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# Resource limitation in sexual vs. asexual *Potamopyrgus antipodarum*

Christina Jenkins  
*University of Iowa*

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RESOURCE LIMITATION IN SEXUAL VS. ASEXUAL POTAMOPYRGUS  
ANTIPODARUM

by  
Christina Jenkins

A thesis submitted in partial fulfillment  
of the requirements for the Master of  
Science degree in Biology  
in the Graduate College of  
The University of Iowa

May 2010

Thesis Supervisor: Assistant Professor Maurine Neiman

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Graduate College  
The University of Iowa  
Iowa City, Iowa

CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

Christina Jenkins

has been approved by the Examining Committee  
for the thesis requirement for the Master of Science  
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To Big Bear, thanks for being there for all these years.

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## ABSTRACT

Why sexual reproduction is so prevalent in natural populations has been called the “queen of questions” in evolutionary biology (Bell, 1982). One potential answer to this question may come from evaluating whether sexuals and asexuals respond differently to a resource-limited environment. If asexuals require more resources to grow and reproduce at the same rate as sexuals, the greater sensitivity of asexuals to resource limitation could ameliorate the two-fold cost of sexual reproduction. Here, I use the New Zealand mud snail, *Potamopyrgus antipodarum*, to empirically address this possibility. First, I used manipulations of the density of experimental sexual and asexual populations of *P. antipodarum* to assess whether asexual females experienced a sharper decline in fitness-related traits under high-density conditions, as expected if higher sensitivity of asexual individuals to food limitation might contribute to the maintenance of sex. Counter to these predictions, I found that sexual females in fact suffered even more in high-density populations than asexual females. Superficially, this result implies that there is a greater cost of sexual reproduction than previously envisioned. However, since the genetic diversity of the sexual populations was likely to have been lower than the asexual populations, this result is actually consistent with the outcome expected under hypotheses for sex postulating that increased genetic diversity within a population alleviates competition for limited resources. I addressed this possibility directly in Chapter 2, evaluating whether genetically identical individuals compete more for the same resources than genetically distinct individuals. Here, I compared fitness-related traits in genetically homogeneous vs. heterogeneous populations of asexual female *P. antipodarum*, and found that the size of individual females relative to other individuals in the population was a more important determinant of individual growth and reproduction than population genetic diversity per se. This has both population and individual level implications, as an individual’s size both positively affects its own reproductive ability and negatively

affects the growth and reproduction of the other individuals in the population. On a population level, this effect may initially increase competition between individuals, but could eventually decrease overall population size. This result also implies that female-biased populations (i.e., highly asexual populations) of *P. antipodarum* could be more resource-limited than sexual populations. In Chapter 3, I addressed whether sexual and asexual *P. antipodarum* differed in their responses to limitation of dietary phosphorous (P). This idea is inspired by ecological stoichiometry, which postulates that organisms need to ingest food that has nutrient ratios similar to their body ratios of those nutrients in order to grow/reproduce at optimal rates. Asexual *P. antipodarum* are triploid, and as a result have more nucleic acid and P per unit mass than their sexual diploid counterparts. The implications are that asexual *P. antipodarum* may need to ingest more P than sexuals to grow and reproduce at the same rate, which could generate an individual-level cost associated with asexuality. I addressed this possibility by manipulating the P content in food provided to sexual and asexual female *P. antipodarum*, and quantifying the effect of dietary P content on fitness-related traits. I detected no effect of dietary P content on growth or reproduction across P treatments or between sexual vs. asexual females. However, I was unable to determine the ploidy of two-thirds of the individuals used in this experiment due to a mechanical breakdown, which may have greatly reduced my statistical power to detect an effect of P treatment. Taken together, my thesis research showed that there could be important differences between genetically identical populations and genetically dissimilar populations of *P. antipodarum* in their response to a resource-limited environment. I also showed that individual size of *P. antipodarum* females strongly influences competitive ability for resources, to the detriment of the other females in the environment. This result also could provide insight into the sensitivity of female-biased (e.g., highly asexual) populations to resource limitation in sexually dimorphic species and generate a cascade of interactions on both the population and individual level that effects how a female interacts with her environment. Moreover, as

asexual individuals only sample a subset of the phenotypic variation exhibited by the ancestral population (“Frozen Niche Variation hypothesis”) and thus will be more susceptible to competitive phenotypic effects.

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## INTRODUCTION

Only females can directly make offspring, with the consequence that an asexual female that only produces daughters will have a two-fold advantage over a sexual female who allocates half her resources to producing sons. Thus, there is a “two-fold cost of males” associated with sex (Maynard Smith, 1978). All else being equal, this cost should quickly result in the elimination of sexual populations, but the predominance of sexual reproduction in natural populations indicates inherent advantages to reproducing sexually and/or inherent disadvantages to reproducing asexually. The predominance of sex despite this cost has been dubbed the “queen of questions” in evolutionary biology (Bell, 1982). Numerous hypotheses addressing the maintenance of sex have been proposed (*e.g.*, Jaenike, 1978; Bell, 1982; Maynard Smith, 1978; Butlin, 2002; Hadany and Comeron, 2008), but why sex is so common remains unresolved.

One class of hypotheses for sex has focused on the potential for a disparity in resource requirements between sexual and asexual individuals such that asexuals are more sensitive to resource limitation than their sexual counterparts (Antonovics, 1978; Maynard Smith, 1978; Bell, 1982; Ellstrand and Antonovics, 1985; Doncaster *et al.*, 2000). There are at least two plausible ways in which asexuals might be more likely to be resource-limited than sexuals: 1) higher per-capita resource needs for asexual individuals (Neiman *et al.*, 2009) and/or 2) genetically similar individuals compete more with each other than with genetically dissimilar individuals (Bell, 1984; Ellstrand and Antonovics, 1984; Weeks *et al.* 1985).

The aim of my thesis is to empirically analyze differences in the response of sexuals and asexuals in a resource-limited environment, and specifically assess whether asexuals experience more severe costs of resource limitation with respect to growth and reproduction than sexual counterparts. In chapter one, I used manipulations of density within experimental populations to address the possibility that sexual and asexual

individuals will differentially respond to food limitation. Chapter two focuses on how genetic diversity influences the outcome of competitive interactions by comparing responses to increased density in genetically identical vs. genetically dissimilar individuals. In chapter three, I consider whether asexual individuals may require higher quality food than sexual counterparts to achieve maximal reproduction and growth rates.

I used *Potamopyrgus antipodarum*, a freshwater snail native to New Zealand, to address these questions. This snail is an excellent and well-established system for studying the maintenance of sex in nature because sexual and asexual individuals often coexist in New Zealand lakes. The relative frequency of sexual individuals varies widely across lake populations, suggesting that there is variation across lakes in the strength of selection for sex (Lively, 1987). There are also multiple origins of new asexual lineages from sexual populations (Neiman *et al.* 2005), generating high asexual genetic diversity and constant competition between novel asexual lineages and sexuals.

A substantial body of theory has addressed whether competition for limited resources might play a role in the maintenance of sex (*e.g.*, Antonovics, 1978; Maynard Smith, 1978; Price and Waser, 1982; Bell 1984; Doncaster *et al.*, 2000). The majority of this work stems from the most obvious difference between sexuals and asexuals, sexual individuals produce genetically variable offspring. One scenario apart from resource limitation in which this unique characteristic of sex can provide an advantage is when the environment imposed by biological enemies changes rapidly over time. For example, under the Red Queen hypothesis, if parasites or pathogens target common host genotypes, rare genotypes – and thus sex - will have a fitness advantage (Van Valen, 1973; Jaenike, 1978).

It is heterogeneity through space rather than time that seems more likely to generate advantages for sex related to resource limitation. Because in a spatially heterogeneous environment, offspring are likely to encounter different environments than their parents and/or siblings will be likely to disperse to different habitat types. Genetic

recombination could be favored in this context by generating genetically distinct offspring some of which might have a better chance of success in environments they encounter as they disperse from their parents. Theoretical studies show that relatively high spatial heterogeneity can in fact favor sex in scenarios where resources are limiting and competition between genetically similar individuals for a particular resource set is intense (Bell, 1982).

The advantage of being genetically dissimilar when there is intense competition for limited resources in a spatially heterogeneous environment is generally known as the Tangled Bank Hypothesis (Bell, 1982). Ghiselin (1974) was the first to address space as a dimension important to consider in the maintenance of sex. As an economist, he explored variation across environmental space in economic terms, utilizing game theory. Graham Bell followed his lead, and compared individuals in an environment to buttons saturating an economy (Bell, 1982): If a given button factory makes only one kind of button, they will saturate the market once everyone already possesses that button. Conversely, if a button factory were to make four marginally different buttons, their market would be broader and would reach saturation more slowly. Bell compares this scenario to organisms in a resource-limited environment where it is better to generate offspring that are genetically different.

Several high-profile empirical studies from the 1980s that seemed to suggest that the Tangled Bank Hypothesis had little to do with the maintenance of sex are a major reason why this once-popular hypothesis has been largely ignored for the last decade. First, Burt and Bell (1987) contrasted the Tangled Bank Hypothesis against the Red Queen Hypothesis, as the two then-prominent ecological explanations for the evolution of sexual reproduction. Under the Red Queen, longer host generation times are expected to increase the strength of selection imposed by parasites, and thus the intensity of selection favoring sex. The basis for this prediction is that longer generations mean that the parasite environment is more prone to evolutionary change because the parasites will

have more time to adapt to exploit particular hosts. By this logic, Burt and Bell predicted that under the Red Queen, recombination rates should be positively correlated with time to maturity. On the other hand, under the Tangled Bank, selection for sex and recombination is expected to be positively correlated with litter size. Here, the more offspring produced, the higher the rate of recombination needed to generate sufficient genetic diversity between siblings to mitigate competition. Burt and Bell assessed whether either set of predictions was met in a set of 40+ mammal species by comparing the amount of recombination within each species (as estimated by the number of chiasmata per chromosome) to time to maturity and litter size. They found that recombination rate was not associated with litter size, as predicted under the Tangled Bank. Instead, recombination rate increased with time to maturity, consistent with their expectations for the Red Queen. Based on these results, Burt and Bell concluded that the Red Queen was more likely to stand as an explanation for the maintenance of sex than the Tangled Bank. This result, along with Lively's (1987) paper implicating the Red Queen as a key player in the maintenance of sex in *P. antipodarum*, was the beginning of the end of empirical exploration of the Tangled Bank.

The rejection of the Tangled Bank Hypothesis may have been slightly premature. For one, Burt and Bell (1987) exclusively examined a group of animals – mammals - where asexuality is entirely absent. In addition, there is a growing sense that sex is both so costly and complex that it will require complex, multi-faceted explanations (West *et al.* 1999, Meirmans and Neiman 2006, Neiman *et al.* 2009). For example, different mechanisms may act simultaneously and/or in a complimentary fashion, and these mechanisms may vary across taxa. Moreover, nearly all of the hypotheses for sex require strict assumptions to operate. In the last decade, several different theoretical studies have shown that synergism between multiple mechanisms can ease the constraints imposed by individual models (Manning and Thompson, 1984; Howard and Lively, 1994, 1998; Lively and Howard, 1994) From this more nuanced perspective, the rejection of the

Tangled Bank Hypothesis by competing two unrelated theories against each other was perhaps too narrow an approach.

A different resource-based cost of being asexual is considered in another prominent hypothesis for sex, the density-dependent selection hypothesis (Maynard Smith, 1978; Price and Waser, 1982; Ellstrand and Antonovics, 1985). This hypothesis is similar to the Tangled Bank in that it treats the genetic similarity between asexuals as a cost because genetically identical individuals will compete more for limited resources than genetically dissimilar individual, but departs from the Tangled Bank in its focus on density. To expand, as the density of a population increases, competition between individuals utilizing similar resources may increase. The density-dependence selection hypothesis views this competition as exacerbated between genetically identical individuals, such that at higher densities, asexual members of the same asexual lineage will compete more with one another than sexually-produced offspring. Maynard Smith was the first to explore this concept theoretically, showing that a parent will leave fewer descendents if there is severe competition between its offspring. If resource requirements have a genetic basis, the intensity of competition will increase with genetic similarity (Maynard Smith, 1978). Other models demonstrated that populations with mixtures of genotypes that utilize different resources deplete the available resources substantially less than populations with genetically identical individuals (Antonovics, 1978). These ideas were combined by Young (1981) in the Density Dependent Selection hypothesis, which suggests that as population density increases, competition between genetically identical asexuals in a population increases more than competition between sexuals in a population, countering – at least in part – the cost of sex (Young, 1981; “Elbow Room Model”).

Increased offspring density could result from increasing population sizes or from decreasing progeny dispersal. On the other hand, increased progeny dispersal will decrease the density of siblings, and thus competition between them. Price and Waser

(1982) took the latter approach in a model-based study, and showed that the increase in density and competition caused by limited progeny dispersal could favor sex.

Ellstrand and Antonovics (1984) used *Anthoxanthum odoratum*, sweet vernal grass, to perform the first – and one of the only – empirical tests of the density-dependence selection hypothesis. They planted sets of genetically identical and genetically dissimilar seedlings generated from the same mother plants in an old field in a design that varied density, measured as the distance between each plant, while holding the number of neighbors surrounding each plant constant. Ellstrand and Antonovics predicted that under the density-dependence selection hypothesis, asexual individuals should suffer more of a reduction in fitness – here, in terms of individual inflorescence number and plant mass - at higher densities than sexuals. While they did find a trend towards a negative effect of density on fitness, their power to detect a difference between sexually- and asexually-produced individuals (and an effect of density) was sharply reduced by high mortality. This study thus generated ambiguous results.

Yet another twist on how resource limitation can influence the maintenance of sex was suggested by Doncaster *et al.* (2000). They used a modeling approach to evaluate the ecological constraints that govern asexual invasion of a sexual population by allowing asexuals and sexuals to coexist as two species competing for an overlapping resource base. Their model aimed to address the idea that the two-fold advantage associated with asexuality is only realized if the asexual population is uninhibited by competitive interactions between sexuals and asexuals. Interestingly, they found that intraspecific competition between asexual individuals is in fact sufficient to slow the invasion of a sexual population by an asexual lineage. This slower rate of increase of the asexual invaders under these conditions could in fact help to maintain sex, since it provides more time for relatively slow mechanisms favoring sex (such as mutation accumulation) to operate. Doncaster *et al.* (2000) also showed that the interspecific interactions between sexuals and asexuals are of negligible importance unless the asexuals have less of an

inhibitory effect on sexuals than asexuals have on themselves. In this case, over a certain population size threshold, the asexual population will self-regulate such that intraspecific competition halts their population growth before extinction of the sexual population occurs. Doncaster *et al.* (2000) concluded that strong intraspecific competition could prolong coexistence between sexuals and asexuals after an asexual invasion, which gives the sexual population more time to realize its long-term evolutionary advantages over asexuals, such as mutation accumulation or escape from parasites.

Evidence for different response to resource limitation in  
sexual vs. asexual *P. antipodarum*

Neiman (2006) discovered that asexual *Potamopyrgus antipodarum* females have a strong negative effect on each others' reproduction at high density, but that males do not confer any such effect. Since this study also showed no evidence of direct benefits to asexual females of male presence (*i.e.*, copulatory dependence), and since female *P. antipodarum* are larger than males (Jokela, unpublished data), Neiman speculated that the strikingly different effects of female vs. male competitors on reproduction in asexual female *P. antipodarum* was mostly likely due to larger competitive influence of asexual females relative to sexual males.

This possibility is particularly intriguing in light of the wide across-lake variation in the relative frequency of sex, hinting that highly asexual (and thus female-biased) populations could be more susceptible to resource limitation. Regardless of the mechanism involved, these results suggest that resource limitation could have important influences upon reproduction in asexual *P. antipodarum*. These data thus set the stage for direct empirical evaluation of the extent to which asexual *P. antipodarum* are in fact susceptible to resource limitation.

My goal was to generate an in-depth understanding of the Neiman (2006) result by directly addressing whether there are differences in resource requirements in sexual vs. asexual *P. antipodarum* that could help to counter the costs of sex. I took three different approaches, each focused on one of the three differences between the sexual males and asexual females studied in Neiman (2006): 1) higher ploidy level of the asexual (triploid) vs. sexual (diploid) snails, 2) higher genetic diversity in sexual vs. asexual populations, and 3), larger size of females vs. males, which I tested indirectly by analyzing the competitive effects of differently-sized asexual snail lineages in mixed-lineage populations.

In Chapter 1, I tested the hypothesis that asexuals need more resources per capita than sexuals by evaluating how reproduction in asexual vs. sexual females responded to increased density. Counter to my expectations, I found that sexual females reproduced less than asexual females when housed in high-density populations. Although this result superficially suggests that sexual individuals may require more resources than asexual individuals, post-hoc analyses revealed that the sexual experimental populations were in fact likely to harbor less genetic diversity. This means that the sexual individuals may in fact have been more genetically similar than the asexual individuals, and perhaps have been subject to more intense competition for limited resources.

I tested the Density Dependence hypothesis in Chapter 2 by measuring the value of fitness-related traits in asexual individuals housed in genetically homogeneous (single lineage) vs. genetically heterogeneous (mixed lineage) populations. I found that the two asexual lineages used in this experiment responded differently to genetic diversity, such that one lineage grew and reproduced more in mixed than single lineage populations, while the other grew and reproduced less in mixed lineage populations than when housed only with other lineage members. I used post-hoc analyses to find that differences in lineage size drove these different responses, with the physically larger asexual lineage reproducing and growing more when housed with the smaller lineage, while the smaller

lineage both grew and reproduced more when housed alone than when housed with the larger lineage.

In Chapter 3, I evaluated whether the higher ploidy level of asexual *P. antipodarum* might result in higher sensitivity to decreased food quality (in terms of decreased dietary phosphorus content) than sexual counterparts. Ecological stoichiometry predicts that P content in an organism is linked to the amount of P available within the environment. Bodily phosphorus (P) content is positively correlated with bodily nucleic acid content in *P. antipodarum*, such that asexual triploid individuals have both higher nucleic acid and bodily P content than sexual diploids (Neiman *et al.*, 2009). Therefore asexual may require more P to grow and reproduce than sexual *P. antipodarum*. I tested the difference in competitive interactions between sexuals and asexuals empirically by housing individual adult female *P. antipodarum* separately and administering one of three food treatments that varied only in P content. I found that food quality is positively correlated with reproduction and growth in *P. antipodarum*. Moreover, there was a trend towards lower reproduction by asexual individuals in the low and mid P treatments. However, a substantial portion of the individuals used were not included in the final analysis due mostly to high mortality, which may have substantially reduced my power to detect significant differences. Although these data are consistent with the possibility that asexual individuals could reproduce less than their sexual counterparts in a resource-limited environment, it is impossible to make any definitive conclusions in the absence of more data.

CHAPTER 1  
EVALUATING THE CONSEQUENCES OF DENSITY-DEPENDENCE  
IN SEXUAL VS. ASEXUAL FRESHWATER SNAILS

1.1 Abstract

Evolutionary theory suggests that if all else is equal, asexual females that produce only daughters will have a two-fold fitness advantage over sexual females that allocate half of their resources to producing sons. This difference in fitness between sexual and asexual females implies that sexual reproduction should be rare, in contrast to biological reality. The predominance of sex implies that this “all else is equal assumption” must be violated such that sexual reproduction confers important benefits and/or that there are critical costs associated with asexuality. One potential cost is increased per capita requirements for needed resources in asexuals such that asexual individuals are more constrained by resource-limited environments than their sexual counterparts. Here, I used the New Zealand mud snail, *Potamopyrgus antipodarum*, to empirically evaluate this possibility. I established replicate populations of asexual females and sexual females in low-, intermediate- and high-density populations. Each population received the same amount of food, thereby reducing the amount of food per capita with increasing density. After two months, I quantified individual female embryo production. I found a significant effect of density, such that embryo production decreased with increasing density. I also found that sexual females produced significantly fewer embryos than asexual females under high-density conditions. Superficially, this result suggests resource limitation will not contribute to the maintenance of sex in *P. antipodarum*. However, the likelihood that my sexual populations were less genetically diverse than asexual populations due to sampling error means that the poorer performance of sexual females in high-density conditions is consistent with expectations of hypotheses suggesting that genetically diverse populations are better at partitioning limited resources than less diverse

populations. For *P. antipodarum*, this result implies genetically similar populations (i.e., members of the same asexual lineage) will compete more with each other than genetically dissimilar populations (i.e., sexually-produced individuals). This could generate individual-level costs associated with asexuality in a resource-limited environment, and could also influence the outcome of competition between asexual individuals from the same vs. different lineages. More broadly across taxa, highly asexual populations may be more likely to be less genetically diverse and thus more resource-limited than highly sexual populations, which could generate a population level cost linked to asexuality.

## 1.2 Introduction

A fundamental question in evolutionary biology is why sexual reproduction is so prevalent. Males do not directly produce offspring, meaning that asexual females who only produce daughters will have a two-fold advantage over sexual females who allocate half of their resources to producing sons. Despite this theoretical “two-fold cost of males” (Maynard Smith, 1971), sexual reproduction is the predominant form of reproduction in eukaryotic organisms. Thus, in order for sex to persist, there must be substantial advantages to sexual reproduction and/or substantial disadvantages to asexual reproduction. Numerous hypotheses have been proposed to resolve the question of why sexual reproduction is so common (Jaenike, 1978; Maynard Smith, 1978; Bell, 1982; Butlin, 2002) but why sex is maintained remains unclear (Hadany and Comeron, 2008).

The argument against sexual reproduction is contingent on the “all else being equal” assumption that asexual and sexual females are identical other than their manner of reproduction. To what extent is this assumption actually met? This assumption has only been tested in limited capacity, given the wide phenotypic variation between asexual taxa (Jokela *et al.*, 1997). However, one potential difference between asexual and sexual individuals is a disparity in resource requirements per capita that may result in asexuals

being more sensitive to resource limitation than their sexual counterparts. If this were indeed the case, costs associated with asexuality could counter the two-fold cost of males in resource-limited environments. There are at least two non-mutually exclusive and plausible ways in which asexual individuals might be especially subject to resource limitation: 1) higher resource needs for individual asexuals (Neiman *et al.*, 2009a) and/or 2) genetically similar individuals compete more with one other than with genetically dissimilar individuals (Maynard Smith, 1978; Bell, 1984; Ellstrand and Antonovics, 1984; Weeks *et al.*, 1985).

There are hints from at least one system that asexual females could be particularly sensitive to resource limitation. Neiman (2006) found that reproductive output in asexual female New Zealand mud snails, *Potamopyrgus antipodarum*, was sharply reduced in the presence of many other asexual females, but that sexual male *P. antipodarum* did not have this effect. The intent of this experiment was to evaluate whether asexual females required access to males to produce embryos at their maximal rate, so the experimental treatments consisted of comparing reproduction in asexual females housed in mixed-gender populations to reproduction in asexual females housed only with other females. Both “high-female” (25 individuals) and “low-female” (17 individuals) treatments were included along with the mixed male/asexual female treatment (8 males, 17 females) to control for the effect of the number of females in a population. While there was no evidence that reproduction was dependent on males, there was a 50% drop in embryo output in the high-female treatment relative to the low-female and mixed treatments. This result indicated that population size interacts with sex ratio in a manner that can profoundly affect fitness in asexual female *P. antipodarum*. Inspired by recent evidence that female mosquitoes exert a larger competitive influence than male mosquitoes due to the larger size of the former (Bedhomme *et al.*, 2003), Neiman (2006) speculated that the larger size of female vs. male *P. antipodarum* might also translate into more severe competition in all-female vs. mixed populations.

Since female and male *P. antipodarum* used in Neiman (2006) also differed in sexuality and ploidy (asexuals are triploid, while sexuals are diploid), sexual size dimorphism leading to higher resource use in females is only one of at least three potential explanations for these results. It is also possible that asexual individuals require more resources per capita than sexuals and/or that triploids need more resources to realize maximum fitness than diploids. Here, I evaluate one of these possibilities: a difference in per capita resource requirements in asexual vs. sexual individuals, by comparing reproduction under low- vs. high-density conditions in asexual vs. sexual female *P. antipodarum*. This snail is an excellent, well-established system for studying the maintenance of sexual reproduction because sexual and asexual individuals coexist at varying relative frequencies of sex in natural populations, indicating that there are some populations in which sexual reproduction is favored more than in others (Lively, 1987). I predicted that asexual female *P. antipodarum* would show a sharper decrease in reproduction under high-density conditions than sexual counterparts, if asexual *P. antipodarum* have higher per-capita resource needs than sexuals.

### 1.3 Materials and Methods

I established separate replicate populations of sexual and asexual female *P. antipodarum* to address the question of whether asexuals are in fact more sensitive to increased density. Because there is wide variation in the rate of growth of *P. antipodarum* asexual lineages in a laboratory setting (Jokela *et al.*, 2003), I sought to minimize sampling biases by using 5 different asexual lineages to generate my experimental populations. I pooled 40 adult females from each of these five lineages, for a total of 200 individuals, and haphazardly selected three (low density), six (intermediate density), or nine (high density) individuals to form each experimental population. Sexual female experimental populations were created in the same manner from a pool of five genetically distinct sexual lines, but constraints of adult female availability meant that I was not able

to sample evenly from each line (Table 1.1). I created 16 replicate experimental populations for each density treatment (8 of each sexuality), for a total of 64 experimental populations. While males do not affect asexual female reproductive success (Neiman, 2006) sexual females need males to reproduce. Thus, I added one male for every three females in each population such that each population was 25% male. By adding males to both the sexual and asexual populations, I controlled for any possible effect of male presence.

Each population was housed in 240 ml water in 800 ml plastic cups and given 0.6 mL of a 0.116mg spirulina/1 mL water solution three times per week. Since each population received the same amount of spirulina (a dried algae commonly used as a laboratory food source for *P. antipodarum*), the amount of food per snail decreased with increasing density (low: 0.0232 mg/snail; intermediate: 0.0116 mg/snail; high: 0.0077 mg/snail). An earlier study indicates that these amounts of spirulina per capita are likely to constitute a resource-limited environment (Krist *et al.*, 2004). I changed water and cleaned each container three times per week prior to feeding to control for algal growth within containers.

All snails were checked daily for mortality; ten snails died prior to the completion of the experiment. To keep population density constant, I replaced each dead individual with marked adult females of the same sexuality. Data from the replacement snails were not included in statistical analyses because these individuals had not been part of the entire experiment. After 58 days (following Neiman, 2006) all remaining live females were dissected so that the number of embryos produced by each female snail, which are ovoviviparous and carry developing embryos inside an internal brood pouch, could be counted. Reproduction is positively correlated with size measured as shell length (Tibbets *et al.*, 2009). Thus, I used individual dry weight to control for the effect of size on reproduction. I quantified dry mass by removing the embryos and shell of each female,

drying the bodies for one week at 32°C, and weighing each body to the nearest 0.00001 g with a microbalance. Dry mass was included as a covariate in all statistical analyses.

A two-factor model was fitted to the data that also accounted for weight and a random replicate effect that allows for correlation of snails housed in the same replicate population. A log transformation was applied to the counts of embryos to avoid violations of assumptions of the model. In the full model there was no significant sex by treatment effect so it was removed from further analysis (Table 1.1)( $F = 0.01, p = 0.990$ ).

I used Tukey-Kramer post-hoc analyses to determine whether particular pairwise combinations of treatments differed in embryo production.

#### 1.4 Results

There was a marked reduction in embryo production (~50 %) with increased density ( $F_{2,51} = 9.90, p < 0.0001$ ) (Table 1.2) after accounting for the positive relationship between size and embryo production. There was also a significant decrease in embryo production in the high-density treatments relative to the low-density treatments (Tukey-Kramer: t-value = 4.26,  $p$ -value < 0.001) and in the low-density treatment relative to the intermediate-density treatment (Tukey-Kramer: t-value = 2.67,  $p$ -value = 0.027). There was no significant difference in embryo output between high-density vs. intermediate density treatments (Tukey-Kramer: t-value = 1.98,  $p$ -value = 0.127).

There was a significant effect of sexuality on embryo production ( $F_{1,38.4} = 9.23, p = 0.004$ ). Asexual females reproduced significantly more than sexual females when averaged across all three treatments (t-value = 3.04,  $p$ -value = 0.004).

#### 1.5 Discussion

In *P. antipodarum*, increased female density within a population sharply decreases reproductive output of both sexual and asexual individuals. This result is particularly striking given the small scale of density increase: a change in population size from three to nine female snails resulted in a 90% decrease in embryo production. Even

more surprisingly, sexual females were affected much more severely by increases in density than their asexual counterparts. On the surface, this result implies that sex confers more than just the cost of males and thus that it is unlikely that density-dependence contributes to the maintenance of sex in *P. antipodarum*. There are other factors besides sexuality, however, which may have influenced the outcome of the experiment.

For one, it is important to consider the quality of the food resources supplied to the snails. Ecological stoichiometry postulates that food quantity is of secondary importance to food quality as a source of resource limitation (Sternler and Elser, 2002). *Spirulina* is very nutrient-rich (Ciferri, 1983), meaning that although the *P. antipodarum* in my experiment were subjected to an increasingly food-limited environment (Krist *et al.*, 2004), high food quality could have masked any differential effects of snail sexuality at the two lower densities used.

Another relevant consideration is the genetic diversity of the sexual vs. asexual experimental populations. Numerous theoretical studies have suggested that costs associated with asexuality might be linked to increased population density when asexual individuals tend to be more genetically similar than sexual competitors (Antonovics 1978; Maynard Smith, 1978; Bell, 1982; Price and Waser, 1982). Assuming resource requirements and the ability to compete for those resources have a genetic basis, individuals that are more genetically similar (*e.g.*, members of the same asexual lineage) might be expected compete more for the same resource than their sexual counterparts. I had attempted to control for differences in genetic diversity between my experimental sexual and asexual populations by generating each set of populations from a pool of 5 genetically distinct lineages (Table 1.2). However, the asexual replicates were established equally from five different populations originally sampled from New Zealand and Europe, while constraints of snail availability meant that the sexual replicates were largely descended from inbred lines generated from snails sampled from a single New Zealand lake. Calculation of Simpson's index of diversity indicates that the asexual

experimental populations were in fact drawn from a pool that was likely to be substantially more genetically diverse than the sexual populations (Equitability: Sexual = 0.522; Asexual = 0.986).

Higher genetic diversity of the asexual pool thus provides a possible explanation for the surprisingly poor performance of sexual *P. antipodarum* at high density, and is in fact consistent with the expectations of hypotheses postulating an advantage for genetically diverse over genetically homogeneous populations under resource-limited conditions (Maynard Smith, 1978; Ellstrand and Antonovics, 1985; Tonsor, 1989; Lopez-Suarez, 1993; Koelewijn, 2004; Tagg *et al.*, 2005; Hughes *et al.*, 2008). Studies of phenotypic variation in asexual *P. antipodarum* confirm that there is a genetic component to phenotypic variation amongst asexual lineages (Jokela *et al.*, 1997). My results thus suggest that genetic diversity and resource partitioning could interact to mediate the outcome of competitive interactions in *P. antipodarum*. This would also mean that sex is less likely to be maintained in populations where asexual diversity is high relative to the diversity of the sexual component of the population.

Regardless of the effect of genetic diversity, my documentation of a sharp decrease in embryo production with increasing female density corroborates an earlier result showing that asexual female *P. antipodarum* negatively impact each others' reproductive output when housed in relatively dense populations (Neiman, 2006), and extends this finding to sexual females.

Table 1.1- Two-way ANOVA table for the full model. Embryos (log transformed) is the response variable.

| Source        | Numerator df | Denominator df | F value | <i>p</i> -value |
|---------------|--------------|----------------|---------|-----------------|
| Density       | 2            | 47.7           | 9.50    | <0.001          |
| Sex           | 1            | 61.9           | 7.67    | 0.007           |
| Sex x Density | 2            | 47.6           | 0.01    | 0.990           |
| Weight        | 1            | 280            | 10.84   | 0.001           |

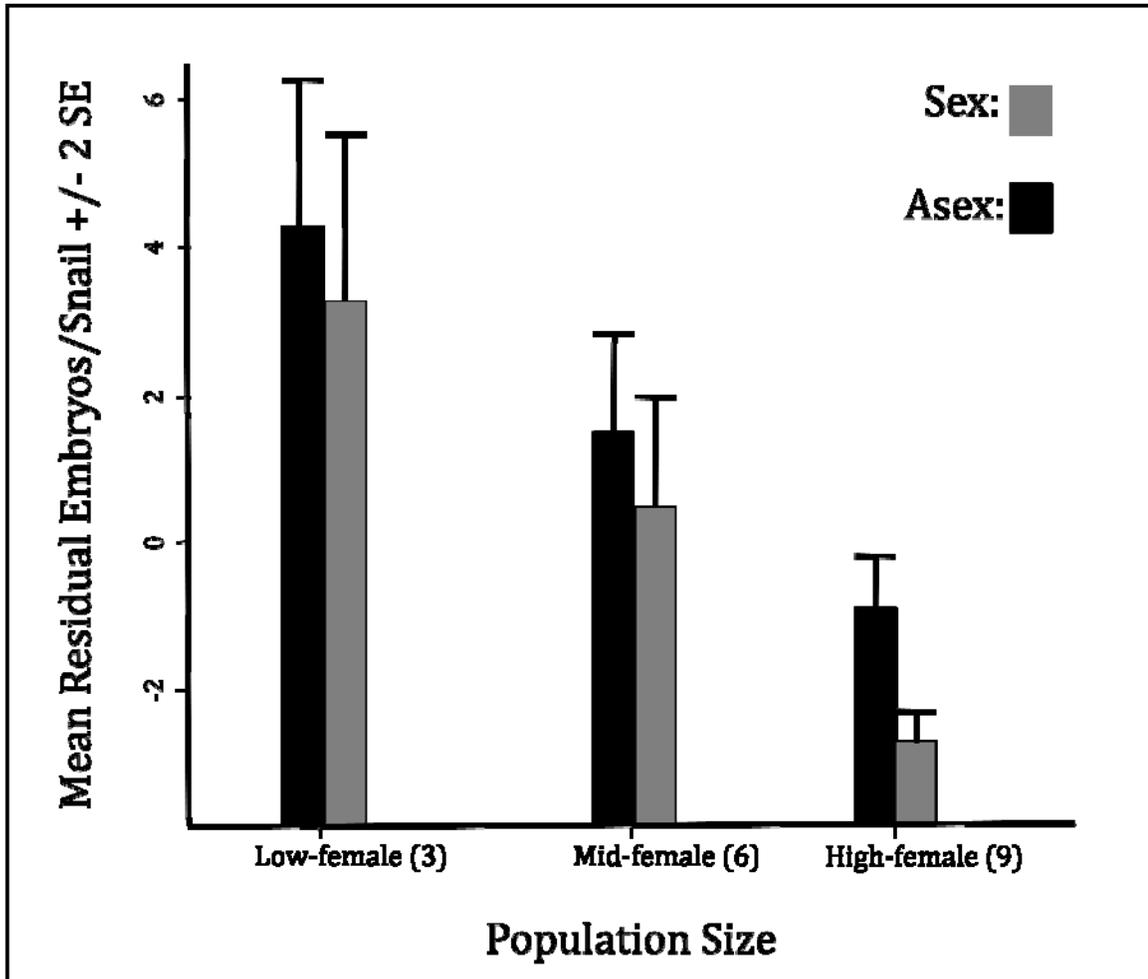
Table 1.2- Two-way ANOVA table with embryos per female as the response variable and density treatment and sexuality as the predictor variables. Weight is included as a random variable.

| Source    | df   | <i>F</i> | <i>p</i> -value |
|-----------|------|----------|-----------------|
| Density   | 51   | 9.90     | >0.001          |
| Sexuality | 38.4 | 9.23     | 0.0043          |
| Weight    | 281  | 10.9     | 0.0011          |
| Total     | 282  |          |                 |

Table 1.3 – Proportional contribution of each of the sexual and asexual source lineages to the sexual and asexual experimental populations. \*Bloomington Aquatic Research Facility

| Sexual Lineage                      | % Sexual Pool | Asexual Lineage                     | % Asexual Pool |
|-------------------------------------|---------------|-------------------------------------|----------------|
| BARF* x Alex Yellow                 | 32            | Map 75                              | 20             |
| Alex Yellow                         | 22            | BARF* Clone 1                       | 20             |
| Alex Sexual                         | 5             | AC51                                | 20             |
| Alex x Map                          | 26            | Denmark B                           | 20             |
| BARF* x Alex Sexual                 | 14            | McGregor                            | 20             |
| Simpson's Equitability Index: 0.522 |               | Simpson's Equitability Index: 0.986 |                |

Figure 1- Mean embryos per female (while controlling for size) in each of the three density treatments. Error bars represent  $\pm 2$  SE. There was also a significant decrease in embryo production in the high-density treatments relative to the low-density treatments (Tukey-Kramer:  $t$ -value = 4.26,  $p$ -value < 0.001) and in the low-density treatment relative to the intermediate-density treatment (Tukey-Kramer:  $t$ -value = 2.67,  $p$ -value = 0.027). There was no significant difference in embryo output between high-density vs. intermediate density treatments (Tukey-Kramer:  $t$ -value = 1.98,  $p$ -value = 0.127).



CHAPTER 2  
THE EFFECT OF POPULATION DIVERSITY ON GROWTH AND  
REPRODUCTION IN ASEXUAL FEMALE *POTAMOPYRGUS*  
*ANTIPODARUM*, A NEW ZEALAND SNAIL

2.1 Abstract

Since only females can directly make offspring, asexual females who produce only daughters should have a two-fold advantage over sexual females who allocate half of their resources to sons. The predominance of sexual reproduction despite this profound “cost of males” indicates that sex must confer major advantages. One class of hypotheses accounting for the prevalence of sex is based on the premise that genetically distinct individuals are better at partitioning limited resources. Under these conditions, genetically identical individuals (e.g., members of an asexual lineage) should be more sensitive to increases in population density than genetically heterogeneous (e.g., sexually-produced) counterparts. I evaluated these predictions by quantifying the effects of density and genetic diversity on fitness-related traits in experimental populations comprised of two different asexual lineages of *Potamopyrgus antipodarum*, the New Zealand mud snail. I found that density had profound negative effects upon growth and reproduction, with females producing ~92% fewer embryos and growing ~70% less when housed in high- vs. low-density conditions. There was an interaction between genetic diversity, density, and lineage such that individuals from one lineage, Map 75, reproduced 50% more when housed at high density with the other lineage (Denmark B) than when housed at high density only with other Map 75. The opposite effect was observed in Denmark B housed at high density, with markedly higher embryo production and growth occurring when Denmark B was housed with other Denmark B. A likely explanation for the different responses of the two asexual lineages is individual size: adult Map 75 females are significantly larger than adult Denmark B females, and I found that larger females

both make more embryos and confer larger negative effects on the growth and reproduction of smaller members of the replicate populations. Taken together, these findings suggest that the success of new asexual *P. antipodarum* lineages might be influenced by the part of the sexual size distribution sampled by the asexual lineage ancestor. Also, since female *P. antipodarum* are usually larger than their male counterparts, my data are consistent with previous work in *P. antipodarum* suggesting that highly asexual (e.g., female-biased) populations may be more likely to suffer from resource limitation than largely sexual populations.

## 2.2 Introduction

The persistence of sexual reproduction has fascinated evolutionary biologists for many decades. Because only females are able to directly contribute to population growth, an asexual female who produces only daughters will have a two-fold advantage over a sexual female who allocates half of her resources toward producing sons (Maynard Smith, 1978). This translates into a much higher rate of growth of the asexual component of the population that should culminate in the rapid elimination of sexual individuals. However, sex predominates in most natural populations. Despite extensive theoretical and empirical research into the evolution and maintenance of sexual reproduction, why sex is so common despite its costs remains a mystery (Jaenike, 1978; Maynard Smith, 1978; Bell, 1982; Butlin, 2002; Hadany and Comeron, 2008).

The two-fold cost of sex is contingent on the assumption that “all else is equal” between sexual and asexual females (Maynard Smith, 1978). However, the rareness of asexuality indicates that there must be other differences between sexual and asexual individuals that counter the costs of sex. The most obvious difference between sexually-produced and asexually-produced offspring is that the former are genetically different from both their siblings and their parents (Bell, 1982). A large body of theoretical work has focused on addressing whether the higher genetic diversity associated with sexual

reproduction might contribute to the maintenance of sex (Williams and Mitton, 1973; Williams, 1975; Lively, 1987). One possibility that has been explored at length is whether sex can be favored if genetically identical individuals experience increased competition for limited resources relative to genetically variable counterparts (Antonovics, 1978; Maynard Smith, 1978; Price and Waser, 1980; Bell, 1982; Doncaster *et al.*, 2000).

The extent that any organism experiences intraspecific competition will be influenced in part by its resource requirements and how it attains these resources. Once limited resources (or a particular means of acquiring them) are exhausted, individuals must compete to acquire the resources that they need to thrive. Assuming a genetic basis for resource requirements, genetically identical individuals will experience more intense resource competition with one another than will genetically dissimilar individuals (Bell, 1982). The implications are that members of the same asexual lineage will compete more for limited resources than sexually-produced individuals.

The Density-Dependent Selection hypothesis for the maintenance of sexual reproduction considers how density might affect the outcome of competition for resources between genetically dissimilar and genetically identical individuals (Young, 1981: "Elbow Room Model"). Competition for limited resources is expected to increase as the density of the population increases (Maynard Smith, 1978) and this model predicts that sex can be more readily maintained under high-density conditions since asexually-produced individuals will experience more severe reduction in fitness than sexually-produced individuals. Although many empirical studies have evaluated whether genetic diversity and density interact to influence fitness-related traits (Perez-Tome and Toro, 1982; Martin *et al.*, 1988; Tonsor, 1989; Koelewijn, 2004), most of this work does not directly consider the implications for the maintenance of sex. One of the only empirical studies addressing the Density Dependence Selection hypothesis was performed by Ellstrand and Antonovics (1984). They quantified the effect of density on correlates to

fitness in asexually-derived and sexually-derived *Anthoxanthum odoratum*, sweet vernal grass. While asexually-produced plants had lower mean fitness than sexually-produced individuals, there was little effect of density on fitness for either type of individual. Ellstrand and Antonovics speculated that this negative result was most likely due to high mortality in the experiment, limiting their power to compare sexually- vs. asexually-produced individuals. This study thus proved inconclusive, and to the best of my knowledge, it remains one of the only tests of the Density Dependence Selection hypothesis.

Here, I used an experimental population approach to evaluate the Density Dependent Selection hypothesis in *Potamopyrgus antipodarum*, the New Zealand mud snail. This snail is a well-established system for studying the maintenance of sexual reproduction (Lively, 1987; Lively, 1992; Neiman, 2006) because obligately sexual (dioecious) individuals and obligately asexual females frequently coexist in New Zealand freshwater lakes and streams. There is wide across-population variation in the relative frequency of sexual individuals, pointing to spatial variation in the strength of selection for sex (Lively, 1987).

In my experiment, I housed two different asexual lineages of *P. antipodarum* either in populations containing only members of one lineage or in populations containing members of both lineages. I replicated the two single- and one mixed-lineage population types at both low and high density. By using exclusively asexual females, I held sexuality and gender constant, and could thus directly evaluate the effect genetic similarity of coexisting individuals has on fitness-related traits in *P. antipodarum* at two different densities. I found that the two asexual lineages responded differently to the increase in genetic diversity, such that one lineage reproduced and grew more when housed in genetically heterogeneous populations, while the other lineage reproduced and grew more when housed genetically homogeneous populations. Post-hoc analyses indicated that these different outcomes was driven by innate difference in

lineage size, and specifically, that *P. antipodarum* fitness decreases as the size of competitors increases.

## 2.3 Materials and Methods

### 2.3.1 Snail maintenance

To address the question of whether increasing density differentially affects fitness-related traits in genetically homogeneous vs. genetically heterogeneous populations of female *P. antipodarum*, I established separate replicate populations of asexual females that varied only in the number and identity of lineages they contained. I used females from two asexual lineages, Denmark B and Mapourika 75 (“Map 75”), that both perform well under laboratory conditions (personal observation). I then used a full-factorial design, where diversity (single lineage vs. mixed lineage) and density (low vs. high density) treatments were applied to the populations, for a total of 8 population types. I used two snails per population for the low-density treatment, and six snails per population for the high-density treatment. The number of snails used is identical to earlier work demonstrating significant negative effects of density on reproduction and growth in *P. antipodarum* (Ch. 1). “Mixed” populations contained either 1 (low-density, 2 snails total) or 3 (high-density, 6 snails total) individuals from each of the two lineages. Individuals were housed in these populations for 10 weeks, following an earlier study that used a similar treatment period and found demonstrably negative effects of density on reproduction in asexual female *P. antipodarum* (Neiman 2006).

I created 12 replicate populations for each density and diversity treatment, for a total of 72 experimental populations. Each population was housed in 240 ml water in 800 ml plastic cups, and given 0.6 mL of an 0.116 mg spirulina/1 ml water solution 3 times per week. Spirulina, a dried algal product, is a common protein-rich food source for laboratory *P. antipodarum* (Ciferri, 1983). Individuals in the low-density treatment received 0.0348 mg spirulina per feeding, and individuals in the high-density treatment

received 0.0116 mg spirulina per feeding. Water was changed and containers were cleaned three times a week to control for algal growth within the containers. An  $\sim 1 \text{ cm}^3$  piece of classroom chalk was added to each container as a source of calcium.

Snails were painted with acrylic nail polish to distinguish between individual snails. In order to quantify individual growth, each individual was photographed with a ruler (mm) next to the snail on the first day of the experiment, and again on the day prior to dissection. Images were imported into the computer software ImageJ, and snails were measured from apex to aperture opening with the “measure” function and with the mm ruler as a standard. Growth was defined as the shell length at the beginning of the experiment subtracted from shell length at the end of the experiment.

I checked all snails daily for mortality. Across all six treatments, six snails died prior to the completion of the experiment, and were immediately replaced with marked snails from the same lineage. Data from these replacement individuals did not contribute to the final analyses. I dissected all remaining live snails after 70 days, and counted the number of embryos brooded by each female. Embryo number per female served as my measure of reproductive output.

### 2.3.2 Statistical analysis

When we consider the number of embryos produced per female, we used a one-quarter power transformation because this was suggested by the Box-Cox method as the optimal transformation. We fit a 3-way ANOVA while accounting for correlation due to population replicate. The predictor factors were clonal lineage, genetic diversity and density, and the response variable is the transformed embryo count. In this model we had a significant three-way interaction ( $F_{1,257} = 4.38, p = 0.037$ ), therefore we approached the data with a one-way ANOVA with eight distinct treatments. We then did Tukey-Kramer post-hoc pairwise comparisons.

When we consider the growth per female we used a log transformation to avoid violating assumptions of the model. We fit a 3-way ANOVA while accounting for a correlation due to population replicates. There was no significant three-way interaction ( $F_{1,257} = 2.41, p = 0.122$ ), but we once again fitted a one-way ANOVA with eight distinct treatments for consistency. We then did Tukey-Kramer post-hoc pairwise comparisons.

To quantify the effect of cohabitating with a larger individual, I calculated the shell length of each snail relative to the shell length of coexisting snails by dividing individual shell length by the total shell length of all other individuals in the population to generate a value hereafter referred to a “relative size.” I then used a post-hoc simple linear regression analysis between the number of embryos produced and relative size to examine how the size of coexisting individuals affects individual growth and reproduction (Fig. 2.5). The proportional contribution of each individual to the combined size of all individuals in a population will necessarily be inversely correlated with density, so low-density treatments and high-density treatments were considered separately.

## 2.4 Results

There are no overall effects of density or diversity because we had a three-way interaction, so we choose to do pairwise comparisons to analyze the differences between the cell means in the one-way ANOVA when considering the effect of diversity and density on embryo production (Table 2.1). Tukey-Kramer post hoc pairwise t-tests revealed that when housed in single lineage, low density populations, Map 75 produced significantly more Denmark B (Tukey-Kramer: t-value = -3.59,  $p = 0.013$ ). There was not a significant difference between embryo production in low-density single vs. mixed lineage populations for either Map 75 (Tukey-Kramer: t-value = 0.67,  $p = 0.998$ ), or Denmark B (Tukey-Kramer: t-value = 1.84,  $p = 0.596$ ). In the high density treatments,

there was not a significant difference in embryo production between the two single-lineage population means (Tukey-Kramer:  $t\text{-value} = -0.67, p = 0.998$ ). Map 75 in the mixed high-density populations produced significantly more embryos than Map 75 housed with other Map 75 (Tukey-Kramer:  $t\text{-value} = -4.60, p < 0.001$ ), but no such effect was detected for Denmark B (Tukey-Kramer:  $t\text{-value} = 2.67, p = 0.148$ ).

Similarly when we considered the effect of density and diversity on individual growth, there are no overall effects because we choose to do pairwise comparisons to analyze the differences between the cell means in the one-way ANOVA when (Table 2.2). Tukey-Kramer post-hoc pairwise t-tests showed that single-lineage populations of Map 75 grew significantly more than single-lineage populations of Denmark B at high (Tukey-Kramer:  $t\text{-value} = 3.32, p = 0.028$ ) but not at low-density (Tukey-Kramer:  $t\text{-value} = 0.85, p = 0.989$ ). There was no difference in growth for either Denmark B (Tukey-Kramer:  $t\text{-value} = 0.06, p = 1.00$ ) or Map 75 (Tukey-Kramer:  $t\text{-value} = -0.22, p = 1.00$ ) housed in low-density single vs. mixed lineage populations. There was also no difference in growth for Map 75 in high-density mixed vs. single lineage populations (Tukey-Kramer:  $t\text{-value} = -0.35, p = 1.00$ ). In contrast, Denmark B in high density mixed populations grew significantly less than Denmark Bin high-density single lineage populations (Tukey-Kramer:  $t\text{-value} = 3.32, p = 0.004$ ).

The different responses of Map 75 and Denmark B to the diversity treatments led us to explore the effect of individual and mean population size on growth and reproduction. These post-hoc analyses were motivated by the noticeable difference in mean shell length between the two lineages (Denmark B: mean = 3.036 mm; SD = 0.484 mm; Map 75: mean = 3.931 mm; SD = 0.476 mm)(Fig. 2.3). A two-tailed t-test comparing shell length between populations revealed that Map 75 were significantly larger than Denmark B ( $t = -15.302, p < 0.0001$ ).

I found that at both low and high density, there was a significant positive correlation between individual size relative to the rest of the population and the number

of embryos the snail produced (Low-density:  $R^2 = 0.144$ ;  $p = 0.001$ ; High-density:  $R^2 = 0.0712$ ;  $p = 0.00015$ ).

## 2.5 Discussion

The two asexual lineages of *P. antipodarum* used in this experiment responded differently to being housed in genetically homogeneous vs. genetically heterogeneous populations. At high density, Map 75 reproduced and grew ~50% more in mixed populations relative to Map 75 housed only with other Map 75. The opposite trend was observed for Denmark B housed in high-density populations, which reproduced and grew more when housed only with other Denmark B than in mixed populations (Figs. 2.1-2.2). Counter to the predictions of the Density-Dependence Hypothesis, these results suggest that there is not a straightforward relationship between genetic diversity and individual performance in *P. antipodarum*. Rather, my results indicate that heritable characteristics of particular asexual lineages – here, adult size – are a critical determinant of how individuals will respond to population composition.

Specifically, I found that inherent differences in size between the relatively small Denmark B and the relatively large Map 75 are likely to underlie the strikingly different responses of the two lineages to increasing genetic diversity. Several lines of evidence support this conclusion. First, individual embryo production and growth across both lineages decreased markedly as the size of cohabitating snails increased. Second, embryo production increased with individual length (also see Tibbets *et al.*, 2009). Third, I also found clear negative effects of increasing density on growth and reproduction, consistent with other studies of *P. antipodarum* (e.g., Neiman, 2006; Ch. 1). Taken together, these results suggest cascading effects of individual size on both individual- and population-level characteristics (Flowchart Figure): larger individuals have larger competitive impacts on other population members, both in terms of growth and reproduction, and also reproduce more than smaller snails.

These relationships between size, growth, and reproduction could have profound consequences for asexual populations (Fig. 2.5) because these results suggest that there could be strong selection favoring rapid acquisition of large adult size. These findings also raise the question of what maintains their remarkable variation in adult size (Winterbourn, 1972; Jokela *et al.*, 1999a) in the face of what would seem to be a clear advantage for large female *P. antipodarum*. This effect could interact with other forces selecting for or against larger adult size and with other factors determining lineage fitness (e.g., resistance to parasites) to determine which asexual lineages are likely to be successful (Jokela and Lively, 1995a; 1995b; Krist *et al.*, 2004).

My results also suggest that Neiman (2006)'s speculation that the larger size of female vs. male *P. antipodarum* may explain what appeared to be a larger competitive influence of females on other females was correct. A similar effect of sexual size dimorphism has been documented in other taxa (e.g., *Aedes aegypti*, Bedhomme *et al.* 2003). More broadly, as suggested by Neiman (2006), the interaction between population density and individual size on fitness-related traits has potential implications for the population dynamics and persistence of mostly asexual (*i.e.*, highly female) populations. Specifically, if the larger size of female vs. male *P. antipodarum* (Jokela, unpublished data) is realized in higher competitive impacts of females, a sexual population that is partially comprised of males will utilize less resources per capita than an asexual population of the same size that is mostly comprised of females. This effect may generate a population-level cost associated with asexuality in sexually-dimorphic species such as *P. antipodarum*. This possibility is bolstered by evidence for severe negative effects of high density and consequent food limitation on natural populations of *P. antipodarum* (Schreiber *et al.*, 1998).

My results are also relevant from the perspective of considering the fate of new asexual lineages. If adult size is heritable, individual asexual lineages will sample a narrow slice of the phenotypic variation in size present in the ancestral sexual population

(Frozen Niche Variation; Vrijenhoek, 1979; 1984). My data imply that, all else being equal, a new, relatively small asexual lineage will be at a disadvantage relative to larger sexual females and larger asexual lineages. This effect could interact with other forces selecting for or against larger adult size and with other factors determining lineage fitness (e.g., resistance to parasites; Jokela and Lively, 1995; Jokela *et al.*, 1997; Jokela *et al.*, 2000, Krist *et al.*, 2004) to influence the outcome of coexistence and competition between asexual lineages and sexual and asexual components of a populations.

Table 2.1 –One-way ANOVA table with embryos per individual female as the response variable the 8 cell means from the three-way interaction as the predictor variable.

| Source    | Numerator df | Denominator df | <i>F</i> | <i>p</i> -value |
|-----------|--------------|----------------|----------|-----------------|
| Treatment | 7            | 78.1           | 56.4     | <0.001          |

Table 2.2 –One-way ANOVA table with embryos per individual female as the response variable the 8 cell means from the three-way interaction as the predictor variable.

| Source    | Numerator df | Denominator df | <i>F</i> | <i>p</i> -value |
|-----------|--------------|----------------|----------|-----------------|
| Treatment | 7            | 80.3           | 6.11     | <0.001          |

Figure 2.1 - Mean number of embryos produced per individual female  $\pm$  2 SE. Tukey-Kramer post hoc pairwise t-tests revealed that when housed in single lineage, low density populations, Map 75 produced significantly more Denmark B (Tukey-Kramer:  $t$ -value = -3.59,  $p$  = 0.013). There was not a significant difference between embryo production in low-density single vs. mixed lineage populations for either Map 75 (Tukey-Kramer:  $t$ -value = 0.67,  $p$  = 0.998), or Denmark B (Tukey-Kramer:  $t$ -value = 1.84,  $p$  = 0.596). In the high density treatments, there was not a significant difference in embryo production between the two single-lineage population means (Tukey-Kramer:  $t$ -value = -0.67,  $p$  = 0.998). Map 75 in the mixed high-density populations produced significantly more embryos than Map 75 housed with other Map 75 (Tukey-Kramer:  $t$ -value = -4.60,  $p$  < 0.001), but no such effect was detected for Denmark B (Tukey-Kramer:  $t$ -value = 2.67,  $p$  = 0.148).

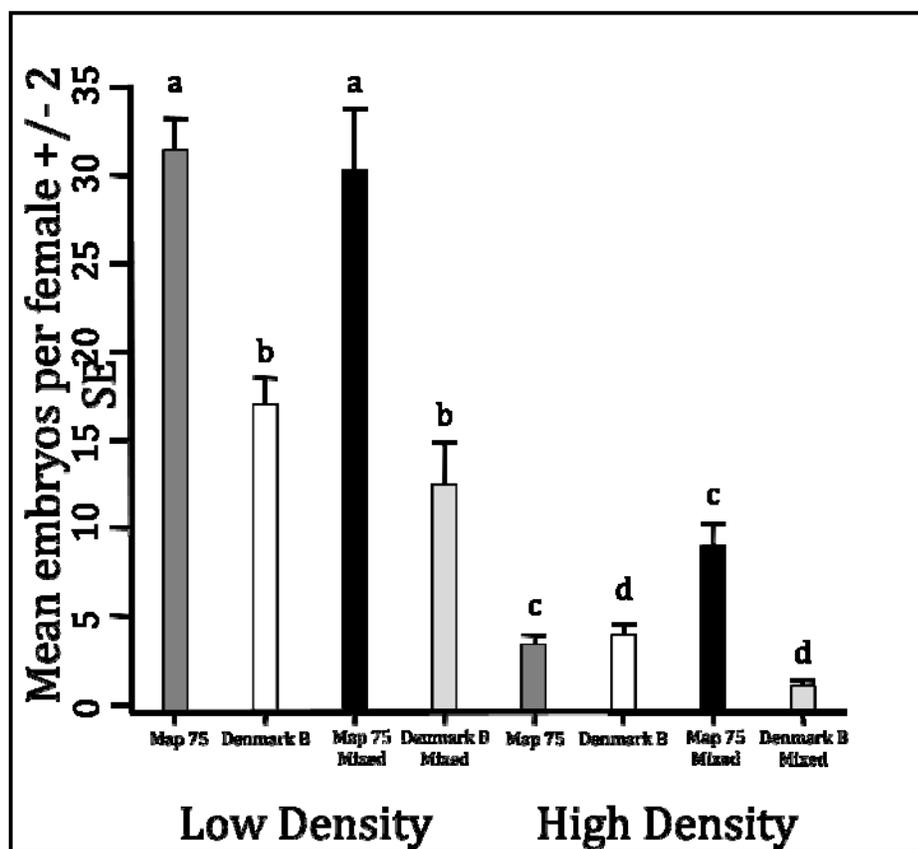


Figure 2.2 - Tukey-Kramer post-hoc pairwise t-tests showed that single-lineage populations of Map 75 grew significantly more than single-lineage populations of Denmark B at high (Tukey-Kramer:  $t$ -value = 3.32,  $p$  = 0.028) but not at low density (Tukey-Kramer:  $t$ -value = 0.85,  $p$  = 0.989). There was no difference in growth for either Denmark B (Tukey-Kramer:  $t$ -value = 0.06,  $p$  = 1.00) or Map 75 (Tukey-Kramer:  $t$ -value = -0.22,  $p$  = 1.00) housed in low-density single vs. mixed lineage populations. There was also no difference in growth for Map 75 in high density mixed vs. single lineage populations (Tukey-Kramer:  $t$ -value = -0.35,  $p$  = 1.00). In contrast, Denmark B in high density mixed populations grew significantly less than Denmark Bin high density single lineage populations (Tukey-Kramer:  $t$ -value = 3.32,  $p$  = 0.004).

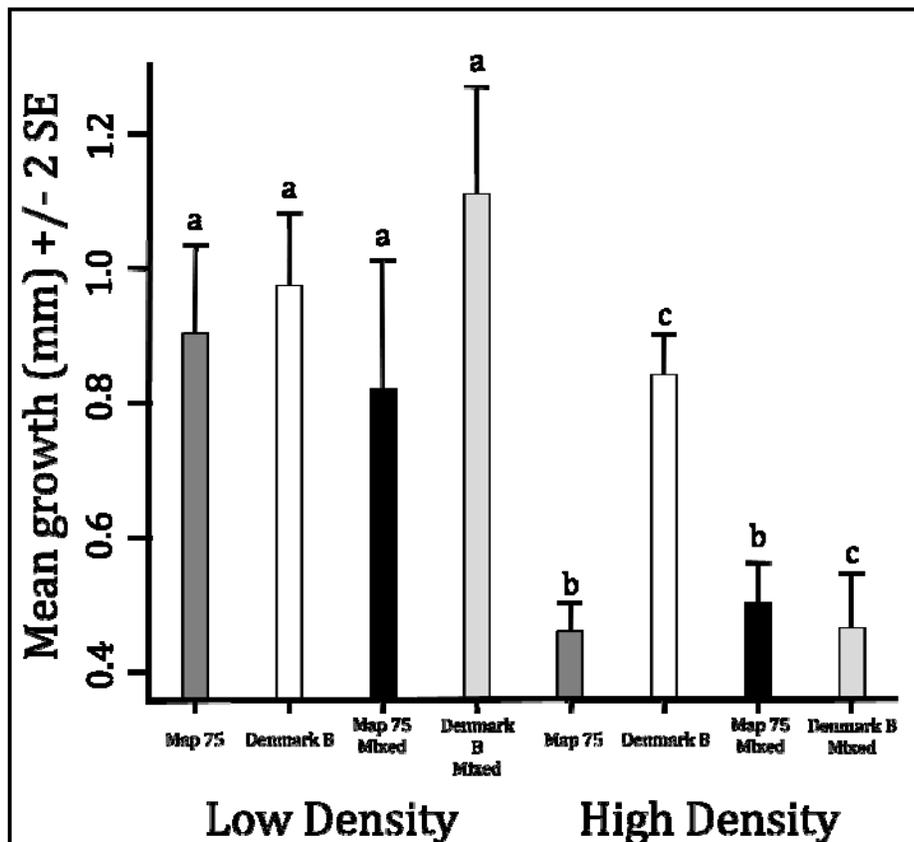


Figure 2.3- Mean shell length (mm) for each individual female. Map 75 females are significantly larger than Denmark B ( $t = -15.302$ ;  $p < 0.0001$ ).

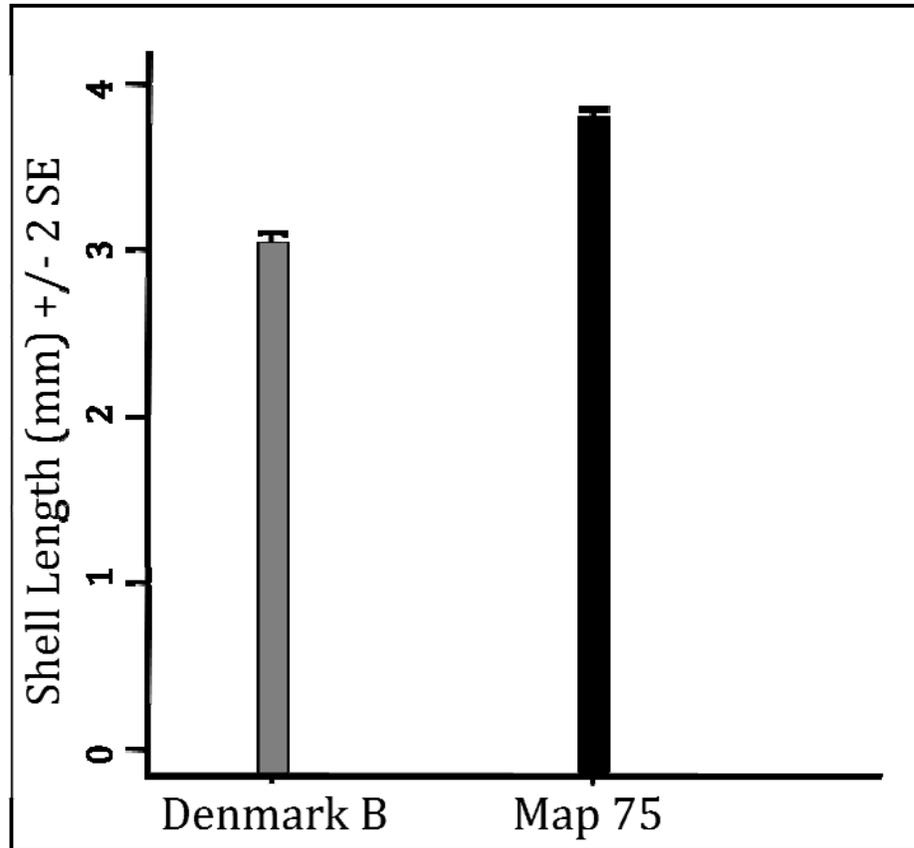


Figure 2.4- Embryos per individual female vs. the size of an individual female relative to the size of the other individuals within the population (“relative size”) at either low density (a) or high density (b). There is a positive correlation between embryo production and relative size, such that large snails in populations with smaller snails reproduce more than small snails that are housed with large snails (Low-density:  $R^2 = 0.144$ ;  $p = 0.001$ ; High-density:  $R^2 = 0.0712$ ;  $p = 0.00015$ )

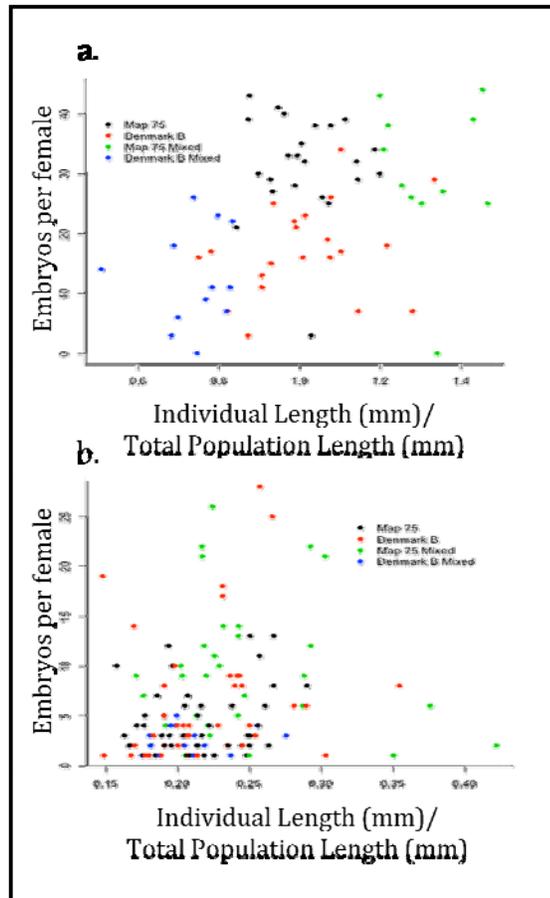
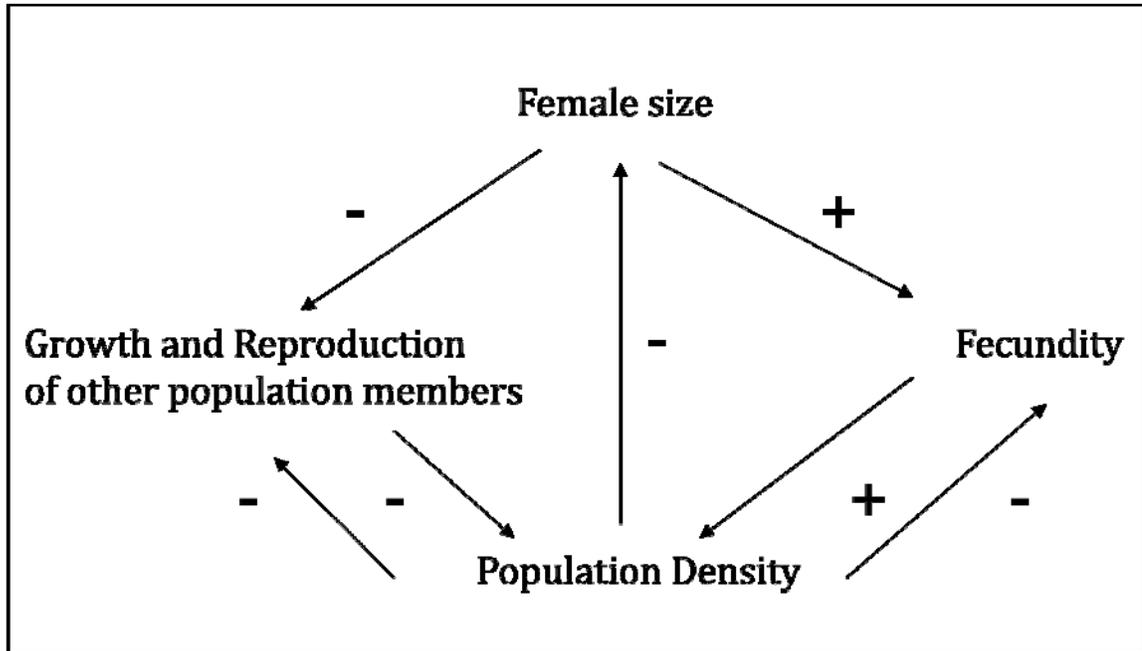


Figure 2.5 – Hypothesized demographic and selection relationships between female size, population density, individual fecundity, and population growth.



## CHAPTER 3

### DO DIFFERENCES IN BODY COMPOSITION GENERATE HIGHER COSTS OF RESOURCE LIMITATION FOR ASEXUAL VS. SEXUAL SNAILS?

#### 3.1 Abstract

Asexual females should have a two-fold advantage over sexual females who allocate half of their resources to producing sons, and yet sexual reproduction is widespread. Ecological stoichiometry postulates that an organism's ability to grow and reproduce is correlated with bodily phosphorous (P) which is in turn linked to the amount of P available within their environment. Asexuality has long been associated with polyploidy, and as nucleic acids are P rich, asexuals that have more bodily P are expected to require more environmental P to grow and reproduce at the same rate as their sexual counterparts. I tested this hypothesis empirically using the New Zealand mud snail, *Potamopyrgus antipodarum*. Each snail was housed individually for two months, and given food that varied only in the amount of P. Snails were measured at the beginning and the end of the experiment to quantify growth. Reproduction was measured as the number of embryos produced at the end of two months. I found that there was no significant difference between P treatments in either growth or embryo production. This relationship remains insignificant when I consider diploid vs. triploid, or sexual vs. asexual. It is entirely possible that low power is the result of this result, as the trend was what I expected, with asexuals reproducing and growing less than sexual in all three treatments.

#### 3.2 Introduction

The question of why sex persists in nature is one of the great mysteries in evolutionary biology. Females are the only individuals that directly contribute to population growth, meaning that asexual females who make only daughters will have a

two-fold advantage over sexual females that allocate half their resources towards making sons (Maynard Smith, 1978). This discrepancy in the rate of daughter production generates a two-fold cost of producing sons that should translate into the rapid invasion of a sexual population by the descendants of an asexual mutant, and is the basis for the expectation that asexuality should be the primary form of reproduction in nature. The predominance of sexual reproduction in natural populations thus indicates there must be inherent advantages to being sexual and/or inherent disadvantages to being asexual that counterbalance the profound costs of sex. More than 20 different hypotheses have been proposed to explain this enigma (Jaenike, 1978; Maynard Smith, 1978; Bell, 1982; Lively, 1987; Butlin, 2002), but why sex is so common continues to elude scientists (Hadany and Comeron, 2008).

The two-fold cost of males is based on the assumption that there are no other differences between sexual and asexual females (“all else equal” assumption (Maynard Smith, 1978)). Identifying violations of this assumption that confer costs to asexuals will thus generate new insights into the maintenance of sex. One common difference between asexuals and sexuals is that asexuals are often polyploid (Bell, 1982; Otto and Whitton, 2000). The transition to polyploidy often influences phenotype (Otto, 2007), such that any phenotypic costs associated with polyploidy also have the potential to influence the maintenance of sex when asexuals are polyploid. For example, if increased ploidy level increases individual resource requirements, as recently suggested by Neiman et al. (2009), polyploidy could generate an individual-level cost associated with asexuality.

The idea that polyploidy could influence dietary resource requirements is rooted in ecological stoichiometry, which addresses how imbalances between environmental availability of carbon (C), nitrogen (N) and phosphorous (P) and consumer requirements for these nutrients influence ecological and evolutionary processes (Sternner and Elser, 2002). One clear message from ecological stoichiometry is that food quality (as measured by C:N:P ratio) is likely to be at least as important as food quantity as a factor limiting

consumer growth and reproduction (Sterner and Elser, 2002). This expectation has found empirical support from studies of freshwater zooplankton showing that imbalances between bodily C:P ratios and dietary C:P content limit reproduction when P in food is low relative to bodily P content (Urabe and Sterner, 2001; Acharya *et al.*, 2006). In addition to reproduction being linked to P, the Growth Rate hypothesis postulates that organismal growth is constrained by the amount of P ingested (Elser *et al.*, 2003). The basis for this expectation is that P is needed to generate ribosomal RNA, which is required for organismal growth.

A recent study found negative effects of high C:P (i.e., low-quality) food on growth and reproduction in asexual *Potamopyrgus antipodarum* (Tibbets *et al.*, 2009), the New Zealand mud snail. These snails are uniquely well-suited to study why sex is so common in nature, since coexistence between obligate sexual individuals and obligate asexual individuals is common in New Zealand lake populations. There is wide across-population variation in the relative frequency of sexual *P. antipodarum*, pointing to variation in the strength of selection for sex (Lively, 1987). Another recent study showed that asexual forms of *P. antipodarum*, which are triploid, have substantially higher bodily P content than their sexual diploid counterparts (Neiman *et al.*, 2009). There is also indirect evidence that asexual *P. antipodarum* may have higher *per capita* resource needs than sexual *P. antipodarum* (Neiman 2006), which could be linked to the different ploidy levels of sexual and asexual *P. antipodarum*. Taken together, the results of Neiman (2006), Tibbets *et al.* (2009) and Neiman *et al.* (2009) set the stage for empirical investigation of whether, as proposed by Neiman *et al.* (2009) the higher P content of triploid asexual *P. antipodarum* will result in their being more sensitive to food quality than sexual diploids.

Here, I use manipulations of dietary phosphorus content to assess whether variation in dietary P content differentially affects fitness-related traits in sexual vs.

asexual *P. antipodarum*. I predicted that the higher P content of the asexuals would result in increased sensitivity to P-limitation relative to their sexual counterparts.

### 3.3 Materials and Methods

#### 3.3.1 Snail maintenance

*Potamopyrgus antipodarum* were collected in January 2009 from rocks and submerged vegetation in shallow regions of one North Island lake and four South Island lakes in New Zealand. All snails were transported to the University of Iowa, and housed in a 16°C room with a 12:12 light-dark cycle until the initiation of the experiment on August 12, 2009. Flow cytometry was performed on other *P. antipodarum* from the same collections indicated that the samples contained between 30% and 67% diploid sexual snails (Neiman, unpublished), such that all 6 samples were comprised of a mix of sexual and asexual individuals.

Adult *P. antipodarum* from each of the five field samples were sexed by visual identification of the presence of a penis (male) or absence (female). I housed 90 adult females from each of the five populations (450 individuals total) individually in 417 cm<sup>3</sup> cylindrical plastic containers containing 240 ml of water. Water was changed three times per week to prevent the growth of algae in the containers. Sexual female *P. antipodarum* need access to males to reproduce, so adult males from each of the five field samples populations were arbitrarily rotated through each container. This allowed each female (sexual and asexual) to experience thirty-five days with a male, and thirty-five days alone.

I used lettuce that was grown in three different fertilizer treatments in greenhouse facility at the University of St. Thomas (St. Paul, MN). After one month of growth, leaves were harvested and dried in a drying oven before being pulverized into powder. The P content was determined at The University of St. Thomas. Ecological stoichiometry hypothesizes that optimal P levels are defined by organismal bodily P content. As a

result, my treatments were defined as low, mid and high relative to bodily P content in sexual (0.8%) and asexual (1.2%) *P. antipodarum* (Neiman et al. (2009). Thus, low-P lettuce leaves contained ~0.01% P, mid-P 1%, and high-P 2% P. Each female was given 0.0232 mg of powdered dried lettuce dissolved into 1 mL water 3 times/week.

I quantified growth by measuring shell length for each female at the beginning and end of the experiment. For each measurement, each individual was photographed under a dissecting scope with their aperture facing down next to a millimeter-ruled ruler. I then used ImageJ software (National Institute of Health, Bethesda MD) to measure the length of each shell from aperture opening to apex of the shell. I subtracted shell length at the end of the experiment from the shell length at the beginning of the experiment to determine growth for each individual.

Snails were checked daily for mortality. Approximately 80 snails (17.3%) died prior to the completion of the experiment, and were not included in further analyses. After 70 days (consistent with Neiman, 2006), on Oct. 14, 2009, the 370 remaining females were dissected. At this time, I found that 5 snails (~2.25 %) were infected by a castrating trematode (*Microphallus*) and were also removed from the analysis. The number of embryos brooded inside each female, if any, were recorded as a measure of individual reproductive fitness.

### 3.3.2 Flow cytometry

Flow cytometry can be used to estimate nuclear DNA content, and thus provides a means of distinguishing diploid (sexual) from triploid (asexual) *P. antipodarum*. Gonadal tissue and undetected parasitic infection can both comprise ploidy estimation, so I used the entire head of each female, which is both spatially distant from the gonads and unlikely to be infested by parasites, for ploidy estimation. The heads were flash frozen in liquid nitrogen during the dissections described above, and then ground in a solution containing 0.2M Tris-HCl (pH 7.5), 4 mM MgCl<sub>2</sub>, 1% TritonX-100, and 4 µg/mL DAPI.

I filtered this solution through a 30 micron nylon sheet to remove remaining cellular debris. All prepared samples were run on a Beckman-Coulter Quanta SC MPL flow cytometer. Nucleic acid content was quantified by the amount of DAPI fluorescence, using the FL1 channel under a UV lamp. Prior to running the snail samples, the flow cytometer was calibrated using 100 µg of chicken red blood cells (Lampire Biological Labs, Pipersville, PA) that were prepared in the same manner as the snail samples. The FL1 channel was adjusted so that the chicken standard peak was centered on 200 FL1 units. Immediately after the chicken sample, heads from known diploid and triploid *P. antipodarum* (prepared as for the experimental heads) were analyzed on the flow cytometer to provide a visual basis for ploidy discrimination of the experimental samples.

### 3.3.3 Statistical analysis

Preliminary analysis of the number of embryos and growth per female showed that both data sets were skewed to the left because of the large number of females that either did not reproduce or grow. Therefore, I used the non-parametric Kruskal-Wallis Rank Sum test to assess the effect of dietary P treatment on the dependent variables of embryo number and growth both with and without respect to ploidy level. I also used Kruskal-Wallis Rank Sum tests to detect across- population differences in response to P treatments. Furthermore, to analyze within-population response to P treatment, I analyzed each population separately.

## 3.4 Results

### 3.4.1 The effect of P treatment on embryo production and growth

Previous studies have found that *Potamopyrgus antipodarum* females stop growing once they reach reproductive maturity (*e.g.*, Winterbourne, 1970). Consistent with these earlier studies, I used a linear regression and found a negative correlation

between individual fecundity and growth ( $R^2 = 0.01$ ;  $p = 0.049$ ) (Fig. 3.5). This result suggests that there could be a tradeoff in resource allocation, where females are either growing or reproducing (Williams, 1966). Accordingly, I used analyzed individuals that did not reproduce separately from individuals that did reproduce. Because of the possibility of a tradeoff between growth and reproduction I only analyzed growth in the 170 females that made no embryos. I did not detect any effect of P treatment on growth (Kruskal-Wallis  $\chi^2 = 1.534$ ;  $p = 0.464$ ) or embryo production (Kruskal-Wallis  $\chi^2 = 2.539$ ;  $p = 0.201$ ) (Fig. 3.1).

#### 3.4.2 The effect of ploidy on embryo production and growth

The flow cytometer malfunctioned prior to the completion of ploidy determination of 148 individuals. As a result, my analyses of the effect of ploidy on embryo production and growth were confined to the 222 individuals for which I had ploidy data (Table 3.1). There was no detectable effect of ploidy on embryo production (Kruskal-Wallis  $\chi^2 = 3.222$ ;  $p = 0.666$ ) (Fig. 3.3) or growth (Kruskal-Wallis  $\chi^2 = 2.341$ ;  $p = 0.800$ ) (Fig. 3.2).

My ability to detect an effect of ploidy on growth may have been confounded by differences in the sizes of individuals within each treatment group at the beginning of the experiment. Specifically, relatively small individuals may be more likely to be allocating resources towards growth instead of reproduction since they may have not reached sexual maturity. This possibility is consistent with the results of a post-hoc simple linear regression using growth (mm) as the dependent variable and initial size (mm) as the independent variable, which showed that there was a significant negative relationship ( $R^2 = 0.0567$ ;  $p = 0.0004$ ) between initial size and growth. In other words, individuals that were smaller at the beginning of the experiment grew more than individuals who were larger at the beginning of the experiment (Fig. 3.4). There was a trend that individuals

from different treatment groups differed in starting size (Kruskal-Wallis  $\chi^2 = 4.089$ ;  $p = 0.101$ ), and this may have biased my results in that smaller individuals may have been allocating resources towards growth instead of reproduction.

### 3.4.3 The effect of P treatment and ploidy on embryo production and growth among lake populations

I used a Kruskal-Wallis test to find that there was significant across-population variation in response to P treatments (Kruskal-Wallis  $\chi^2 = 38.907$ ;  $p = 0.0004$ ). I also analyzed variation in growth and reproduction across the P treatments and within each ploidy level for each of the five lake populations (summarized in Tables 3.2, 3.3). There was a significant positive effect of P treatment on embryo production in only one population (Kruskal-Wallis  $\chi^2 = 9.262$ ;  $p = 0.00975$ ) (Fig. 3.3a), Lake Clearwater. This relationship remained significant after removing the effect of ploidy level (Kruskal-Wallis  $\chi^2 = 11.429$ ;  $p = 0.0435$ ) (Fig. 3.3b), such that asexual individuals reproduced less than sexual individuals in both the low-P and mid-P treatments.

## 3.5 Discussion

There was not a significant effect of P treatment on embryo production or growth. While low sample size due to relatively high mortality (>30% vs. 1.5% in Ch. 1 and <1% in Ch. 2) and my inability to determine ploidy for a large fraction of experimental individuals indicates that these results must be interpreted with caution. Our results are the converse of expectations of a scenario where 1) reproduction in *P. antipodarum* is limited by food with P content that is lower than their bodily P content, and 2) the higher bodily P content of asexual *P. antipodarum* translates into greater sensitivity to P-limited conditions.

Under the Growth Rate hypothesis (Elser *et al.*, 2003), there should be a positive relationship between growth and % P in food. Contrary to these predictions, there was no significant effect of P treatment on growth. Since I detected a significant negative

correlation between initial size and growth, one possible explanation for the lack of any apparent effect of P treatment on growth was across-treatment variation in the individual size at the beginning of the experiment (Fig. 3.4).

I detected a negative correlation between reproduction and growth in this study. This result is consistent with resource allocation theory suggesting that organisms allocate resources to growth until they reach reproductive age, and then reallocate the majority of their resources to reproduction (Williams, 1966). Earlier studies of *P. antipodarum* also suggest that females stop growing when they reach reproductive maturity (Winterbourne, 1970). Thus, it is possible that a growth/ reproduction tradeoff could have confounded my ability to detect significant effects of P treatment on growth and reproduction because some individuals were primarily allocating resources towards reproduction, while others were primarily allocating resources towards growth. The negative correlation I detected between growth and reproduction would also almost certainly reduce statistical power, since I had to analyze these individuals separately.

There was wide-across population variation in response to the P treatments. Ecological stoichiometry predicts that variation in an organism's bodily nutrient content will be in part driven by environmental variation in the availability of these nutrients (Frost and Elser, 2002; Sterner and Elser, 2002; Perkins *et al.*, 2004). ES also predicts that adequate dietary P is determined by an organism's body composition. Thus, the population-level variation I detected could reflect of across-population variation in P availability, which is common in New Zealand lakes (Ministry for the Environment, 2007). For example, one would predict that *P. antipodarum* sampled from a high-P lake may respond differently to the low-P treatment than snails from a low-P lake. In this context, information on P availability from Lake Clearwater could be quite illuminating, since asexual females within the population reproduced significantly less than sexual females in the low- and mid- P treatments. This result suggests that asexual individuals may have been more P-limited in this experiment than sexual individuals. However, the

other populations in this experiment did not respond to the P treatment in a similar manner, which does not allow me to make firm conclusions.

Another potentially confounding factor is that though the P-treatment levels in my experiment were based on bodily P content in *P. antipodarum*, there is wide across-lineage variation in % P even within a given ploidy level (Neiman et al., 2009a). Therefore, the different response of each of these populations to the P treatment could have also been a function of across-lineage variation in bodily P content.

Overall my results were mixed and my analysis probably had low statistical power. Despite these complications these results hint that there are some environments where there could be a resource-based cost associated with asexuality in *P. antipodarum*. The trade-off between growth and reproduction was particularly informative, and needs to be considered if someone were to attempt to replicate this experiment. Future research that, for example, quantifies across-lineage variation in bodily P in field-collected *P. antipodarum* and evaluates whether this variation is correlated with the amount of P available in the sampling environment could be quite illuminating.

Table 3.1 - The number of diploid and triploid individuals within each population used in this experiment. The natural frequency of sexual individuals is the percentage of field- collected snails determined to be diploid immediately after field collection in January 2009.

| Population  | # 2n | # 3n | Natural frequency of sexual individuals |
|-------------|------|------|---|
| Alexandrina | 19   | 38   | 50%                                     |
| Clearwater  | 39   | 24   | 30%                                     |
| Heron       | 21   | 21   | 30%                                     |
| Selfe       | 13   | 13   | 66.67%                                  |
| Wairarapa   | 32   | 2    | <50%                                    |
| Total       | 124  | 98   |   |

Table 3.2- Results of the Kruskal-Wallis test of significance for each of the 5 populations. The independent variable is P treatment and the dependent variables are female growth and embryo production.

| Population  | Embryos  |          | Growth   |          |
|-------------|----------|----------|----------|----------|
|             | $\chi^2$ | <i>p</i> | $\chi^2$ | <i>p</i> |
| Alexandrina | 0.786    | 0.675    | 2.277    | 0.3202   |
| Clearwater  | 9.262    | 0.00975  | 0.575    | 0.75     |
| Heron       | 1.118    | 0.571    | 3.996    | 0.136    |
| Selfe       | 2.597    | 0.273    | 2.0704   | 0.355    |
| Wairarapa   | 4.0001   | 0.135    | 5.783    | 0.0555   |

Table 3.3- Results of the Kruskal-Wallis test for each of the 5 populations. Independent variables are P treatment and ploidy and the dependent variables are female growth and embryo production.

| Population  | Embryos  |          | Growth   |          |
|-------------|----------|----------|----------|----------|
|             | $\chi^2$ | <i>p</i> | $\chi^2$ | <i>p</i> |
| Alexandrina | 1.532    | 0.909    | 3.8011   | 0.578    |
| Clearwater  | 11.429   | 0.0435   | 1.324    | 0.933    |
| Heron       | 4.666    | 0.458    | 5.797    | 0.327    |
| Selfe       | 4.570    | 0.471    | 2.597    | 0.273    |
| Wairarapa   | 4.025    | 0.259    | 5.808    | 0.121    |

Figure 3.1– Boxplots show a) the median embryos per female (horizontal line in each box) for each P treatment and b) the median growth. The interquartile range is shown by the upper and lower edges of each box. There is not a significant difference in embryo production (Kruskal-Wallis  $\chi^2 = 2.539$ ;  $p = 0.201$ ) or growth (Kruskal-Wallis  $\chi^2 = 1.534$ ;  $p = 0.464$ ) across P treatments.

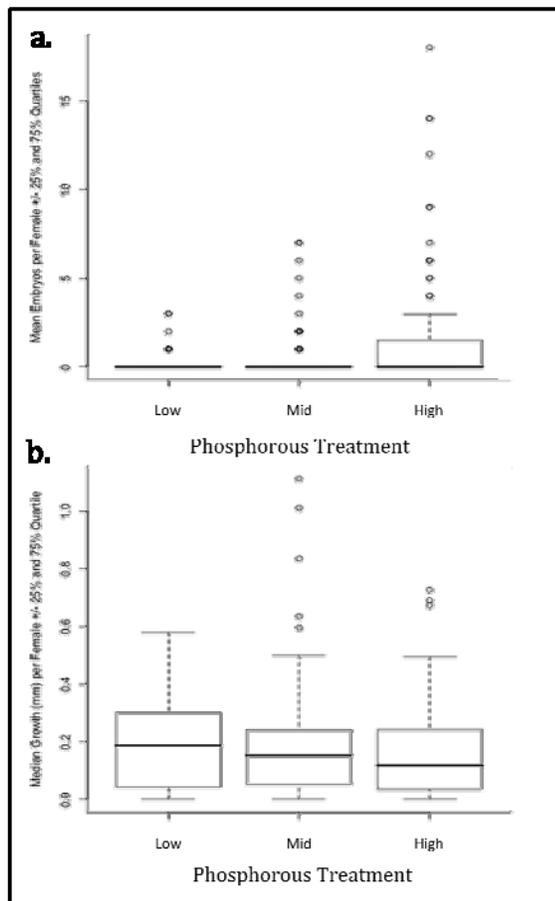


Figure 3.2 - Boxplots show a) the median embryos per individual female (horizontal line in each box) for either diploid or triploid b) the median growth for either diploid or triploid. The interquartile range is shown by the upper and lower edges of each box. There is no significant difference in embryo production (Kruskal-Wallis  $\chi^2 = 2.539$ ;  $p = 0.201$ ) or growth (Kruskal-Wallis  $\chi^2 = 1.534$ ;  $p = 0.464$ ) between P treatments.

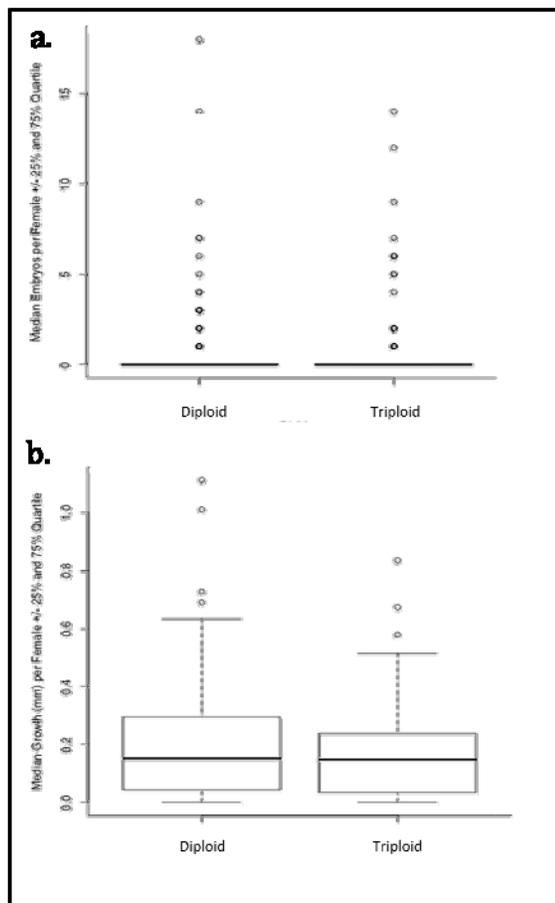


Figure 3.3- Boxplots show the a) the median embryos per individual female (horizontal line in each box) for all three P treatments in Lake Clearwater (Kruskal-Wallis  $\chi^2 = 9.262$ ;  $p = 0.009$ ); b) the median growth for all three P treatments when considering ploidy in Lake Clearwater (Kruskal-Wallis  $\chi^2 = 11.429$ ;  $p = 0.0435$ ). The interquartile range is shown by the upper and lower edges of each box.

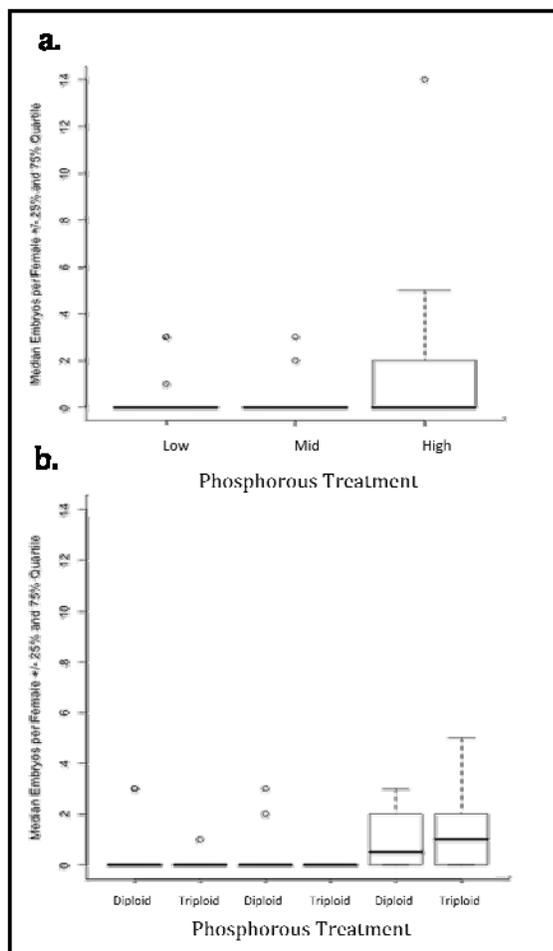


Figure 3.4- Plot of beginning shell size (mm) and growth (mm) between both diploid individuals and triploid individuals. There is a significant negative correlation between shell size and growth ( $R^2 = 0.0567$ ;  $p = 0.0004$ )(best fit line shown).

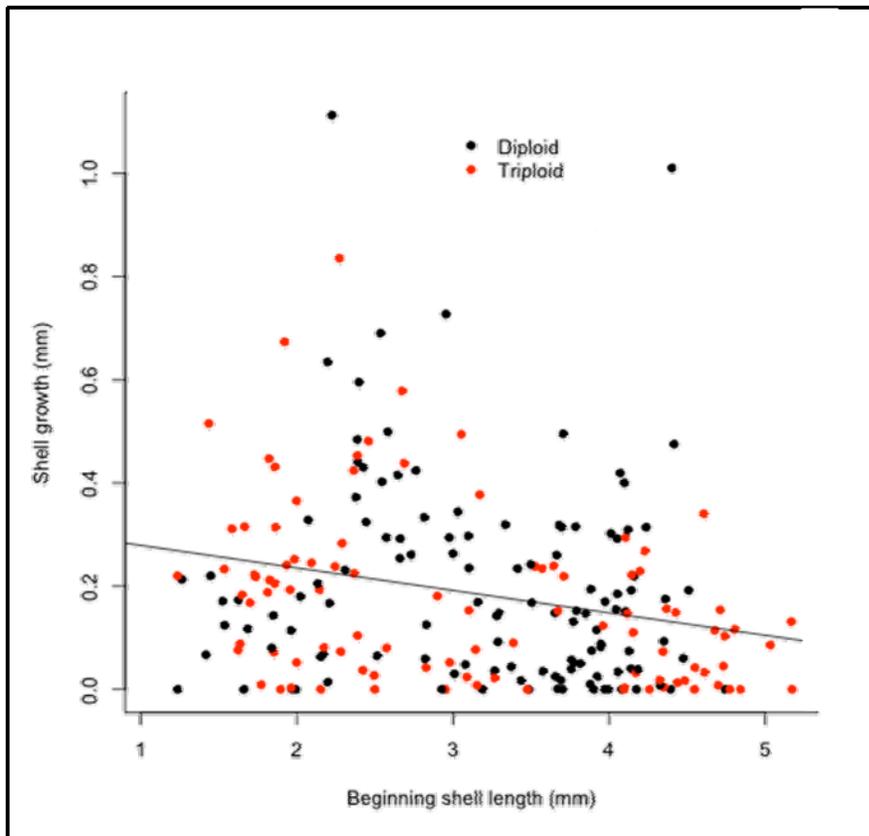
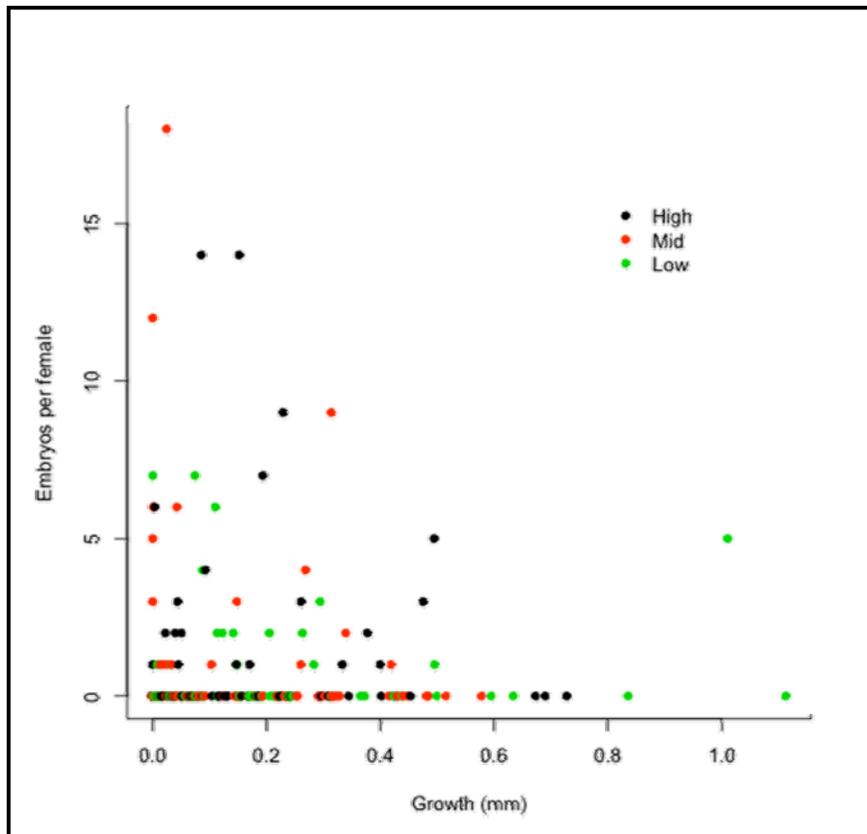


Figure 3.5- Plot of growth (mm) vs. embryos per female. There is a significant negative correlation between growth and embryos ( $R^2 = 0.01; p = 0.049$ ).



## CONCLUSIONS

The aim of my thesis is to compare the value of fitness-related traits of sexual and asexual individuals in resource-limited environments. To address this, I used three different experiments with *Potamopyrgus antipodarum*, a snail system that is the subject of a large body of research into the maintenance of sex in natural populations.

In chapter one, I used density treatments to evaluate whether reproduction and growth in sexual and asexual female *P. antipodarum* responded differently to decreased per capita food availability. I expected that asexuals would show a sharper decline in the values of these fitness-related traits than sexuals, if they were in fact more sensitive to food limitation. While increased density had marked negative effects on growth and reproduction in both sexual and asexual females, sexual females were affected much more severely by high density than their asexual counterparts. Superficially, this result suggests that sex is unlikely to be maintained via greater sensitivity to limited food resources in asexuals. There are other factors, however, which may have influenced the outcome of the experiment.

One such factor is the genetic diversity of the sexual vs. asexual experimental populations. Some evolutionary models suggest that sex can be maintained if there is increased competition for resources between genetically identical (*e.g.*, asexually produced) relative to genetically dissimilar (*e.g.*, sexually produced) individuals (Bell, 1982). Thus, the advantages of sex in this context are generated by genetic diversity, rather than direct consequences of sex (*i.e.*, recombinant genotypes). In the experiment described above, I attempted to control for across-lineage genetic variation by generating the experimental populations from two common pools of genetically distinct lineages. However, constraints of lineage availability meant that while asexual pool comprised lineages from five different lake populations, the sexual populations were established from a pool containing five inbred lines largely derived from one New Zealand lake. As a

result, the sexual experimental populations may have had lower genetic diversity than the asexual populations. If this lower genetic diversity translated into more intense competition between individuals, it could explain why the sexual populations were affected more by increased density than the asexual populations. This explanation is in fact consistent with hypotheses suggesting that high genetic diversity can generate advantages for sex. My results also point to a possible role for resource partitioning in mediating the outcome of competitive interactions in *P. antipodarum*, such that the genetically similar individuals competed more with each other for the same resource than the genetically dissimilar individuals.

In chapter 2, I used the framework of the Density Dependence Selection hypothesis, which suggests that disparities in intensity of competition between genetically identical vs. genetically distinct individuals will increase with density, to directly address whether genetic diversity could generate an advantage for sexual *P. antipodarum*. I housed females from two asexual lineages (Map 75 and Denmark B) in either genetically homogeneous (“single lineage”) or genetically heterogeneous (“mixed lineage”) populations and at either high or low density. Map 75 and Denmark B responded differently to the diversity and density treatments, such that at high density, Map 75 reproduced and grew more in mixed vs. single lineage populations, while the converse was observed in Denmark B. Counter to the predictions of the Density-Dependence Selection hypothesis, these results suggest that that differences in genetic diversity per se may not wholly explain the higher performance of asexual populations of *P. antipodarum* under high density conditions in chapter 1 and that different genotypes respond differently to increases in density in genetically uniform or genetically variable environments.

Post-hoc analyses indicated that inherent differences in size between the smaller Denmark B and the larger Map 75 were likely to contribute to the different responses of the two lineages to increased genetic diversity. For one, there is an established positive

relationship between adult female size and fecundity in *P. antipodarum*, which was also documented in each of the two lineages. Second, embryo production and growth decreased in individuals from both asexual lineages as the size of other individuals within the population increased. These results indicate that adult female size is a critical determinant of both individual-level fecundity and population-level competitive impact. Taken together, these results also imply that there may be cascading and complex effects of individual and lineage size on both individual- and population-level characteristics in *P. antipodarum*.

Evidence for relatively high competitive impacts of coexistence with large *P. antipodarum* is consistent with an earlier study speculating that the sharp drop in embryo production in all-female asexual populations vs. mixed male-asexual female populations was most likely due to the larger size of female vs. male *P. antipodarum* (Neiman, 2006). More broadly, as suggested by Neiman (2006), the apparently major influence of adult female size on potential competitors could mean that female-biased populations (such as highly asexual populations) could be more subject to resource limitation than populations with a higher relative frequency of sex. Since invertebrate females are often larger than males, and since there is evidence from mosquitos for a larger competitive impact of females than males (Bedhomme *et al.*, 2003), this phenomenon could generate a population-level cost associated with asexuality in sexually dimorphic species.

In the third chapter, I addressed whether asexual and sexual *P. antipodarum* respond differently to varying food quality. Ecological stoichiometry considers the balance of elements between an organism and its environment, and specifically predicts that organismal body composition should be influenced by environmental availability of key nutrients. The most basic premise of ES is that fitness can be limited by imbalances between nutrient availability and organismal body composition, such that environments that are too high or too low in key nutrients can impose fitness costs.

The Growth Rate hypothesis (Elser *et al.*, 2003) is based on the principles of ecological stoichiometry, and predicts that the amount of phosphorus (P) in the environment will determine the rate of organismal growth by influencing its ability to produce ribosomal RNA, which is needed to make new proteins. The reason why growth is thought to be especially susceptible to P-limitation is that nucleic acids such as RNA are both P rich relative to other major biomolecules and often make up a substantial fraction of invertebrate dry mass (Sterner and Elser, 2002).

Asexuality has long been linked to polyploidy, and recent data show that asexual (triploid) *P. antipodarum* have higher bodily nucleic acid content and thus higher bodily P content than their sexual diploid counterparts (Neiman *et al.*, 2009). I used manipulations of the P content of food given to sexual and asexual *P. antipodarum* to assess whether the higher P content of asexual individuals translated into more sensitivity to low-P food. I also considered how *P. antipodarum* sampled from different natural populations would respond to varying dietary P content. I expected that sexual females would be better able to grow and reproduce in the low-P treatment than asexual females. While there was not a significant overall effect of P treatment on growth or embryo production, and there did not appear to be a difference between sexual and asexual response to the food treatments.

Some of the sample populations used in my experiment responded differently to the P treatments. This result may be consistent with the expected link between bodily composition of consumers and the available ratios of C:N:P within their environments. For example, if the source lakes for these populations vary in available environmental P, I might expect to see some populations responding more drastically to the P treatment than other populations.

Overall, my thesis shows that *P. antipodarum* are profoundly affected by resource limitation and by the phenotype of competitors. First, low genetic diversity in sexual experimental populations could explain why sexual individuals appeared to respond more

negatively to increased density than asexual counterparts (Ch. 1). This result merits further investigation of the influence of genetic diversity on intraspecific competition in *P. antipodarum*, and especially with respect to sexual vs. asexual individuals and largely sexual vs. largely asexual populations. I also showed that that the intensity of intraspecific competition increases with adult size in asexual female *P. antipodarum*. This result is relevant to considering the fate of new asexual lineages, which sample only particular parts of the sexual phenotypic spectrum. On a population level, the fact that asexual lineages only sample a fraction of the phenotypic variation in the sexual population could increase the likelihood of resource limitation in female-biased populations (*i.e.*, highly asexual) relative to mostly sexual populations that also contain a high relative frequency of the smaller males. Such a phenomenon, if generalized to other systems where females are consistently larger than males, could generate a population-level cost associated with asexuality in sexually dimorphic species. Furthermore, it is clear that in at least one of the lake populations examined, there is a positive correlation between P treatment and reproduction. Moreover, in this population, sexuals experienced less of a decrease in growth and reproduction than asexuals as amount of P decreased. However, there was no clear relationship between reproduction and/or growth in the majority of the populations examined. Because P availability varies across New Zealand lakes (Ministry for the Environment, 2007), it is possible that the threshold for P limitation differs across populations, which could explain why the populations in my study responded differently to the P treatments.

Taken together, my thesis both demonstrates the potential for an important role of resource limitation in determining the outcome of competitive interactions in *P. antipodarum* populations, and sets the stage for several avenues of future research. For one, a better understanding of the influence of genetic diversity on competitive interactions between individuals and how size differences generate different competitive interactions between males and females will clearly generate important new insights into

the success of new asexual lineages and the susceptibility of largely sexual vs. largely asexual populations to resource limitation. The extent to which differences in the elemental body composition of individuals differing in both ploidy and sexuality generates differences in resource requirements still remains unclear, but constitutes a promising avenue for future study.

The statistical data analysis for this paper was generated using SAS software. Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

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