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Plastic and Genetic Determination of Population, Community, and Ecosystem Properties in Freshwater Environments

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PLASTIC AND GENETIC DETERMINATION OF POPULATION, COMMUNITY, AND ECOSYSTEM PROPERTIES IN FRESHWATER ENVIRONMENTS

by

Leigh C. Latta IV

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ABSTRACT

Plastic and Genetic Determination of Population, Community

and Ecosystem Properties in Freshwater Environments

by

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The hierarchy of biological organization, from molecules to ecosystems, describes the relationships among various biological systems. Of particular interest is assessing how the factors that primarily determine the nature of one hierarchical level also have transcendent qualities that affect the ecology and evolution of higher hierarchical levels. The goal of this dissertation was to use a bottom-up approach to examine the transcendent effects of two factors that strongly determine the nature of their associated level of biological organization. The first, phenotypic plasticity, is a primary factor that determines the phenotype of an individual. The second factor, genetic diversity, largely determines the phenotypic distributions associated with populations. Controlled laboratory experiments on taxa from a freshwater tri-trophic food web were employed to examine the transcendent effects of phenotypic plasticity and genetic diversity on the biological hierarchy because relationships between individuals and populations from different trophic levels are well documented for numerous freshwater species. The results show that phenotypic plasticity can induce changes in population means and variances that promote population persistence and evolvability, and that plasticity provides a mechanistic explanation of community stability in response to changing environments. Similarly, genetic diversity may act

as a signal that induces phenotypic plasticity in individuals, modulates community richness and ecosystem properties, and suggests a potential mechanism for the changes in biodiversity. Thus, results from this dissertation show that plasticity and genetic variation can shape the attributes of other biological groups higher in the biological hierarchy, and, in some cases, may also provide a mechanistic explanation for variability observed in higher levels of the biological hierarchy. These results highlight the importance of integrating traditionally disparate biological disciplines and may help to unify biology as a field.

(124 pages)

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CHAPTER 1

INTRODUCTION

The hierarchy of biological organization, from molecules to ecosystems, describes the relationships among various biological systems. By definition, one level of hierarchy cannot exist unless all the levels of hierarchy below it are intact. More importantly, a hierarchical level will display some emergent properties not explained simply by the summation of each of the levels of hierarchy below it. There are two basic approaches for investigating the factors that determine the nature of each level of hierarchy. A top-down approach involves the direct examination of a hierarchical level of interest and subsequent decomposition into the important lower level hierarchical components. Alternatively, a bottom-up approach involves the indirect examination of a hierarchical level of interest through the synthesis of observations on lower hierarchical levels. Both experimental approaches have yielded insight into how specific biological systems are assembled and maintained. One area that is particularly interesting, and the focus of this dissertation, is determining how the factors that primarily determine the nature of one hierarchical level also have transcendent qualities that affect the ecology and evolution of higher hierarchical levels.

As a generic example, the phenotype of an individual is determined by several factors including: the specific set of alleles the individual carries, the pattern of gene expression during ontogeny, and the modulation of gene expression by the local biotic and abiotic environment. Thus, a single individual (i.e., a fixed genotype) has the potential to display numerous phenotypes through developmental plasticity during ontogeny and phenotypic plasticity in response to the local environment. Similarly, a phenotypic distribution of a population, and the metrics that describe the distribution such as the mean and variance, will reflect the level and quality of genetic diversity in the population (i.e., the differences in the genotypes of individuals that arise due to the evolutionary forces of mutation, selection, drift, and gene flow), as well as the variation

that arises through individual-based effectors such as developmental and phenotypic plasticity. Finally, population-based effectors, such as the level and quality of genetic diversity in a focal population, and individual-based effectors like phenotypic plasticity often determine the species composition of a community. Therefore, a single factor that primarily influences the phenotype of an individual, phenotypic plasticity, can also have transcendent effects on the phenotypic distribution of a population and the species composition of a community.

The general goal of this dissertation is to use a bottom up approach to examine the bottom-up effects of two factors that strongly determine the nature of their associated level of biological organization on higher levels of biological hiearchy. The first, phenotypic plasticity, is a primary factor that determines the phenotype of an individual. Phenotypic plasticity has permeated a variety of topics in ecology and evolutionary biology related to higher levels of the biological hierarchy. At the level of populations, the transcendent properties of phenotypic plasticity can effect the amount and quality of genetic variation visible to selection when a population is presented with a changing environment and influence the amount of genetic change a population undergoes in response to the challenge (Price et al. 2003). At the level of communities, plasticity can effect species composition and community structure (Pohnert et al. 2007) by modulating the success of invasive species (Strauss et al. 2006) and species interactions (Agrawal 2001). The second factor, genetic diversity, largely determines the phenotypic distributions associated with populations. However, genetic diversity can also influence community level properties such as composition and structure, and ecosystem level properties such as stability (Hughes et al. 2008). Thus, phenotypic plasticity and genetic diversity are of primary importance in determining the attributes of individuals and populations, respectively, but also exert some degree of influence on higher levels of biological organization.

Freshwater food webs present an ideal model system to examine the transcendent effects of phenotypic plasticity and genetic diversity on the biological hierarchy because relationships

between individuals and populations from different trophic levels are well documented for numerous freshwater species. The taxa used in this dissertation, two species of fish, three species of *Daphnia*, two species of algae and numerous microbial species, are characteristic of a simple tri-trophic food web that includes secondary consumers (fish), primary consumers (*Daphnia*), and primary producers (algae and microbes). Chemicals produced by species of higher trophic levels often mediate phenotypic plasticity in focal individuals in freshwater systems. For example, individual algae will produce defensive morphological structures through phenotypic plasticity in response to chemicals produced by *Daphnia* (Hessen and Van Donk 1993; Lürling 2003) and *Daphnia* will produce defensive morphological structures through phenotypic plasticity in response to chemicals produced by fish (Stibor 1992; Reede & Ringleberg 1995; Spaak et al. 2000; Sakwinska 2002; Reede 2003). Genetic diversity in freshwater populations is influenced by direct interactions among trophic levels such as fish predation on *Daphnia* and *Daphnia* grazing on algal populations. Thus, the effects of phenotypic plasticity and genetic diversity on higher levels of biological hierarchy may also be tied to the relative trophic status of the interacting species.

Three chapters of this dissertation deal with the effects of individual phenotypic plasticity on populations and communities while a fourth data chapter concerns the effects of population genetic diversity on community and ecosystem properties. Specifically, chapter 2 describes the results from a common-garden experiment designed to assess the nature of phenotypic plasticity in individuals of *Daphnia* in response to chemicals produced by fish and then discusses how phenotypic plasticity has influenced the ability of *Daphnia* populations to respond evolutionarily to a novel selective challenge. Chapter 3 depicts the results of microcosm experiments that describe the nature of phenotypic plasticity in algal cells in response to chemicals produced by both fish and *Daphnia* and then discusses the potential consequences for populations of algae and *Daphnia* in the context of simple freshwater communities. Chapter 4 is grounded in the results

obtained in chapter three and describes results from a common-garden experiment that illustrate phenotypic plasticity in response to resource depression in *Daphnia*. The final data chapter of this dissertation is based on results from microcosm experiments designed to assess the impact of varying genetic diversity in a focal population on microbial communities and ecosystem metabolism. A concluding chapter summarizes the important results from this dissertation and places these results in a broader biological context.

CHAPTER 2

RAPID EVOLUTION IN RESPONSE TO INTRODUCED PREDATORS: THE CONTRIBUTION OF ADAPTIVE PHENOTYPIC PLASTICITY

Abstract

Introductions of non-native species can significantly alter the selective environment for populations of native species, which can respond through phenotypic plasticity or genetic adaptation. We examined phenotypic and genetic responses of *Daphnia* populations to recent introductions of non-native fish to assess the relative roles of phenotypic plasticity versus genetic change in causing the observed patterns. The *Daphnia* community in alpine lakes throughout the Sierra Nevada of California (USA) is ideally suited for investigation of rapid adaptive evolution because there are multiple lakes with and without introduced fish predators. We conducted common-garden experiments involving presence or absence of chemical cues produced by fish and measured morphological and life-history traits in *Daphnia melanica* populations collected from lakes with contrasting fish stocking histories. The experiment allowed us to assess the degree of population differentiation due to fish predation and examine the contribution of adaptive plasticity to the response to predator introduction. Our results show reductions in egg number and body size of *D. melanica* in response to introduced fish. These phenotypic changes have a genetic basis but are partly due to a direct response to chemical cues from fish via adaptive phenotypic plasticity. Body size showed the largest phenotypic change, on the order of nine phenotypic standard deviations, with approximately 11% of the change explained by adaptive plasticity. Both evolutionary and plastic changes in body size and egg number occurred but no changes in the timing of reproduction were observed. Native *Daphnia* populations exposed to chemical cues produced by salmonid fish predators display adaptive plasticity for body size and fecundity. The magnitude of adaptive plasticity was insufficient to explain the total phenotypic change, so the realized change in phenotypic means in populations exposed to introduced fish

may be the result of a combination of initial plasticity and subsequent genetic adaptation. Our results suggest that immediately following the introduction of fish predators, adaptive plasticity may reduce the impact of selection through "Baldwin/Bogart effects" by facilitating the movement of populations toward new fitness optima. Our study of the response of a native species to an introduced predator enhances our understanding of the conditions necessary for rapid adaptive evolution and the relationship between rapid evolution and adaptive phenotypic plasticity.

Introduction

Introductions of non-native species can result in strong selective challenges for native populations. The strength of selection in this case is determined by the size of the environmental shift, which imposes a fitness cost on the population proportional to the squared distance between the population mean phenotype and the position of the new optimum (Lynch & Lande 1993; Lande & Shannon 1996). If the optimum moves far enough, the fitness cost will be sufficiently high to reduce the intrinsic rate of increase of the population to <1. Unless the population can rapidly advance toward the new optimum phenotype, it will not persist (Gomulkiewicz & Holt 1995). Two processes can facilitate persistence of populations challenged with a rapidly changing environment: adaptive phenotypic plasticity and genetic adaptation.

Adaptive phenotypic plasticity allows individuals within a population to accommodate a changing environment (Dudley & Schmitt 1996; Price et al. 2003) by facilitating rapid movement to a new fitness optimum. This movement occurs through changes in the mean value of a trait and/or changes in the genetic and phenotypic variance/covariance structures. In the extreme case, plastic changes in the mean value of a trait are able to completely move a population to a new fitness optimum and no genetic adaptation is required (Price et al. 2003). In cases where a plastic change in the mean is not sufficient to shift a population to a new optimum it can allow a population to persist until sufficient adaptive genetic changes occur (Baldwin 1896; Yeh & Price

2004). An incomplete shift in the population mean towards a new selective optimum that facilitates population persistence is called the "Baldwin effect" (Baldwin 1896) and the resulting reduction in the intensity of selection is referred to as the "Bogart effect" (Huey et al. 2003) or adaptive buffering (Strauss et al. 2006). Plastic changes in the (co)variance matrix may result in increased levels of expressed genetic variance (*i.e,*. variance that is context-dependent and arises only in response to specific environmental cues) and changes in covariances between traits that increase the response to selection (Schlichting 1986; Boersma et al. 1998; Pigliucci et al. 1999; Pigliucci 2005).

Populations may also adapt genetically to new environmental conditions when there is no pre-existing adaptive phenotypic plasticity or plasticity is insufficient to completely shift a population to a new phenotypic optimum. The rate of genetic adaptation toward a new optimum is determined by a number of factors, including the amount of additive genetic variation present for the traits under selection (Fisher 1958), the rate at which mutation produces new adaptive variation (Falconer & Mackay 1996; Lynch & Walsh 1998), and genetic correlations among characters (Arnold 1992; Lynch & Walsh 1998; Etterson & Shaw 2001; Etterson 2004).

A common source of rapid environmental change arises from the introduction of novel predator species. In a notable example, non-native fishes have been widely introduced into naturally fishless alpine lakes throughout the world and have had profound effects on native zooplankton species, including *Daphnia*. *Daphnia* have a long history as a model system to study the consequences of introduced fish predators (Dodson 1970; Wells 1970; Werner & Hall 1974; Kitchell & Kitchell 1980). *Daphnia* adapt to introduced fish through changes in traits related to detection avoidance, including alterations in patterns of diel vertical migration (DVM) (Pijanowski et al. 1993; Cousyn et al. 2001) and reduced body size (Galbraith 1967; Wells 1970; Kitchell & Kitchell 1980). *Daphnia* also display significant adaptive phenotypic plasticity in response to chemical cues produced by fish that can facilitate persistence during changes in

selection regime. Plastic changes that reduce pigmentation (Tollrian & Heibl 2004) and body size (Stibor 1992; Reede & Ringleberg 1995; Spaak et al. 2000; Sakwinska 2002; Reede 2003) in *Daphnia* decrease the ability of fish to detect their prey resulting in higher survivorship, while plastic increases in fecundity (Stibor 1992; Reede & Ringleberg 1995; Reede 2003) result in higher intrinsic rates of population increase.

Daphnia melanica (identified as *Daphnia middendorfiana* in previously published studies (*e.g.*, Bradford et al. 1998; Knapp et al. 2001; Knapp et al. 2005), but recently classified as *D. melanica* based on molecular analyses [M. Pfrender, unpublished data]) populations located in alpine lakes throughout the Sierra Nevada in eastern California, USA provide a unique opportunity to study the effects of introduced predators on naive populations. These alpine lakes have been the subjects of extensive ecological study (Bradford et al. 1998; Knapp et al. 2001; Knapp et al. 2005) in part because the history of fish introductions is well documented. In lakes where *D. melanica* and fish co-occur, *D. melanica* have smaller body sizes and reproduce earlier relative to those in lakes without fish (Fisk et al. 2007). These differences were attributed to rapid adaptive evolution. However, because *Daphnia* are often highly plastic in response to chemical cues from fish, the differences in morphology and life-histories observed previously may not be solely due to underlying genetic alteration. Differences in morphology and life-history could be entirely due to phenotypic plasticity or a combination of plastic and genetic modification.

To determine the relative contributions of adaptive plasticity and genetic adaptation during rapid evolution in response to introduced fish we conducted common-garden experiments on clonally reproducing females of *D. melanica* populations collected from four lakes in the Sierra Nevada with contrasting fish stocking histories. Two lakes were never stocked and remain in their natural fishless condition and two lakes have contained introduced fish populations during the last several decades. We measured morphological and life-history traits of clonally

reproducing females from each population in the presence and absence of chemical cues from fish *(i.e.,* fish kairomone). Because *D. melanica* can be maintained in a state of constant clonal reproduction in the lab, it is straightforward to utilize standard quantitative genetic techniques to estimate the contribution of genetic and plastic phenotypic effects underlying adaptive traits. Our chief working assumption in this experiment is that the phenotypic states of fishless populations are representative of the ancestral phenotypic states of populations that currently contain fish. Given our assumption of equality between currently fishless populations and ancestral states of fish populations is true, our study design allowed us to determine the degree of morphological and life-history differentiation due to selection by fish predation and quantify the contribution of phenotypic plasticity in determining adaptive responses to the introduction of fish.

Materials and Methods

Study Populations

Individual genotypes used in the life-table assay were collected from four permanent lakes in the central Sierra Nevada during the summer of 2004. These lakes are located in the Humphreys, French Canyon, and Vogelsang basins at elevations ranging from 3150-3632 meters. Frog Lake (ID# 52103; UTM Zone 11: 351079 E, 4124432 N) and Source Lake (UTM Zone 11; 349988 E 4125708 N), remain in their natural fishless condition (referred to collectively as fishless populations). Puppet and Evelyn Lakes were naturally fishless but were stocked with trout during the past century. Puppet Lake has been stocked with golden trout (*Oncorhynchus mykiss aguabonita*) every other year since 1951 (California Dept. of Fish and Game, unpublished stocking records), resulting in 53 years of fish predation on the resident *D. melanica* population at the time of collection. Evelyn Lake (UTM Zone 11; 295393 E, 4186659 N) was initially stocked with brown trout (*Salmo trutta*) in 1913. Brook trout (*Salvelinus fontinalis*) were introduced in 1928, 1946, 1947, 1949, 1951, 1954 and 1958, and rainbow trout (*Oncorynchus mykiss*) were

introduced in 1939, 1942, 1944, 1957, 1962, and 1966 (Elliot & Loughlin 2005). No stocking has occurred since 1966, and the resident rainbow trout population is self-sustaining. In total, *Daphnia* in Evelyn Lake were exposed to 91 years of fish predation at the time of collection (Puppet Lake and Evelyn Lake are referred to collectively as fish populations).

Clone Establishment and Maintenance

Daphnia were collected from each of the study lakes and maintained at 4˚C for a period of 1-2 weeks prior to isolation in the lab. To capture the maximum amount of genetic variation from each population, mature females from the original field collection were isolated and placed singly in 250 mL beakers containing 200 mL of filtered well-water. This procedure ensures that no isolates were genotypically identical juveniles produced in the period from collection in the field until isolation in the lab. Isolated individuals were maintained by clonal reproduction under constant conditions of temperature (15˚C) and 16L:8D photoperiod for approximately 20 generations prior to experimentation. Water levels in the beakers were kept constant with the periodic addition of double-distilled water. *Daphnia* were fed a vitamin supplemented pure culture of the green alga *Scenedesmus obliquus* every 3-4 days.

Life-Table Assay

Morphological and life-history characteristics were assayed using a standard experimental design (Lynch 1985; Pfrender & Lynch 2000). Briefly, single immature females were taken from the stock isolates, each representing an experimental line. The lines were then maintained as single asexually produced progeny for two generations. In third generation individuals, we measured a suite of traits upon reaching maturity (defined as the first instar with the deposition of eggs into the brood pouch). Two traits, number of eggs in the brood pouch and size at maturity are directly related to visibility and potential for survival in the face of visually-feeding predators. The two remaining traits, age at maturity and number of viable offspring

produced are related to the intrinsic rate of population increase. Each experimental line was maintained in a 250 mL beaker containing 150 mL of filtered well-water supplemented with a constant concentration (135,000 cells/mL) of *S. obliquus*. Upon reaching maturity, second generation lines assigned to the fish kairomone treatment were placed in filtered well-water aged with a 20-25 cm bull trout (*Salvelinus confluentus*) for 24 hours. (the kairomone treatment is referred to as kairomone(+) and the non-kairomone treatment as kairomone(-)) Exposing second generation individuals to fish kairomone post-maturity ensures that maternal effects due to fish kairomone are minimized. All beakers in the life-table assay were maintained in a controlled temperature room with a 16L:8D photoperiod at 18˚C and their position in the chamber changed every two days to minimize micro-environmental differences. The food/water mixture in all beakers was replaced with food/water of the appropriate type, kairomone(+) or kairomone(-), every other day.

Statistical Procedures

We performed linear regression on egg number and number of surviving offspring upon release of first clutch to determine if egg number serves as a proxy for the more general fitness character of fecundity. Regressions were run on four separate subsets of the data: 1) fishless populations in the kairomone(-) treatment; 2) fishless populations in the kairomone(+) treatment; 3) fish populations in kairomone(-) treatment; and 4) fish populations in the kairomone(+) treatment. Analyzing the subsets separately aided in determining whether a correlation between egg number and viable offspring is sensitive to environmental and/or genetic differences between populations.

Nested analysis of variance (NANOVA) in which covariance parameters were estimated using restricted maximum likelihood was performed on three traits (body size at maturity, age at maturity, and egg number at maturity) to test for fixed effects of treatment (kairomone(+) or kairomone(-)), lake type (fish or fishless), population nested within lake type, and interactions

between environment and lake type, and between environment and population nested within lake type (PROC MIXED; SAS Institute, Inc). The model was designed to account for heterogeneity in covariance matrices across treatments because variance and covariance estimates can vary across environments.

Interpretation of results based on our model is relatively straightforward. A significant treatment effect is evidence for phenotypic plasticity in a given trait, irrespective of a population's fish stocking history. A significant lake type effect implies phenotypic differences between populations in historically fishless and fish-containing lakes. The strength of conclusions about the actual level of genetic differentiation underlying phenotypic divergence is based on the level of significance of the interaction term. For example, a significant effect of lake type in conjunction with non-significant interaction terms would indicate underlying genetic differences among populations regardless of treatment effects.

Plasticity in the expressed genetic variance of traits was assessed by calculating coefficients of variation (CV) for each population across treatments separately. We then constructed 95% Modified McKay confidence intervals for each CV (McKay 1932; Vangel 1996) and assessed differences between estimates based on the degree of overlap of confidence intervals.

Results

Number of Eggs and Fecundity

Due to occasional mortality in the life-table prior to release of first clutch we measured egg number as an index calibration for fecundity to increase our sample sizes. For individuals that had both egg number and number of live offspring measured egg number was a highly significant predictor of the number of viable offspring (all regressions: $p<0.01$). All regressions

(described in more detail in the methods section) showed a positive correlation between the two variables. Correlation coefficients for the data subsets were between 0.43 and 0.57.

Levels of Phenotypic Plasticity

Fish kairomone caused significant reductions in mean body size at maturity for all populations (Fig. 2.1A). In the kairomone(-) treatment, mean body size at maturity for all genotypes was 1.78 mm, while the average size at maturity in the kairomone(+) treatment was 1.70 mm. Non-significant interaction terms suggest that a population's response to fish kairomone is independent of its history of fish introductions. Mean age at maturity did not change in response to fish kairomone (Fig 2.1B). This result appears largely as a consequence of the large variances associated with this trait. The number of eggs in the brood pouch increased in response to fish kairomone (Fig. 2.1C). The number of eggs increased significantly from 4.1 in kairomone(-) to 5.2 in kairomone(+) in response to fish kairomone (Table 2.1). Although there is a tendency for fishless populations to produce more eggs in response to kairomones than fish populations, the difference in reaction norms between fishless and fish populations is not significant so changes in clutch size are also independent of the history of fish introductions.

Because levels of variation for a trait are often context dependent we calculated coefficients of variation for body size, age at maturity, and egg number to determine if the amount of variance in these traits is dependent upon the presence/absence of fish kairomone. Coefficients of variation were lowest for body size at maturity (range 1.5 - 9.8), intermediate for age at maturity (range 10.5 - 22.1), and highest for egg number (range 32.8 – 51.4). Expressed variance showed little response to fish kairomone based on our criterion of non-overlapping confidence intervals. Variance in body size increased significantly only in the Frog Lake population in the presence of fish kairomone (Table 2.2).

Genetic Differentiation Among Populations

Genotypes from fishless populations had significantly larger body sizes at maturity than did genotypes from populations that co-exist with fish (Table 2.1). The average body size of genotypes from fishless lakes was 1.97 mm while genotypes from fish-containing lakes averaged 1.51 mm in size. Post hoc pairwise comparisons based on *t* values between all populations showed that genotypes from fishless populations ("Source" and "Frog") did not differ from one another ($p=0.47$), but are significantly larger than genotypes from Puppet Lake ($p<0.0001$) and Evelyn Lake ($p<0.0001$). Puppet Lake genotypes are also significantly larger than genotypes from Evelyn Lake ($p<0.0001$). A non-significant interaction term implies that these differences are not sensitive to the presence of fish kairomone (Table 2.1).

There was a significant reduction in the number of eggs in the brood pouch, from 5.27 eggs per individual in fishless populations to 4.05 eggs per individual in populations co-occurring with fish (Table 2.1). However, this result appears largely influenced by one population. Post hoc comparisons show that Evelyn Lake genotypes produce significantly fewer eggs than genotypes from Frog, Source, and Puppet Lake ($p=0.0015$, 0.0298, and 0.0241, respectively), but Frog, Source, and Puppet Lakes do not differ in egg production (all possible pairs: $p>0.2602$). A non-significant interaction suggests the difference in egg production between Evelyn Lake genotypes and all others did not depend on the assay environment. Age at maturity did not differ among fish and fishless populations (Table 2.1).

Discussion

Rapid evolution is an important component of the success of invading species (Lee 2002) and the response of organisms in invaded communities (Strauss et al. 2006) because it ameliorates the selective cost imposed by a shift in the phenotypic optimum and enhances the probability of long-term population persistence. Similarly, adaptive plasticity may be an important component of rapid evolution as it can allow short-term population persistence

following changes in the selective environment (Baldwin 1896; Huey et al. 2003) that in turn provides time for evolutionary mechanisms to operate. However, disentangling actual cases of rapid evolution from purely plastic changes in response to a changing environment can be problematic because adaptive phenotypic plasticity is a common feature in many organisms (Pigliucci 2001).

In this study we examined the rapid changes of *Daphnia* morphology and life histories in response to a single abrupt change in the environment. Specifically, we investigated the relative role of genetic and plastic phenotypic changes in moving populations exposed to a novel predator toward a new fitness optimum. Our results show that reductions in egg number and body size of *D. melanica* genotypes from the Sierra Nevada, in response to introduced fish, are largely adaptive evolutionary responses and not due entirely to adaptive phenotypic plasticity. We do find evidence for adaptive plasticity, in the form of increases in clutch size and reductions in body size, in these populations that could facilitate short-term persistence and subsequent rapid evolution. We caution, however, that our interpretation of these results is predicated on the assumption that our measured phenotypes of currently fishless populations are representative of the ancestral phenotypes of populations that currently co-exist with fish.

Our results suggest that naive *D. melanica* populations in the Sierra Nevada may initially respond to fish introductions through adaptive phenotypic plasticity brought about by chemical cues from fish, which facilitates movement towards the new phenotypic optimum. First, plastic reductions in body size make *D. melanica* less visible to fish and constitute evidence for a "Baldwin/Bogart effect" (Baldwin 1896; Huey et al. 2003). Fish are highly effective size-selective predators and their efficiency is primarily linked to prey visibility (Brooks et al. 1965; Galbraith 1967; Zaret & Kerfoot 1975; O'Brien et al. 1979). Thus, *D. melanica* that are less visible have a fitness advantage (via increased survivorship) through movement towards the new phenotypic optimum and the resulting reduction in selection intensity due to decreased predator

efficiency. Concomitant with a decrease in body size, *D. melanica* also show adaptive phenotypic increases in fecundity that could lead to higher intrinsic rates of population increase. Although the rate at which fish remove individuals from these *Daphnia* populations is unknown, our observed increase in clutch size of approximately one is quite significant. Estimates of *D. melanica* population sizes in the Sierra Nevada are on the order of hundreds of millions to billions (R. Knapp, unpublished data), thus, an increase of one individual at first reproduction might substantially offset any losses due to predation. An interesting aspect of our findings is that our naive *Daphnia* populations, those without any history of fish exposure, are responsive to chemicals produced by fish. This observation suggests that *D. melanica* may be pre-adapted to fish predation, and that the genetic machinery responsible for adaptive phenotypic plasticity in response to fish kairomone is ancestral in this species.

Although we find evidence for adaptive phenotypic plasticity that would facilitate short-term population persistence in the face of novel predation, it is not sufficient to explain the difference in body-size and egg number between populations that are historically fishless and those that co-occur with fish. For example, a comparison of the average body size in the Evelyn Lake population (1.34 mm in the kairomone(-) and 1.24 mm in the kairomone(+) treatments) with the average in the fishless populations (2.00 mm in the kairomone(-) and 1.95 mm in the kairomone(+) treatments) shows that the mean phenotype in Evelyn Lake has diverged by 9.4 phenotypic standard deviations. The change in body size attributable to plasticity in fishless populations is approximately one standard deviation. In other words, the change in body size due to plasticity accounts for only about 11% of the total difference observed between Evelyn and fishless populations. Thus, the phenotypic differences observed in our study appear largely due to changes in the underlying genetic components controlling phenotype.

Our observation that the body-size response in Evelyn Lake was much higher than that in Puppet Lake could arise for three reasons. First, the difference in body size could simply reflect

the different amounts of time each population was exposed to fish predation (Puppet Lake – 53 years; Evelyn Lake – 91 years). Second, *Daphnia* populations may have experienced differing levels of fish predation resulting in varying selection intensities, with the selection intensity in Evelyn Lake substantially higher. Finally, given our observation that expressed levels of genetic variance for body size increased approximately 6-fold in response to fish kairomone in one fishless population (Frog Lake) but not in the other (Source Lake) our fish populations may have differed in the initial levels of standing genetic variation, with the Evelyn Lake population harboring more standing genetic variation than Puppet Lake.

Our observation that Evelyn Lake was the only population to display a significant evolutionary reduction in egg number is likely due to the ability of *D. melanica* to deposit large amounts of melanin in the carapace. Melanin production in the carapace would initially "blind" selection to changes in egg number. Thus, an evolutionary response in egg number should occur only after reductions in melanin deposition. Fish predation produces strong selection on melanin production in other *Daphnia* populations (Saegrov et al. 1996), and there is evidence for reduced melanin expression in *Daphnia* from our fish populations relative to fishless populations (M. Pfrender, unpublished data). Therefore, the apparent delayed onset of selection on egg number could be due to initial selection on melanin production and subsequent selection on egg number.

Traditional views of character evolution typically involve trade-offs among traits that can limit the adaptive potential of a population (Roff 2002). However, several selection experiments involving *Daphnia* suggest adaptive evolutionary changes in one trait are not necessarily associated with concomitant maladaptive changes in others (Spitze 1991; Spitze et al. 1991; Baer & Lynch 2003). We observe a similar result here, where evolutionary and plastic changes in body size and fecundity occur in the absence of changes in the timing of maturity and reproduction. Our results, and those of other researchers that imply the absence of a trade-off, could be attributed to assay conditions in which food is not a limiting resource (Reznick et al.

2000). *Daphnia* morphology and life-history can display food concentration-dependent reactions to the presence of fish kairomone (Weetman & Atkinson 2002).

In conclusion, we investigated the relative contributions of selection and adaptive phenotypic plasticity to the rapid evolution of morphology and life histories in response to an introduced predator. We conclude that adaptive plasticity could facilitate short-term population persistence through "Baldwin/Bogart effects," but that long-term persistence was achieved through subsequent genetic adaptation. Further investigation into other traits that may have also undergone rapid change in selective regime as a consequence of fish introductions, such as pigmentation and DVM behavior, examined under differing kairomone and food conditions, will provide a more detailed view of the traits and processes involved in the overall evolution of the *Daphnia*/fish predator-prey system in the Sierra Nevada.

Numerous studies have examined the contributions of plasticity and selection to rapid adaptation in non-native species following their introduction into a novel environment (*e.g.*, Dybdahl & Kane 2005). In contrast, few studies have examined the phenotypic and evolutionary response of native species to introduced species that pose strong novel selective challenges. Thus, this study and a growing body of others investigating the response of native communities to introduced species should enhance our understanding of the conditions necessary for rapid adaptive evolution and the relationship between rapid evolution and population persistence (Strauss et al. 2006).

Table 2.1. Results from NANOVA for morphological (size) and life-history (age and egg number) traits. Shown are the degrees of freedom (df), F-values (F) and p-values (p). Significant results ($p<0.05$) are indicated in bold.

	Trait								
	Size			**Age**			**Egg #**		
Effect	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Environment	1/60	6.87	**0.0111**	1/60	0.81	0.3726	1/60	5.41	**0.0235**
Type	1/60	278.56	**<0.0001**	1/60	0.42	0.5196	1/60	5.85	**0.0186**
Pop(Type)	2/60	66.06	**<0.0001**	2/60	1.79	0.1760	2/60	3.11	0.0519
Environment X Pop(Type)	2/60	0.02	0.9776	2/60	3.13	0.0507	2/60	0.12	0.8890
Environment X Type	1/60	0.71	0.4014	1/60	0.00	0.9945	1/60	3.35	0.0723

Table 2.2. Estimates of phenotypic means and coefficients of variation. The units for body sizes are mm and for ages at maturity are days. Standard errors for the mean and 95% Modified McKay confidence intervals for coefficients of variation are given in parentheses.

				Treatment			
				Kairomone (-)		Kairomone (+)	
Population	**N**	**Exposure**	**Trait**	**Mean (SE)**	**CV (95% CI)**	**Mean (SE)**	**CV (95% CI)**
Source	7	0	*Size*	2.01 (0.04)	5.0 (3.2-10.9)	1.96 (0.04)	5.1 (3.3-11.3)
			Age	9.82 (0.50)	13.4 (8.6-30.3)	9.65 (0.38)	10.5 (6.7-23.4)
			Egg #	4.00 (0.72)	47.8 (28.9-165.2)	5.86 (0.74)	33.3 (20.8-86.9)
Frog	9	0	*Size*	1.98 (0.01)	1.5 (1.0-2.9)	1.93 (0.06)	9.8 (6.6-19.0)
			Age	10.18 (0.65)	19.2 (12.8-38.1)	9.63 (0.38)	11.7 (7.9-22.8)
			Egg #	4.44 (0.60)	40.8 (26.3-95.2)	6.78 (1.09)	48.2 (30.7-125.4)
Puppet	10	53	*Size*	1.77 (0.03)	6.2 (4.3-11.4)	1.68 (0.04)	7.7 (5.3-14.2)
			Age	10.94 (0.64)	18.6 (12.7-34.5)	9.21 (0.53)	18.2 (12.4-34.3)
			Egg #	4.60 (0.48)	32.8 (22.0-66.6)	5.10 (0.72)	44.7 (29.3-101.5)
Evelyn	8	91	*Size*	1.34 (0.03)	6.0 (3.9-12.2)	1.24 (0.02)	5.6 (3.7-11.5)
			Age	8.54 (0.46)	15.2 (10.0-31.8)	9.55 (0.75)	22.1 (21.5-47.6)
			Egg #	3.25 (0.59)	51.4 (31.8-163.2)	3.25 (0.45)	39.4 (25.0-99.1)

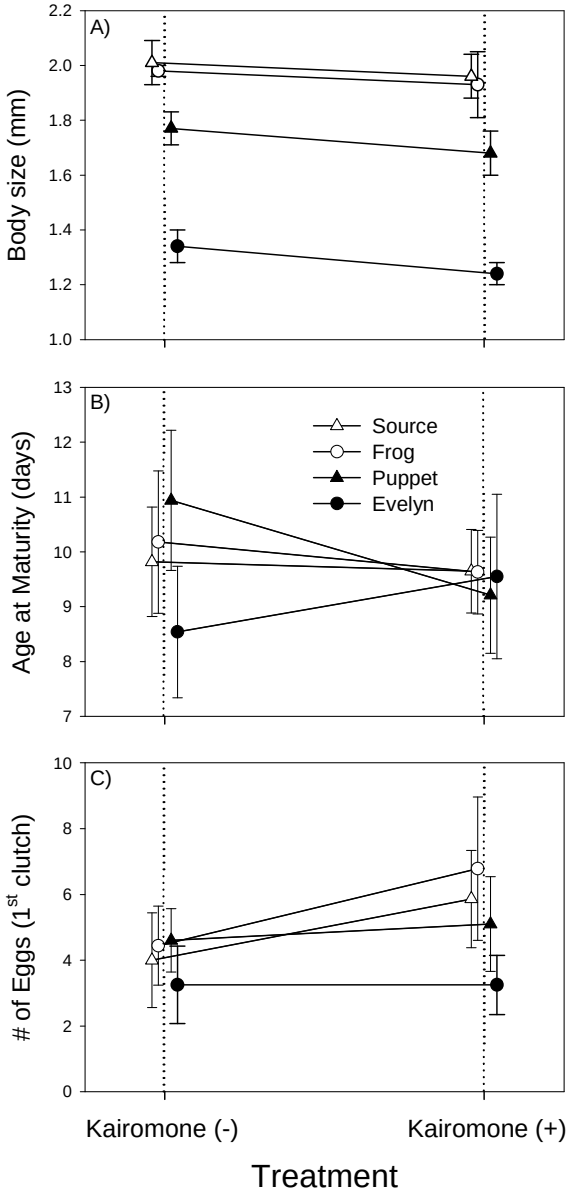


Figure 2.1. Reaction norm plots for a) body size, b) age at maturity, and c) number of eggs in response to presence (+) or absence (-) of fish kairomone. Open symbols left of the vertical dotted lines are values for fishless populations, filled symbols right of the vertical dotted lines are values for fish-containing populations. Error bars are +/- one standard error of the mean.

CHAPTER 3

VERTICAL DISTRIBUTION OF CHLAMYDOMONAS CHANGES IN RESPONSE TO GRAZER AND PREDATOR KAIROMONES

Abstract

Individuals in aquatic communities frequently assess their biotic environment through infochemicals. In particular, kairomones are commonly involved in interactions between predator and prey. However, the relationship between individuals and chemicals produced by other organisms that are not direct predators, but may indicate the presence of a predator, is not well characterized. We used experimental microcosms to test whether the unicellular green alga *Chlamydomonas reinhardtii* alters vertical migration patterns in response to kairomones produced by zooplankton (*Daphnia*) and planktivores (fish). Our results suggested that phototaxis in *C. reinhardtii* was strongly affected by the type of kairomone present, the concentration of the kairomone, and the duration of exposure to the kairomone. Kairomones generally increased phototaxis in *C. reinhardtii*. The adaptive significance of such behavioral changes in natural settings would depend largely on local community composition. The similarity in phototactic responses of *C. reinhardtii* to *Daphnia* and fish kairomone suggest that, in at least this species of phytoplankton, the underlying genetic elements responsible for kairomone detection may be responsive to a broad range of chemical stimuli, allowing this species to adjust its phototaxis in response to not only the presence of its grazers, but also to predators of its grazers.

Introduction

In many communities, infochemicals provide a means for individuals to assess the biotic environment. Kairomones are a class of infochemicals that benefit the receiver but do not benefit the sender. Kairomones have received extensive attention because the changes in behavior,

morphology, and life history they elicit are often easily characterized (Lass and Spaak 2003; Pohnert et al. 2007; Van Donk 2007).

Kairomones frequently manifest in predator-prey interactions whereby prey chemically detect the presence of potential predators. A well characterized kairomone-mediated interaction between predator and prey is that between *Daphnia* and their vertebrate and invertebrate predators. For example, in response to fish kairomone *Daphnia* reduce body size and increase fecundity (Stibor 1992; Reede 1995; Chapter 2), display increased escape ability (Brewer et al. 1999), and reduce the amplitude of diurnal vertical migrations (De Meester 1993).

Kairomones produced by zooplankton such as *Daphnia* elicit adaptive responses in phytoplankton. Unicellular green algae belonging to the genera *Scenedesmus* and *Desmodesmus* exposed to kairomones produced by numerous zooplankton species form colonies and, in some species, long rigid spines, both of which increase resistance to grazing by zooplankton (Hessen and Van Donk 1993; Lürling 2003). *Daphnia* kairomones also induce behavioral changes in some phytoplankton species. *Gonyostomum semen* and *Peridinium* sp. exhibit lower rates of recruitment into the water column in the presence of *Daphnia* (Hansson 2000).

The vertical distribution of phytoplankton in naturally occurring freshwater lakes also varies in response to the resident zooplankton community (e.g. Arvola et al. 1992), suggesting behavioral responses to zooplankton kairomones. However, studies in natural lakes are difficult to interpret because the distribution of phytoplankton is both indirectly affected by kairomones and directly affected by grazing. Thus, the vertical distribution of phytoplankton may be a reflection of zooplankton consumption and not a direct response to zooplankton kairomones.

Largely ignored in kairomone research is the response of individuals to kairomones produced by organisms that are not direct consumers, but that may indicate the presence of a consumer, such as the relationship between fish kairomones and phytoplankton. There is evidence that the cryptomonad *Plagioselmis prolonga* var. *nordica* produce longer tails in the

presence of the silver carp *Hypophthalmichthys molitrix* Val. (Kim et al. 2003). However, these experiments were conducted in mesocosms that also contained zooplankton, and thus the morphological response in *P. prolonga* may have been a direct effect of changes in zooplankton density and not related to the presence of fish kairomone.

The unicellular green alga *Chlamydomonas reinhardtii* provides an ideal organism with which to investigate kairomone-mediated changes in vertical distribution. They are distributed worldwide in freshwater ecosystems and are a common food source for naturally occurring zooplankton populations. They are approximately 10 µm in length and swim using two flagella. Wild-type strains display positive phototaxis during the day to maximize photosynthesis (Bruce 1970) and use chemotaxis at night to acquire nitrogen sources (Byrne et al. 1992). In environments with ideal temperatures (20-25° C), constant light, and sufficient nitrogen availability haploid vegetative cells reproduce mitotically to produce clonal haploid daughter cells every 5-8 hours. Cultures of *C. reinhardtii* can be entrained, using regular light:dark photoperiods, to liberate daughter cells once every 24 hours by exploiting the underlying circadian control of the cell division cycle (Goto and Johnson 1995).

To investigate the effects of kairomones produced by grazers and predators we examined the response of *C. reinhardtii* to kairomones produced by zooplankton (grazers) and zooplanktivores (predators). In the lab we constructed microcosms containing a population of *C. reinhardtii* in water aged with grazers (*Daphnia*), predators (fish), and both. We then measured the response of the phytoplankton by assessing their vertical distribution after 3 and 24 hours of exposure to kairomones. We were particularly interested in assessing whether or not phytoplankton can respond to kairomones produced by organisms that have direct effects on phytoplankton population dynamics (zooplankton), as well as kairomones produced by organisms that have indirect effects on phytoplankton population dynamics (fish). We discuss our results in

the context of the potential advantages changes in behavior may offer natural populations. We also offer ideas on the chemical nature of kairomones suggested by our results.

Material and methods

Organisms

The subject of this study was strain CC-1928 of *C. reinhardtii*, acquired from the Chlamydomonas Culture Collection (www.chlamy.org). The strain was semi-continuously cultured in an aerated 5 liter (L) carboy containing 4 L of modified Bold's Basal Medium (BBM; Stein 1973). Every 2-3 days 2 L of fluid were removed from the carboy and replaced with fresh BBM. Algal cultures were maintained in an 18L:6D photoperiod at 20° C in order to entrain our cultures to liberate mitotically-produced daughter cells once every 24 hours. Because we clonally propagated a single strain of *C. reinhardtii* with normal phototactic responses, there is essentially no genetic variation among our treatments. This lack of genetic variation is convenient for the primary purpose of our investigation, because it eliminates the potential confounding effects of genetic variation among treatments.

Grazer kairomone water was created by isolating several hundred individual *Daphnia pulex*, whose diet consisted of the unicellular green alga *Scenedesmus obliquus*, and placing them in 4 L of filtered well-water for 24 hours. Predator kairomone water was obtained from a 110 L aquarium containing two tinfoil barbs (*Barbonymus schwanenfeldii*; 10-12 cm length), a potential predator of *Daphnia*. The diet of the fish was comprised of fish flakes and fish pellets that did not contain *Daphnia*. Prior to use in microcosms the kairomone water was filtered through 165 µm nitex mesh to remove particulate matter, or in the case of grazer kairomone water, the *Daphnia*.

Experimental Design

The experiment consisted of five treatments: 1) a control treatment of pure filtered well-water, 2) a 100% grazer kairomone water treatment, 3) a 50% grazer kairomone treatment consisting of half grazer kairomone water and half filtered well-water, 4) a 100% predator kairomone treatment, and 5) a grazer/predator kairomone treatment consisting of 50% grazer kairomone water and 50% predator kairomone water. To prepare treatments, we centrifuged 2 L of our *C. reinhardtii* culture at 3000 rpm for 3 min and 15 sec. This centrifugation concentrates the algae into a slurry at the bottom of the sample. Based on previous experiments in which we centrifuged cells and then exposed them to top-lighting to promote phototaxis we determined that centrifugation does not cause significant mortality or injure *C. reinhardtii* flagella substantially enough to cause a noticeable reduction in motility at the population level (unpublished data). We removed the supernatant and added 100 mL of distilled water to this slurry and resuspended the algal cells by gentle mixing. We then added 10 mL of this concentrated *C. reinhardtii* into 500 mL of each kairomone treatment or control water.

Microcosms were established in 25 mL Falcon serological pipettes filled with 25 mL of control or treatment water mixed with *C. reinhardtii*, and sealed at the bottom with parafilm. Initially, the density of *C. reinhardtii* in each microcosm was equal and individuals were evenly distributed throughout the water column. Over the course of the experiment the microcosms were maintained at 18° C in a top-lit controlled temperature room.

Ten replicates for each treatment were divided evenly into two sampling periods. After three hours of exposure to top lighting, five replicates were randomly chosen and destructively sampled by placing the bottom, middle, and top 2 mL of fluid from each microcosm in eppendorf tubes using a pipette pump. The remaining replicates were exposed to a 10L:8D:6L photoperiod. The dark period reset phototactic responses in each microcosm sample. After 24 hours (6 hours after the lights turned on in the morning) these five replicates for each treatment were destructively sampled in the same manner as the first five.

We froze samples immediately after collection to kill the individual cells and prevent *C. reinhardtii* from a normal phototactic response towards a spectrophotometer beam. This protocol ensures accurate estimates of cell density in a spectrophotometer. We thawed and mixed each sample and used a ThermoSpectronic Genesys 20 spectrophotometer to measure % light transmittance. An index of *C. reinhardtii* density was estimated as 1 - (% transmittance). We also measured % light transmittance in treatment water samples prior to seeding with *C. reinhardtii* and used these values to correct for differences in baseline transmittance due to water aged with live organisms.

Statistical Analyses

For each group of samples corresponding to the top, middle, or bottom layer in the microcosms we used two-factor ANOVA, with treatment and time as main effects, for analysis (SAS Institute Inc. 2004). We performed ANOVA on the entire dataset corresponding to each microcosm level. We then compared the control group to the 100% grazer treatment to test whether *C. reinhardtii* responds directly to kairomones produced by a grazer. We also compared the 50% grazer treatment to the 100% grazer treatment to determine whether the behavioral response is concentration dependent.

We tested whether *C. reinhardtii* responds directly to kairomones produced by predators, and whether the presence of predator kairomones alters *C. reinhardtii* response to grazer kairomones. We compared the control group to the predator treatment to test for a direct response to predator kairomones. We also compared the 50% grazer treatment to the grazer/predator treatment, both of which had equal concentrations of grazer kairomone.

Results

Cell Density at the Surface

Cell density of *C. reinhardtii* in the top 2 mL of fluid in the microcosms was significantly affected by the type of kairomone and the duration of exposure to the kairomone (ANOVA; $p<0.0001$). Specifically, grazer kairomones induced phototactic movement that resulted in higher cell density at the surface than in untreated water (Fig 3.1A); however, this response did not differ over a doubling of concentration (Figure 3.2A; Table 3.1). Predator kairomone did not directly affect cell density, but predator and grazer kairomone combined significantly reduced cell density relative to grazer kairomone alone (Figure 3.2A; Table 3.1). This effect of multiple kairomones was only manifest in the 6 h sampling period.

Cell Density in the Middle

Density of *C. reinhardtii* in the middle 2 mL of fluid in the microcosms was significantly affected by the type of kairomone present (ANOVA; $p=0.0002$). *C. reinhardtii* density in the presence of grazer kairomones was higher than untreated water (Figure 3.1B) and was independent of time and kairomone concentration (Figure 3.2B; Table 3.1). Cell density in the middle sample was not significantly changed by predator kairomone or a combination of grazer and predator kairomone (Figure 3.2B; Table 3.1).

Cell Density at the Bottom

Cell density in the bottom 2 mL of fluid in the microcosm was significantly affected by the type of kairomone present and the duration of time exposed to the kairomone (ANOVA; $p<0.0001$). In response to grazer kairomone, density estimates decreased relative to controls and the difference in density between control and grazer kairomone treatments was highest after 24 h of exposure (Figure 3.1C; Table 3.1). Density was also significantly reduced as grazer kairomone concentration increased and the difference was most pronounced after 24 h of exposure (Figure 3.2C; Table 3.1). Density was also significantly reduced by predator kairomone and a

combination of grazer and predator kairomone. As in the surface sample, these differences were most pronounced after prolonged exposure to kairomone (Figure 3.2C; Table 3.1).

Discussion

The vertical distribution of *C. reinhardtii* in our microcosms was strongly affected by the type of kairomone(s) present, the concentration of the kairomone, and the duration of exposure to the kairomone. Overall, the general response of *C. reinhardtii* to kairomones was increased cell density in the water column or near the surface as evidenced by significantly high density estimates at the middle and top of microcosms containing kairomone and/or significantly low density estimates at the bottom of microcosms containing kairomone. Although we did not specifically measure the rate of movement of individual cells, the estimated swimming speed of *Chlamydomonas* cells in response to light is approximately 0.5 m/hr (Berthold et al. 2008). Thus, our results suggest that natural populations of *Chlamydomonas* may undergo vertical shifts of several meters due to kairomone-dependent phototaxis.

Our interpretation of these results is that kairomones induce a stronger phototactic response in *C. reinhardtii* than water that does not contain kairomones. Phototaxis in *C. reinhardtii* is controlled through an underlying circadian rhythm (Bruce 1970). During the day, individuals swim maximally towards light sources in order to optimize photosynthesis. Kairomones produced by a potential grazer, in this case *Daphnia*, have the effect of increasing phototaxis resulting in more individuals in the water column or at the surface. The response to *Daphnia* kairomone also showed concentration dependence in the lowest level of our microcosms with higher concentrations of kairomone inducing a stronger phototactic response. Such a response may appear adaptive in that more individuals would be exposed to light sources for use during photosynthesis. However, the response is only adaptive in specific ecological settings.

For example, in lake communities that contain only zooplankton and phytoplankton, increased phototaxis in phytoplankton may be maladaptive. In the absence of visually-feeding

predators, zooplankton frequently do not exhibit diel vertical migration and are able to exert continuous grazing pressure which can reduce the growth rate of phytoplankton populations (Reichwaldt et al. 2004). Alternatively, in lake communities with populations of planktivorous fish, zooplankton, and phytoplankton, increased phototaxis in phytoplankton may be an adaptive strategy because fish induce DVM in zooplankton such that during the day, when visual predators are active, zooplankton reside near the bottom (De Meester 1993). Thus, daytime phytoplankton movement into the water column and away from resident zooplankton populations should act to reduce individual mortality because of the discontinuous grazing pressure that results from the daily migration of phytoplankton away from zooplankton.

Phototaxis in *C. reinhardtii* also increased when exposed to predator kairomones. For natural populations, this result suggests that phytoplankton may be able to detect the presence of their grazers indirectly through predators of their grazers. This behavior could be of great utility when zooplankton population density varies seasonally as individuals could still detect the presence of grazers even when grazer density is low.

The photosensory and chemosensory pathways in *C. reinhardtii* share common elements as the addition of specific chemoeffectors can inhibit phototaxis (Ermilova et al. 1997; Govorunova and Sineshchekov 2003). These results bear on two aspects of our study. First, a caveat to our study is that we cannot rule out that differences in nutrient concentrations and ratios among treatments may have contributed to the different phototactic responses we observed. Water used in our experimental treatments had been previously inhabited by live animals and thus kairomones as well as nutrients, such as nitrogen and phosphorous compounds, may have differed among treatments. If the phototactic response in *C. reinhardtii* is influenced by nutrient levels then the responses we observed in our experiment may not solely reflect differences in the type of kairomone.

Second, our conclusion that the similarity in phototactic response to *Daphnia* and fish is due to kairomones suggests a few possibilities on the nature of the chemoreceptor and photosensory systems in *C. reinhardtii*. *Daphnia* and fish kairomones may have a similar chemical structure that can be detected by the same chemoreceptor, or different chemoreceptors are involved in detection, but the transduction pathways converge at some junction and result in the same response. The chemical nature of *Daphnia* and fish kairomones is not well resolved, but aliphatic sulfates have been identified as a candidate class of *Daphnia* chemicals known to induce morphological defenses in phytoplankton (Yasumoto et al. 2005; Yasumoto et al. 2006). Given the vast array of genomic tools and complete genome sequence available for *C. reinhardtii*, a functional genomic approach using microarray experiments could be utilized to address the effect of nutrients on phototaxis as well as the nature of the signal transduction cascade that arises from exposure to different kairomones.

In conclusion, phototaxis in *C. reinhardtii* is responsive to kairomones produced by both grazers and predators. However, the adaptive significance of the behavioral change would be context dependent varying with the community composition in natural settings. The similarity in response to kairomones produced by different organisms may also lend insight into the characterization of the specific chemicals that induce morphological, life history, and behavioral changes in other taxa known to respond to kairomones. Furthermore, the similarity in phototactic response to *Daphnia* and fish kairomone suggest that, in at least this species of phytoplankton, the underlying genetic elements responsible for kairomone detection may be responsive to a broad range of chemical stimuli and endow the individual with a broader knowledge of the prevailing biotic environment, allowing this species to adjust its phototaxis in response to not only the presence of its grazers, but also to predators of its grazers.

Table 3.1. Results from Two-Factor ANOVA. Degrees of freedom (df), F values, and level of significance (p) for Type III sums of squares for each source of variation corresponding to specific comparisons for the top (T), middle (M), and bottom (B) sample collected from the microcosms.

	Source of Variation								
	Treatment			Time			Interaction		
Comparison	df	F value	p	df	F value	p	df	F value	p
All (T)	4	10.3	<0.0001	1	12.0	0.0013	4	3.1	0.0254
All (M)	4	9.0	<0.0001	1	1.0	0.3361	4	1.7	0.1794
All (B)	4	23.3	<0.0001	1	30.0	<0.0001	4	27.2	<0.0001
Control v 100% Daphnia (T)	1	7.9	0.0128	1	2.2	0.1583	1	4.1	0.0601
Control v 100% Daphnia (M)	1	11.0	0.0044	1	2.7	0.1227	1	1.3	0.2763
Control v 100% Daphnia (B)	1	72.4	<0.0001	1	0.2	0.7014	1	106.6	<0.0001
50% Daphnia v 100% Daphnia (T)	1	2.6	0.1241	1	0.1	0.7443	1	0.0	0.9171
50% Daphnia v 100% Daphnia (M)	1	2.9	0.1100	1	0.0	0.9900	1	0.2	0.6353
50% Daphnia v 100% Daphnia (B)	1	35.1	<0.0001	1	18.3	0.0006	1	19.4	0.0004
Control v Fish (T)	1	1.6	0.2194	1	25.6	0.0001	1	0.1	0.7534
Control v Fish (M)	1	0.0	0.9628	1	0.2	0.6756	1	4.4	0.0512
Control v Fish (B)	1	231.7	<0.0001	1	7.3	0.0155	1	319.2	<0.0001
50% Daphnia v Fish/Daphnia (T)	1	9.4	0.0074	1	5.1	0.0380	1	6.4	0.0225
50% Daphnia v Fish/Daphnia (M)	1	4.2	0.0586	1	0.8	0.3937	1	1.8	0.1979
50% Daphnia v Fish/Daphnia (B)	1	8.5	0.0103	1	5.5	0.0324	1	5.9	0.0271

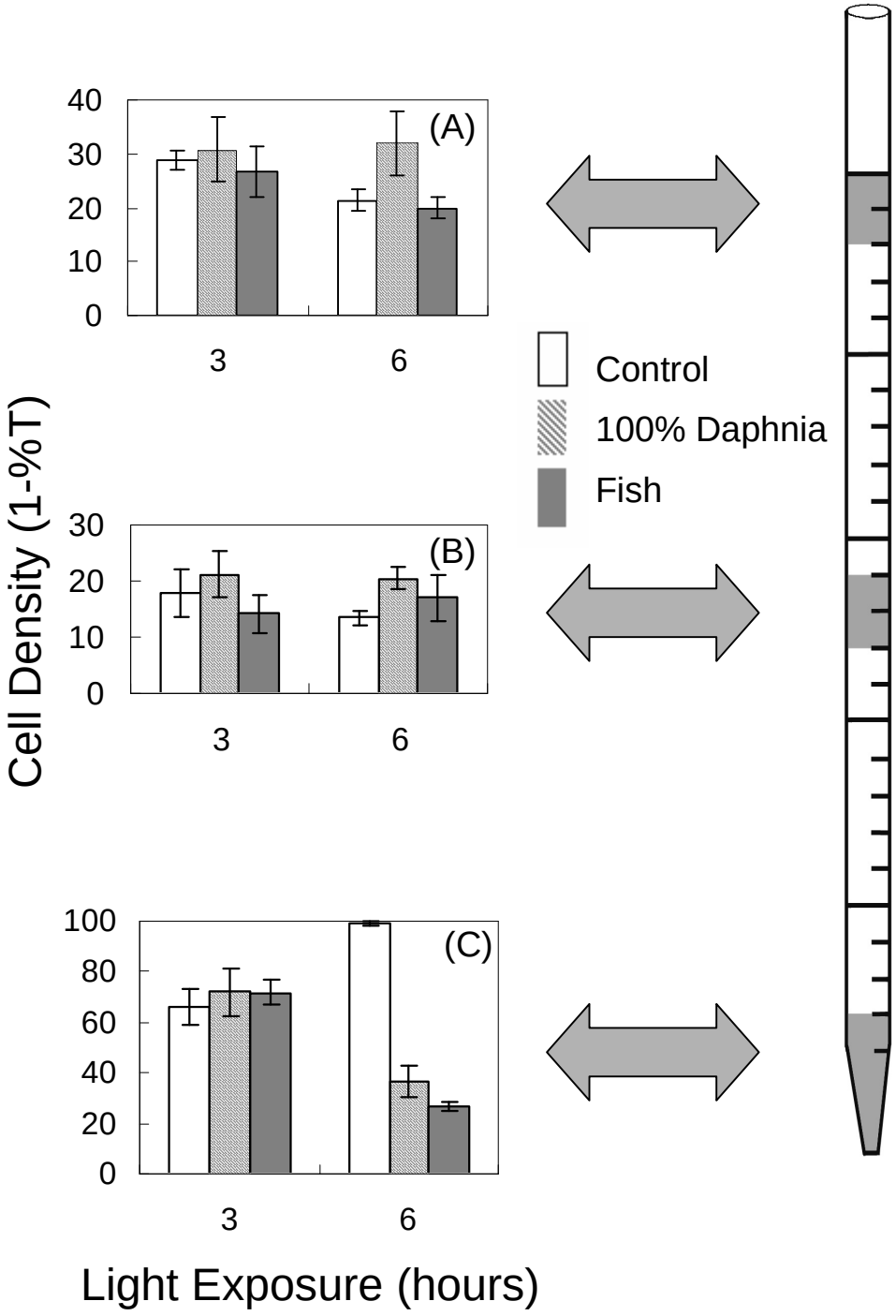


Figure 3.1. Cell density estimates for the A) top, B) middle, and C) bottom of the experimental microcosms depicting the direct response to *Daphnia* and fish kairomone. Error bars are ± 2 SE.

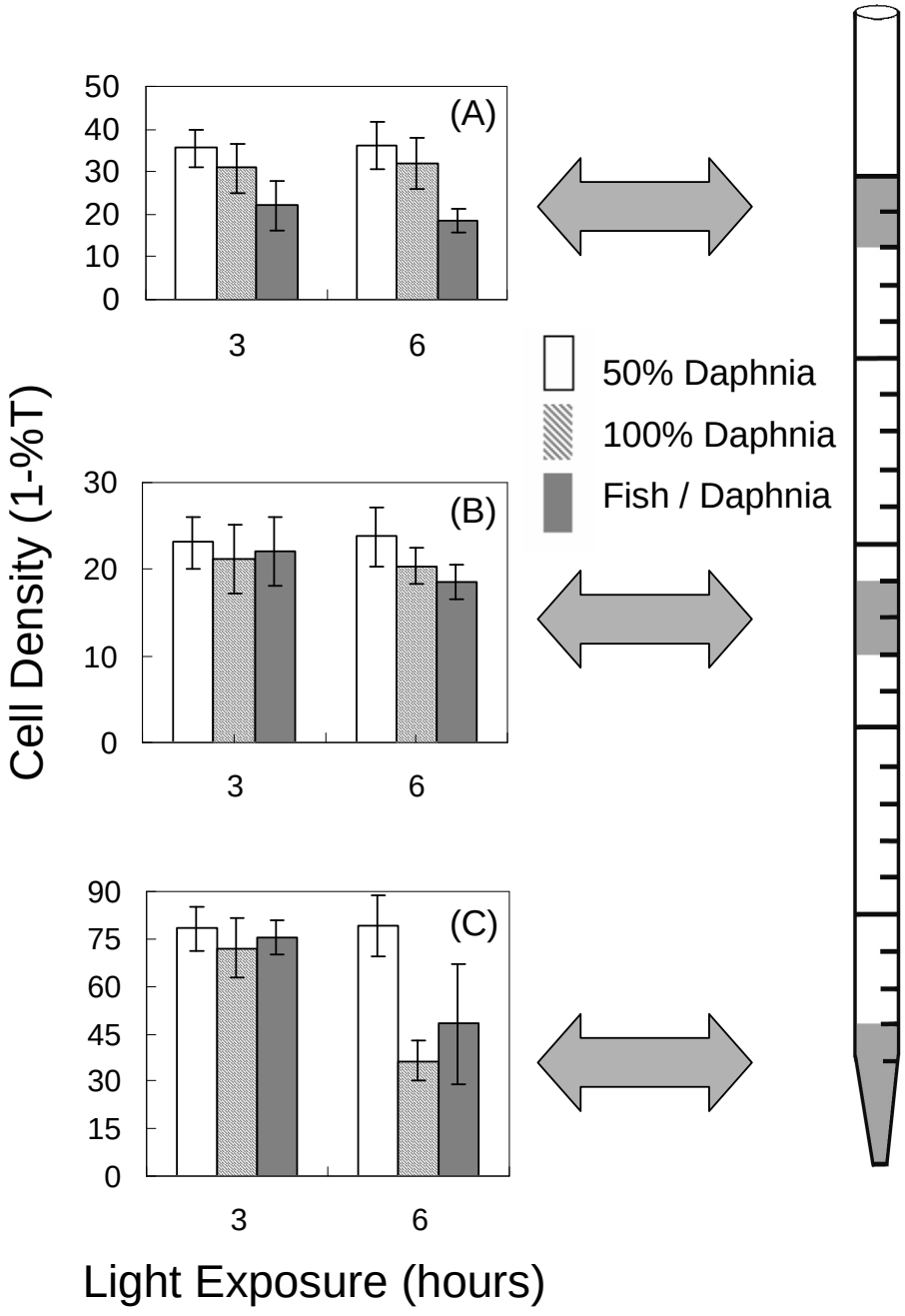


Figure 3.2. Cell density estimates for the A) top, B) middle, and C) bottom of the experimental microcosms depicting concentration dependence and the effect of simultaneous exposure to multiple kairomones. Error bars are ± 2 SE.

CHAPTER 4

DIET RESTRICTION AND LONGEVITY IN SHORT-AND LONG-LIVED SPECIES

Abstract

The life-extending effects of caloric restriction are well-documented in numerous short-lived taxa. Unresolved is whether enhanced longevity mediated by caloric restriction extends to long-lived taxa, such as humans. To address this issue a majority of studies have used observational and epidemiological data on humans or non-model organisms, particularly rhesus monkeys. In this study, we propose an alternate approach to address the effects of caloric restriction on long-lived organisms by using short-lived model organisms with widely divergent lifespans that parallel the different lifespans in the currently utilized non-model organisms. We conducted a common-garden experiment that included two sister-species of *Daphnia* where the lifespan of one species, *D. pulicaria*, is two to three times greater than that of its sister species, *D. pulex*. Our study provides clear evidence that the short-lived species in our study, *D. pulex*, shows the classically observed relationship of enhanced lifespan in response to reduced caloric intake. However, we find no evidence that the long-lived species in our study, *D. pulicaria*, gains any life-extending effects through diet restriction. Our results suggest that the manipulation of lifespan through diet intervention in long-lived taxa, such as humans, may not be plausible.

Introduction

The life-extending effects of caloric restriction are well documented in numerous short-lived taxa including rats, mice, dogs, hamsters, fish, invertebrates, and yeast (Masoro 2002). Unresolved is whether enhanced longevity mediated by caloric restriction extends to long-lived taxa, such as humans. To address the question of dietary effects on human longevity, research largely relies on using closely related long-lived primates, in particular rhesus monkeys (Roth et al. 2004), or observational and epidemiological studies in humans.

Evidence from on-going studies on rhesus monkeys suggests that caloric restriction provides many of the beneficial changes in body composition, metabolism, and maturation and reproduction (Lane et al. 1997; Mattison et al. 2003) observed in caloric restricted short-lived taxa such as mice. Preliminary evidence also suggests caloric restriction in monkeys increases survivorship late in life (Colman et al. 2009). Evaluation of the human population in Okinawa (Willcox et al. 2006), alternate day feeding trials on human males (Vallejo 1957), and preliminary results from controlled trials on non-obese humans (Redman et al. 2008), also suggest that caloric restriction may extend lifespan in humans. Although the information gathered from these studies is obviously most closely relevant for humans, the drawback is that monkeys and humans are long-lived, and thus controlled experiments that accurately describe the relationship between diet and lifespan require several decades to complete.

This study proposes an alternative to these long-term studies on monkeys and humans by utilizing closely related pairs of short-lived taxa with different lifespans. In particular, we utilize a common-garden experiment that includes two sister-species of *Daphnia* where the lifespan of one species, *D. pulicaria*, is two to three times greater than that of its sister species, *D. pulex*. *Daphnia* are ideally suited for the investigation of dietary effects on longevity. First, there is a rich history documenting the relationship between food concentration and life-history strategies for numerous species of *Daphnia* (*e.g.* Ingle 1933; Ingle et al. 1937; Vijverberg 1976; Lynch 1989). Second, closely related hybridizing species of *Daphnia* often employ widely divergent life-history strategies that translate into large differences in lifespan. Finally, *Daphnia* are easily cultured in the laboratory and can be reared clonally so that specific genotypes of interest can be maintained indefinitely.

Our goal in this study was to examine the reaction norms that describe the relationship between food concentration and morphological or life-history traits in clones of short- and long-lived sister species of *Daphnia*. By manipulating food concentration we effectively produced

treatments of varying caloric content while the measurement of several fitness and fitness-related traits allowed us to evaluate potential trade-offs that may have facilitated differences in lifespan. Our results show that the life-extending effects of dietary restriction are manifest in the short-lived species, *D. pulex*, and that there were no apparent tradeoffs to explain the increase in lifespan. Conversely, the long-lived species, *D. pulicaria*, showed no relationship between caloric content and lifespan. These results suggest that caloric restriction may not extend life in long-lived taxa, and raises questions about the potential for increasing human lifespan through dietary restriction.

Material and Methods

Study Organisms

We used a single clone from each of two species of *Daphnia* collected from the Midwestern USA in our common-garden experiment. One clone represented a short-lived *Daphnia* species, *D. pulex*, while the second clone represented a long-lived *Daphnia* species, *D. pulicaria*. These two species are sister species in the *Daphnia* subgenus and can readily hybridize both in the wild and in the laboratory.

The primary factor that explains the wide divergence of lifespan in these organisms is their unique habitat use. *Daphnia pulex* is found in temporary ponds while *D. pulicaria* inhabits permanent lakes. The *D. pulex* clone used in this study was collected from a pond that is only habitable for a few months during the year, but that typically has high food levels. Because the pond dries by summer, the maximum lifespan of this population is constrained to a few months. Alternatively, the *D. pulicaria* clone used in this study is from a permanent lake with much lower food availability. In this population, lifespan is not constrained because the lake is habitable year-round.

Common garden experiments

Morphological and life-history characteristics were assayed using a standard experimental design (Lynch et al. 1999; Pfrender & Lynch 2000). Briefly, single immature females of each clone were taken from the stock isolates, each representing an experimental line. The lines were then maintained as single asexually-produced progeny for two generations. In third generation individuals we measured three traits (body size, egg number, and time) upon reaching maturity, defined as the first instar with the deposition of eggs into the brood pouch, and longevity. Individuals that did not reach maturity were not included in estimates of longevity. Some of the traits we measured (specifically, body size and egg number) required handling of the *Daphnia*. Thus, in order to minimize the potential effects of handling on longevity we randomly divided individuals into two groups. One group was used to assay the number of eggs in the brood pouch and body size upon reaching maturity, while the second group was used to assay longevity. Because the primary focus of our investigation was the effects of diet restriction on longevity we inflated the sample size for the second group so that approximately two-thirds of the individuals were assigned to this group.

To establish varying food concentrations each experimental line was maintained in a 250 mL beaker containing 100 mL of 10% Bold's Basal Medium (Stein 1973) supplemented with a controlled concentration of the unicellular green alga *Scenedesmus obliquus*. We exposed individuals of each clone to a total of six different food concentrations using five different dilutions of a high food treatment. We used a spectrophotometer to assess the level of food at each concentration and these corresponded to 95, 96.4, 97.8, 98.7, 99.3, and 99.8 % light transmittance. All beakers in the life-table assay were maintained in a controlled temperature room with a 16L:8D photoperiod at 18°C and their position in the chamber changed every two days to minimize micro-environmental differences. The food/water mixture in all beakers was replaced every other day.

Statistical Analyses

To examine the differences in morphology and life-history between *D. pulex* and *D. pulicaria* we used two-factor analysis of variance (ANOVA). Our model was designed to test for the main effects of species (2 levels) and food treatment (4 levels), as well as the interaction between these effects. We excluded the two treatments with the highest food levels (95 and 96.4 % light transmittance) due to reduced juvenile survivorship that resulted in small sample sizes for both clones, particularly the *D. pulex* clone. In the context of this model a significant effect of species would suggest inherent differences between the two clones used in our experiment while a significant treatment effect would suggest variation in morphology or life-history with varying food concentration. A significant interaction term would suggest that the responses of *D. pulex* and *D. pulicaria* differ across food treatments.

To assess the nature of the responses to food concentration in these species, particularly for those traits in which a significant interaction term based on ANOVA was recovered, we performed linear regression on estimates of each species-specific trait and food concentration. For these analyses, a significant regression should yield information on the directionality and strength of the relationship between a species-specific trait and food concentration. All analyses were performed in Program R (R Development Core Team 2008).

Results

Two-way ANOVA showed that our *D. pulex* clone differed significantly from our *D. pulicaria* clone for three of the four traits we measured (Table 4.1). Specifically, *D. pulex* achieves a larger body size and produces more eggs upon reaching maturity than the *D. pulicaria* clone (Table 4.2). Also, *D. pulex* has a lifespan that is approximately 53% that of the *D. pulicaria* clone. There was no difference between the species in the time to maturity. When both species are considered jointly, ANOVA also revealed a significant increase in body size and number of eggs produced at maturity in response to decreasing food concentrations (Table 4.3).

We detected significant species by treatment interaction terms for longevity and number of eggs produced at maturity (Table 4.1). Regression analyses for these traits corroborated these results and showed that for *D. pulex* reduced food concentrations result in longer lifespans (Fig. 4.1A; $R^2 = 0.23$, $p < 0.001$) while food concentration had no effect on the lifespan of *D. pulicaria* (Fig. 4.1A; $R^2 = 0.01$, $p = 0.202$). Egg number also increased with reduced food concentration in *D. pulex* (Fig. 4.1B; $R^2 = 0.44$, $p < 0.001$), but not in *D. pulicaria* (Fig. 4.1B; $R^2 = 0.04$, $p = 0.113$). Regression also corroborated the observed lack of a significant interaction term for time to maturity as neither *D. pulex* (Fig. 4.1C; $R^2 = 0.01$, $p = 0.235$) nor *D. pulicaria* (Fig. 4.1C; $R^2 = 0.03$, $p = 0.082$) displayed significant relationships between time to maturity and food concentration. Despite the lack of a significant interaction term for body size, regression analyses suggested that *D. pulex* achieves larger body sizes in response to reduced food concentrations (Fig. 4.1D; $R^2 = 0.31$, $p < 0.001$) whereas *D. pulicaria* does not (Fig. 4.1D; $R^2 = 0.06$, $p = 0.067$).

We should note that regression results for *D. pulicaria* presented previously included individuals from the two food treatments excluded from the ANOVA. We did conduct regressions for *D. pulicaria* with these treatments removed and obtained the same results. Given our inability to find a significant relationship between *D. pulicaria* traits and food concentration we chose to analyze and present the complete data to emphasize the breadth of food concentrations over which the lack of a relationship exists.

Discussion

Assessment of the relationship between diet restriction and lifespan in long-lived species, especially in humans, is currently at the forefront of research in biogerontology. Several observational and epidemiological studies on humans, and experimental studies on closely related rhesus monkeys suggest that caloric restriction may indeed increase lifespan in humans (Mattison et al. 2003; Redman et al. 2008). The drawback to such studies is that they require a decades long

commitment to investigation due to the long lifespan of monkeys and humans and thus current conclusions based on these studies is speculative.

In this study we propose that an alternate approach to using long-lived non-model organisms is to use short-lived model organisms with widely divergent lifespans that parallel the different lifespans in the currently utilized non-model organisms. In particular, we use sister species of *Daphnia, D. pulex* and *D. pulicaria,* whose evolutionary relationships between one another parallel many of the evolutionary relationships between rhesus monkeys and humans. Similar to the approximate three-fold difference in the rate of aging between monkeys and humans (Roth et al. 2004), *D. pulex* senesces two to three times as fast as *D. pulicaria* (Dudycha 2003). Our data further supports the different lifespans in these *Daphnia* species with an observed difference in lifespan that is nearly two-fold (Table 4.2). Humans and rhesus monkeys are recently diverged, approximately 25 million years ago (Kumar & Hedges 1998), and share 93% sequence identity (Gibbs et al. 2007). *Daphnia pulex* and *D. pulicaria* are also recently diverged, with an estimated divergence time of less than 5 million years, and these species share 99% sequence identity (Colbourne & Hebert 1996). The obvious assumption our methodology makes is that the diet-mediated patterns of senescence between closely related pairs of species with different lifespans hold across taxonomically distinct groups (*i.e.* crustaceans and mammals). While such an assumption is unlikely to be strictly true, evidence suggesting the underlying physiological and genetic elements responsible for diet-mediated longevity are remarkably similar in yeast, nematodes, fruit flies, and mammals (Bishop & Guarente 2008) provides support for our approach.

Our study provides clear evidence that the short-lived species, *D. pulex*, shows the classically observed relationship of enhanced lifespan in response to reduced caloric intake. In fact, diet restriction increased lifespan by more than 30% over the range of food concentrations we used. This result is qualitatively similar to results obtained in other studies on the same

species (Lynch & Ennis 1983; Lynch 1989) and other *Daphnia* species with a similar life-history strategy (Ingle 1933; Vijverberg 1976; Martinez-Jeronimo et al. 1994). Concomitant with the increase in lifespan, *D. pulex* also displays increased fecundity and body size at reduced food levels, providing little evidence for tradeoffs to explain the changes in lifespan. However, the apparent lack of a tradeoff is a common observation in experimental studies of *Daphnia* life-histories (Spitze 1991; Spitze et al. 1991; Baer & Lynch 2003; Chapter 2).

In contrast to our results for *D. pulex*, we find no evidence that the long-lived species in our study, *D. pulicaria*, gains any life-extending effects through diet restriction. This result is disheartening for research on diet-mediated life-extension in humans because our data suggest that manipulation of human lifespan through dietary intervention may not be plausible. We concede the possibility that we did not test a broad enough range of high food concentrations. However, food concentrations at levels higher than we tested typically induce mortality in *Daphnia* through other non-dietary mechanisms (*e.g.* algae attached to the limbs which causes an inability to swim properly) and thus disentangling longevity effects directly related to food consumption from those not related to food may introduce unwanted bias into the experiment. It is also possible that the reaction norms we observed for *D. pulicaria* are unique to the particular clone we used. Subsequent assays that utilize more genotypes should adequately address this issue.

In summary, we utilize the novel approach of comparing short- and long-lived sister species of short-lived taxa to assess the potential relationship between diet restriction and longevity in long-lived taxa. Our results reinforce the concept that diet restriction enhances longevity in a short-lived species by providing evidence that longevity in *D. pulex*, a short-lived crustacean, is inversely related to food concentration. Furthermore, the enhanced longevity in *D. pulex* occurs in the absence of tradeoffs with other key life-history traits. Alternatively, our long-lived species, *D. pulicaria*, experiences no changes in longevity in response to reduced caloric

intake. Thus, interpretation of current results from long-lived species, such as monkey and humans that are based on as-of-yet incomplete data that suggest diet restriction can prolong life, may be tenuous.

Table 4.1. Results from two-way ANOVA. The total sample size (N), degrees of freedom (df), F-value (F), and p-value are listed under ANOVA Parameters. The model includes main effects of species type (S), food concentration (T), and the interaction (S*T). P-values less than 0.05 are in bold.

ANOVA	Longevity			Eggs			Age at Maturity			Body Size		
Parameters	S	T	S*T	S	T	S*T	S	T	S*T	S	T	S*T
N	119			61			118			73		
df	1	1	1	1	1	1	1	1	1	1	1	1
F	77.53	0.07	5.36	81.05	16.64	24.34	1.15	0.24	0.47	147.16	20.49	2.61
p-value	**<0.001**	0.799	**0.022**	**<0.001**	**<0.001**	**<0.001**	0.285	0.627	0.495	**<0.001**	**<0.001**	0.111

Table 4.2. The main effect of species. The sample size (N) and mean (± 2 SE) for each life-history trait measured in each species. The mean trait values are averaged over the four treatments included in the two-way ANOVA.

Species	Longevity		Eggs		Age at Maturity		Body Size	
	N	mean	N	mean	N	mean	N	mean
D. pulicaria	65	51.2 (4.7)	30	2.0 (0.4)	65	12.0 (0.8)	30	1.26 (0.05)
D. pulex	54	27.2 (2.3)	31	6.4 (1.2)	53	11.5 (0.5)	43	1.61 (0.04)

Table 4.3. The main effect of treatment. The sample size (N) and mean (± 2 SE) for each life-history trait measured in each treatment. The mean trait values are averaged over both species.

	Longevity		Eggs		Age at Maturity		Body Size	
Treatment	**N**	**mean**	**N**	**mean**	**N**	**mean**	**N**	**mean**
97.8	24	46.0 (8.3)	11	2.5 (0.8)	25	11.2 (1.1)	11	1.34 (0.27)
98.7	26	41.2 (10.1)	14	4.1 (1.7)	25	12.5 (1.4)	16	1.41 (0.11)
99.3	32	34.2 (4.6)	15	4.1 (1.7)	32	12.2 (0.9)	20	1.46 (0.11)
99.8	47	41.2 (4.9)	21	5.4 (1.7)	36	11.4 (0.8)	26	1.55 (0.08)

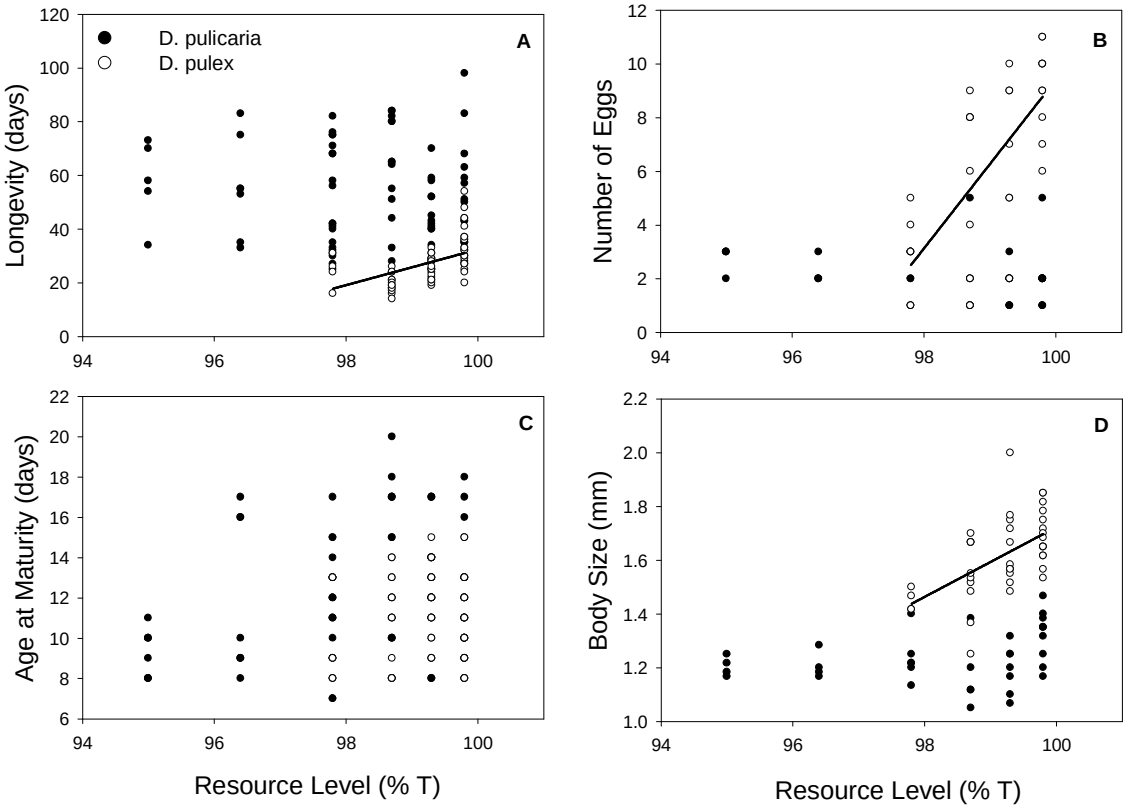


Figure 4.1. The effect of resource level on A) longevity, B) number of eggs at maturity, C) age at maturity, and D) body size at maturity. Closed circles denote the long-lived species, *D. pulicaria*, and open circles denote the short-lived species, *D. pulex*. Regression lines are fitted in those instances where there was a significant relationship ($p < 0.05$) between food concentration and a life-history trait for the short-lived *D. pulex*. No significant regressions were found for the long-lived *D. pulicaria*. Note that resource level proceeds from highest concentration (95% T) to lowest concentration (100% T).

CHAPTER 5

SPECIES AND GENOTYPE DIVERSITY DRIVE COMMUNITY AND ECOSYSTEM PROPERTIES IN EXPERIMENTAL MICROCOSMS

Abstract

Species diversity is important to ecosystems because of the increased probability of including species that are strong interactors and/or because multiple-species communities are more efficient at using resources due to synergisms and resource partitioning. Genetic diversity also contributes to ecosystem function through effects on primary productivity, community structure and resilience, and modulating energy and nutrient fluxes. Lacking are studies investigating the relationship between ecosystem function and diversity where hierarchical levels of biological diversity are systematically varied during experimentation. In this experiment, we manipulated both species and genotypic diversity of two *Daphnia* species in microcosms initially seeded with *Chlamydomonas* and measured community- and ecosystem-level properties to determine which level of diversity was most important for explaining variation in the property. Our results show that species diversity alters microbial community composition while genotypic diversity reduces microbial richness and primary productivity. In addition, the highest level of genotypic and species richness appeared to increase community and ecosystem stability. These findings revealed that species and genotypic diversity were significant drivers of community and ecosystem properties and stability.

Introduction

Understanding the interaction between organisms and their environment and the relationship between these interactions and ecosystem functions such as productivity and stability is the central goal of ecosystems ecology. The concept that species diversity is functionally important to ecosystem performance is widely accepted. Two mechanisms have been identified;

increased probability of including species that are strong interactors (Hooper & Vitousek 1997; Huston 1997; Tilman et al. 1997), and/or increased efficiency of resource use via complementary functional traits (Tilman et al. 2001) and resource partitioning (Chapin et al. 1997). Although not as well documented as the relationships between species diversity and ecosystem function, population genetic variation also affects community and ecosystem-level processes (Hughes et al. 2008) such as the biodiversity of communities (Wimp et al. 2004), nutrient flux (Schweitzer et al. 2005; Madritch et al. 2006), and ecosystem resilience (Hughes & Stachowicz 2004).

Changes in the composition or number of species alter ecosystem processes through species-specific traits that govern the rates, efficiencies and pathways that process nutrients and energy. This premise has led ecologists to identify suites of traits that are likely to be important in modulating energy and nutrient flows (Vitousek 1990: Vitousek et al. 1987). Species with traits that alter biogeochemical cycles in similar ways, or species that extract energy from the same trophic levels are often combined into functional groups to determine how alterations in this higher level of biodiversity might affect ecosystem responses (Naeem et al. 1995). In contrast, changes in genotypic diversity alter functional diversity through changes in genetically based phenotypic variation (Johnson et al. 2006) or changes in community susceptibility to invasion (Crutsinger et al. 2008). Genetic diversity can influence ecosystem-level properties via impacts on interacting species. For instance, high above-ground net primary productivity associated with genetically variable populations of *Solidago* determines arthropod abundance (Crutsinger et al. 2006) and genetic variation in leaf litter determines the decomposer community and hence the rates of decomposition and nutrient release (Schweitzer et al. 2005; Madritch et al. 2006). Genotypic diversity also enhances the ability of ecosystems to resist global disturbance (Hughes & Stachowicz 2004).

One shortcoming of the current literature on the relationship between ecosystem function and diversity is that often only one level of biological diversity, either species or genetic, is

experimentally varied despite the recent call to focus on the connections between genetic and species diversity (Vellend & Geber 2005). Such designs preclude the ability to assess how different types of diversity influence ecosystem properties and the degree of correlation between types of diversity. As a first step in addressing the correlation between genetic and species diversity, our goal with this study was to determine the relative contribution of genotypic and species diversity in a novel experimental design to vary both variables and subsequently measure community and ecosystem properties in aquatic microcosms.

First, we tested which level of biological diversity, genetic or species, was the best predictor of community and ecosystem properties. Second, we tested which level of diversity was important for community and ecosystem stability. We defined properties as mean trait values and stability as the coefficient of variation for those traits.

Here, we used two model systems, *Daphnia* and *Chlamydomonas*, which were previously used in microcosm experiments to assess fundamental ecological hypotheses (McCauley et al. 1999: Nelson et al. 2005). The phytoplankton *Chlamydomonas reinhardtii* was utilized as a model system for understanding the effects of genetic diversity on productivity (Bell 1991) and for studies of experimental evolution in microcosms (Collins & Bell 2004). The microcrustacean *Daphnia* has emerged as one of the most tractable and ecologically relevant of genetic model systems (*e.g.*, Eads et al. 2007). In our experiment, we systematically manipulated the species and genotypic diversity of *Daphnia* in microcosms while employing a single genotype of *Chlamydomonas* as a food resource. After two weeks we measured the means and variances of community and ecosystem traits, and then determined which level of diversity, genotypic or species, was most important for explaining the observed patterns.

Our results show that species diversity is an important predictor of microbial community composition while genotypic diversity is an important predictor of microbial community richness and ecosystem productivity (gross primary production and community respiration). The highest

level of genotypic and species diversity increased community and ecosystem stability; however, the diversity-stability relationship is not consistent across traits. Our findings reveal that species and genotypic diversity were significant drivers of community and ecosystem properties and stability.

Material and Methods

Study Organisms

Phytoplankton – The alga used in the microcosms was strain CC-1928 of *C. reinhardtii*, acquired from the *Chlamydomonas* Culture Collection (www.chlamy.org). The strain was semi-continuously cultured in three aerated 5 liter (L) carboys containing 4 L of modified Bold's Basal Medium (BBM; Stein 1973). Every 2-3 days 2 L of fluid were removed from the carboy and replaced with fresh BBM. Algal cultures were maintained in a 16L:8D photoperiod at 20°C in order to synchronize our cultures to liberate mitotically produced daughter cells once every 24 hours. Because we clonally propagated a single strain of *C. reinhardtii* there was essentially no population genetic variation among our treatments.

Zooplankton – Two clones of *Daphnia pulex* (Px1 and Px2) and two clones of *Daphnia pulicaria* (Pu1 and Pu2) were used in this study. The clones of *D. pulex* were collected from a temporary pond in Michigan while the clones of *D. pulicaria* were collected from a permanent lake in Michigan. Stock cultures of each *Daphnia* clone were maintained by clonal reproduction in 19 L plastic buckets containing 15 L of filtered well-water under constant temperature (18°C) and light (16L:8D). Water levels were maintained by periodic addition of double-distilled water. *Daphnia* cultures were fed a pure culture of *C. reinhardtii* every 3–4 days.

To ensure that clones from each species constituted unique genotypes we used two methods. First, we used a common garden experiment (Lynch et al. 1999; Pfrender and Lynch 2000) to assay quantitative genetic variation. Briefly, five single immature females of each clone

were taken from the stock isolates, each representing an experimental line. The lines were maintained as single asexually-produced progeny for two generations. In third generation individuals we measured two traits, number of eggs in the brood pouch and body size, upon reaching maturity (defined as the first instar with the deposition of eggs into the brood pouch). Each experimental line was maintained in a 250 mL beaker containing 150 mL of filtered well-water supplemented with a constant concentration (98.5 % light transmittance) of *C. reinhardtii*. All beakers in the life-table assay were maintained in a controlled temperature room with a 16L:8D photoperiod at 18°C and their position in the chamber changed every two days to minimize micro-environmental differences. The food/water mixture in all beakers was replaced every other day.

Second, we screened each clone for molecular genetic variation with 16 microsatellite markers. We extracted genomic DNA from 10 individuals of each clone with a standard proteinase-K digestion followed by phenol/chloroform/isoamyl alcohol extraction (Sambrook and Russell 2002). DNA was amplified with primers using the following PCR conditions: 95ºC for 5 min, and 30 cycles of 94ºC 0.5 min, 54ºC 0.5 min, 72ºC .75 min followed by 5 min at 72ºC. PCR products were diluted 10-fold and sequenced using a 3730 DNA analyzer (Applied Biosystems, Foster City CA, USA). Microsatellites were typed using ABI prism software (Applied Biosystems). We found a single microsatellite locus (P7 H4) that differentiated three of the four clones using three alleles (169, 189, 194) with Px1 identified as a 189/194 heterozygote, Px2 a 189/189 homozygote, and both *D. pulicaria* clones 169/169 homozygotes. Despite screening the clones with 16 microsatellite loci we were unable to find a marker that differentiated the two *D. pulicaria* clones.

Microbial Community

The microbial community was assayed in a subset of the microcosms using tRFLP. Specifically, a 300 ml water sample was taken from 2-3 replicates of each treatment at the end of

the experiment and filtered onto a 22 µm cellulose nitrate filter. Community DNA was extracted from each filter using the DNeasy® DNA extraction kit (QIAGEN, Hilden, Germany). The 16S rRNA genes were amplified using universal bacterial primers 27f (FAM labeled) and 1392r with the following PCR conditions, 95ºC for 9 min, and 25 cycles of 95ºC 1 min, 59ºC 1 min, 72ºC 1.67 min followed by 10 min at 72ºC. Reconditioning PCR followed the same conditions with only 3 cycles. PCR products were purified using the Qiaquick® PCR purification kit (QIAGEN). PCR-amplified sequences were digested using HhaI (New England Biolabs, Ipswich MA, USA) and visualized using a 3730 DNA analyzer (Applied Biosystems). We used *E. coli* K12 as a positive control. The restriction fragments were analyzed using FragSort software (http://www.oardc.ohio-state.edu/trflpfragsort/index.php).

Microcosm Establishment and Maintenance

Microcosms were established in 3.8 L glass jars containing 2.7 L of filtered well-water, 0.3 L of 100% BBM (Stein 1973) for growth, and a uniform concentration of *C. reinhardtii* cells (98.5 % light transmittance). Microcosms were maintained in a controlled temperature room with a 16L:8D photoperiod at 18°C and their position was haphazardly rotated daily to minimize micro-environmental differences.

Twelve experimental treatments were established (Table 5.1). The design was not fully factorial, with four possible treatments not included due to ecosystem property sampling limitations. One treatment contained only *C. reinhardtii* and was replicated nine times to establish baseline measurements of community and ecosystem properties but was not included in any analyses conducted to assess the effects of genotype and species diversity. The remaining 11 treatments were replicated six times and each replicate contained 84 mature *Daphnia*. Treatments were established to cover a range of species (0-2) and genotypic (0-4) diversity. Each replicate contained the same initial density of *Daphnia*. Individual *Daphnia* were divided equally among genotypes and/or species to maintain this density in all treatments. Ecosystem properties were

sampled in three blocks of two replicates, with one block sampled per day for the first three days (Days 1-3) and the last three days (Days 14-16) of the experiment. At the end of the experiment all individuals in microcosms from one randomly chosen replicate in each block of each treatment, representing a total of three replicates, were filtered through nitex mesh, placed in 95% ethanol and subsequently counted to obtain an estimate of total abundance. At the end of the experiment we also screened 25-50 adult *Daphnia* from multi-genotype treatments with a single microsatellite marker (P7 H4) that distinguished the clones to estimate relative abundance and ensure that competition among clones did not result in the extinction of a clonal line during the course of the experiment. The variability in the number of individuals screened with microsatellites was due to variability in adult availability in each of the microcosms. Our protocol was to screen up to 50 adult *Daphnia* from each microcosm, but in cases where there were fewer than 50 adults we screened all available adults.

Characterization of Ecosystem Function

Ecosystem Metabolism - We measured net productivity (NEP) and community respiration (CR) in microcosms by monitoring dissolved CO_2 concentrations during light and dark incubations, respectively. Each microcosm was sealed with a gas-tight lid that was fitted with a rubber septum to allow sampling of the 1.1 L headspace. Incubations were performed at 20 °C on a shaker table at 50 RPM to allow for equilibration between the water and headspace within the microcosm (Kling et al. 1992). CO_2 samples were collected from the headspace every 30-40 minutes over the course of 1-2 hours, and stored in evacuated glass vials (Vacutainer, Franklin Lakes NJ, USA) for later analysis by gas chromatography. CO_2 was quantified on a calibrated SRI 8610 gas chromatograph (Torrance, CA) with thermal conductivity detector. NEP was calculated as the slope of the line relating CO_2 concentration and time during light incubations, and CR was similarly calculated using samples collected during dark incubations. Gross primary productivity (GPP) was calculated from these values as NEP-CR.

Water Chemistry - At the end of each set of light/dark incubations, we collected 60 ml of water from each microcosm. A known volume (30-60 mL) was filtered onto a precombusted glass fiber filter (Whatman GF/F, nominal pore size 0.7 µm). The filter was preserved by freezing for analysis of chlorophyll *a*. The remaining water was similarly filtered into acid-rinsed HDPE bottles and frozen for analysis of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP). Total dissolved N was quantified using a potassium persulfate digestion (Nydahl 1978) followed by cadmium reduction for measurement of NO_3+NO_2 (APHA 1998). Measures of TDP were made using a potassium persulfate digestion followed by an ascorbic acid molybdenum reaction for soluble reactive phosphorus (Murphy and Riley 1962). Both colorimetric analyses were done using an automated analytical system with FASPac II data acquisition software (Astoria Pacific International, Portland OR, USA). Chlorophyll *a* on filters was extracted using 90% acetone and quantified fluorometrically (AquaFluor Turner Designs, Sunnyvale CA, USA). Samples were corrected for phaeophytin using 0.1 N HCl (Steinman et al. 2006).

Statistical Analyses

Clonal Identity – Clone-specific estimates of body size and fecundity from the common-garden experiment were analyzed using one-way analysis of variance (ANOVA) with clone representing the single fixed main effect. We also used two-way ANOVA with clone and block as main effects on a data subset that consisted only of single genotype microcosms to determine if the clones differed with respect to each of the community and ecosystem properties.

Characterization of Microbial Community - Two metrics were used to characterize the microbial community in the subset of microcosms for which the microbial community was sampled. First, the number of unique 16s rRNA fragments was used as in estimate of microbial richness. Second, we used non-metric multidimensional scaling (NMDS) as implemented by the vegan package (Oksanen et al. 2008) in Program R (www.R-project.org) to estimate microbial

community composition. Initial input was a presence/absence matrix that characterized microbial community composition. A community dissimilarity matrix based on the presence/absence matrix was constructed using the Bray-Curtis index. The community dissimilarity matrix was then subjected to NMDS and the scores for each microcosm were used as a quantitative estimate of the microbial community for use in subsequent analyses. Preliminary results indicated that the microbial communities associated with the *Chlamydomonas*-only treatments were substantially different from any of the treatments that contained *Daphnia*. Therefore, we restricted our analyses of microbial community composition to only those microcosms that included *Daphnia* .

Description of Hypotheses and Datasets – We tested three hypotheses regarding the effects of genotypic and species diversity on community and ecosystem properties. First, we tested the joint contributions of genotypic and species diversity on zooplankton and phytoplankton abundance, ecosystem productivity, and water chemistry using data from the microcosms that contained *Daphnia* (hereafter referred to as the full dataset). To test the joint effect of genotypic and species diversity on microbial diversity we used the subset of the full dataset for which the microbial community was sampled. Second, we tested the specific effects of genotypic diversity on community and ecosystem properties by using a subset of the data that included only those microcosms in which the level of species diversity was constant (2 species), but levels of genotype diversity varied (2-4 genotypes; referred to as the genotype dataset). To test the effects of genotype diversity on the microbial community we used the subset of the genotype dataset for which microbial communities were sampled. Finally, to assess the specific effects of species diversity on community and ecosystem properties, we used a subset of the data that included only those microcosms in which the level of genotype diversity was constant (2 genotypes), but levels of species diversity varied (1-2 species; referred to as the species dataset).

To test the effects of species diversity on the microbial community we used the subset of the species dataset in which microbial communities were sampled.

Ecosystem Properties During the First Sampling Interval - Based on the experimental design we were certain that a majority of the ecosystem properties (zooplankton abundance, chlorophyll and phaeophytin levels, dissolved nitrogen and phosphorous, and nitrogen:phosphorous ratio) were identical across treatments at the beginning of the experiment. However, we could not be certain that measures of ecosystem productivity were equal during the first sampling interval. Therefore, we used species number, genotype number, and block as fixed main effects in two-way (for the genotype and species datasets) and three-way (for the full dataset) ANOVA to test for effects of these factors on community respiration, net productivity, and gross primary productivity.

Analyses for the Full Dataset - To examine the relative importance of genotypic and species diversity we used stepwise regression procedures to build general linear models to explain variation in our community and ecosystem response variables. *Daphnia* species richness, *Daphnia* genotypic richness, block effects, and all possible interactions were included as candidate predictor variables. The best model for each response variable was selected based on Akaike's information criterion (AIC), where the smallest AIC indicates the best model. Our protocol for determining which variables were significant predictors of a response variable was first to take the best regression model based on AIC values and examine the p-value associated with the regression model. If the p-value of the best regression model was greater than 0.05 then we concluded that no predictor variables were important for explaining variation in the response variable. If the p-value of the best regression model was less than 0.05 we then examined the importance of each predictor variable individually in the context of the best regression model using ANOVA that only included variables included in the best regression model. If the p-value associated with the predictor variable in the context of the ANOVA model was greater than 0.05

then we did not consider the variable a significant predictor of variation in the response variable. If the p-value of the predictor variable in the context of the ANOVA model was less than 0.05 then we concluded that the predictor variable was a significant contributor to variation in the response variable. To test the relationship between diversity and stability we estimated coefficients of variation (CV) for each community and ecosystem property measured at each level of genotypic or species diversity. The CV's were calculated by dividing the standard deviation of replicates within a treatment by the mean of the replicates within a treatment for the second sampling interval only. A CV estimated in this manner yields a dimensionless measure that facilitates comparisons across treatments and properties. In this context, stability is inversely correlated with CV such that low estimates of CV suggest high stability. All analyses were conducted in Program R.

Analyses for the Genotype and Species Datasets – To examine the specific importance of genotype or species diversity we used stepwise regression procedures to build models to explain variation in the community and ecosystem response variables. Our approach was identical to that described for the full dataset except our initial candidate predictor variables differed. For the genotype dataset our predictor variables were *Daphnia* genotypic richness, block effects, and their two-way interaction. For the species dataset, our predictor variables were *Daphnia* species richness, block effects, and their two-way interaction. Our criteria for determining significant predictor variables and exploring the diversity-stability relationship were the same as those used for the full dataset.

Results

Clonal Uniqueness

Body size and fecundity varied significantly among clones reared in the common garden experiment (Fig. 5.1A, ANOVA $p < 0.001$, df = 3, F=38.37; Fig. 5.1B, ANOVA p=0.018, df = 3,

F= 4.47). At the species level, *D. pulex* clones are larger (t-test p=0.001, df=13, t=4.04) and produced fewer offspring (t-test p=0.046, df=18, t=2.15) than *D. pulicaria* clones. At the genotypic level, Pu2 was smaller than the other three genotypes while Pu1 produced more eggs than the other three genotypes. Examination of the single genotype microcosms showed that the Px1 genotype treatment had significantly higher levels of chlorophyll (Fig. 5.1C; ANOVA df=3, F=15.35, p<0.001) and phaeophytin (Fig. 5.1D; ANOVA df=3, F=16.14, p<0.001), and lower levels of total dissolved nitrogen (ANOVA df=3, F=4.06, p=.021) relative to the other three clones.

Predictors of Community and Ecosystem Properties

First Sampling Interval–Neither level of diversity significantly affected ecosystem productivity (CR, NEP, GPP) in any of the data subsets during the first sampling interval. However, there were significant block effects in the full dataset for GPP (df=1, F=4.68, p=0.034) and NEP (df=1, F=12.31, p<0.001). There was also a significant block effect in the species dataset for NEP (df=1, F=8.71, p=0.008). We interpret these block effects as evidence for our anticipated ecological changes in the microcosms because blocks 1-3 represent sampling during days 1-3 of the experiment, respectively.

Microbial Community Richness and Composition – For the full dataset, the best predictive model of microbial community composition included species diversity, genotypic diversity, block and the genotype*block interaction (R^2=0.70, p<0.0001). However, based on ANOVA results, only species diversity and block were significant predictors of microbial community composition. In the genotype dataset, the best predictive model was not significant (R^2=0.14, p=0.090). In the species dataset, the best predictive model included species diversity and block (R^2=0.70, p=0.003) and both were significant based on ANOVA results (Fig. 5.2).

For microbial richness, the best predictive model from the full dataset included species diversity, genotype diversity, block, a species*genotype interaction, and a species*block

interaction (R^2=0.28, p=0.022). However, only species diversity was a significant predictor based on ANOVA. In the genotype dataset, genotype diversity and block were included in the best model (R^2=0.44, p=0.013), and both were significant predictors based on ANOVA with high levels of genotype diversity resulting in reduced microbial richness (Fig. 5.3A). The best predictive model for the species dataset included species diversity and block, but the overall model was not significant (R^2=0.21, p=0.161).

Zooplankton Abundance – The total abundance of *Daphnia* in the microcosms did not vary as a function of species diversity, genotype diversity, or block in any of the data subsets.

Community Respiration – In the full dataset, all single variables, two way and three way interactions were included in the best model describing CR (R^2=0.31, p=0.0002),with only block and the species * genotype interaction explaining a significant amount of variation in respiration (ANOVA). In the genotype dataset, genotype and block were the only terms in the best model (R^2=0.27, p=0.014), and both were significant predictor variables based on ANOVA with high levels of genotypic diversity resulting in low levels of CR (Fig. 5.3B). The best model of CR for the species dataset was not significant (R^2=0.23, p=0.111)

Net Productivity – Species and genotype diversity were included in the best model describing NEP in the full dataset but the overall model was not significant (R^2=0.04, p=0.156). None of the candidate predictor variables were important for describing variation in NEP in both the species and genotype datasets.

Gross Primary Productivity – All single variables, two way, and three way interactions were included in the best model describing variation in GPP in the full dataset (R^2=0.27, p=0.007). Block and the species * genotype interaction were the only significant predictors based on ANOVA. Genotype diversity and block were also significant predictors of GPP in the genotype dataset (R^2=0.24, p=0.025). Genotype diversity was the only significant predictor of gross primary productivity based on ANOVA and showed that high genotypic diversity resulted

in low GPP (Fig. 5.3C). The best model describing GPP for the species dataset was not significant (R^2=0.32, p=0.066).

Algal Pigments– In the full dataset, genotype diversity was the only predictor included in the best models explaining variation in chlorophyll and phaeophytin content (R^2=0.09, p=0.008, R^2=0.10, p=0.007, respectively) and was a significant predictor for both pigments based on ANOVA. In the genotype dataset, genotype diversity and block were included in the best fit models (R^2=0.24, p=0.009, R^2=0.20, p=0.020), but block was the only significant predictor for both pigments based on ANOVA. In the species dataset the best models for both pigments included only block, but neither model was significant (R^2=0.10, p=0.069, R^2=0.05, p=0.142).

Dissolved Nutrients – Dissolved nitrogen was predicted by block in the full data set, although genotype and block were included in the best model (R^2=0.10, p=0.013). In contrast, genotype diversity was included in the best model describing dissolved phosphorous and the N:P ratio in the full dataset (P: R^2=0.09, p=0.017, N:P: R^2=0.05, p=0.042) and in both cases was significant based on ANOVA. In the genotype dataset, block was the only significant predictor in the best model for nitrogen, (R^2=0.19, p=0.010). Similarly, in the species dataset, block was the only significant predictor of dissolved nitrogen (R^2=0.19, p=0.019). The best models of dissolved phosphorous were not significant in the genotype dataset (R^2=0.06, p=0.210) and the species dataset (R^2=0.02, p=0.360). The best models of N:P ratio were not significant in the genotype dataset (R^2=0.06, p=0.205) and the species dataset (R^2=0.04, p=0.289).

Ecosystem Stability

Our results concerning the diversity-stability relationship were hindered due to the experimental design. Specifically, we only obtained a single estimate of the CV for each treatment and thus were unable to perform any statistical tests of the diversity-stability relationship. In light of this limitation, our results and conclusions regarding diversity-stability relationships are purely descriptive in nature.

In general, there is a pattern of lower CVs at higher levels of both genotype and species diversity (Table 5.2). The pattern of low CVs at high diversity is clearest for zooplankton abundance, and to a lesser extent, chlorophyll content, phaeophytin levels, and dissolved nitrogen. Several properties (net productivity, gross primary productivity, dissolved phosphorous, and N:P ratio) show a pattern of the lowest CVs at low (1 genotype) and high (4 genotypes) levels of genotype diversity with high CVs occurring at intermediate levels of genotype diversity. Community respiration shows a pattern of increasing CVs with increasing species and genotype diversity.

Discussion

Research that jointly considers ecological and evolutionary principles has enjoyed a surge in the recent literature. This is perhaps most prominently displayed by the contributions of the burgeoning fields of community and ecosystem genetics that integrate the disciplines of evolution, ecology, and population genetics (Whitham et al. 2006). While numerically few, these studies convincingly show that varying levels of genetic diversity can profoundly influence community structure (*e.g.*, Wimp et al. 2005; Johnson & Agrawal 2005; Johnson et al. 2006;) and ecosystem function (*e.g.*, Hughes & Stachowitz 2004; Crutsinger et al. 2006). Our goal was to elaborate on the traditional studies of community- and ecosystem-level consequences of species and genetic diversity by examining the importance of variation in one hierarchical level of diversity while simultaneously maintaining a constant level of diversity in the other hierarchical component. Our results suggest that species diversity is important for determining the composition of microbial communities while genotypic diversity is a significant predictor of microbial community richness and ecosystem metabolism in experimental microcosms (Table 5.3).

An important consideration for investigations of the effects of genetic and species diversity on communities and ecosystems is ensuring that there is enough functional variability

among genotypes and species. Experiments in which genetic variation among genotypes is low, or different species are functionally redundant, may lead to the potentially false conclusion that genetic and species diversity do not influence communities and ecosystems. In this experiment we obtained four different sets of results that suggest there was sufficient variation among genotypes and species to warrant inclusion in our study.

First, a common-garden experiment that tested the quantitative genetic differences among genotypes and species showed that *D. pulex* is larger and produces fewer offspring than *D. pulicaria*, and that among *D. pulicaria* clones Pu1 is larger and produces more offspring than Pu2 (Fig. 5.1 A,B). These results show clear quantitative genetic differences between the two species used in this experiment, and also between the genotypes of *D. pulicaria*. Second, screening with microsatellite markers showed that *D. pulex* and *D. pulicaria* differ at the neutral molecular genetic level. Of the 16 microsatellite loci tested, 7 amplified in all four clones, and none of the alleles present in *D. pulex* were present in *D. pulicaria*. Among genotypes, there was no molecular genetic variation between Pu1 and Pu2, but Px1 and Px2 differed for 4 of the seven loci that amplified in all four clones. These results then suggest that there are neutral molecular genetic differences between the species, and also among genotypes of *D. pulex*. Third, results from the single genotype microcosm treatments showed that Px1 treatments had higher levels of chlorophyll and phaeophytin, and lower levels of dissolved nitrogen, compared to the other three genotypes (Fig. 5.1 C-E). These results imply that Px1 is an inefficient grazer relative to the other genotypes. Finally, in treatments that contained a single genotype from each species, estimates of relative abundance suggest that *D. pulicaria* is a superior competitor to *D. pulex* (Table 5.1). Taken together, these results provide clear evidence that the species and genotypes used in this experiment differ through some combination of quantitative genetic, neutral molecular genetic, resource utilization, and competitive ability.

The most significant findings from our microcosm experiment are that *Daphnia* genotype diversity determines the richness of microbial communities and governs estimates of ecosystem metabolism. Our first result, that genotype diversity drives microbial richness, parallels numerous other studies that have documented the relationship between genotypic diversity and community structure (*e.g.*, Johnson et al. 2006). We initially hypothesized there would be a positive relationship between *Daphnia* genotype diversity and microbial richness because we presumed that each genotype harbored a unique community of microbes, and that the successive addition of *Daphnia* genotypes would lead to an increasingly rich microbial community. However, our results suggested the opposite, where increases in genotype diversity resulted in less rich microbial communities. We are aware of few studies that documented a reduction in community biodiversity with increasing levels of genetic diversity (Kanaga et al. 2009). One potential explanation for this result is that each *Daphnia* genotype occupies a unique filter-feeding niche in the microcosms and that the system-wide rate of filter feeding in the high genotype diversity treatments was higher than low genotype diversity treatments. We observed a reduction of phytoplankton abundance (as measured by chlorophyll content) in the high genotype diversity treatments, compared to low genotype treatments (Fig. 5.3D). Although phytoplankton abundance corroborates an explanation of higher feeding rates in both the full and genotype dataset, it was statistically significant only for the full dataset.

A separate line of evidence that supports our hypothesis that the four-genotype treatment has an overall high rate of biological filtration is our observation that *Daphnia* genotype diversity determines the rate of ecosystem metabolism. The relationship between two- and three- genotype diversity treatments and gross primary productivity is consistent with the notion of a balance between phytoplankton reproduction and zooplankton grazing. In contrast, the four-genotype treatment suggests grazing pressure from the zooplankton community outpaces the reproductive capabilities of the phytoplankton population, resulting in a net loss of primary productivity.

In some cases, relationships between experimentally manipulated eukaryotic diversity and ecosystem parameters are mediated by prokaryotic organisms that were not manipulated (Zak et al. 2003). Thus, one caveat to our observed relationship between genotypic diversity and ecosystem metabolism is that we cannot rule out the possibility that microbial diversity also drives the relationship between genotype diversity and ecosystem productivity since genotypic diversity and microbial richness were correlated. Another factor that we cannot rule out is the potential effect of microbial biomass on ecosystem function. It is highly likely that the genetic composition and abundance of the microbes present jointly influence estimates of ecosystem productivity due to normal growth and metabolism during the experiment. Given our results that microbial community structure and richness are related to levels of species and genotypic diversity, respectively, future endeavors should seek to quantify microbial abundance in addition to indices of microbial diversity.

Our finding that species diversity was only important for predicting microbial community composition and not other ecosystem properties is not particularly surprising because we only examined two levels of species diversity (one and two). Most studies that have documented significant effects of species diversity on communities and ecosystems have examined levels of species diversity that exceed the levels used in our experiments (reviewed in Hooper et al. 2005). Furthermore, despite our strong evidence for phenotypic, molecular and competitive differences between the two species utilized in this experiment, and the fact that they have evolved decidedly different life-history strategies to deal with their native environments (*D. pulex* resides in temporary ponds while *D. pulicaria* inhabit permanent lakes), they are sister taxa. Thus, the close phylogenetic relationship between these species likely makes functional differences between these species much more subtle than functional differences between more distantly related crustaceans.

The possible functional redundancy in our chosen taxa may also explain the weak patterns of increased ecosystem stability with increasing genotype and species diversity we observed. Only zooplankton abundance displayed a convincing positive correlation between stability and diversity. The remaining properties showed essentially no change, or a pattern of low stability at intermediate levels of diversity. Further microcosm research that utilizes phylogenetically diverse taxa would help in addressing the importance of species diversity and the true nature of diversity-stability relationships in such systems.

One important goal of investigations that systematically vary both genetic and species diversity is to resolve the relationship between these two types of diversity. A majority of the characters investigated in this experiment were influenced by only one type of diversity, genetic or species. However, two ecosystem properties, community respiration and gross primary productivity, were best explained by models in which a significant genotype*species interaction term was included in the model. Although our experimental design (only two species and two genotypes per species) is not entirely conducive to a thorough statistical treatment of this interesting result due to non-overlapping reaction norms, some discussion on the nature of genetic effects on ecosystems at different levels of species diversity is warranted. For the two ecosystem properties in question, the nature of the interaction between genotypic diversity and species diversity changes depending on the level of species diversity (Fig. 5.4). Specifically, at low species diversity (one), increasing genotype diversity does not significantly effect gross primary productivity (t-test $p=0.25$, $df=8$, $t=1.25$) or community respiration (t-test $p=0.23$, $df=8$, $t=1.28$). Conversely, at high species diversity (two), increasing genotypic diversity is associated with a reduction in gross primary productivity (regression $p=0.06$, $R^2=0.16$) and a significant increase in community respiration (regression $p=0.05$, $R^2=0.17$). Overall, it appears as if the effects of genotypic diversity on ecosystem properties are dependent on the level of species diversity, although due to the limitations imposed by our experiment we cannot be certain that this pattern

is robust to higher levels of genotypic and species diversity. If this observed pattern is a common feature of natural systems it suggests, at least, that the design of conservation strategies aimed at preserving local ecosystems may be guided by the relative amounts of genetic and species diversity contained therein. In speciose ecosystems, the manipulation of genetic diversity may have large impacts on ecosystems, whereas in genotypically depauperate ecosystems the manipulation of species diversity will lead to more pronounced ecosystem change.

To conclude, we found that genotype diversity is an important predictor of microbial community richness and ecosystem metabolism. The likely mechanism that drives these relationships is the enhanced filter-feeding capability of a genetically-rich assemblage of unique *Daphnia* genotypes. We also found that species diversity was important in shaping the composition of the microbial community but not important for other ecosystem properties. We also provide tentative evidence that the impacts of manipulating genetic diversity are dependent on the level of species diversity. Future research that utilizes a phylogenetically rich assemblage of zooplankton and quantifies microbial abundance will more accurately address the relative importance of species vs. genotype diversity in aquatic microcosms. Overall, these results highlight the importance of examining basic ecosystem properties in systems where genetic and species diversity can be controlled and strongly suggest that declines in species and genetic diversity can substantially alter ecosystem performance.

Table 5.1. Experimental design indicating the specific clonal mixtures used in the experiment (Treatment), the species and genotypic diversity associated with each treatment (Species and Genotypes, respectively), and the initial and final relative abundances (%) of each genotype. "U" indicates genotypes for which we were unable to determine relative abundance because we did not identify a microsatellite marker that distinguished the two *D. pulicaria* clones.

Treatment	Species	Genotypes	Initial Relative Abundance	Final Relative Abundance
No Daphnia	0	0	0	0
Px1	1	1	1	1
Px2	1	1	1	1
Pu1	1	1	1	1
Pu2	1	1	1	1
Px1/Px2	1	2	50:50	53:47
Pu1/Pu2	1	2	50:50	U:U
Px1/Pu1	2	2	50:50	18:82
Px2/Pu2	2	2	50:50	36:64
Px1/Px2/Pu1	2	3	33:33:33	14:17:69
Px2/Pu1/Pu2	2	3	33:33:33	13:U:U
Px1/Px2/Pu1/Pu2	2	4	25:25:25:25	9:21:U:U

Table 5.2. Coefficients of variation for each ecosystem property as a function of diversity levels for each of the three datasets.

Dataset	Diversity	Richness	Composition	Abundance	CR	NEP	GPP	Chlorophyll	Phaeophytin	N	P	N:P
	1	0.31	2.83	0.71	1.33	1.40	6.20	0.89	0.87	0.07	0.13	0.10
Full	2	0.33	10.24	0.51	1.04	3.26	1.53	0.53	0.51	0.03	0.09	0.08
(Genotype Diversity)	3	0.26	2.57	0.33	1.17	2.73	1.71	0.55	0.54	0.04	0.14	0.11
	4	0.26	1.45	0.25	3.46	0.67	1.06	0.44	0.42	0.02	0.11	0.10
Full	1	0.30	3.89	0.69	1.26	1.82	3.03	0.86	0.84	0.06	0.12	0.09
(Species Diversity)	2	0.32	3.31	0.32	1.67	6.53	4.47	0.59	0.58	0.03	0.13	0.10
	2	0.32	14.46	0.35	1.06	2.08	1.15	0.59	0.59	0.03	0.09	0.09
Genotype	3	0.26	2.23	0.33	1.17	2.73	1.71	0.55	0.54	0.04	0.14	0.11
	4	0.26	1.45	0.25	3.46	0.67	1.06	0.44	0.43	0.02	0.11	0.10
Species	1	0.30	0.52	0.69	1.04	5.08	1.70	0.46	0.44	0.03	0.09	0.07
	2	0.32	1.48	0.35	1.06	2.08	1.15	0.59	0.59	0.03	0.09	0.09

Table 5.3. Summary of levels of diversity (Genotype, Species) and their interaction (G * S) that are significant predictors of ecosystem and community properties based on stepwise regression results from the full, genotype and species datasets. F, S, G refer to the datasets (full, species, genotype, respectively) in which variation in the specific level of diversity is a significant predictor of variation in the ecosystem property based on inclusion in a significant best regression model and significant at $P<0.05$ based on ANOVA that included only variables in the best regression model. "N" indicates no relationship between the level of diversity and ecosystem property.

	Level of Diversity		
Property	**Genotype**	**Species**	**G * S**
Microbial Community Composition	N	**F,S**	N
Richness	**G**	**F**	N
Zooplankton Abundance	N	N	N
Community Respiration	**G**	N	**F**
Net Productivity	N	N	N
Gross Primary Productivity	**G**	N	**F**
Chlorophyll	**F**	N	N
Phaeophytin	**F**	N	N
Dissolved Nitrogen	N	N	N
Dissolved Phosphorous	**F**	N	N
N:P Ratio	**F**	N	N

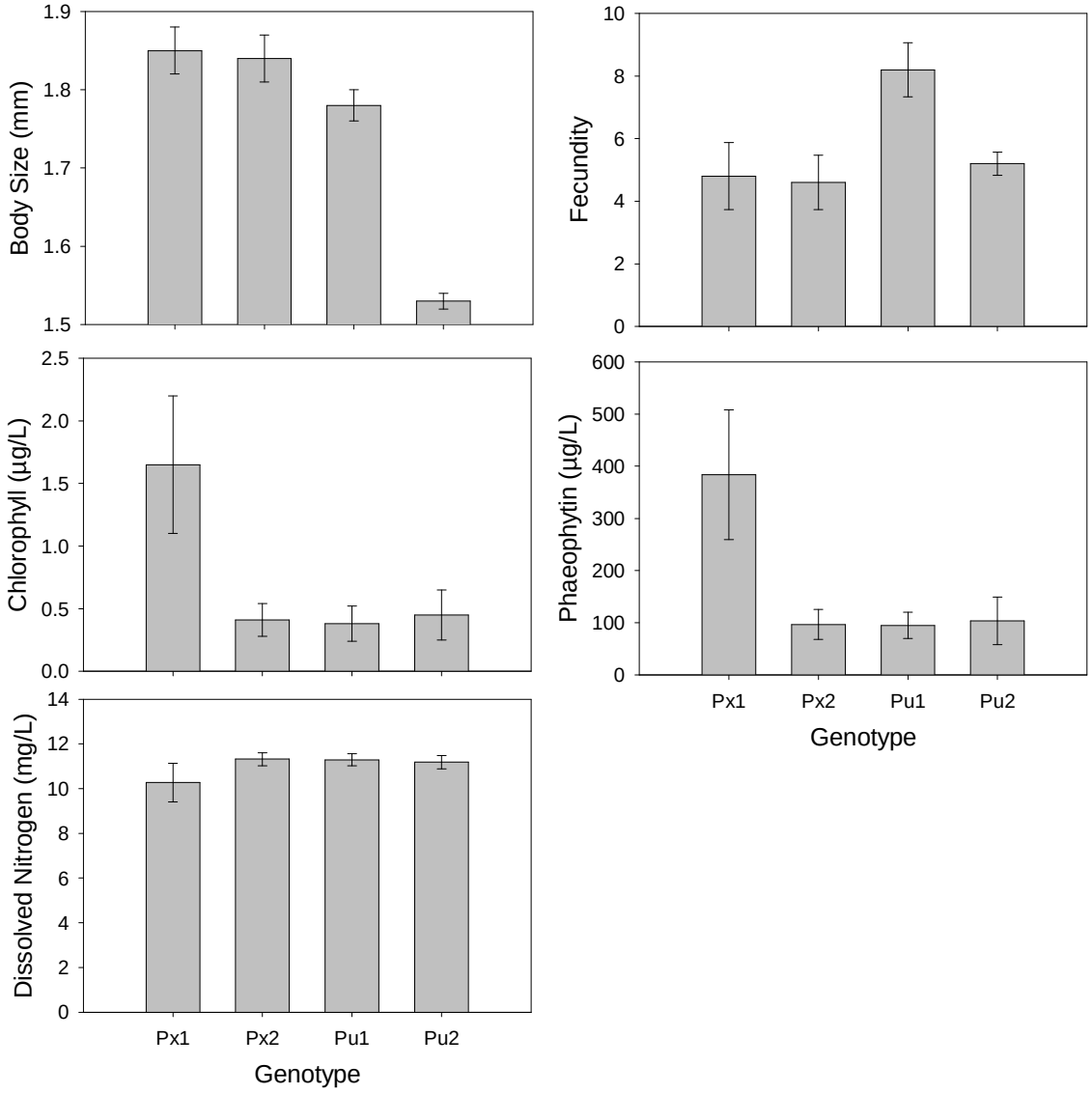


Figure 5.1. Life-history traits of each clone used in the microcosms based on a common-garden experiment (A and B), and clonal differences after the second sampling interval (C-E). Error bars are ± 2 SE.

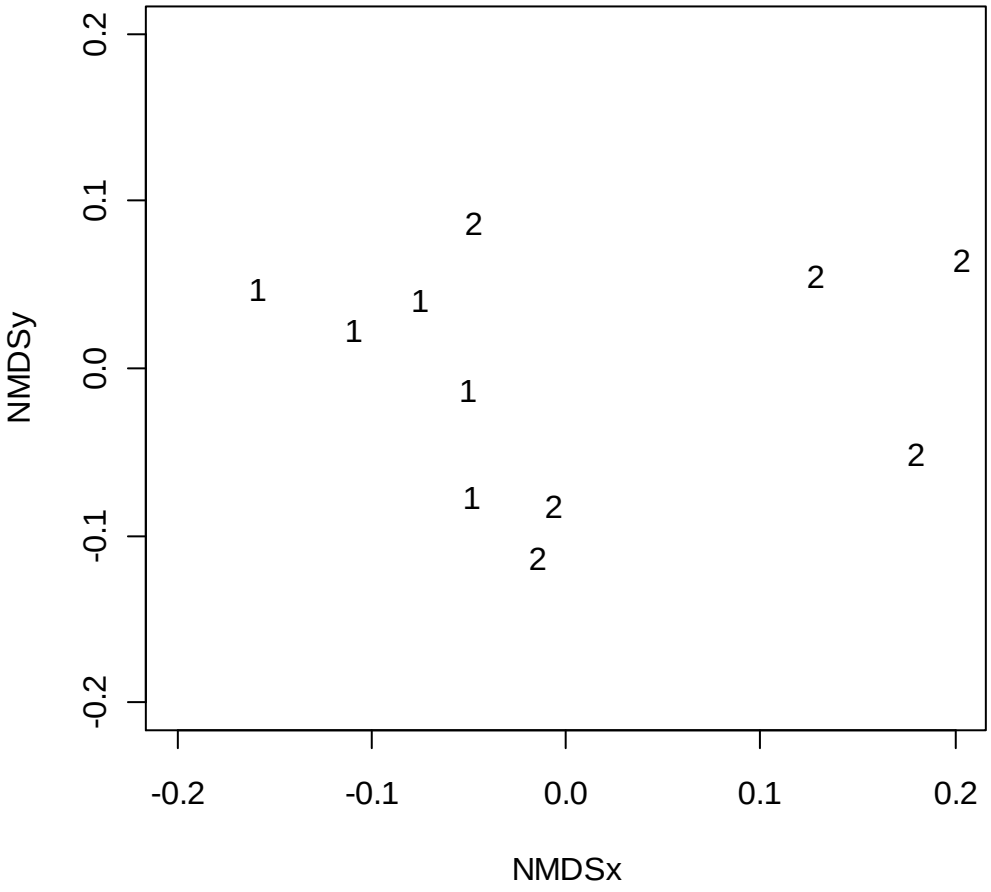


Figure 5.2. NMDS plot of microbial community composition for two levels of species diversity (1 and 2).

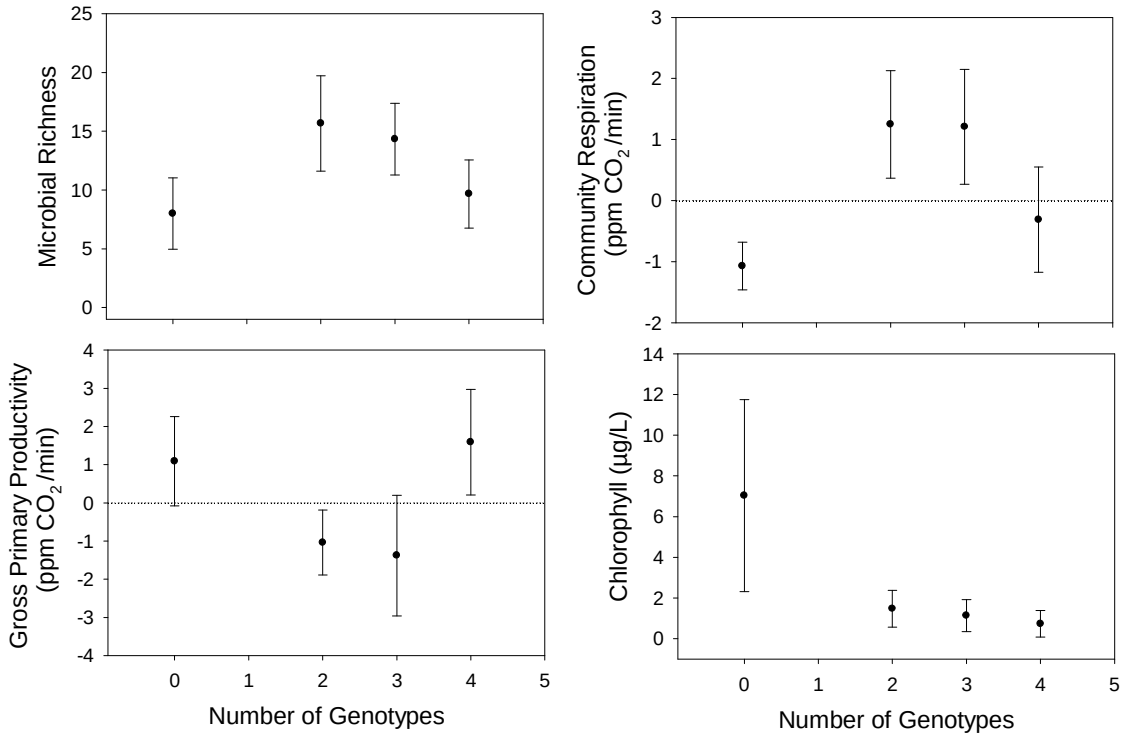
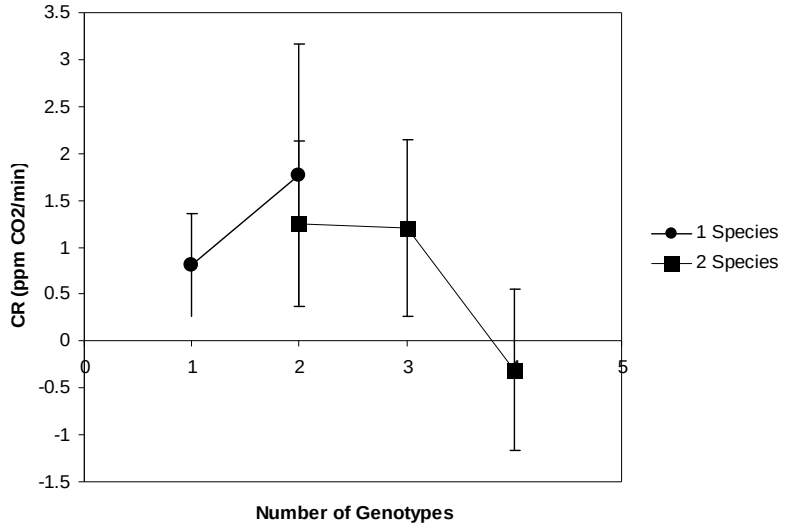


Figure 5.3. The effect of genotypic diversity on community (A) and ecosystem properties (B-D) when species diversity is constant. Note that the y-axis for two ecosystem properties (B and C) are given in units of ppm CO_2/min so that positive values suggest a net loss of primary productivity while negative values suggest a net gain in primary productivity. The *Chlamydomonas*-only treatment (Number of Genotypes=0) was not included in the analyses and is provided only as a reference. Error bars are ± 2 SE.

A



B

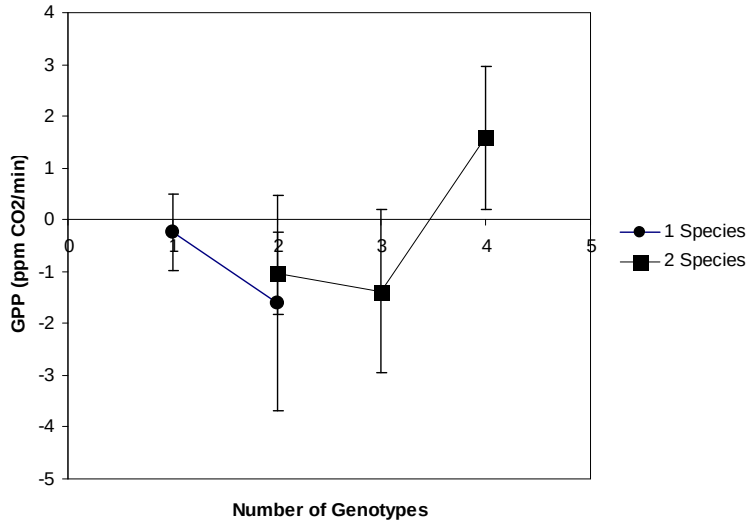


Figure 5.4. The interaction between species diversity and genotype diversity for community respiration (CR; A) and gross primary productivity (GPP; B). Note that the y-axis is given in units of ppm CO_2/min so that positive values suggest a net loss of primary productivity while negative values suggest a net gain in primary productivity. Error bars are ± 2 SE.

CHAPTER 6

CONCLUSIONS

Characterizing the variation within and among groups in a biological hierarchical level has long been a goal of biologists. To that end, numerous metrics have been developed to quantify the variability among hierarchical groups. At the level of populations, biologists measure the amount of phenotypic or genetic variance within populations, while communities are frequently quantified using the total number of individuals (abundance), the number of species present (richness), or metrics that incorporate both abundance and richness (e.g. the Shannon-Weaver Index). The experimental derivation of these types of metrics provides a valuable tool for biologists attempting to test fundamental ecological and evolutionary hypotheses pertaining to variation among groups within a biological hierarchical level.

However, there is a growing awareness that the ecological and evolutionary metrics that describe variability among groups within a hierarchical level are, at least in part, determined by factors that are fundamental to other levels of the biological hierarchy. Traditionally, these factors have been completely ignored or described only in qualitative terms, but it is exactly these factors that may provide specific mechanistic explanations for the observed attributes of groups within hierarchical levels. For example, a single community can be described by metrics that quantify the biodiversity of a community (richness, abundance, and the Shannon-Weaver Index). These metrics, and manipulations thereof, can then be compared to theoretical expectations that describe different processes of community assembly, such as niche partitioning or neutrality. Suppose one identifies a community that conforms to the expectations of niche partitioning, this result still says nothing about the specific mechanisms through which the partitioning occurred. In the case of comparisons among multiple communities, biodiversity indices can be used to determine to what extent two communities differ, but provide little explanation as to why they differ, other than the generic conclusion that they assembled differently. These generalities can

be resolved, however, through the examination of the attributes of lower levels of biological hierarchy.

The goal of this dissertation was to examine how primary determinants of two separate biological hierarchies, phenotypic plasticity at the level of individuals and genetic variation at the level of populations, determine the ecological position and evolutionary potential of hierarchical levels above them. Through the use of a simple freshwater tri-trophic food web and controlled laboratory experiments the results summarized subsequently highlight the importance of phenotypic plasticity and genetic variation for the ecology and evolution of populations, communities and ecosystems.

Phenotypic Plasticity

Phenotypic plasticity is the ability of an individual genotype to modify its phenotype in response to variable biotic or abiotic factors. Based on results from this dissertation, it is clear that in freshwater environments phenotypic plasticity can influence several attributes of a population or community. First, phenotypic plasticity can alter the mean phenotype of a population, and the change in phenotype results from the combined effects of individual genotypes whose plastic response to a cue is in the same direction. In freshwater systems, *Daphnia* morphology and life-history, and *Chlamydomonas* behavior displayed significant plastic shifts in response to chemical cues produced by organisms that occupy higher trophic levels. Furthermore, *Daphnia* morphology and life-history can exhibit significant plastic shifts in mean phenotype in response to changes in the abundance of organisms that occupy lower trophic levels. Second, phenotypic plasticity can alter the variance in the mean phenotype. Specifically, the variance in *Daphnia* body size changed in response to chemical cues produced by organisms of higher trophic status.

The changes in population mean phenotype due to phenotypic plasticity also have ramifications for community level properties. The plastic responses documented in both *Daphnia*

and *Chlamydomonas* were in a direction that would be considered adaptive. Thus, the adaptive plastic changes in population mean phenotype would facilitate population persistence in the face of a changing environment. At the community level, population persistence serves to maintain the species richness of zooplankton and phytoplankton populations which might otherwise perish in the absence of adaptive phenotypic plasticity and reduce the richness of the associated community. The significant adaptive plastic increases in *Daphnia* fecundity may also help maintain the total zooplankton abundance in freshwater environments where organisms of higher trophic status may utilize *Daphnia* as prey, or organisms of lower trophic status that would be utilized as food by *Daphnia* are not abundant. Overall, phenotypic plasticity, in part, determines adaptive changes in population means and variances, and provides a mechanistic explanation of community stability in response to changing environments.

Genetic Variation

Genetic variation describes the allelic variation in a population and is a primary factor that determines the phenotypic mean and variance of a population. The results presented here suggest that genetic variation also influences community and ecosystem properties in freshwater environments. In particular, high genetic diversity in a population of primary consumers, *Daphnia*, results in the reduction of species richness in associated microbial communities. High levels of genetic diversity also result in a net loss of ecosystem gross primary productivity and a concomitant increase in community respiration through significant reductions in phytoplankton abundance. Both the reduction in microbial diversity and change in ecosystem can be attributed to the increased grazing capabilities of a genetically diverse zooplankton assemblage. Overall, genetic diversity modulates communities and ecosystems, and also provides a mechanistic basis for the changes.

Summary

Phenotypic plasticity and genetic variation are important factors that determine the nature of individuals and populations, respectively. Results from this dissertation show that plasticity and genetic variation can shape the attributes of other biological groups higher in the biological hierarchy. In some cases, plasticity and genetic variation also provide a mechanistic explanation for variability observed in higher levels of the biological hierarchy. As biology grows as a discipline, the integration of investigations from various fields that cover concepts that have been historically viewed as disparate will undoubtedly yield new insights and ultimately unify the biological sciences.

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March 11, 2010

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.

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APPENDIX B

SAS and R code for Selected Analyses

Chapter 2 Analyses

```
data plastic;
input  Pop $ Time Genotype $ Adapted $ Environment $ SM AM NE;
cards;
proc mixed data=plastic method=reml;
class Environment Pop Adapted;
model SM = Environment  Adapted Pop(Adapted) Environment*Adapted
     Environment*Pop(Adapted);
     repeated / group=Environment;
     lsmeans Adapted/ pdiff;
     run;
     proc mixed data=plastic method=reml;
class Environment Pop Adapted;
model AM = Environment  Adapted Pop(Adapted) Environment*Adapted
     Environment*Pop(Adapted);
     repeated / group=Environment;
     lsmeans Adapted/ pdiff;
     run;
     proc mixed data=plastic method=reml;
     class Environment Pop Adapted;
     model NE = Environment  Adapted Pop(Adapted) Environment*Adapted
     Environment*Pop(Adapted);
     repeated / group=Environment;
     lsmeans Adapted/ pdiff;
     run;
```

Chapter 3 Analyses

```
data cb;
input Treatment $ Time $ Depth $ T TC;
cards;
data top; set cb; if Depth = 'T';
data middle; set cb; if Depth = 'M';
data bottom; set cb; if Depth = 'B';
data dapht; set top; if Treatment = 'Control' or Treatment = 'Daph';
data daphm; set middle; if Treatment = 'Control' or Treatment = 'Daph';
data daphb; set bottom; if Treatment = 'Control' or Treatment = 'Daph';
data concentrationt; set top; if Treatment = 'HalfDaph' or Treatment = 'Daph';
data concentrationm; set middle; if Treatment = 'HalfDaph' or Treatment = 'Daph';
data concentrationb; set bottom; if Treatment = 'HalfDaph' or Treatment = 'Daph';
data fisht; set top; if Treatment = 'Control' or Treatment = 'Fish';
data fishm; set middle; if Treatment = 'Control' or Treatment = 'Fish';
data fishb; set bottom; if Treatment = 'Control' or Treatment = 'Fish';
data predatort; set top; if Treatment = 'HalfDaph' or Treatment = 'FishDaph';
data predatorm; set middle; if Treatment = 'HalfDaph' or Treatment = 'FishDaph';
```

```
data predatorb; set bottom; if Treatment = 'HalfDaph' or Treatment = 'FishDaph';
proc glm data=top;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment / pdiff stderr;
run;
proc glm data=middle;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment / pdiff stderr;
run;
proc glm data=bottom;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=dapht;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=daphm;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=daphb;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=concentrationt;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=concentrationm;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=concentrationb;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
```

```
proc glm data=fisht;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Time / pdiff stderr;
run;
proc glm data=fishm;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=fishb;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=predatort;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=predatorm;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
proc glm data=predatorb;
class Treatment Time;
model TC = Treatment Time Treatment*Time;
lsmeans Treatment*Time / pdiff stderr;
run;
```

Chapter 5 Analyses for Genotype Dataset

```
microbe=read.table("MGenotypes.txt", header=T, na.strings=".")
NMDS1=lm(NMDS1 ~ Genotypes*Block1, data=microbe)
NMDS1step=stepAIC(NMDS1, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
Richness=lm(Richness ~ Genotypes*Block1, data=microbe)
Richnessstep=stepAIC(Richness, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
summary(NMDS1step)
anova(NMDS1step)
summary(Richnessstep)
anova(Richnessstep)
nomicrobe=read.table("NMGenotypes.txt", header=T, na.strings=".")
TotalN1=lm(TotalN1 ~ Genotypes*Block1, data=nomicrobe)
```

```
TotalN1step=stepAIC(TotalN1, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
CR1=lm(CR1 ~ Genotypes*Block1, data=nomicrobe)
CR1step=stepAIC(CR1, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
NEP1=lm(NEP1 ~ Genotypes*Block1, data=nomicrobe)
NEP1step=stepAIC(NEP1, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
GPP1=lm(GPP1 ~ Genotypes*Block1, data=nomicrobe)
GPP1step=stepAIC(GPP1, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
ChlA=lm(ChlA ~ Genotypes*Block1, data=nomicrobe)
ChlAstep=stepAIC(ChlA, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
Phaeophytin=lm(Phaeophytin ~ Genotypes*Block1, data=nomicrobe)
Phaeophytinstep=stepAIC(Phaeophytin, scope = list(upper=~Genotypes*Block1,
    lower=~1, direction="both"))
TDN=lm(TDN ~ Genotypes*Block1, data=nomicrobe)
TDNstep=stepAIC(TDN, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
TDP=lm(TDP ~ Genotypes*Block1, data=nomicrobe)
TDPstep=stepAIC(TDP, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
Npratio=lm(Npratio ~ Genotypes*Block1, data=nomicrobe)
Npratiostep=stepAIC(Npratio, scope = list(upper=~Genotypes*Block1, lower=~1,
    direction="both"))
summary(TotalN1step)
anova(TotalN1step)
summary(CR1step)
anova(CR1step)
summary(NEP1step)
anova(NEP1step)
summary(GPP1step)
anova(GPP1step)
summary(ChlAstep)
anova(ChlAstep)
summary(Phaeophytinstep)
anova(Phaeophytinstep)
summary(TDNstep)
anova(TDNstep)
summary(TDPstep)
anova(TDPstep)
summary(Npratiostep)
anova(Npratiostep)
```

Chapter 5 Analyses for Species Dataset

```
microbe=read.table("MSpecies.txt", header=T, na.strings=".")
NMDS1=lm(NMDS1 ~ Species*Block1, data=microbe)
NMDS1step=stepAIC(NMDS1, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
Richness=lm(Richness ~ Species*Block1, data=microbe)
Richnessstep=stepAIC(Richness, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
summary(NMDS1step)
anova(NMDS1step)
summary(Richnessstep)
anova(Richnessstep)
nomicrobe=read.table("NMSpecies.txt", header=T, na.strings=".")
TotalN1=lm(TotalN1 ~ Species*Block1, data=nomicrobe)
TotalN1step=stepAIC(TotalN1, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
CR1=lm(CR1 ~ Species*Block1, data=nomicrobe)
CR1step=stepAIC(CR1, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
NEP1=lm(NEP1 ~ Species*Block1, data=nomicrobe)
NEP1step=stepAIC(NEP1, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
GPP1=lm(GPP1 ~ Species*Block1, data=nomicrobe)
GPP1step=stepAIC(GPP1, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
ChlA=lm(ChlA ~ Species*Block1, data=nomicrobe)
ChlAstep=stepAIC(ChlA, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
Phaeophytin=lm(Phaeophytin ~ Species*Block1, data=nomicrobe)
Phaeophytinstep=stepAIC(Phaeophytin, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
TDN=lm(TDN ~ Species*Block1, data=nomicrobe)
TDNstep=stepAIC(TDN, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
TDP=lm(TDP ~ Species*Block1, data=nomicrobe)
TDPstep=stepAIC(TDP, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
Npratio=lm(Npratio ~ Species*Block1, data=nomicrobe)
Npratiostep=stepAIC(Npratio, scope = list(upper=~Species*Block1, lower=~1,
    direction="both"))
summary(TotalN1step)
anova(TotalN1step)
summary(CR1step)
anova(CR1step)
summary(NEP1step)
```

```
anova(NEP1step)
summary(GPP1step)
anova(GPP1step)
summary(ChlAstep)
anova(ChlAstep)
summary(Phaeophytinstep)
anova(Phaeophytinstep)
summary(TDNstep)
anova(TDNstep)
summary(TDPstep)
anova(TDPstep)
summary(Npratiostep)
anova(Npratiostep)
```

Chapter 5 Analyses for Full Dataset

```
microbe=read.table("MBoth.txt", header=T, na.strings=".")
NMDS1=lm(NMDS1 ~ Species*Genotypes*Block1, data=microbe)
NMDS1step=stepAIC(NMDS1, scope = list(upper=~Species*Genotypes*Block1),
    lower=~1, direction="both")
Richness=lm(Richness ~ Species*Genotypes*Block1, data=microbe)
Richnessstep=stepAIC(Richness, scope = list(upper=~Species*Genotypes*Block1),
    lower=~1, direction="both")
summary(NMDS1step)
anova(NMDS1step)
summary(Richnessstep)
anova(Richnessstep)
nomicrobe=read.table("NMBoth.txt", header=T, na.strings=".")
TotalN1=lm(TotalN1 ~ Species*Genotypes*Block1, data=nomicrobe)
TotalN1step=stepAIC(TotalN1, scope = list(upper=~Species*Genotypes*Block1,
    lower=~1, direction="both"))
CR1=lm(CR1 ~ Species*Genotypes*Block1, data=nomicrobe)
CR1step=stepAIC(CR1, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
NEP1=lm(NEP1 ~ Species*Genotypes*Block1, data=nomicrobe)
NEP1step=stepAIC(NEP1, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
GPP1=lm(GPP1 ~ Species*Genotypes*Block1, data=nomicrobe)
GPP1step=stepAIC(GPP1, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
ChlA=lm(ChlA ~ Species*Genotypes*Block1, data=nomicrobe)
ChlAstep=stepAIC(ChlA, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
Phaeophytin=lm(Phaeophytin ~ Species*Genotypes*Block1, data=nomicrobe)
Phaeophytinstep=stepAIC(Phaeophytin, scope =
    list(upper=~Species*Genotypes*Block1, lower=~1, direction="both"))
```

```
TDN=lm(TDN ~ Species*Genotypes*Block1, data=nomicrobe)
TDNstep=stepAIC(TDN, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
TDP=lm(TDP ~ Species*Genotypes*Block1, data=nomicrobe)
TDPstep=stepAIC(TDP, scope = list(upper=~Species*Genotypes*Block1, lower=~1,
    direction="both"))
Npratio=lm(Npratio ~ Species*Genotypes*Block1, data=nomicrobe)
Npratiostep=stepAIC(Npratio, scope = list(upper=~Species*Genotypes*Block1,
    lower=~1, direction="both"))
summary(TotalN1step)
anova(TotalN1step)
summary(CR1step)
anova(CR1step)
summary(NEP1step)
anova(NEP1step)
summary(GPP1step)
anova(GPP1step)
summary(ChlAstep)
anova(ChlAstep)
summary(Phaeophytinstep)
anova(Phaeophytinstep)
summary(TDNstep)
anova(TDNstep)
summary(TDPstep)
anova(TDPstep)
summary(Npratiostep)
anova(Npratiostep)
```

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Advisor: Dr. Michael Lynch

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Employment:

Patineur. Parks Bronze. Enterprise, OR. 1999-2001.
Apply color to bronze sculptures using heat and chemicals or airbrush. Packaging and shipping of finished sculptures.

Head Brewer. Terminal Gravity Brewing Enterprise, OR 2001-2005.
Head of brewing operations, train new employees, quality control, representative at brewing science conferences.

Peer Review Publications:

Latta IV, L.C., S. Frederick, M.E. Pfrender. Diet restriction and longevity in short- and long-lived species. *In preparation for Journal of Gerontology*.

Latta IV, L.C., M. Baker, T. Crowl, J.J. Parnell, B. Weimer, D. DeWald, and M.E. Pfrender. Species and Genotype Diversity Drive Community and Ecosystem Properties in Experimental Microcosms. *In preparation for Evolutionary Ecology*.

Parnell, J.J., G. Rompato, **L.C. Latta IV**, M.E. Pfrender, J. Van Nostrand, Z. He, J. Zhou, G. Andersen, P. Champine, B. Ganesan, B.C. Weimer. Functional biogeography as evidence of gene transfer in hypersaline microbial communities. *Submitted to mBio.*

Latta IV, L.C., D.L. Fisk, R.A. Knapp, and M.E. Pfrender. Genetic resilience of *Daphnia* populations following experimental removal of introduced fish. *Accepted by Conservation Genetics*.

Kanaga, M.K., **L.C. Latta IV,** and M.E. Pfrender. 2009. Plant genotypic diversity and environmental stress interact to negatively affect arthropod community diversity. Arthropod-Plant Interactions 3: 249-258.

Latta IV, L.C., R.P. O'Donnell and M.E. Pfrender. 2009. Vertical distribution of *Chlamydomonas* changes in response to grazer and predator kairomones. Oikos 118: 853-858.

Latta IV, L.C., J.W. Bakelar, R.A. Knapp, and M.E. Pfrender. 2007. Rapid evolution in response to introduced predators II: the contribution of adaptive plasticity. BMC Evolutionary Biology: 7:21.

Fisk, D.L., **L.C. Latta IV**, R.A. Knapp, and M.E. Pfrender. 2007. Rapid evolution in response to introduced predators I: rates and patterns of morphological and life-history divergence. BMC Evolutionary Biology: 7:22.

Morgan, K.K., J. Hicks, K. Spitze, **L. Latta**, M.E. Pfrender, C.S. Weaver, M. Ottone, and M. Lynch. 2001. Patterns of genetic architecture for life-history traits and molecular markers in a subdivided species. Evolution 55: 1753-1761.

Pfrender, M.E., K. Spitze, J. Hicks, K.K. Morgan, **L. Latta**, M. Lynch. 2000. Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels. Conservation Genetics 1: 263-269.

Lynch, M., M.E. Pfrender, K. Spitze, N. Lehman, J. Hicks, D. Allen, **L. Latta**, M. Ottone, F. Bogue, and J. Colbourne. 1999. The quantitative and molecular genetic architecture of a subdivided species. Evolution 53: 2016-2016.

Lynch, M., **L. Latta**, J. Hicks, and M. Giorgianni. 1998. Mutation, selection, and the maintenance of life-history variation in a natural population. Evolution 52: 727-733.

Teaching Experience:

Utah State University-Logan, UT
Graduate Instructor, Fall 2009
Coevolution
Evolutionary Genetics (Co-Instructor)

Utah State University-Logan, UT
Teaching Assistant, 2005-2010
Human Physiology – Lecturer: Reproductive Physiology
Evolution – Lecturer: Conservation Genetics, Molecular Evolution, Speciation
Introductory Biology

University of Oregon-Eugene, OR
Teaching Assistant, 1996-1999
Introductory Biology

Presentations:

Rapid evolution in response to introduced predators. 2009. Invited speaker - Lester Newman Biology Seminar Series. Portland State University.

Evolutionary transitions to saline environments in *Daphnia*. 2009. Talk – Center for Integrated Biosystems Research Program. Utah State University.

Environmental effects on spontaneous deleterious mutation parameters. 1998. Talk - Society for the Study of Evolution Annual Meeting.

Effects of spontaneous deleterious mutation on behavior in *C. elegans*. 1998. Poster - Society for the Study of Evolution Annual Meeting.

Grants:

The Evolution of Salinity Tolerance in Daphnia. Utah State University Center for Integrated Biosystems, $7000, 2008

Reviewer:

Journal of Plankton Research, 2010
Journal of Crustacean Biology, 2008-2009
Biology Letters, 2007

Service:

Biology Graduate Programs Committee, USU, 2007-2010
Biology Graduate Student Association, Vice President, USU, 2006-2007

Laboratory and Field Experience:

Field Assistant, March 1999.
Contact: Dr. William Bradshaw (University of Oregon)

Laboratory Manager, June 1995 – December 1995.
Contact: Dr. Michael Lynch (University of Indiana)

Personal References

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