the response of chironomids to resource depletion, a component of average fitness as defined by Chesson (2000). Under high density treatments, 8 larvae per 15 ml were used; under low density, only 4 larvae per 15 ml were used (Figure 4.2b). Densities were based on the range of natural larval densities found in bromeliads surveyed in the study area in 2008 (D. S. Srivastava & G. Q. Romero, unpubl. data).

Fine particulate organic matter (FPOM), collected originally from bromeliads in situ, was added to tubes at a concentration of 0.008 g ml^{-1}. FPOM

provides food and habitat for chironomid larvae (Oliver, 1971; Walshe, 1951). The concentration added was again based on surveys of natural bromeliads in the study area (D. S. Srivastava & G. Q. Romero, unpubl. data). Before adding FPOM to tubes, it was boiled to ensure that no invertebrate larvae or eggs remained alive. Samples of boiled FPOM were dried on filter paper and weighed to determine the wet volume equivalent of 0.008 g dry mass. FPOM was added to tubes in liquid form because dried FPOM does not dissolve well in water.

In addition to FPOM, dead arboreal leaves were added to tubes to create habitat structure as would be found in a natural setting. Leaves were collected from bromeliads, cleaned thoroughly, and oven dried for sterilization. They were then standardized by size. A small leaf was added to small tubes, and a large leaf was added to large tubes.

Chironomid assemblages were placed in 15 or 50 ml centrifuge tubes for small or large plants, respectively. In preparation for the experiments, an 8.5 mm hole was drilled through both sides of each tube. Holes were covered with 80 μm Nytex mesh, which allowed bromeliad water and micro-organisms to flow into the tube, while preventing chironomids from escaping. Bromeliads play an important role in affecting the oxygen and nutrients within their tanks, and we wanted to ensure our experiment allowed for these processes (Benzing *et al.*, 1972; Lopez *et al.*, 2009). Tubes were placed in bromeliad leaf wells with one replicate of each treatment per plant. Thus, each plant held 4 (ontogeny experiment) to 6 (equivalence experiment) tubes. The top of each tube was covered with a mesh emergence trap to catch emerging insects and prevent oviposition into the tubes. The emergence traps were checked daily. Following identification, adult insects were released.

Performance was measured in terms of adult emergence (counted daily) and overall survival of each species. The experimental phase for each repli-

cate lasted 8 weeks, which should have given both species sufficient time to develop to adulthood (Canteiro and Albertoni, 2011; Oliver, 1971). At the end of the experiment, all remaining larvae were identified. Survival was calculated as the sum of emergences and remaining larvae.

4.2.4 *Data Analysis*

Four models were created for each experiment: a model for each of the two species with chironomid emergence as the response variable, and a model for each of the two species with chironomid survival as the response variable. The full model was used as a starting point for model fitting. The full model included all treatments and all possible interactions between them. For each model, insignificant terms were removed one-by-one, starting with the three-way interaction term, followed by the two-way interactions, and finally the individual terms, if needed. Each simplified model was compared with the more complex version using a likelihood ratio test (Zeileis and Hothorn, 2002). When the likelihood-ratio test returned a significant p-value, the more complex model was retained.

In the equivalence experiment, four additional models were created, fitting survival and emergence against total abundance. Models with total abundance of species and total abundance of conspecifics as explanatory variables were compared using Akaike's Information Criterion (AIC). Model fits including plant size were also tested, and compared to the simpler models using likelihood-ratio tests.

In all cases, a binomial GLM was used with plant identity set as a random effect. Model fitting was done with the statistical software, *R* (R Core Team, 2014) using the package *lme4* (Bates *et al.*, 2014). Models were assessed for goodness of fit by examining the deviance residuals.

4.2.5 *Fitness Proxy Estimates*

Average fitness in the context of Chesson's (2000b) coexistence framework refers to the ability of a species' population to grow quickly despite resource competition. Importantly, this ecological definition differs from Darwinian fitness. Empirically determining fitness differences is widely acknowledged as one of the most difficult aspects of applying Chesson's (2000b) framework to real systems. For example, the approach advocated by Adler et al. (2007) is to first fit a demographic model to each species and then force competitive equivalence upon the model to reveal fitness differences. This approach has been successfully implemented for an annual plant community (Godoy *et al.*, 2014; Godoy and Levine, 2014; Kraft *et al.*, 2015). However, such an approach is not feasible for a system such as bromeliad chironomids, where it is extremely difficult to obtain the necessary information on fecundity and adult mortality to fit dynamic models. Instead, we return to the original definitions of average fitness and first note that any definition of fitness as insensitivity to resource competition requires that the density of both conspecifics and heterospecifics be considered; for example, one term of the Godoy and Levine (2014) equation for fitness considers the inverse of the product of the intraspecific and interspecific competition coefficients. Therefore we expect that, in general, a species with high average fitness should show insensitivity in its vital rates to changes in total density (combining conspecifics and heterospecifics). We therefore estimate a proxy of average fitness, density resistance, as:

$$\textit{Density resistance} = \frac{\textit{proportion survival at high density}}{\textit{proportion survival at low density}} \tag{4.1}$$

Here survival includes both larval survival and emergence, as both will contribute to the growth potential of the population. In practice, we added one to the remaining survival proportions in order to avoid dividing by zero and

removed replicates with no survival at both high and low density. We follow Kraft *et al*. (2015) in expressing average fitness differences between species as a ratio:

$$Average\ fitness\ ratio = \frac{density\ resistance\ i}{density\ resistance\ j} \tag{4.2}$$

High values of this ratio indicate that species i is able to better resist the deleterious effects of resource competition than species j, whereas a ratio not different that one indicates fitness equivalence. We calculate 95% CI as 1.96 x SE to see if the ratio included the value one.

We acknowledge that we have implicitly assumed that there are no fecundity differences between species that contribute to fitness differences. This is analogous (although not numerically identical) to collapsing the Kraft *et al*. (2015) equation for average fitness to the term they refer to as "competitive response". We consider this assumption further in the Discussion. In the equivalence experiment, we evaluate the average fitness difference between species over a range of proportions. As the response of a species to total density can depend on the relative proportion of conspecifics and heterospecifics (except in the case of competitive equivalence), estimates of fitness over a range of relative abundances allow us to evaluate how robust our conclusions are. True fitness differences should persist over all relative abundances (Kraft *et al*., 2015).

4.2.6 *Observed Plant Size Preferences*

We used survey data (D. S. Srivastava & G. Q. Romero, unpubl. data) to assess the observed plant size preferences of the study chironomids under natural conditions. Regionally rare species are more likely to be found in larger bromeliads due to the fact that larger bromeliads hold more individuals. To correct for this, we created a null model that placed individuals of each

chironomid species in bromeliads one at a time (based on Amundrud and Srivastava 2015). Probabilities of placing an individual in a particular bromeliad (P_b) were based on the total abundance of all species in the bromeliad:

$$P_b = \frac{n_b}{N} \tag{4.3}$$

where n_b is the number of individuals in bromeliad b and N is the total number of individuals in the data set. Next, the two target species were distributed into bromeliads using the calculated probabilities, until all individuals of the species were placed. The abundance-weighted mean volume (in ml) in which each species was found was calculated after all individuals were placed, generating a mean expected bromeliad volume if species were distributed randomly among bromeliads. This procedure was repeated 9,999 times to generate a distribution of mean expected bromeliad sizes for each species. Finally, overall means were calculated by taking the mean of means for each species. 95% confidence intervals were calculated based on the distribution of means. Mean observed and mean expected plant sizes were compared using a z-score to establish significance and determine whether or not observed plant size preferences differed from the null expectation.

4.3 RESULTS

4.3.1 *Equivalence Experiment*

Chironomus detriticola responded to all manipulations of initial proportion, relative abundance and plant size in terms of both survival and emergence (Table 4.2, Figure 4.3). *C. detriticola* had the highest survival at low density, in small plants, and when it encountered more heterospecifics than conspecifics. The effects of these treatments were purely additive, that is, there were no interactions. These patterns in survival largely translated into emergence rates,

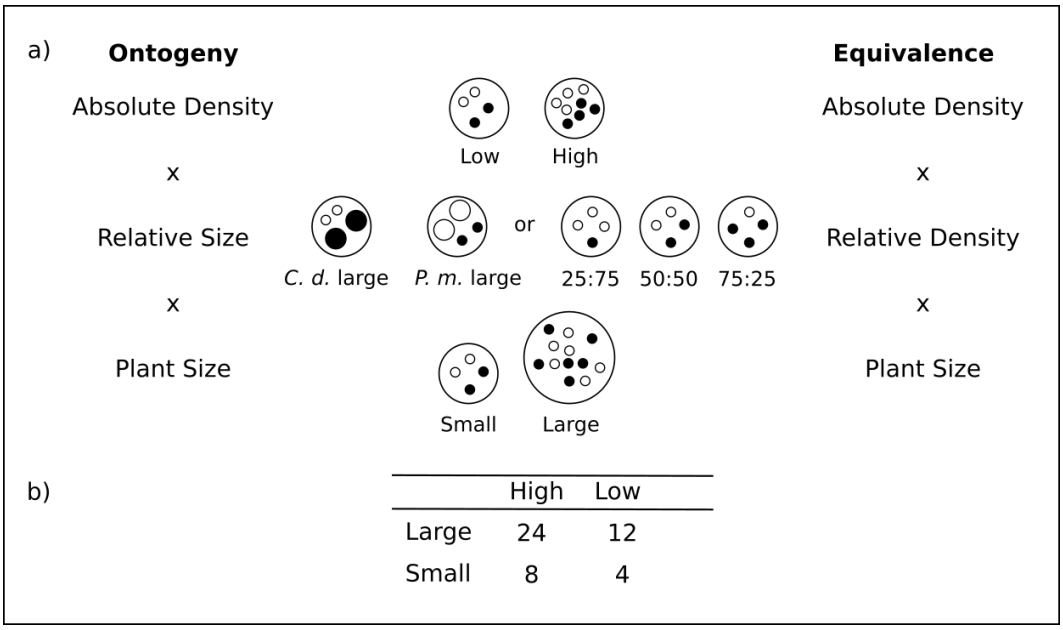

FIGURE 4.2: Experimental design of the ontogeny and equivalence experiments. a) Treatments for both experiments are shown. Circles represent a top-view of the tubes used in the experiment. Black and white dots represent the two chironomid species: *Chironomus detriticola* (C. d.) and *Polypedilum marcondesi* (P. m.). b) Total numbers of chironomids used, where "High" and "Low" refer to absolute density and "Large" and "Small" refer to plant size.

except the advantage of being in small plants disappeared for chironomids at low density. That is, in addition to the main effects of treatments on *C. detriticola* emergence there were also interactions with density.

Considering the response to total species abundance, *C. detriticola* emergence decreased with total abundance of conspecifics (Figure 4.4; Z = -4.469, p < 0.0001) as well as with plant size (Z = -2.175, p = 0.0296). The effect of plant size disappeared when survival was assessed, such that survival was lowest at highest total abundance of conspecifics (Figure 4.4; Z = -3.982, p < 0.0001).

Polypedilum marcondesi (Table 4.2, Figure 4.5) was unaffected, either in survival or emergence, by the initial proportion of conspecifics. Like *C. detriticola*, *P. marcondesi* had highest survival and emergence at low absolute densities. Additionally, *P. marcondesi* had higher emergence - but not survival - in small plants. These effects of absolute density and plant size were additive, that is, there were no interactions. The results of likelihood-ratio tests for both species can be found in the supplemental materials (Appendix A.2; Table A.1).

P. marcondesi survival (Z = -3.575, p = 0.00035) and emergence (Z = -4.152, p < 0.0001) both declined with total abundance of all species (Figure 4.6). Models including plant size showed no improvement over these abundance-only models.

4.3.2 *Ontogeny Experiment*

In the ontogeny experiment, *C. detriticola* larvae survived best as small instars cohabiting with large instars of *P. marcondesi* (Table 4.3, Figure 4.7), rather than the reverse. The effects of plant size on *C. detriticola* survival depended on the absolute density of larvae. At high densities, survival in small plants was double that in large plants, but at low densities the reverse was true: survival was double that in large plants than small plants. Unlike survival, emergence of

C. detriticola larvae was only affected by absolute density: survival decreased slightly as absolute density increased.

Larvae of *P. marcondesi* had the highest survival and emergence rates when they were large instars cohabiting with small instars of *C. detriticola* (Table 4.3, Figure 4.8). Survival was also greater in small, as opposed to large, plants. Results of likelihood tests for both species can be found in the supplemental materials (Appendix A.2; Table A.2).

4.3.3 *Fitness Proxy Estimates*

We estimated density resistance as a proxy for fitness in both experiments. In the equivalence experiment, increasing density in small plants reduced the survival of both species at the highest relative abundance, and of *P. marcondesi* also at the lowest relative abundance (Figure 4.9). The effects of density were similar for both species so the average fitness ratios were near one, indicating fitness equivalence.

In the ontogeny experiment, density dependence was unaffected by treatment for *P. marcondesi*, but dependent on both plant size and relative body size for *C. detriticola*. In small plants and at small body size, *C. detriticola* showed strong density resistance; however, in large plants, *C. detriticola* was more sensitive to density. Consequently, neither species had a fitness advantage when they were the smaller species, but at large body sizes, *P. marcondesi* had a fitness advantage in large plants and *C. detriticola* had a fitness advantage in small plants (Figure 4.9). Therefore, when the species differ in their relative body sizes, fitness differences emerge in different plant sizes at large but not small body sizes.

4.3.4 *Observed Plant Size Preferences*

Both *C. detriticola* ($z = 25.39$, $p < 0.0001$) and *P. marcondesi* ($z = 10.38$, $p < 0.0001$) naturally occurred in larger plants than expected by chance (Figure 4.10). However, *C. detriticola* was found on average in even larger plants than *P. marcondesi*.

4.4 DISCUSSION

In this study, we deconstructed the competitive relationship between two chironomid species to determine whether the species experienced ecological (symmetric or asymmetric) equivalence, and how this relationship was affected by context (habitat and ontogeny). We found that competition affected the performance of both species differently, with signs of asymmetric equivalence and competitive stabilization, as we explain shortly. Furthermore, habitat size and ontogenetic differences in body size both had an effect on survival or emergence in one or both species, underlining a dependency of the outcome of competition on context.

The results of both experiments confirm that competition plays a role in this system. In the equivalence experiment, both species experienced reduced survival and emergence in response to increased absolute densities of organisms (Table 4.2). This was also true for *C. detriticola* in the ontogeny experiment, although *P. marcondesi* was largely unaffected by absolute density (Table 4.3). To explore the competitive relationship between the species and the mechanisms driving this relationship (Question 1), we first examine the response to relative abundance in the equivalence experiment. Under stabilization, levels of intraspecific competition should be higher than interspecific competition for one (asymmetric equivalence) or both species (Table 4.1A). Therefore, we would expect to see reduced performance (emergence or survival) in response

	Chironomus detriticola		*Polypedilum marcondesi*	
	Emergence	Survival	Emergence	Survival
Plant Size	$Z_{113} = 144.0$ $p < 0.0001$	$Z_{115} = 3.268$ $p = 0.00108$	$Z_{116} = 2.601$ $p = 0.009288$	$Z_{115} = 1.572$ $p = 0.1159$
Relative Abundance	$Z_{113} = 18.4$ $p < 0.0001$	$Z_{115} = 3.441$ $p = 0.00058$	$Z_{115} = 0.523$ $p = 0.6012$	$Z_{116} = -1.576$ $p = 0.1151$
Density	$Z_{113} = 431.8$ $p < 0.0001$	$Z_{115} = 2.318$ $p = 0.02044$	$Z_{116} = 3.464$ $p = 0.000532$	$Z_{117} = 4.792$ $p < 0.0001$
Relative Abundance x Density	a) $Z_{113} = -4.3$ $p < 0.0001$	$Z_{112} = 0.122$ $p = 0.9032$	$Z_{113} = 0.769$ $p = 0.442$	$Z_{113} = 0.410$ $p = 0.6819$
Relative Abundance x Plant Size	$Z_{112} = -1.1$ $p = 0.251$	$Z_{113} = 0.452$ $p = 0.6516$	$Z_{112} = 0.351$ $p = 0.7252$	$Z_{112} = -0.094$ $p = 0.9253$
Plant Size x Density	b) $Z_{113} = -167.3$ $p < 0.0001$	$Z_{114} = 1.081$ $p = 0.2798$	$Z_{114} = 0.899$ $p = 0.3688$	$Z_{114} = 1.564$ $p = 0.1179$
Plant Size x Relative Abundance x Density	$Z_{111} = 17.1$ $p < 0.0001$	$Z_{111} = -1.643$ $p = 0.1004$	$Z_{111} = 0.706$ $p = 0.4805$	$Z_{111} = 1.679$ $p = 0.0933$

TABLE 4.2: Summary of models for the equivalence experiment. Values highlighted in red indicate a negative relationship between the treatment and response variables while those highlighted in blue indicate a positive relationship. Green values are involved in an interaction (described below). Values in black were not significant. Shaded values were removed from the model. Interactions are described here: a) Emergences were higher at low density and high relative abundance than at low density and low relative abundance; b) At high densities, emergence was higher in large than small plants, while the reverse was true at low density.

	Chironomus detriticola		*Polypedilum marcondesi*	
	Emergence	Survival	Emergence	Survival
Plant Size	$Z_{81} = 1.597$ $p = 0.1103$	$Z_{82} = 2.160$ $p = 0.0307$	$Z_{84} = 0.887$ $p = 0.375$	$Z_{84} = 2.390$ $p = 0.0168$
Body Size	$Z_{81} = 1.877$ $p = 0.0606$	$Z_{82} = 2.223$ $p = 0.0262$	$Z_{85} = 7.706$ $p < 0.0001$	$Z_{84} = 6.204$ $p < 0.0001$
Density	$Z_{81} = 2.059$ $p = 0.0395$	$Z_{82} = 4.516$ $p < 0.0001$	$Z_{83} = 0.565$ $p = 0.572$	$Z_{83} = 1.594$ $p = 0.1110$
Plant Size x Body Size	$Z_{81} = -1.895$ $p = 0.0581$	$Z_{81} = -1.755$ $p = 0.0792$	$Z_{82} = 0.818$ $p = 0.414$	$Z_{82} = 1.143$ $p = 0.253$
Plant Size x Density	$Z_{81} = -1.747$ $p = 0.0806$	a) $Z_{82} = -2.748$ $p = 0.0060$	$Z_{81} = -0.697$ $p = 0.486$	$Z_{80} = -0.123$ $p = 0.9018$
Density x Body Size	$Z_{80} = -0.017$ $p = 0.9866$	$Z_{80} = -0.537$ $p = 0.5910$	$Z_{80} = -0.213$ $p = 0.832$	$Z_{81} = 0.440$ $p = 0.6570$
Plant Size x Density x Body Size	$Z_{79} = -0.030$ $p = 0.976$	$Z_{79} = -0.742$ $p = 0.4578$	$Z_{79} = -0.517$ $p = 0.605$	$Z_{79} = 0.161$ $p = 0.8718$

TABLE 4.3: Summary of models for the ontogeny experiment. Values highlighted in red indicate a negative relationship between the treatment and response variables while those highlighted in blue indicate a positive relationship. Green values are involved in an interaction (described below). Values in black were not significant. Shaded values were removed from the model. Interactions are described here: a) At low densities, survival was higher in large plants whereas survival was higher in small plants at high densities.

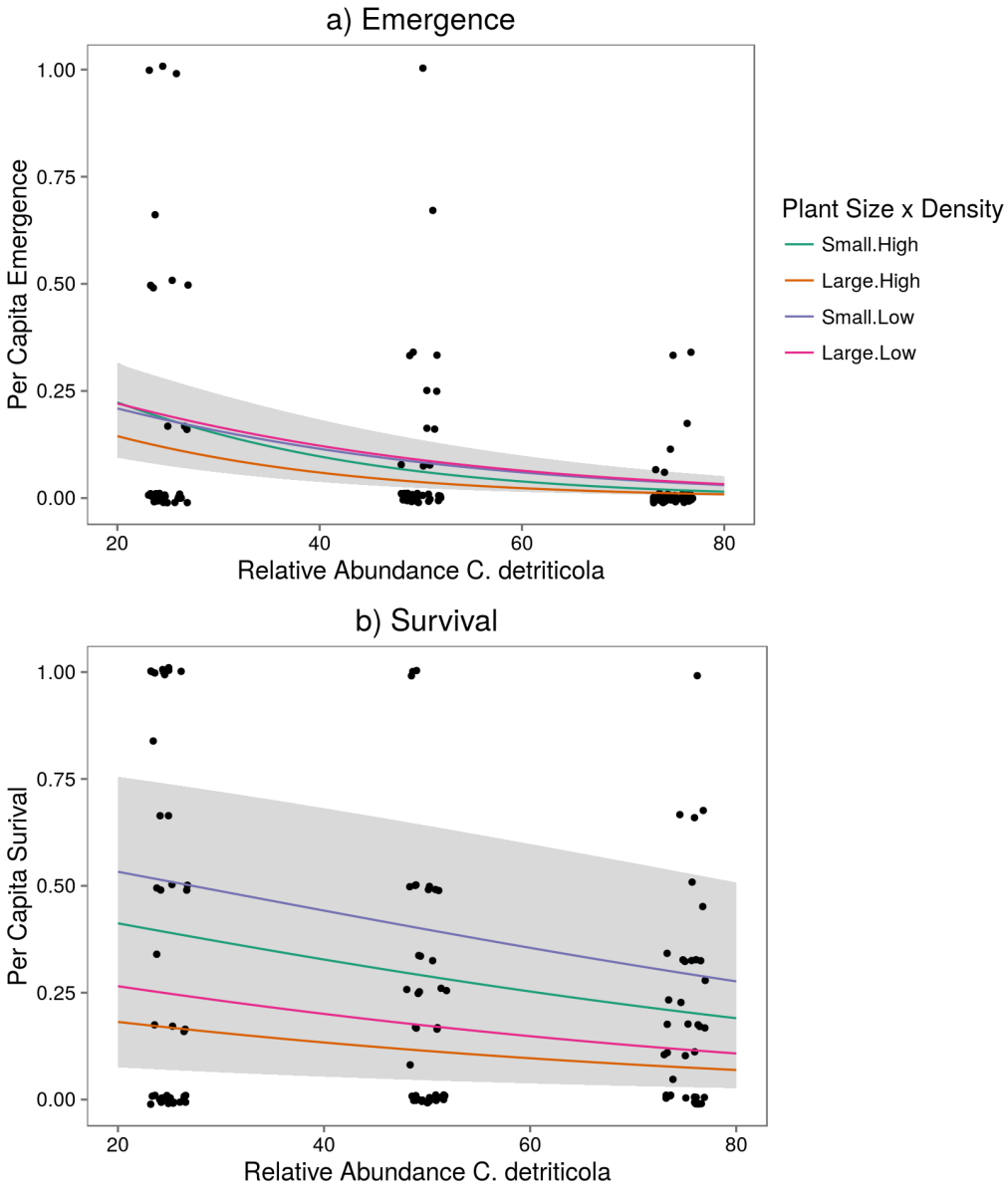

FIGURE 4.3: Response of *C. detriticola* in the equivalence experiment. Lines show the model predictions with variance due to the random effect of plant identity in gray. Points represent the actual data (jittered). Under both response variables, *C. detriticola* showed a stabilizing relationship with higher intraspecific competition at higher densities. a) Emergence Density*** + Pct Chironomus*** + Plant Size*** + Density:Pct Chironomus*** + Density:Plant Size*** + (1 | Plant ID). b) Survivors Plant Size** + Pct Chironomus*** + Density* + (1 | Plant ID).

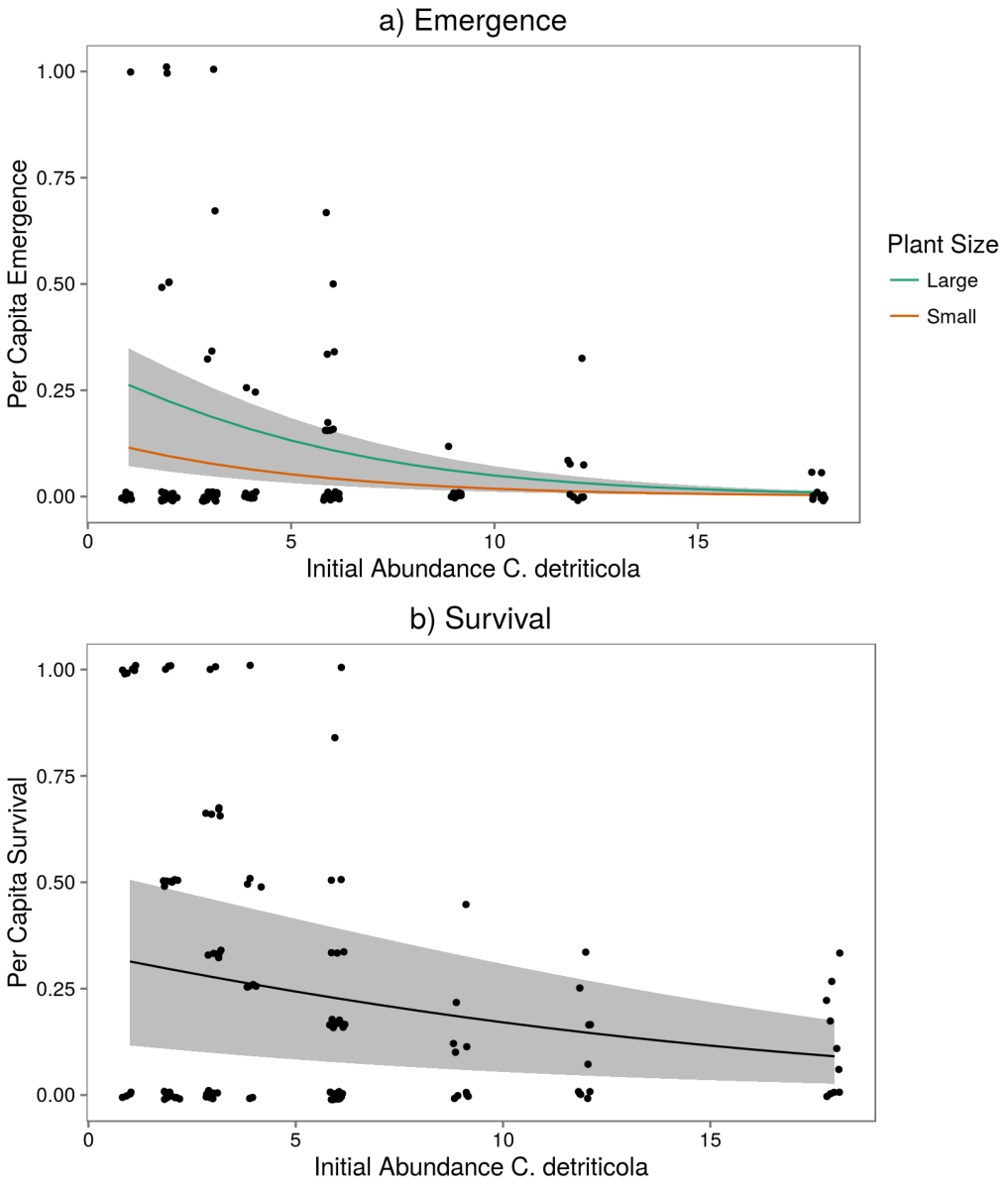

FIGURE 4.4: *C. detriticola* had decreased survival and emergence at higher abundances of conspecifics. Lines show the model predictions with the variance due to plant identity in gray. Points show the actual data (jittered). a) Emergence Abundance of Conspecifics*** + Plant Size* + (1 | Plant ID). b) Survival Abundance of Conspecifics*** + (1 | Plant ID).

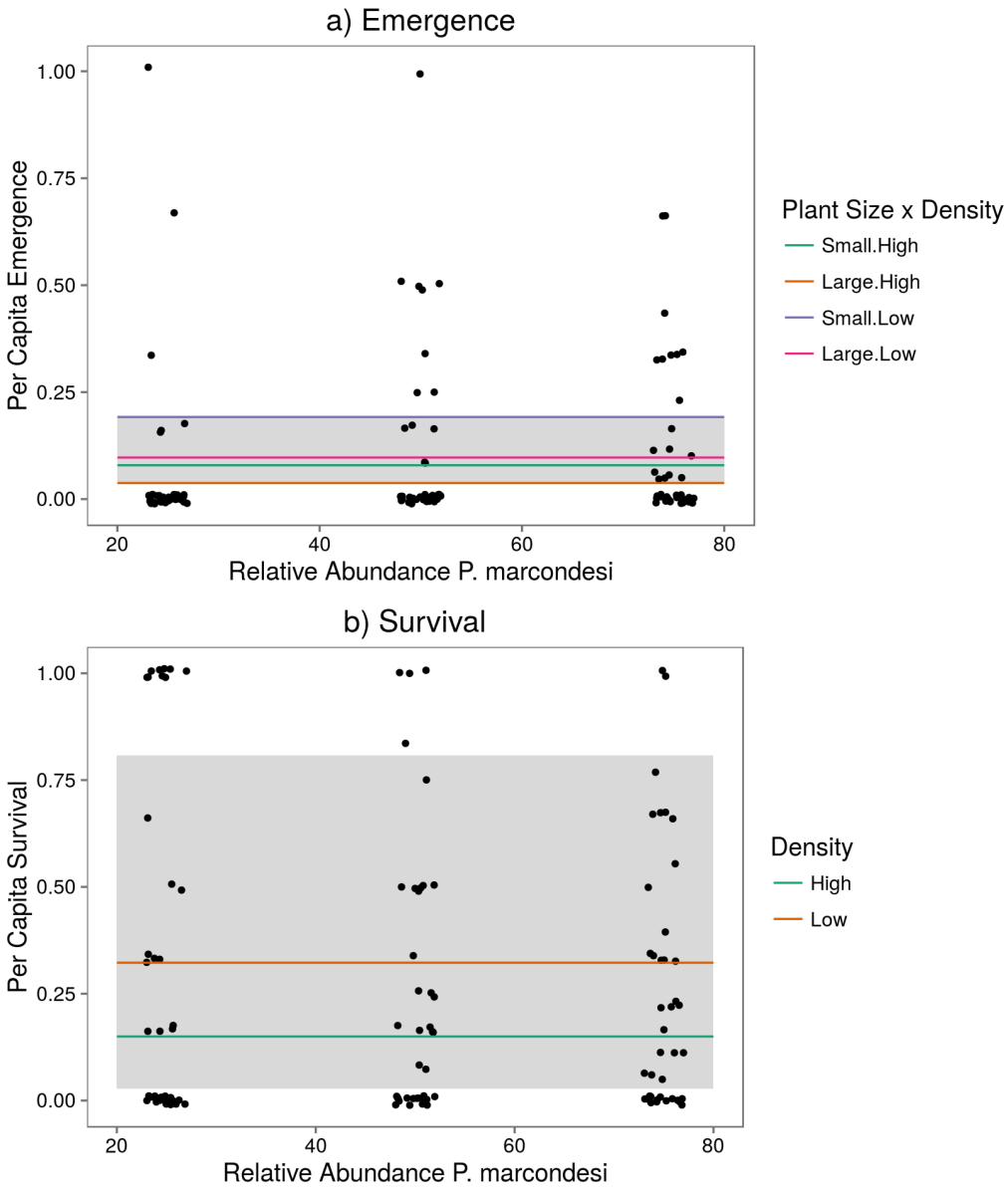

FIGURE 4.5: Response of *P. marcondesi* in the equivalence experiment. Lines show the model predictions with variance due to the random effect of plant identity in gray. Points represent the actual data (jittered). Relative abundance was not significant in either response, suggesting that *P. marcondesi* experiences competitive equivalence. a) Emergence Density*** + Plant Size** + (1 | Plant ID). In this model, plant identity had a very weak effect so box plots are narrow and appear as lines. b) Survival Density*** + (1 | Plant ID).

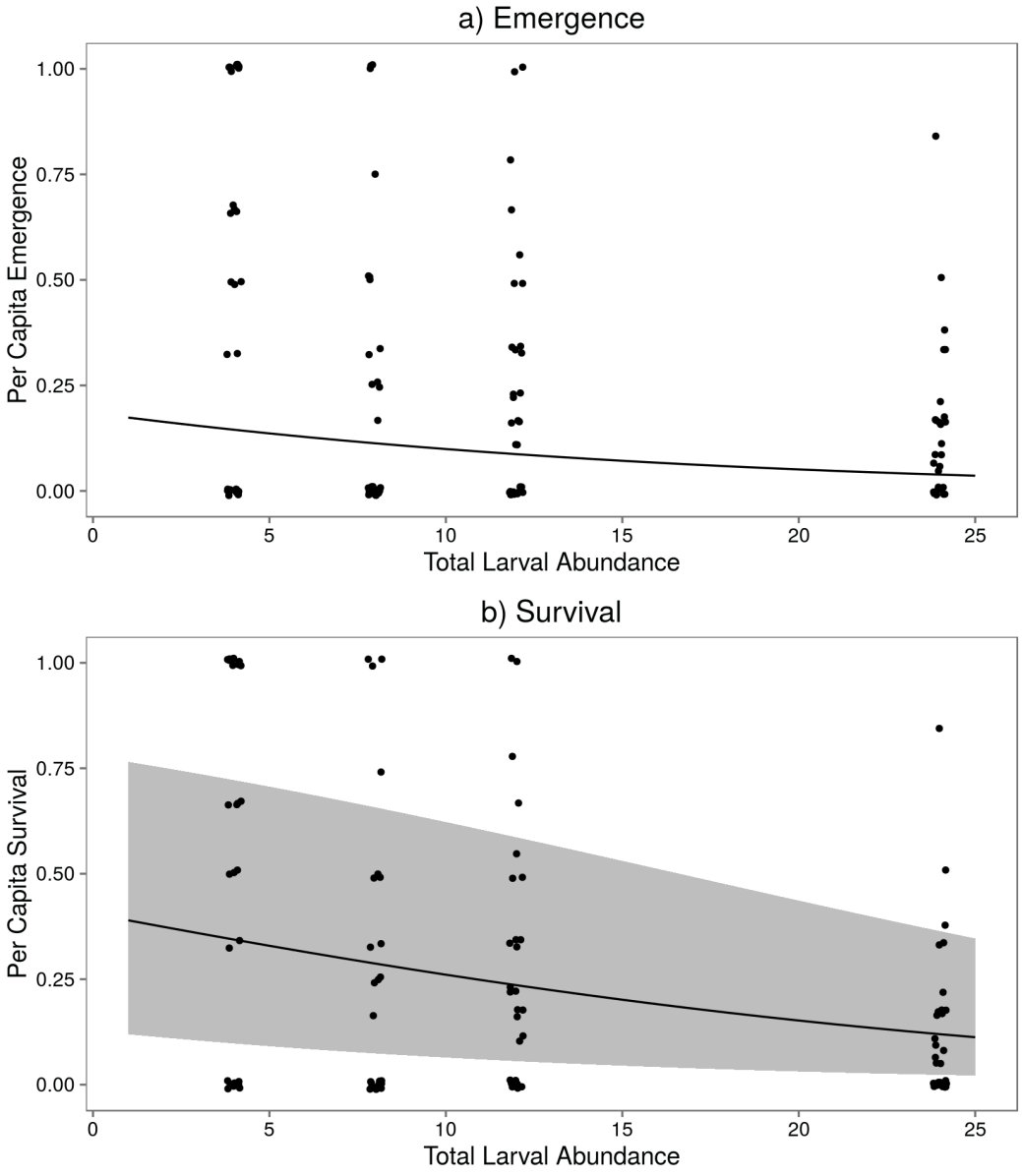

FIGURE 4.6: *P. marcondesi* had decreased survival and emergence at higher abundances of larvae. Lines show the model predictions with the variance due to plant identity in gray. Points show the actual data (jittered). a) Emergence Total Abundance*** + (1 | Plant ID). b) Survival Total Abundance*** + (1 | Plant ID).

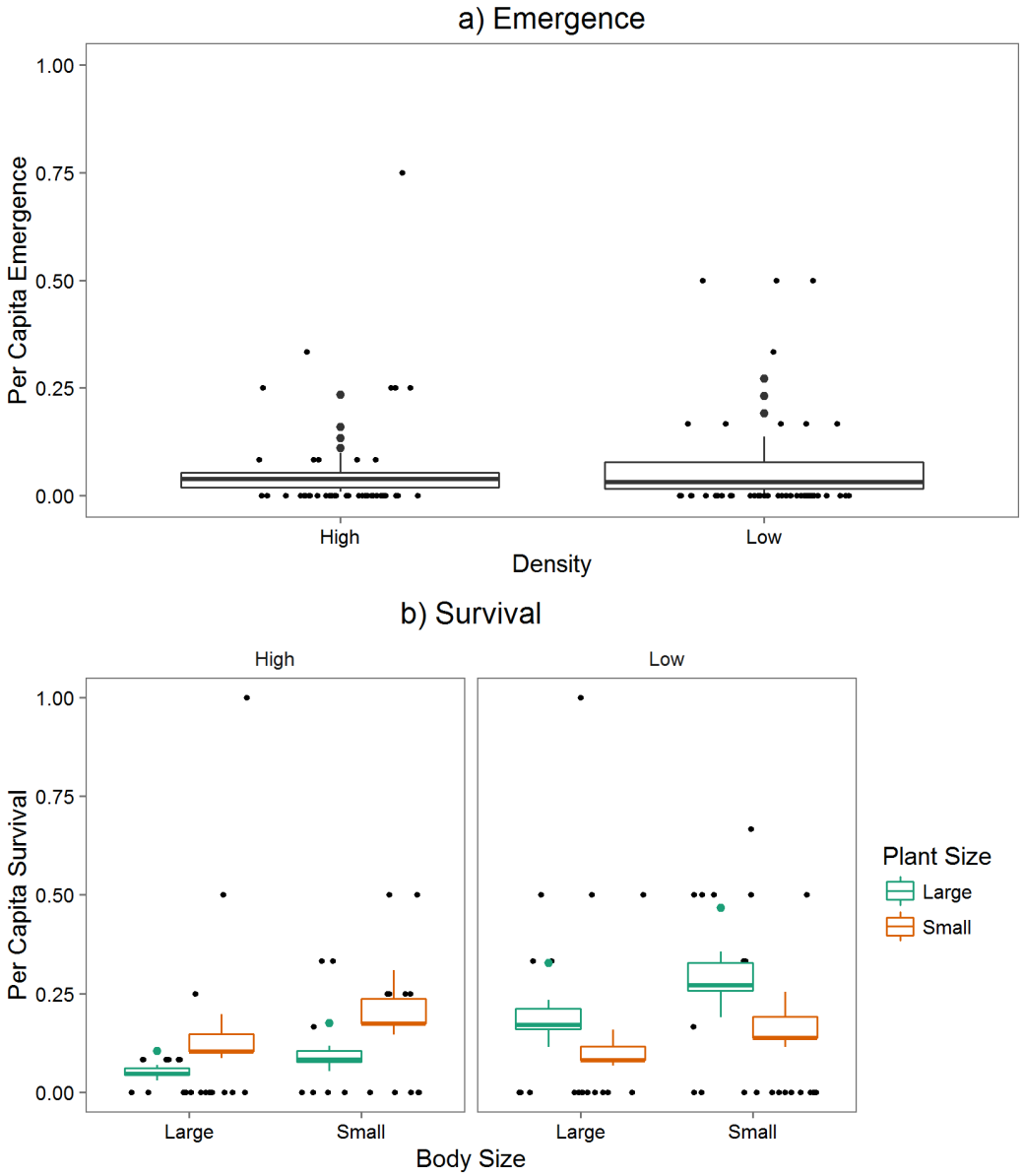

FIGURE 4.7: Response of *C. detriticola* in the ontogeny experiment. Boxes show the range of predicted values based on the random effect of plant identity. Points represent the actual data. *C. detriticola* performed worse at large body sizes. a) Emergences Plant Size* + Body Size + Density*** + Plant Size:Density + Plant Size:Body Size + (1 | Plant ID). b) Survival Plant Size* + Body Size* + Density*** + Plant Size:Density** + (1 | Plant ID).

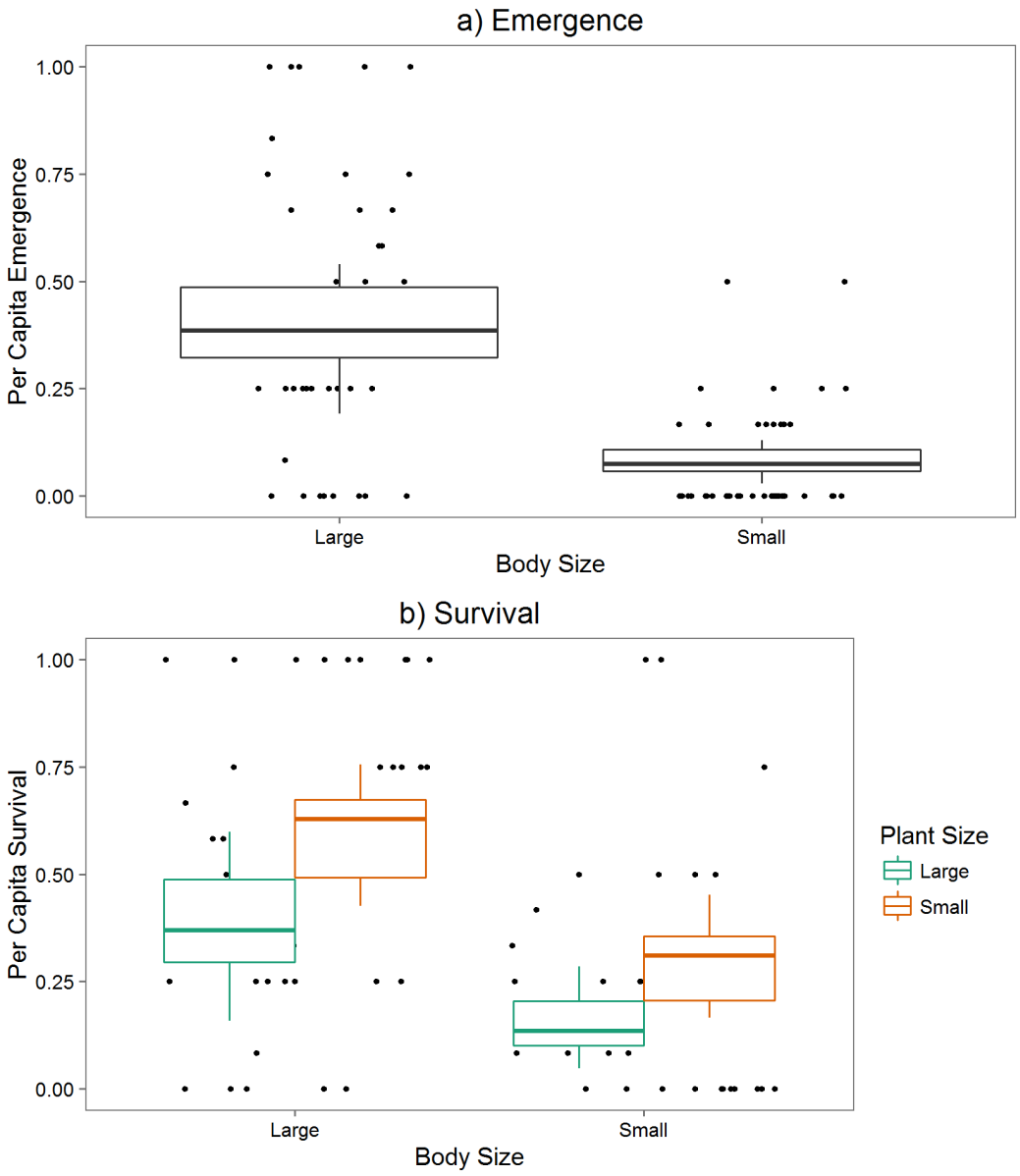

FIGURE 4.8: Response of *P. marcondesi* in the ontogeny experiment. Boxes show the range of predicted values based on the random effect of plant identity. Points represent the actual data. *P. marcondesi* performed worse at small body sizes (when *C. detriticola* was larger). a) Emergence Body Size*** + (1 | Plant ID). b) Survival Plant Size* + Body Size*** + (1 | Plant ID).

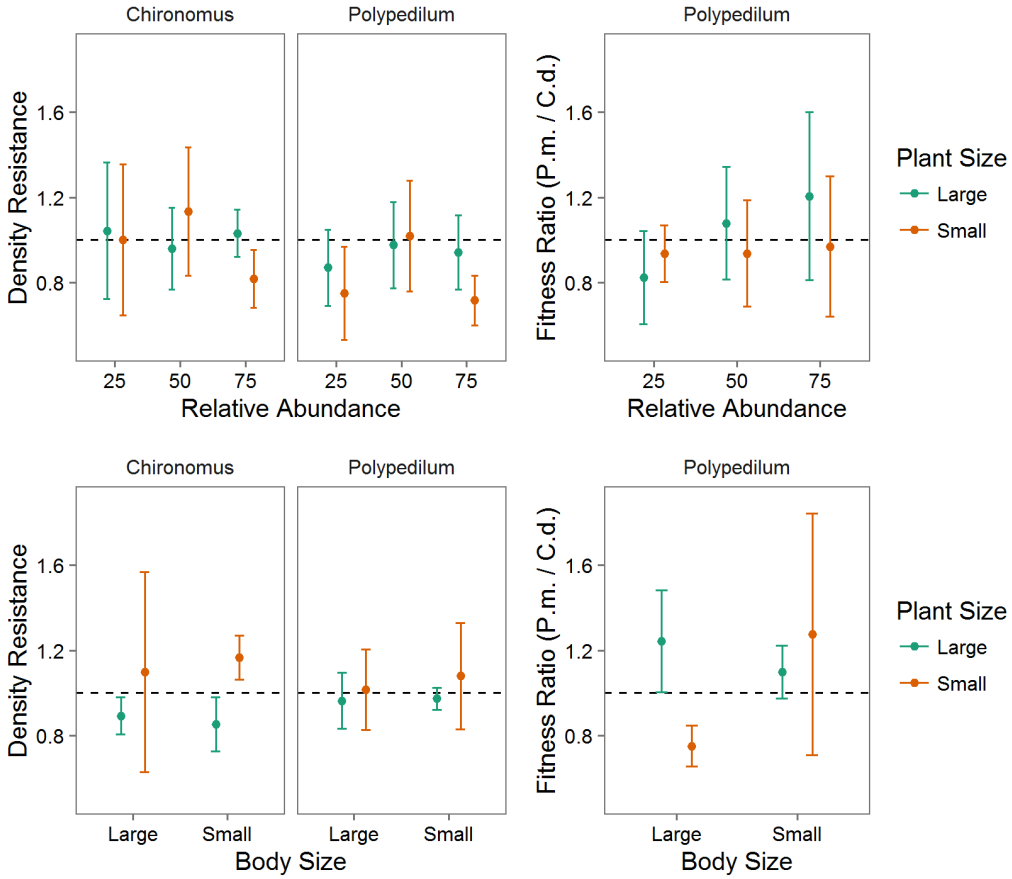

FIGURE 4.9: Density resistance and fitness ratio in the equivalence (top) and ontogeny (bottom) experiments. In the equivalence experiment, species tended to have similar density resistance in the same treatment and consequently showed no mean fitness ratios different from 1. In the ontogeny experiment, *P. marcondesi* had mean fitness just above 1 in large plants when it was large and *C. detriticola* was small. When *P. marcondesi* was large in small plants, *C. detriticola* had higher fitness. When *P. marcondesi* was small, there were no fitness differences. Error bars are 95% CI calculated as mean +/- 1.96 SE.

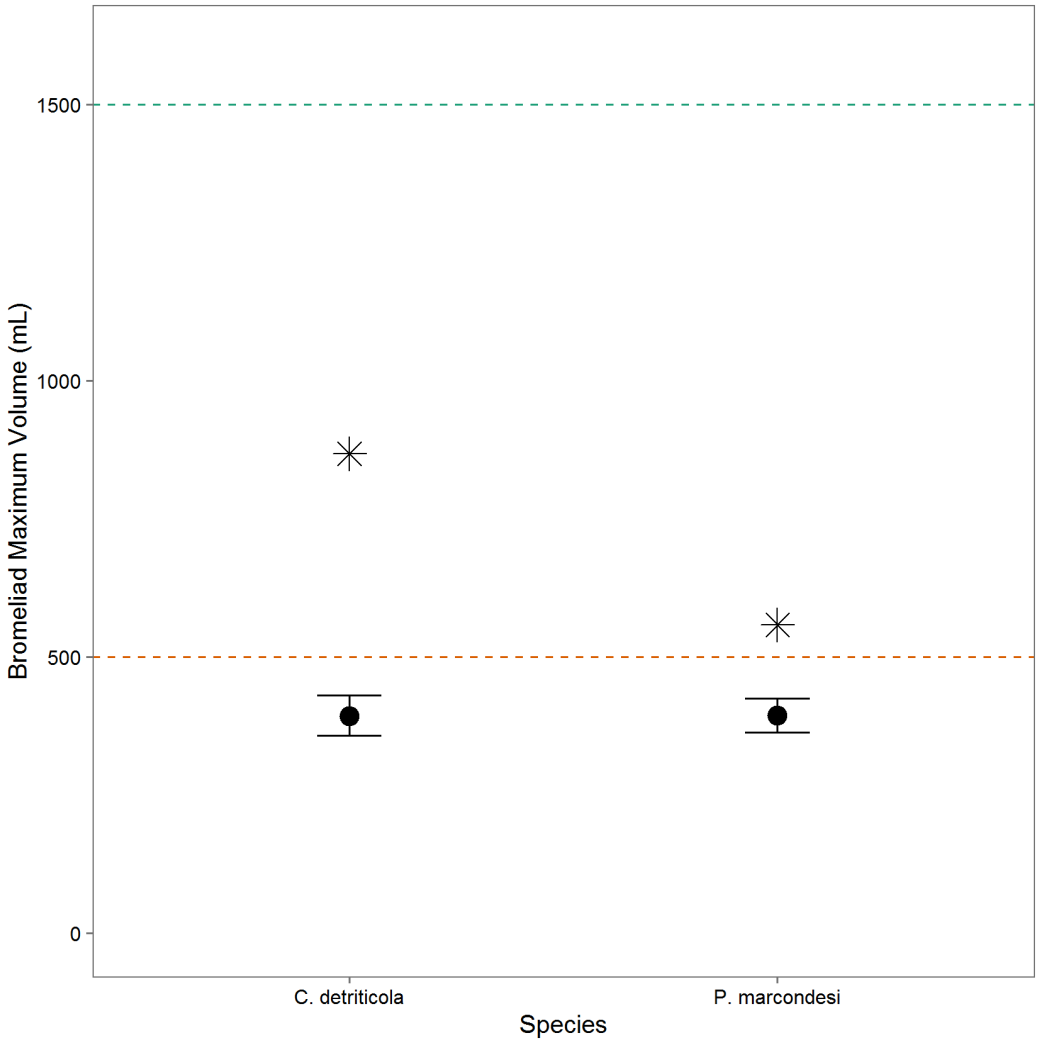

FIGURE 4.10: Null and observed plant sizes for study chironomids. Stars show the observed, abundance-weighted mean, representing the chironomid plant size preference. Points show the null model expectation if species are randomly placed in bromeliads with 95% CI. Dashed lines show the plant size for "small" (orange) and "large" (green) plants as we defined them in the experiment. Both species were observed in plants significantly larger than the null expectation, with *P. marcondesi* being found in plants close to the size of the "small plants" used in these experiments.

to increased relative abundance. For *C. detriticola*, both emergence and survival decreased at higher relative abundances (Table 4.2, Figure 4.3), revealing an increase in intraspecific competition with higher densities of conspecifics. This suggests that *C. detriticola* abundance is mediated by competitive stabilization. However, *P. marcondesi* (Table 4.2, Figure 4.5) did not exhibit the same increase in intraspecific competition. *P. marcondesi* emergence depended only on plant size and absolute density, and survival depended on density alone. *P. marcondesi* therefore appears to experience a competitively equivalent relationship with *C. detriticola* - responding negatively to higher densities of organisms in general, regardless of the identities of the organisms.

Though we assumed the strength of competition would depend on the density of organisms - which was kept constant across treatments within a given density treatment, model fits to absolute abundances suggested otherwise. Both *P. marcondesi* and *C. detriticola* had lower survival and emergence at higher absolute abundances (Figures 4.4, 4.6). In the case of *C. detriticola*, only the abundance of conspecifics affected performance, while *P.marcondesi* responded to total larval abundance - a pattern consistent with the results of the original models. It is unclear why abundance and not density might be important for the survival and emergence of these species. Possibly our assumption that total volume of the experiment tubes would correspond to increased usable habitat for the chironomids was faulty. Chironomids rely on detritus to construct cases and are often found deep in the bromeliad leaf well. It is possible that they remain at the bottom of the water column, which was roughly the same size in both large and small tubes. However, further empirical research is needed to delve into the mechanisms causing this relationship with total abundance rather than density.

Changing the relative body sizes of the two species (Question 2) also affected the nature of the interspecies relationship in an asymmetric way: Both

species had lower performance when *C. detriticola* was the larger-bodied species than when *P. marcondesi* was the larger species (Table 4.3; Figures 4.7, 4.8). The negative response of *C. detriticola* to a larger biomass of conspecifics is perhaps not surprising given the demonstration of strong intraspecific competition for this species in the equivalence experiment (Figure 4.3). Asymmetry in the competitive abilities of small and large versions of conspecifics has been observed frequently, often with larger individuals being superior competitors (Alcock, 2013; Bolund *et al.*, 2007; Cameron *et al.*, 2007; Livdahl, 1982; Werner, 1994), although occasionally with smaller individuals being better (Bolund *et al.*, 2007; Marshall and Keough, 1994). In our experiment, not only did large *C. detriticola* suppress the performance of conspecifics, but also that of small *P. marcondesi*. The lack of a symmetric response when *P. marcondesi* was large suggests a change in relative competitive advantages, violating competitive equivalence, and suggesting a context-dependency in the way *P. marcondesi* and *C. detriticola* interact.

One possible reason for the negative response to *C. detriticola* by both species is that *C. detriticola* may change trophic level as it grows, shifting from a detritivore to a cannibalist and predator. This would be detrimental to the small-bodied *P. marcondesi*, but *C. detriticola* might also be expected to fare poorly when faced with a combination of intraspecific competition and cannibalism. Facultative predation has been observed in other species of the genus *Chironomus* (Pinder, 1986). Furthermore, some models suggest that species that engage in cannibalism often switch from competitive to cannibalistic behaviour as they develop (Claessen *et al.*, 2000; Persson *et al.*, 2000). This may occur when smaller instars are better competitors for shared resources because the switch to cannibalism can allow large instars to coexist with small ones. If this were the case, the larger *C. detriticola* larvae might reduce survival and emergence of all other larvae by consuming both conspecifics

and heterospecifics. Larger *P. marcondesi* larvae, on the other hand, would not have such an adverse effect on other organisms because they would retain their detritivorous diet throughout the larval stage.

In addition to body size we also manipulated plant size (Question 2), which seemed at first to play a prominent role in the relationship between the two chironomid species. In both experiments, there was a general trend of higher performance in smaller plants. However, the effect of plant size was mostly lost once total abundance was considered (Figures 4.4, 4.6), though *C.detriticola* did have higher emergences in large plants. Our analysis of observational data (Figure 4.10), shows *P. marcondesi* most frequently in bromeliads of similar volumes to those used in the "small plant" bromeliads in the experiments, even when we corrected for sampling effects with a null model, and *C. detriticola* is found in much larger plants ($\sim$ 870 ml) than expected by chance ($\sim$ 393 ml). Thus, there is some suggestion that plant size may be a factor in chironomid coexistence, but our experiments give no definite answers about what the effect may be and when it occurs. A follow-up experiment done in whole bromeliads rather than tubes might help clarify some of the potential issues with our design.

While one of our experiments suggested a relationship of asymmetric competitive equivalence, and the second suggested that competitive equivalence is context-dependent, it is only with information about the relative fitness of the two species that we can make conjectures about whether or not ecological equivalence is present and make predictions regarding local and regional coexistence (Question 3). Conclusions about coexistence based on our fitness proxy estimates come with some caveats. Most importantly, in our fitness estimates, we did not consider the "demographic ratio" of Godoy & Levine (2014; see also Appendix A.1). The demographic ratio incorporates fecundity in the absence of competition, information which we do not have and would

be difficult to obtain. Thus, our inferences about local and regional coexistence will be most useful for illustrative purposes, and for directing future research into the particularities of chironomid coexistence.

In the equivalence experiment, fitness estimates for chironomid species were equal in all treatments (Figure 4.9), garnering support for fitness equivalence. This suggests that these species experience fitness equivalence, and by extension, asymmetric ecological equivalence. According to our analysis (Appendix A.1), coexistence in such cases is dependent on the dynamics of the non-equivalent species. In this case, because *C. detriticola* experienced competitive stabilization, the two species are expected to be able to coexist (Table 4.1A). In the ontogeny experiment, species differed in their relative body sizes. Here we found a definite shift away from fitness equivalence. In large plants, large *P. marcondesi* had higher fitness than small *C. detriticola* (fitness ratio above one), and in small plants, large *P. marcondesi* had lower fitness than small *C. detriticola* (fitness ratio below one; Figure 4.9). When the focal species was the smaller of the two, there was no compensating effect of plant size on fitness differences. Because each species had higher performance than the other in a given context (small vs. large plants), the fitness data could be interpreted to suggest that when there is a relative size difference between species, metacommunity coexistence is achieved via habitat partitioning along a bromeliad size gradient (Table 4.1A, cell labelled "Coexistence via habitat partitioning or priority effects") . However, note that (1) the effect of plant size on fitness is exactly opposite to that observed in nature, and (2) any fitness advantage dependent on the species being the smaller of the two will presumably be lost as the species grows in size, and thus is inherently transient in nature. It is therefore premature to conclude that habitat partitioning in this system is key to coexistence of these species.

Overall, this research suggests that context shifts the relationship between chironomids away from one of asymmetric competitive equivalence. While theory (Adler *et al.*, 2007; Haney *et al.*, 2015; Leibold and McPeek, 2006) and experiment (e.g. Almeida *et al.*, 2015; Cadotte, 2007; Dumbrell *et al.*, 2010; Prado and Rossa-Feres, 2014; Rominger *et al.*, 2009) support the conclusion that niche and neutral processes exist concurrently, it is still unknown in which conditions one or the other type is more important. Existing research tends to focus on the importance of neutral processes (i.e. dispersal) in colonization (Cadotte, 2007; Chu *et al.*, 2007; Prado and Rossa-Feres, 2014; Püttker *et al.*, 2015) or community structure (Almeida *et al.*, 2015; Rominger *et al.*, 2009). However, the local conditions that lead to ecological equivalence between species have not been widely investigated. One of the best examples of a possible shift from equivalence may be the competition experiments of Thomas Park (1948; 1954; 1957), which pre-date neutral theory (Hubbell, 1997, 2001). In these experiments, the outcome of competition between *Tribolium* spp. beetles was sometimes stochastic (suggesting ecological equivalence; Park 1948) and sometimes deterministic (Park, 1954, 1957), depending on initial conditions, similar to what we have found here.

Research that manipulates relative abundances is invaluable for understanding the role of competitive interactions in a community. While increasing numbers of experiments address the relative importance of neutral and non-neutral forces in communities, few have determined what range of conditions lead to neutral or stabilizing dynamics. Furthermore, no research that we know of has encountered asymmetric equivalence, or at least identified it as such. Here, we found that what looked at first like coexistence via asymmetric competitive equivalence shifted in response to ontogeny. It is clear that considering only one set of ecological conditions is not sufficient for understanding competition and the presence or absence of ecological equivalence

in a community. The interaction between ontogeny and competition is particularly important to understand because changes in species interactions over development time could completely shift the relative success of the species concerned. A stable or neutral relationship in one ecological context could shift in a different context to a destabilizing one, thus ending in the exclusion of one species. We recommend that future research concerning neutrality in particular should include exploration of the role of organism development and environmental context on the outcome of species interactions.

CHAPTER 5

Conclusion

Species distributions can be understood in terms of the environmental conditions and biotic interactions that allow coexistence at local and regional scales. In this thesis, I set out to determine which factors are important for coexistence in a community of bromeliad-dwelling invertebrates by identifying species with strong negative co-occurrence patterns (Chapter 2), and studying the effects of predation and competition on the coexistence of these species (Chapters 3, 4), as well as the effects of habitat and body size (Chapter 4). Using multiple approaches, including null model analysis of observational data, demographic modelling, and empirical tests, I found that:

1. Three species of chironomid have high rates of negative co-occurrence compared to other species pairs in the community;
2. Predators negatively affect performance, primarily through emergence rates, but do not mediate coexistence;
3. Ontogenetic differences in body size manifested differently depending on the identity of the larger species, indicating a context dependency to the outcome of competition;
4. Habitat size manipulations had an effect on species response, but habitat partitioning is not expected to explain metacommunity coexistence.

At the field site of Ilha do Cardoso (Chapter 1), several species pairs exhibit statistically high negative co-occurrence rates (Chapter 2). Three species of

chironomid (Diptera: Chironomidae) — *Polypedilum marcondesi*, *Polypedilum kaingang*, and *Chironomus detriticola* — were chosen for experiments because they are relatively common (A. D. Letaw, pers. obs.; D. S. Srivastava and G. Q. Romero, unpubl. data) and easy to identify at the larval stage (A. D. Letaw, pers. obs.). While all three species experience direct and indirect negative effects of predators (Chapter 3), one species (*P. kaingang*) is competitively superior whether or not predators are present, suggesting that predator mediation is not responsible for the coexistence of these three species in nature. Two of the three study species (*P. marcondesi* and *C. detriticola*) were reared at different body sizes and in different bromeliad sizes (Chapter 4). Relative abundance manipulations of these species suggested an asymmetric relationship in which one species (*P. marcondesi*) appears to experience the world neutrally, while the other (*C. detriticola*) does not (Chapter 4), a relationship we term here asymmetric equivalence. Both species respond negatively to the presence of large *C. detriticola*, but not to large *P. marcondesi*, indicating a shift away from neutrality when ontogeny is manipulated. As for habitat size, both species generally have improved performance in smaller plants (Chapter 4).

Taken together, the results suggest that the chironomids studied have a competitive relationship the nature of which is changed primarily when ontogenetic differences in body size are present. While other factors contribute to performance (i.e. predators and habitat size), only ontogeny shifts the competitive outcome. To date very little is known about the importance of ontogeny in the coexistence of bromeliad invertebrates or whether any temporal colonization patterns are present, making it difficult to predict how important colonization order is at the community or metacommunity levels. In other systems, ontogenetic differences in body size have been found to effect competitive outcome (Eichenberger *et al.*, 2009; Hurd and Eisenberg, 1990; Serrano-Meneses *et al.*, 2007; Werner, 1994; Werner and Gilliam, 1984)

and are often present naturally due to differences in colonization timing (Hurd and Eisenberg, 1990; Yang and Rudolf, 2010). Phenology has been pinpointed as a linking factor between species that can be broken when species respond differently to environmental changes, such as those that occur due to climate change (Both *et al.*, 2009; Parmesan, 2006; Tylianakis *et al.*, 2008; Visser and Both, 2005; Yang and Rudolf, 2010). For example, many pollinators have temporal coordination with the plants they pollinate (Hegland *et al.*, 2009; J Memmot, 2007). However, differences in ontogeny generated by phenology can also effect the nature of antagonistic interspecific interactions, such as predation and competition (Kordas *et al.*, 2011; Miller-Rushing *et al.*, 2010; Tylianakis *et al.*, 2008; Yang and Rudolf, 2010). Research conducted here provides the first evidence in this system in support of a relationship between phenology, ontogeny and the outcome of competition.

In addition to insights gained from individual empirical results, here I apply a variety of approaches to the study of coexistence in bromeliad-dwelling chironomids. Taken separately, each approach offers a new framework or a new way to analyse data. In Chapter 2, AWCA was developed to find negative co-occurrence patterns in observational abundance data; in Chapter 3, a new method was used to model population growth on censored data while also gaining insight about emergence and death rates; in Chapter 4, a theoretical framework was developed, leading to new ideas about how ecological equivalence can be manifested. Each of these methods could be applied to any other system. For instance, there are many examples of checkerboard analysis being used to find signs of competition structuring communities (e.g. Barberán *et al.*, 2012; Beaudrot *et al.*, 2013; Bik *et al.*, 2010; Horner-Devine *et al.*, 2007; Presley *et al.*, 2010; Vernes *et al.*, 2001). As pointed out in Chapter 2, traditional checkerboard analysis is not sufficient to determine whether competition is responsible for community structure. However, using AWCA, it is possible to

identify species pairs driving the checkerboard structure, to use background information about those species to further postulate on whether competition or something else (e.g. predation) is occurring, and to conduct experiments that support or refute the existence of competition as a structuring mechanism. For example, AWCA could be used to analyse other bromeliad-invertebrate datasets, which are available from multiple years and countries (Bromeliad Working Group, unpubl. data). Analyzing these data sets could help us find generalities in which species or functional groups drive community structure in the bromeliad system. Demographic models like the one used here (Chapter 3) could be applied to other experiments to find out how growth, death, or other rates vary between species in response to experimental treatments. Predators are known to reduce growth rates in many cases (McKie and Pearson, 2006; Stoks, 2001; van Uitregt *et al.*, 2012), however, besides predators, demographic models could predict the effect of any experimental factor on any response. Finally, ecological equivalence could be studied in other systems to see if there are other instances of asymmetry in species response, and how these change depending on context. In particular, it would be interesting to know what range of conditions lead to niche or neutral processes. For example, chironomids exist across the geographic range of bromeliads, but the richness of invertebrates tends to decline from South to North (Bromeliad Working Group, unpubl. data). Does overall community richness affect the presence of neutral processes? Do these change when experiments are done at the community, rather than population, scale?

There are still many questions to be asked about how predators, habitat, and ontogeny effect the coexistence of chironomids and other bromeliad invertebrates. Increasingly, research on bromeliad-invertebrate communities is revealing the importance of bromeliad size on species performance and coexistence (Amundrud and Srivastava, 2015; Hammill *et al.*, 2015a; Gilbert

et al., 2008; Jocque and Field, 2014; Marino *et al.*, 2011; Petermann *et al.*, 2015). Further understanding of the role of predators in coexistence could be gained by crossing predator-presence with a plant size manipulation. Another area of exploration could involve looking at the effects of adding more predator species or alternate prey; predators in bromeliads can interfere with one another, reducing their overall consumption rates (Atwood *et al.*, 2014; A.A.M. MacDonald, unpubl. data), and alternate prey are preferred over chironomids by odonates at the field site (LeCraw, 2014). A deeper look into the indirect effects of predators is also warranted. At other field sites, predators reduce colonization (Hammill *et al.*, 2015b), a phenomenon that may cascade down to affect resource distribution and ecosystem function. Analyses performed in Chapter 4 suggest that chironomid species have different plant size preferences. Differences in performance at different plant sizes may indicate differences in drought tolerance between the species (e.g. Amundrud and Srivastava, 2015), a supposition which could be tested experimentally. Results of the manipulations to ontogenetic stage, revealed a decline in performance when *C. detriticola* was larger. Experiments to study the temporal pattern in oviposition could determine whether chironomids tend to colonize in a specific order to avoid the negative effects of arriving second.

Here I showed that multiple factors affect competition between chironomid species, and that ontogenetic body size differences in particular are crucial in determining the outcome of competition. Further, I used a suite of methods to answer each question. There is great value in applying a variety of approaches to the question of coexistence. Because nature is comprised of complex systems, it is necessary to reduce these to just a few components when studying them. However, by removing the complexities, we are likely losing a lot of information about how these systems would behave naturally. Furthermore, manipulating several variables singly can give different results

from those same variables manipulated in conjunction (Geange and Stier, 2010; Le Bagousse-Pinguet *et al*., 2013). Therefore, one way to ensure that we are getting a full understanding of natural systems is to ask a variety of related questions, using different approaches, as was done here.

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APPENDIX A

Supplementary Information to Chapter 4

A.1 How do relationships between competition coefficients effect coexistence?

We aimed to solve equations for coexistence in terms of all possible combinations of intra- and interspecific competition. To do so, we follow Godoy and Levine (2014; see Appendix) in their model of coexistence, which is a modified version of Chesson's formulation of Lotka-Volterra competition (2000b; 2008):

$$\rho_{ij} = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} \tag{A.1}$$

where ρ_{ij} is a measure of niche overlap, and $1 - \rho_{ij}$ describes the strength of stabilization. In this model, fitness (λ_i , λ_j) differences are measured as:

$$\frac{\lambda_j}{\lambda_i} = \left(\frac{\eta_j - 1}{\eta_i - 1}\right) \sqrt{\frac{\alpha_{ij}\alpha_{ii}}{\alpha_{ji}\alpha_{jj}}} \tag{A.2}$$

Here, η_i , η_j are demographic terms incorporating fecundity into the fitness calculation. This inclusion of a "demographic ratio" is the primary difference between Godoy and Levine (2014) and Chesson (2000b). Following Kraft *et al*. (2015), if we assume λ_j has the fitness advantage, species coexistence occurs when:

$$\rho_{ij} < \frac{\lambda_j}{\lambda_i} \tag{A.3}$$

Which, when combined with Eqn. A.2, is equivalent to:

$$\frac{\alpha_{jj}}{\eta_j - 1} > \frac{\alpha_{ij}}{\eta_i - 1} \tag{A.4}$$

This is the classic result requiring intraspecific competition to exceed interspecific competition, rescaled to include the species' effect on growth and fecundity (Godoy and Levine, 2014). If fitnesses are equal, however, we need not know the value of λ_j/λ_i because Eqn. A.3 simplifies to:

$$\rho_{ij} = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} < 1 \tag{A.5}$$

Because of our experimental design, we can determine the relative strengths of intra- and interspecific competition on a given species (i.e. α_{ii} by α_{ij} ; α_{jj} by α_{ji}), and use these relationships to solve Eqn. A.5 and find out whether coexistence can occur (Table 4.1A). It is of note that the formula for ρ_{ij} is identical in both Godoy and Levine (2014) and Chesson (2000b). Thus, our conclusions about coexistence apply to either model.

A.2 RESULTS OF LIKELIHOOD-RATIO TESTS FOR MODEL SIMPLIFICATION

	Chironomus detriticola		*Polypedilum marcondesi*	
	Emergence	Survival	Emergence	Survival
Plant Size	–	–	–	$p = 0.1378$ $\chi^2(4) = 2.2024$
Relative Abundance	–	–	$p = 0.5985$ $\chi^2(4) = 0.2773$	$p = 0.1206$ $\chi^2(3) = 2.4097$
Density	–	–	–	–
Relative Abundance x Density	–	$p = 0.9039$ $\chi^2(7) = 0.0146$	$p = 0.4415$ $\chi^2(6) = 0.5923$	$p = 0.6849$ $\chi^2(6) = 0.1646$
Relative Abundance x Plant Size	$p = 0.8636$ $\chi^2(7) = 0.0295$	$p = 0.6527$ $\chi^2(6) = 0.2025$	$p = 0.7252$ $\chi^2(7) = 0.1236$	$p = 0.9258$ $\chi^2(7) = 0.0087$
Plant Size x Density	–	$p = 0.281$ $\chi^2(5) = 1.1624$	$p = 0.3663$ $\chi^2(5) = 0.8162$	$p = 0.1171$ $\chi^2(5) = 2.4558$
Plant Size x Relative Abundance x Density	–	$p = 0.012$* $\chi^2(8) = 2.6873$	$p = 0.4799$ $\chi^2(8) = 0.499$	$p = 0.0942$ $\chi^2(8) = 2.8004$

TABLE A.1: Results of likelihood-ratio tests for removal of specified terms in the equivalence experiment. Low p-values ($p < 0.05$) indicate that the more complex model fits significantly better than the less complex one. * Although the p-value was low, this term was removed because the model fit very poorly when the three-way interaction was retained.

	Chironomus detriticola		*Polypedilum marcondesi*	
	Emergence	Survival	Emergence	Survival
Plant Size	–	–	$p = 0.3796$ $\chi^2(3) = 0.7719$	–
Body Size	–	–	–	–
Density	–	–	$p = 0.5737$ $\chi^2(4) = 0.3165$	$p = 0.1125$ $\chi^2(4) = 2.5187$
Plant Size x Body Size	–	$p = 0.0803$ $\chi^2(6) = 0.0582$	$p = 0.4058$ $\chi^2(5) = 0.6911$	$p = 0.25$ $\chi^2(5) = 1.3233$
Plant Size x Density	–	–	$p = 0.4849$ $\chi^2(6) = 0.4877$	$p = 0.919$ $\chi^2(7) = 0.0152$
Density x Body Size	$p = 0.9866$ $\chi^2(7) = 0.0003$	$p = 0.5922$ $\chi^2(7) = 0.287$	$p = 0.8327$ $\chi^2(7) = 0.0446$	$p = 0.6602$ $\chi^2(6) = 0.1933$

TABLE A.2: Results of likelihood-ratio tests for removal of specified terms in the ontogeny experiment. Low p-values ($p < 0.05$) indicate that the more complex model fits significantly better than the less complex one.