INTRODUCTION AND PROBLEM STATEMENT

Understanding the impacts of climate change is one of the most important challenges facing ecologists today (Thuiller 2007). Species confronted with a changing environment have three possible responses to prevent local extinction: 1) range shifts (Parmesan et al. 1999, Walther et al. 2002), 2) adaptation through phenotypically plastic responses (Gienapp et al. 2008), or 3) adaptation through genetically-based microevolutionary responses (Bradshaw and Holzapfel 2001, Etterson and Shaw 2001, Reale et al. 2003). Although many studies have demonstrated phenotypic changes of organisms in response to climate change (Mysterud et al. 2001, Stenseth and Mysterud 2002, Parmesan and Ue 2006, Pulido 2007), far fewer have addressed genetic responses at the population level. Of those studies that have detected microevolutionary changes, most have relied on space-time substitutions (Etterson 2004) or long term data sets (>10 years) (Bradshaw and Holzapfel 2001, Reale et al. 2003). Only a handful of studies have used an experimental approach of manipulating abiotic variables to mimic future climate scenarios. These experimental studies have successfully detected both microevolution due to selection (Franks and Weis 2008, Jump et al. 2008) and changes in community composition (Suttle et al. 2007).

Although molecular methods for detecting a response to selection are improving, identifying candidate genes is still expensive and time-consuming (Hoffmann and Willi 2008). Quantitative genetic methods offer a reliable tool to detect a response to selection (Conner and Hartl 2004, Falconer 1989). These methods have been used to successfully identify microevolutionary responses in both plant (Franks and Weis 2008) and insect (Bradshaw and Holzapfel 2001) populations. Furthermore if multiple traits are measured, then direct and indirect selection (Lande 1979, Conner 1988) and evolutionary constraints (Etterson and Shaw 2001) can also be explored. **The aim of my study is to quantify life history and morphological responses of *Daphnia pulex* to changes in vernal pond hydroperiod that mimic climate change scenarios, to partition those responses into genetic and phenotypically plastic components and detect a microevolutionary response to climate change.**

BACKGROUND

Most studies of microevolutionary responses have used plants (e.g. Etterson 2004, Jump et al. 2008) because estimating heritabilities in animals is difficult without long pedigrees or large populations of marked and measured individuals (Gienapp et al. 2008) However, Bradshaw and Holzapfel (2001) reared mosquito larvae in a common garden experiment and demonstrated a response in photoperiod to selection along a latitudinal gradient. Reale et al (2003) measured parturition date in a 10-year pedigree of 325 marked individuals of Yukon red squirrels Although most of the variance was a plastic response, there was heritable variation and directional selection for earlier parturition.

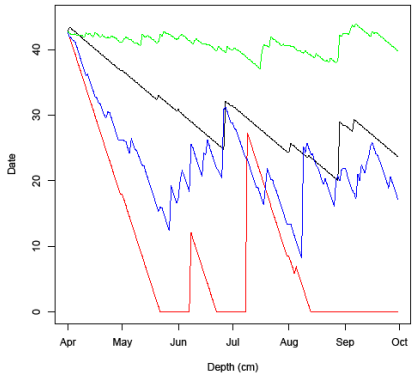

Figure. 1 Four extreme hydrological treatments. The y-axis is pond depth and the x-axis is date, ranging from April to October Green = long hydroperiod, regular precipitation, blue = short hydroperiod, regular precipitation, black = long hydroperiod, extreme precipitation, red = short hydroperiod, extreme precipitation

Gienapp et al (2007) outline three necessary components for a study to demonstrate a genetic response to climate change in a population. (1) There must be selection on a trait, which can be demonstrated by satisfying the three conditions for selection: (a) phenotypic variation, (b) heritability of variation, (c) a fitness differential

between phenotypes (Endler 1986). (2) There must be evidence that climate change is linked to selective agents (change in phenotype in different climate environments). (3) There must be evidence that the change is at least in part genetically based.

RESEARCH QUESTIONS

Since June of 2008, I have maintained a large scale field experiment in central Vermont consisting of 81 experimental ponds to examine the impacts of variable hydrology on insect population dynamics (Fig. 1). These ponds were seeded with pond detritus from a nearby vernal pool that contains invertebrate eggs and resting stages. Each treatment pond was also seeded with a zooplankton sample that included the cyclically parthenogenic cladoceran *Daphnia pulex*. Since the experiment originated, these *Daphnia* populations have persisted and grown in over 90% of the pond replicates. Because they have extremely low migration rates due to passive dispersal (Bilton et al. 2001), the response of *Daphnia* to climate change will either be phenotypically plastic or genetically-based microevolution. Cyclical parthenogens have been used to successfully study local adaptation in *Daphnia spp* (DeMeester 1996, Cousyn et al. 2001, DeClerck et al. 2001) and in pea aphids (Via 1991b, a). Using well-defined quantitative genetic methods (Lande and Arnold 1983, Arnold and Wade 1984) I propose to study natural selection in the context of climate change by asking the following questions.

Question 1. Is the response of *Daphnia pulex* to climate change manipulations dominated by phenotypically plastic or genetically based microevolutionary changes? I will isolate different *Daphnia pulex* clones from the field experiment and raise them in a controlled uniform environment to measure phenotypic variation, and estimate heritability. Trait means and variances can then be regressed against the treatment levels to see if the shift is related to climate variables.

Question 2. What is the direction of selection in *Daphnia pulex* in relation to climate change? Is selection direct or indirect, and is there any evidence for evolutionary constraints? I will repeat the same experiment as above but using *Daphnia pulex* from the source pond from which all the original *Daphnia* came from. This will allow me to estimate fitness and the direction of selection. Using the multivariate breeder's equation I will estimate the direct and indirect components of selection. I will calculate the G-matrix of additive genetic covariance and examine it for evolutionary constraints.

STUDY SYSTEM.

Study organism: Daphnia pulex (Branchiopoda: Cladocera) is a filter feeding zooplankter that consumes bacteria and phytoplankton, and is common in a wide variety of temporary and permanent aquatic habitats (Riessen 1999). *Daphnia pulex* usually survive for 16 instars (Lynch 1980), and exhibit a sigmoid growth function for body size with rapid growth to first reproduction between the fourth and sixth instars, and then asymptotic growth thereafter (Green 1956, Lynch 1980). Reproduction is cyclically parthenogenic with mature diploid females producing a clutch eggs every instar (Green 1956). After each molt, a female releases small free swimming clones (Green 1956). When the environment becomes stressful, females will produce males, and a round of sexual reproduction will occur (Lynch 1983) in which resting eggs are produced that withstand desiccation and freezing (DeClerck et al. 2001). Female body size, clutch size, and neonate size are all correlated within a clonal line, but there is substantial variation among clones (Lynch 1983, Green 1956). Because of the cyclical nature of reproduction, survival of highly fit clonal lineages can lead to highly adapted local populations (Lynch 1983).

Effects of climate change on vernal ponds: Vernal ponds are fishless habitats that fill in the spring (vernal) or fall (autumnal) and hold water for at least 4 months, but dry with regular intervals (Zedler

2003). Regional climate change models predict a warming of 3.5° to 6.5° F by 2100 under low emissions scenarios, and an even larger increase under high emission scenarios (Frumhoff et al. 2006). Climate change scenarios for the Northeast also predict an increased water budget in the winter/spring and increased deficit in the summer / fall (Moore et al. 1997). Precipitation events are likely to become more variable, with longer periods of drought followed by more intense deluges (Sun et al. 2007, Kendon et al. 2008). Two effects of climate change will alter these systems: increased evapotranspiration and more variable precipitation. Increased winter precipitation will increase initial pond volume, and more variable spring summer rainfall combined with greater evapotranspiration will increase the variance of how an individual pond dries down (hydroperiod) (Bauder 2005). By simultaneously varying the rate at which a pond dries, and the frequency and intensity of rainfall events, an entire range of climate scenarios can be experimentally generated.

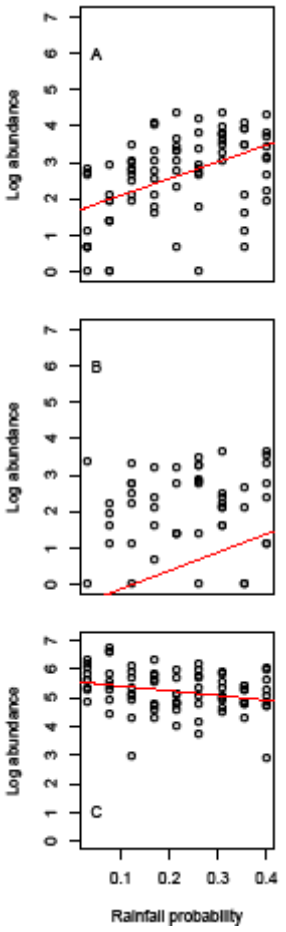

Figure 2. Regressions of log of maximum sampled abundance against rainfall probability for A). Chironomidae, B). Chaoboridae C.). Culicidae. All regression lines are $p < .05$

PRELIMINARY RESULTS

In 2007 I began a small mesocosm experiment to determine the effects of changing precipitation regimes on ephemeral ponds at the University of Vermont's Jericho Research Forest (UVM JRF) (44.45° N, 73.00° W). Using forty-nine fifty- four gallon plastic bins as experimental units, I continuously varied mean water level and the variance around that mean in a 7 x 7 response surface design. Throughout the summer, I made weekly changes to water level and counted and identified insects in each unit to family level. By the end of the summer I had collected time series data for three rapidly-colonizing insect families: Culicidae, Chironomidae, and Chaoboridae. Each time series was then analyzed using a discrete logistic growth function (Dennis and Taper 1994), fitted to the data with a hierarchical Bayesian model. I hypothesized that different taxa would respond differently to the same climate variable (Stenseth and Mysterud 2002). I found that density dependence decreased for Chaoboridae and Chironomidae, but increased for Culicidae the deeper the mean water level.

Encouraged by these results, I constructed eighty-one artificial pond mesocosms, 60 cm deep and 1.75 m in diameter, open for colonization in a mixed deciduous hardwood forest at the UVM JRF. Each pond represents a cross between nine different drying rates, and nine different precipitation probabilities in a response surface design (Inouye 2001). Because of the uncertainty of climate models, a response surface design allowed me to cover the greatest number of possible future scenarios.

Because vernal ponds are hydrologically simple systems of water inputs and outputs, their hydrology can easily be modeled (Pyke 2004). In my field experiment, each pond mesocosm is assigned a unique combination of drying rate and rainfall probability. Each rainfall treatment also has a probability of an extreme precipitation event associated with it. All these variables were combined in stochastic model to create a unique hydrology and water profile for each pond (Fig. 1). I designed these treatments to cover a range of possible climate scenarios. The drying rate treatments mimic the increased evapotranspiration

expected with global warming, and the precipitation treatments mimic the expected changes in precipitation (Frumhoff et al. 2006) .

Pond construction began on May 1st 2008 and the ponds were completed by June 7th. During construction, each pond was seeded with 10 L of pond detritus containing macroinvertebrates and a 50 mL plankton tow, which included *Daphnia pulex* (the dominant zooplankter). Weekly water-level modifications were made according to the hydrological model treatments, as well as weekly sweep net samples identified to family. During the first season, a total of 11 different families of insects colonized these ponds, including representatives from six insect orders with major aquatic representatives: Coleoptera, Diptera, Hemiptera, Megaloptera, Odonata, and Trichoptera. Each pond was also seeded with 200 wood frog (*Rana sylvatica*) tadpoles. All tadpoles successfully metamorphosed, and many of the ponds were inhabited by adult bullfrogs (*Rana catesbeiana).*

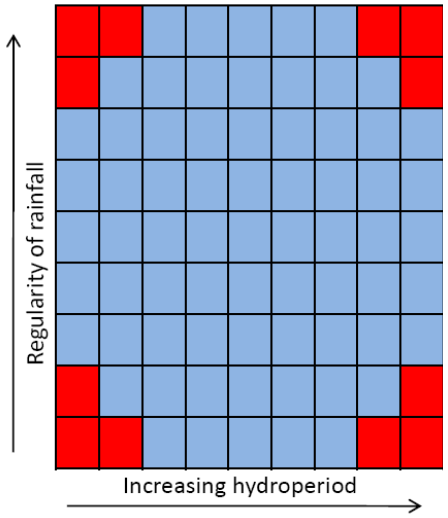

Figure 3. Graph of the total parameter space from the 81 treatments. Each square represents a pond mesocosm. The red square in each corner represent the three replicates for the 2x2 design. Although the replicates are not exactly the same they are very close to each other.

I saw similar results to the previous year's study. Chaoboridae and Chironomidae population sizes were greater in ponds with a higher mean water level, whereas Culicidae populations grew the largest in shallower habitats (Fig. 2). My experimental results have demonstrated that altered precipitation regimes expected with climate change will alter population dynamics and community structure of vernal ponds. Although these results document an ecological response to climate change, we do not know if there have been evolutionary responses as well. Highly mobile organisms that will only complete a few generations a season are poor model organisms to study these potential responses, but the passively dispersing *Daphia pulex* represents a model organism to study evolutionary responses in my climate change experiment because they will have undergone at least 24 generations when the proposed study begins.

PROPOSED STUDY

Question 1. Is the response of Daphnia pulex to climate change manipulations dominated by phenotypic plastic or genetically based microevolutionary changes? I will examine the phenotypic response of *Daphnia pulex* in vernal ponds to climate change manipulations and determine its genetic and environmental components using multigenerational controlled breeding experiments. I predict that there will be heritable variation in traits such as body size, clutch size, and growth rate (Havel and Dodson 1984, Tollrian 1995, Riessen 1999, Riessen and Young 2005) .

Sampling design: I will subsample the extreme combinations of my parameter space to create a 2 x 2 factorial design. In order to create replicates, I will sample two additional ponds close to each extreme (Fig. 3). Although these will not be exact replicates, the hydrological profiles within the 4 clusters in Figure 3 will be very similar to one another. By sampling the extremes, I will be able to encompass a range of contrasting conditions– including extremely high variability in hydroperiod and drying rate– that are to be expected with climate change. At the end of August 2009, I will collect ten females from each pond carrying eppiphia (sexually reproduced resting eggs). Because the eggs are all sexually reproduced, I can be sure that all clonal lines are unique individuals (Ebert et al. 1993). *Daphnia* eggs will be stored

at 4° C in the dark to incubate them before inducing hatching (Ebert et al. 1993, De Meester et al. 1998). I will induce hatching by exposing eggs to continuous light and 18° C temperature in a growth chamber (Schwartz and Hebert 1987, Ebert et al. 1993, De Meester et al. 1998). I will randomly select 4 clones from the 10 initially collected clones. For each clone, I will have five clonal replicates for a total of 240 individuals (2 x 2 factorial x 3 replicates x 4 different clones x 5 clonal replicates = 240). In order to control for maternal effects, a clonal lineage will be established from the granddaughters of the initially hatched females (Tollrian 1995).

Common environment and measurement: Each clone will be raised from a single *Daphnia* maintained in a 500 ml beaker in a growth chamber under constant conditions of 12:12 light:dark and 20° C (Spitze 1993) . All *Daphnia* will be cultured in artificial medium to ensure uniform water conditions (Kluttgen et al. 1994) and fed a constant volume of dried algae (Caroline Biological Supply). The water will be changed every other day with a peristaltic pump fitted with a 30 µm Nitex mesh filter (Riessen and Sprules 1990). For each clone, I will measure a variety of life history and morphological traits. Clones will be monitored daily and measurements will be made after each clone molts to a new instar or when sexual maturity is reached, depending on the trait of interest (Table 1).

Trait	**Measurement Frequency**	**Reference**
Body length (not including spine)	M+4	(Ebert et al. 1993)
Head width	M+4	(Havel and Dodson 1984)
Tail spine length	M+4	(Havel and Dodson 1984)
Size as neonate	M+4	(Ebert 1991)
Time to maturity	1	(Tollrian 1995)
Instar at maturity	1	(Ebert 1991)
Clutch size	All	(Spitze 1991)
Size of neonates	All	(Lynch et al. 1989)
Survivorship/ life span	1	(Orcutt and Porter 1984)
Growth rate	1	(Spitze 1993)
Instars with neck teeth	All	(Riessen and Young 2005)

Table 1. The different traits I will measure and the methodological citation. The first 4 are morphological, the remaining life history. M+4 = Instar at maturity plus 4 adult instars, 1 = once, all = all instars the clone survives.

Data analysis: Because *Daphnia* are clonal, phenotypic variance can be partitioned into $V_P = V_E + V_G$ without complications from other types of gene action such as dominance or epistasis (Conner and Hartl 2004). The structure of my experiment is similar to that of common garden experiments in the plant literature: within a given pond, V_G in the variance among clones and $V_E$is the variance within clonal replicates (Ebert et al. 1993, Silvertown and Charlesworth 2005). Variance components will be estimated using hierarchical Bayesian linear random effects models (Gelman 2005; Collins, Hart and Molofsky 2008), and broad sense heritabilitiess can be estimated as $h^2 = \frac{V_G}{V_P}$ (Conner and Hartl 2004). Finally I will estimate Q_{st}, the analog to Wright's F_{st} for quantitative traits rather than neutral markers, as: $Q_{st} = \sigma^2_{GB}/\sigma^2_{GB} + 2\sigma^2_{GW}$ (Spitze 1993). Here σ^2_{GB} is the average among population variance component, and σ^2_{GW} is the average within population variance component, which will be estimated by bootstrap resampling. Fitness will be calculated $w = \sum_x l_x m_x e^{-\hat{r}x}$ where l_x is survival at age x, m_x is the fecundity at age x and $\hat{r}$ is the population growth rate of the entire date set (Spitze 1993, Charlesworth 1994).

Hypothesis and predictions: I predict that some fraction of the variance in life history and morphological traits will be environmental and some will be genetic. I predict heritabilities comparable to those reported

in the literature of between 0.2 and 0.5 (Lynch and Spitze 1994). I expect a shift in trait means will result from two selective agents correlated with my treatments: *Chaoborus* predation and *Daphnia* food levels. Riessen and Young (2005) examined fitness landscapes for *Daphnia* in the presence of *Chaoborus* at different food levels and found that at low food levels there was a fitness trough for intermediate sizes, and that very small or very large *Daphnia* had the highest fitness. Therefore in ponds with higher numbers of *Chaoborus* I hypothesize that selection will lead to larger *Daphnia* body size, larger tail spines, younger age at maturity, and larger clutch sizes (Spitze 1991, Tollrian 1995). *Daphnia* responses will be effected directly by the changing rainfall altering the nutrient dynamics of the ponds, but indirectly by predation from *Chaoborus* and the presence of competition from *Rana sylvatica* tadpoles (Leibold and Wilbur 1992). I will test three possible hypotheses for each trait with this experiment to detect a response to climate change (Fig. 4). The first is that any differences in a trait are environmental; therefore in the common environment all the trait means will be the same. This implies all the response so far has been plastic (Fig. 4A). The second outcome is a genetic response but with no relationship to the treatments, possibly reflecting some unmeasured selective agent, or founder effects (Fig. 4b). The final outcome is a genetic response in a trait that corresponds to the treatments. In this example, larger populations of *Chaoborus* select for larger body size in *Daphnia* in the ponds that have regular rainfall and a longer hydroperiod (Fig. 4C). Finally I expect that Q_{st} values will show that ponds with similar treatments will be closer to each other. Because I have a full factorial design, I will also be able to determine which of the two treatments causes more similarity between populations and quantify both the additive and interactive effects of drying rate and hydroperiod. These results will represent one of the first empirical studies to parse the variance components of phenotypic responses to climate change into components of genetic change and phenotypic plasticity.

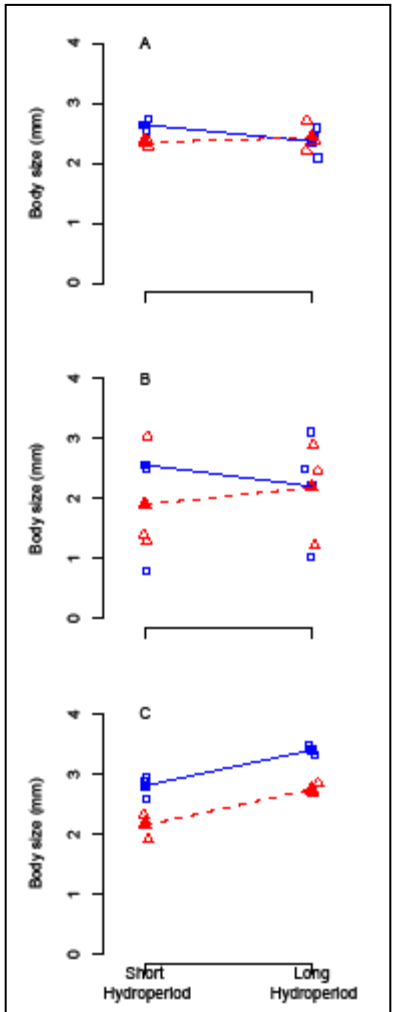

Figure 4. Three possible hypotheses relating climate change to mean genetic trait values of body size, blue squares are regular rainfall treatments, red triangles are infrequent intense treatments. A). All variability is environmental B.) Genetic variability but no response to the treatments. C.) Genetic variability shows an additive response to both treatments

Question 2. What is the direction of selection in Daphnia pulex in relation to climate change? Is selection direct or indirect, and is there any evidence for evolutionary constraints? To answer this question, I will compare the phenotypes of *Daphnia* from the experiment with the phenotypes of *Daphnia* sampled from the original vernal pond. Assuming that conditions in the source pond have remained relatively constant over the past two years, I will then be able to bootstrap estimates the direction of selection. I will also be able to construct a G-matrix for each treatment and measure direct and indirect selection, and detect evolutionary constraints.

Data analysis: Fitness will be calculated for all clonal lineages the same as in the first experiment. I will determine the direction of selection using linear regression of fitness on the trait means (Conner and Hartl 2004). I can construct the multivariate breeder's equation $\Delta\bar{z} = G\beta$ (Arnold and Wade 1984, Conner and

Hartl 2004) where G is the variance-covariance matrix of additive genetic variances (V_G),. Total selection on a character can be estimated with a simple linear regression of fitness on a single character. To estimate direct selection, (e.g. β from above), I will use a multiple regression of fitness on all characters in which each estimate of β is the partial regression slope of all the measured traits on fitness. Numerous studies (Conner 1988, Janzen 1993, Grant and Grant 1995) have shown that because of correlated traits, apparent positive selection on a trait may actually be insignificant or negative due to stronger selection on a correlated trait. Evolutionary constraints can also be detected from this information in two ways. One is if the diagonal elements of the G-matrix are close to 0, in which case there is very little additive genetic variance to work with, so that phenotypic change will be very slow even with strong selection. A second way evolutionary constraints can be detected is if two (or more) traits are negatively correlated and the direction of selection is the same on them. In this case, evolutionary response to climate change will be slowed, or may even become maladaptive (Etterson and Shaw 2001).

Hypothesis and predictions: I predict that there will be there will be positive directional selection for body size, clutch size, size as neonate, size of neonates, and time to maturity in ponds that have regular rainfall and longer hydroperiods. I also hypothesize that selection will operate on only a few of these traits because many of them are strongly correlated. For example, maternal body size, growth rate, and neonate size are all correlated in *Daphnia* (Green 1956, Spitze et al. 1991). Evolutionary constraints are also possible in this system. For example, in populations experiencing heavy predation, there will be positive selection for large body size, but also earlier age at first reproduction (Spitze 1991). Earlier age of first reproduction means smaller neonates, and therefore smaller adult body size (Ebert 1991). Predation may therefore reveal evolutionary constraints on body size and development time.

The results from Q1 and Q2 satisfy the logic requirements laid out by Geinapp et al (2007) to demonstrate a genetically based response to climate change. My measurements of clonal lines of *Daphnia* from different experimental hydroperiods will allow me to partition phenotypic variation into components of genetic change and phenotypic plasticity (Q1), and allow me to quantify the selection differential on those traits (Q2). I will then map the changes in traits onto the different climate change scenarios that are simulated with my experimental treatments (Q1 Fig. 4).

INTELLECTUAL MERIT

The rate at which anthropogenic climate change is occurring is faster than anything the planet has previously experienced (Parry et al. 2007). In aquatic communities, these changes will alter hydroperiods and drying rates, two variables that are critical to the life history of many invertebrate species in vernal ponds. Understanding the ecological and evolutionary responses of these communities to climate change is a critical challenge for environmental science. My current research has focused on how climate change alters aquatic community structure and population dynamics. Ultimately, it is these altered ecological processes that select for evolutionary change. Organisms with short generation times and cycles of clonal growth such as *Daphnia* present a unique opportunity to study evolutionary responses to climate change. To date, only a few studies have documented a genetic response of populations to climate change in the context of a manipulative field experiment. The proposed research will leverage an ongoing climate change experiment to document the genetic and phenotypically plastic responses of *Daphnia* to different hydroperiod manipulations. Measurements from clonal lines of *Daphnia* collected from different experimental treatments and from the ancestral population will allow me to estimate the G-matrix and then the direction, and direct and indirect selection in *Daphnia pulex* in relation to climate change. By simulating climate change in a realistic field experiment, I can explore a number of different climate

change scenarios. Understanding the genetic and plastic responses of populations to climate change will help us to forecast whether these populations are at risk of extinction or whether they can adapt and persist in different climate regimes.

BROADER IMPACTS

Funding of this research program will provide opportunity for students from advanced undergraduates to high school students. I have experience mentoring both undergraduate and high school students which I will only continue with this project. I have lead a week-long workshop for 25 local high school students and 15 undergraduates through the outreach components of the Vermont EPSCoR STREAMS program (http://www.uvm.edu/~streams/). This workshop trained students in aquatic invertebrate sampling and water quality sampling. I am currently mentoring two undergraduates (Jackie Hubbard, and Kaylyn Hawkes) through the same program and helping them develop research projects. I also have close connections with teachers at Mississquoi Valley Union High School, an underprivileged rural school, where I have given 3 guest presentations on venal pond ecology. Finally I have recently volunteered as a science mentor for a high school student at Burlington High School. For the proposed research, I will provide the following specific training opportunities.

Undergraduate training: During my dissertation research, I have trained four undergraduates (Cyrus Mallon, Chris Graves, Erin Hayes-Pontius, and Autumn Amici) in field and laboratory skills. I will hire at least five undergraduates through the Federal workstudy program during the academic year to assist me with rearing *Daphnia*. I will teach these students quantitative genetic methods and the design of evolutionary experiments. I encourage all my undergraduates to take advantage of University of Vermont grants for students, the Helix (www.uvm.edu/~helix/) and URECA program (www.uvm.edu/~provost/ureca/). I will help these students to submit their own grants to fund side projects that they will initiate and develop while working on this project.

High school student training. In the late summer of 2009 I will recruit local high school students into the project by visiting high school science classes and taking them into the field for a full day of intensive collecting and sampling. I will complement these collecting days with a preceding presentation on my research. I will teach students about climate change, evolution and experimental design. In rural, financially-strapped high schools such as Mississquoi Valley Union High School these kinds of opportunities are only possible through such volunteer efforts. For those classes that I do not work with in the field, I will come into the classroom with a presentation on climate change and its effects on ecology and evolution, as well as bringing in sample *Daphnia* for an experiential learning unit on *Daphnia* ecology and microscopy.

Dissemination of work: I regularly present my research at regional and national conferences. I will present my research at the Ecological Society of America conference, as well as the regional Northeastern Natural History conference. I plan to submit this work to journals such as: *Evolution, Ecology,* or *Global Change Biology*. Finally the facility where my research takes place is open to and regularly used by the public. I will lead public presentations by giving a talk at the field site combined with a hike around the site.

Funding of this project will allow me to provide training to undergraduates and high school students, publish and present my work to my academic peers, and reach out and educate the public about both my research and the broader issues of global climate change.