

THE EVOLUTION OF LIFE HISTORIES AND PHENOTYPIC PLASTICITY ACROSS
ENVIRONMENTAL GRADIENTS IN *DAPHNIA* SP.

by

MICHELLE RENEE SWAN PACKER

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Supervising Committee:

Dr. Matthew Walsh, Supervising Professor

Dr. Todd Castoe

Dr. James Grover

Dr. Laura Mydlarz

Dr. Sen Xu

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Dedication

This dissertation is dedicated to my husband, Bill Packer. Your work ethic, dedication, drive and willpower inspire me. You have encouraged me to pursue my dreams, made me laugh, and most of all provided love and support. “You make me want to be a better (wo)man”.

Abstract

TESTING THE INFLUENCE OF PHENOTYPIC PLASTICITY ON THE RATE OF EVOLUTIONARY CHANGE *DAPHNIA SP.*

Michelle Renee Swan Packer, PhD

The University of Texas at Arlington, 2020

Supervising Professor: Matthew R. Walsh

Understanding how environmentally-induced variation ultimately leads to speciation is a main component of evolutionary ecology. This dissertation uses *Daphnia* in experimental and comparative studies to address several theoretical questions concerning the role of phenotypic plasticity in the evolutionary process. Specifically, I explore novel environmental factors which induce plasticity and investigate the plastic response across systems to determine general hypotheses for understanding the mechanisms which may be involved. Additionally, this dissertation provides empirical results which add to the body of research investigating the transition between environmentally induced phenotypes and genetic adaptation. The results of this body of work show that ancestral plasticity can predict the direction of adaptation, and that location-specific biotic factors may change the mechanisms by which plasticity leads to patterns of local adaptation. This work demonstrates the value of *Daphnia* as a model system for addressing empirical evolutionary questions and provides insight on the complex function of plasticity in evolutionary transitions.

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Chapter 1

Introduction

Many organisms are inherently responsive to changing environmental conditions, and it is accepted that such 'phenotypic plasticity' can be adaptive. This ability to modify the expression of traits is often responsible for increasing standing phenotypic variation. Yet, it is still unclear if and how environmentally induced phenotypes influence adaptive evolution. Currently, two opposing theories explain how individual responses to novel environments could affect how a population responds to a change in environmental conditions. On one hand, theory predicts that environmentally induced plasticity buffers an individual from selection. If plastic responses are large in magnitude, and consistently shift reaction norms toward phenotypes that enhance fitness in a new environment, then locally fit phenotypes require minimal genetic modification.

Conversely, it has also been proposed that plasticity can facilitate rapid adaptation by promoting population persistence. The plasticity first hypothesis suggests that novel traits initially arise as environmentally-induced phenotypes and are then refined by selection ultimately resulting in 'genetic accommodation'. Divergent forms of the plastic response also increase the complexity of identifying the role of plasticity in the evolutionary process. This is because responses to a change in environmental conditions are not always adaptive and can be considered maladaptive (i.e., plastic responses that reduce fitness). Conflicts exist over the influence that these differing forms of plasticity (adaptive or maladaptive) have on the rate and direction of evolution.

Furthermore, it is also possible that selection can favor phenotypes with contrasting magnitudes of plasticity; those with increased environmental sensitivity, leading to increased plasticity and to the evolution of polyphenisms, or phenotypes with decreased environmental sensitivity, loss of

plasticity and trait canalization. Tests of these theories are difficult because this requires systems in which ancestral plasticity can be assayed and then tracked as populations adapt to environmental change.

My dissertation focused on a system that yields several opportunities to empirically investigate such questions. This work focuses on waterfleas (*Daphnia*, specifically: *Daphnia pulicaria*, *Daphnia rosea*, and *Daphnia middendorffiana*) which have a rapid generation time (producing clutches of offspring every couple of days) and exhibit well-known patterns of plasticity. These features make them exceptionally well suited to evolutionary studies focusing on the importance of plasticity in the process of adaptation. The subsequent chapters detailed in this dissertation aim to understand if consistent plastic responses to novel environments can influence trade-offs in life history traits and lead to local adaptation. Two unique comparisons of wild populations of *Daphnia* highlight the challenges associated with unraveling phenotypic plasticity from other biotic influences. A comparison of responses to food availability led to the consistent development of an increased body size, yet the mechanisms (evolution of faster growth vs. delayed maturation) differed based upon the nature of the biotic interactions in each system (Chapter 2). The influence of divergent fish predator ecotypes highlighted the importance of including habitat specific variation of a predator, as comparisons between two similar systems which contain stickleback and *Daphnia* revealed divergent evolutionary outcomes (Chapter 3). The last chapter of this dissertation directly tested the influence of different forms of ancestral plasticity on the rate and direction of adaptation (Chapter 4). I found empirical evidence demonstrating that evolutionary divergence follows the direction of ancestral plasticity, yet the mechanisms by which plasticity interacts with the genetic architecture to enact heritable change

are still elusive, as we did not find evidence to support theories of genetic accommodation, nor that different forms of plasticity (adaptive, maladaptive) change the rate of adaptation.

Finally, this dissertation aims to show the immense potential of the *Daphnia* system as a model for detailed investigations into plasticity led evolution as environmentally induced phenotypes can progress rapidly through the evolutionary sequence due to short generation times. Thus, the ancestral population can be directly compared to contemporary populations. Furthermore, the alternation between sexual and asexual reproduction allows for sampling from multiple evolutionary time points during sexual reproduction, yet also provides opportunity to “halt” the process by isolating individuals by evaluating the traits of asexual lineages at specific time points. Most interesting is the potential for the use of *Daphnia* in genomic biology. This is because the environmental sensitivity of ancestral and contemporary alleles can be tested alongside experimental evolution studies to better understand the link between plasticity and heritable characteristics. The work in this dissertation only begins to scratch the surface of understanding the influence of phenotypic plasticity, and poses as many questions as it answers. It is likely that further research using the *Daphnia* system will reveal significant insights into the impacts of phenotypic plasticity on the evolutionary landscape.

Chapter 2

The influence of long-term lake productivity on life history evolution in *Daphnia*: Comparing natural vs. experimental systems

Michelle Packer¹, Kaitlyn J. Howell¹, and Matthew R. Walsh¹

¹Department of Biology, 501 S. Nedderman Drive, University of Texas at Arlington, Arlington, TX 76019 USA

Abstract

Resource availability has long been considered a key selective force on the evolution of life histories. However, our understanding of the link between resource availability and the specific trajectory of evolution of these traits is still not well understood. Here, we tested the connection between natural and experimental increases in lake productivity and evolutionary shifts in the life history traits of waterfleas (*Daphnia sp.*) from two separate ecosystems. We specifically evaluated the life history traits of *Daphnia* from two distinct long-term ecological research (LTER) sites to determine if resource allocation tradeoffs result in parallel patterns of trait evolution. We found a strong correlation between increased resource availability and the evolution of a larger body size in *Daphnia*, however the underlying mechanisms that promoted this change differed between systems. Natural increases in productivity within Wisconsin lakes were associated with increased rates of juvenile growth, resulting in the evolution of a larger size. Experimental increases in productivity in Alaskan lakes were associated with a slower rate of development, leading to the evolution of a larger size. We propose that distinct biotic interactions in each system were responsible for the contrasting response mechanisms observed.

Introduction

Resource availability has long been considered an important selective force on the evolution of life history traits (Noordwijk and Jong 1986, Lynch 1989, Hairston *et al.* 1999, Grether *et al.* 2001, Walsh and Reznick 2008). Efforts to predict how organisms will allocate resources to maximize fitness have been ongoing since Cole first suggested that comparisons among life history traits can be used to determine patterns of evolution (Cole 1954). Early work predicting the optimal allocation of resources postulated that increasing resources dedicated to reproduction would result in a decrease in somatic growth and survival (Gadgil and Bossert 1970). Specifically, that reproductive effort or the amount of nutrients and energy dedicated to reproduction is inversely related to the survival and mortality of parents (Stearns 1976, 1980, Martin 1987). While there is some empirical and theoretical support for this model, these allocations patterns are not universal (Ricker 1975, Lester *et al.* 2004). Differences in these allocation strategies are important because they are responsible for individual trait variation, community biodiversity and structure, as well as population dynamics and ecosystem function (Schoener 1986, Elser *et al.* 1997, 2000, Hooper *et al.* 2005).

The manner in which variation in resources drives the evolution of specific life history strategies remains unresolved. For instance, life history theory predicts that increases in productivity can drive the evolution of earlier (Gadgil and Bossert 1970) as well as delayed maturation (Kozłowski and Uchmanski 1987, Kozłowski and Wiegert 1987). There are also contradictory predictions for the evolution of reproductive effort (Gadgil and Bossert 1970, Kozłowski and Uchmanski 1987, Kozłowski and Wiegert 1987). Part of the challenge with evaluating the link between resource availability and life history evolution in natural systems is

that there are often correlated impacts due to other selective pressures (i.e., predation, population density) as well as species specific nutrient requirements (Williams 1966, Gadgil and Bossert 1970, Law 1979, Reznick 1985, Kozłowski and Uchmanski 1987, Kozłowski and Wiegert 1987, Stearns 1989, Zera and Harshman 2001).

In addition to its direct influence on life history traits, the connections between fitness and resource levels (resource rich vs. resource poor) have also been documented in a diverse array of taxa (Falconer and Latyszewski 1952, Fredrickson and Stephanopoulos 1981, Lynch 1989, Lambers and Poorter 1992, Winemiller and Rose 1992, Boersma and Vijverberg 1994, Boersma 1995, Schmitt 1996, Bronikowski and Arnold 1999, Barrett *et al.* 2005, Hall and Colegrave 2007, Walsh and Reznick 2010). Fitness is often defined in terms of multiple life history traits (Schaffer 1974a, 1974b, Stearns 1977, 1989, Law 1979, Stearns and Koella 1986, Litchman *et al.* 2013). However, body size as an individual trait is often used as a proxy for fitness as it covaries with several other traits such as fecundity, survival, and competitive ability (Brooks and Dodson 1965, Peters 1983, Stearns 1983, 1992, Smith and Brown 1986, Ebenman and Persson 1988, Honěk 1993, Serrano-Meneses *et al.* 2007, Litchman *et al.* 2013). Body size, and thereby fitness, has been shown to be strongly influenced by resources levels, yet empirical research often revealed contradictory responses to shifts in resource quantity. Some studies have shown that organisms with an increased body size have a competitive advantage when faced with low resource conditions due to increased energy storage, decreased metabolic maintenance costs, assimilation efficiency, attack rate and handling time (Threlkeld 1976, Gliwicz 1990). However, several other studies showed that increased resource availability enhances fitness due to increases in body size and reproduction. For example, body mass of rotifers was found to be

positively related to food concentration (Stemberger and Gilbert 1985), while body size and reproductive output increased in *Daphnia middendorffiana* exposed to increased resource availability (Yurista and O'Brien 2001). Similarly, when investigating food limitation in cladocerans, Duncan (1989) found that body size was reduced and age of maturation increased when females were reared in low food conditions. Identifying such tradeoffs between growth and reproduction to optimize fitness in individuals is important for understanding the evolution of divergent life history strategies (Schaffer 1983, Reznick 1985, Noordwijk and Jong 1986, Stearns 1989). Few studies have examined the influence of resources on evolutionary change using a cross-ecosystem approach to compare findings between closely related species in both experimental and natural settings (but see Elser *et al.* 2000, Tessier *et al.* 2000).

Here we evaluate patterns of life history evolution in water fleas (*Daphnia*) in response to long-term variation in resource availability. *Daphnia* are a suitable model organism for understanding responses to shifts in resources as they are commonly found in lakes that vary in productivity (McCauley and Murdoch 1987, Ebert 2011, Lampert 2011, Miner *et al.* 2012). In this study, we assessed responses in replicated populations of *Daphnia* within two independent lake systems which have experienced similar variation in productivity: a temperate natural lake system, and an Arctic whole-lake experimental system. We measure interactions between resource levels and fitness to determine if *Daphnia* from two different systems are utilizing similar trade-off strategies in response to shifting resources.

We leveraged existing data sets which span more than 30 years to identify lake pairs consisting of nutrient enriched, highly productive lakes, and sentinel non-eutrophic partner lakes

within two Long Term Ecological Research (LTER) sites. Our “natural” lake system is located in the North Temperate Lakes (NTL) LTER in Wisconsin. Lakes in the southern region of the NTL LTER, near Madison, Wisconsin have a prolonged history of cultural eutrophication. That is, lake productivity has significantly increased over the past several decades. Their sentinel partners from northern Wisconsin have not experienced external nutrient enrichment. Our “experimental” system includes lakes within the Arctic (ARC) LTER in Northern Alaska, which were part of a long-term nutrient enrichment project (Luecke *et al.* 2014, Daniels *et al.* 2015), and are paired with nearby unmanipulated lakes of similar morphometry. We used populations of related species within the *D. pulex* complex: *D. middendorffiana* from Alaskan lakes, and *D. pulicaria* from Wisconsin lakes in our experiments. While these systems are geographically and ecologically distinct, our primary interest in comparing these systems was to test the degree to which they demonstrate parallel responses to long-term resource enrichment. We evaluated responses to high versus low productivity using three resource treatments arranged in a factorial design. We predicted that increased primary productivity would alter selection on growth rates, as well as the ability of *Daphnia* to exploit declines in resources (Frisch *et al.* 2014). Specifically, that *Daphnia* from lakes with a history of high productivity would experience selection for faster growth, resulting in an earlier maturation at a smaller size, while those from lakes with a history of low productivity would exhibit slower growth, resulting in later maturation at a larger size (Stearns and Koella 1986). We also expected that the life histories of *Daphnia* from lakes with a history of low productivity would be less negatively impacted by declines in controlled food in the lab. That is, rates of growth and reproductive outputs would be less impacted by low food levels in *Daphnia* from low productivity lakes. This would manifest as treatment by lake history interactions. However, our results suggest that increases in resource

availability consistently selects for increased body size at maturity, but the mechanism by which this increase was acquired differed between systems.

Materials and Methods

Lake Systems and Focal Lakes

Arctic Long-Term Ecological Research site: Experimental lake system

This site is located on the North Slope of the foothills in the Brooks Mountain Range in Northern Alaska, U.S.A. Original research at this site began in 1975. Between the years of 2001 and 2013, researchers associated with the Arctic Long-Term Ecological Research (ARC LTER) performed whole-lake nutrient manipulations to test the influence of nutrients on community and ecosystem processes (Luecke *et al.* 2014, Daniels *et al.* 2015). The level of enrichment employed in these experiments resulted in a fourfold increase over the background-loading rate of nitrogen and phosphorus found in the nearby reference lake, Lake Toolik, and an annual increase in chlorophyll *a* biomass for our focal lake (E6) (Gettel *et al.* 2013). Nearby ultra-oligotrophic lakes were chosen as sentinel control partners for each manipulated lake. In the summer of 2016, *Daphnia middendorffiana* were collected using an 80 μm mesh plankton net from one experimental lake E6 and its sentinel partner Fog 4 (F4). Tows were performed at a minimum of five locations to ensure genetic diversity among individuals collected.

North Temperate Lake Long-Term Ecological Research site: Natural lake system

Comprehensive lake characteristic data has been collected by North Temperate Lakes Long-Term Ecological Research (NTL LTER) researchers for over 30 years. Lakes within the NTL LTER site are located within two distinct lake districts. We sampled four lakes within this

system. Lakes Mendota (MD) and Monona (MN) (located in south-central WI, U.S.A) are situated in the Yahara District (YD), which is characterized by highly urbanized and agricultural surroundings. Big Muskellunge (BM) and Allequash (AL) (situated in northern WI, U.S.A) are within the Northern Highland Lake District (NHL) surrounded by rural forested lands. The NTL data shows significant variation in the degree of nutrient input between the lake districts as a result of variation in land-use patterns (Carpenter *et al.* 2007). Phosphorus levels are 3 times higher in lakes in the YD versus the NHL (Mean total P in $\mu\text{g/l}$: YD = 61.4, NHL = 21.2) (Stanley 2014). Increased concentrations of nutrients have had profound effects on the eutrophication of the lakes in the YD region, with lakes Mendota and Monona being classified as eutrophic since the late-1800's (Birge and Juday 1922, Stewart 1976, Brock 1985, Lathrop 2007). Conversely, external influences on primary production in lakes within the NHL (i.e., Big Muskellunge and Allequash) have been considerably limited due to geographic isolation, which has resulted in these lakes typically being classified as oligotrophic (Hanson *et al.* 2003, Lauster *et al.* 2006). While it is important to note that both increased human population size and agricultural land-use are raising concerns about potential eutrophication of NHL lakes in northern Wisconsin (Peterson *et al.* 2003, Waller and Rooney 2008), for the purposes of this study, we will be focusing on the historic differences in productivity. *D. pulicaria* from this system were collected in May of 2016 via plankton tows (80 μm mesh net). Multiple tows from varying locations (minimum of 10) in each lake were taken to increase the likelihood that individuals were genetically distinct.

Experimental protocols

Adult females from both systems were isolated in the field (hereafter referred to as ‘clones’), placed in 90 ml jars containing 60 ml of their respective lake water, and shipped back to the laboratory at UT Arlington (n size: AK: E6=30, F4=30; WI: MD=20, MN= 5, AL=13, BM=12). Individuals were slowly acclimated to COMBO media (Kilham *et al.* 1998) and cultured in the same jars under controlled temperature ($12 \pm 1^\circ\text{C}$) and light (12:12 light-dark cycle) conditions while fed a non-limiting supply of *Scenedesmus obliquus* (concentration: 1.0 mg carbon C L⁻¹ day⁻¹) in order to establish populations of individual clone lines in high-quality conditions.

Common garden Experiment

All clones were reared in a common garden setting for two generations before initiating the experiments. Individuals were transferred to fresh media and algae every other day. Three adult females of each clonal line were reared individually in a 90 ml jar to establish the parental generation (G0). All subsequent generations (G1, G2, F0, F1) were taken from the second clutch of the previous generation and consisted of neonates less than 12 hrs old. Starting with the first laboratory generation (G1), two neonates were reared together in a 90 ml jar, with each clone having 3 replicates. All *Daphnia* were checked twice daily for maturity and the production of the first and second clutches of offspring. Generations G1 and G2 were reared under the same conditions as the parents, consistent with the high-quality acclimation protocol conditions listed above.

We evaluated patterns of within- and across-generation plasticity starting with the third-generation (F0) lab reared clones from all lakes. Six neonates (<12 h old) were collected from

each clone (i.e. multiple females were available to produce the experimental treatments), subdivided into pairs, and randomly assigned to one of three resource treatments: (i) “low” (0.2 mg C L⁻¹), (ii) “medium” (0.5 mg C L⁻¹), and (iii) “high” (1.0 mg C L⁻¹) (Fig. 1). These food levels reflect the full range of varying food conditions likely to be experienced by *Daphnia*, with 0.2mg C L⁻¹ slightly below the incipient limiting level, and 1.0 mg C L⁻¹ being above predicted net carbon intake levels for *D. pulicaria* species (Taylor and Gabriel 1985, Lynch *et al.* 1986, Lampert *et al.* 1988). We converted biovolume to carbon using an established formula (Rocha and Duncan 1985). The experiment was run for two experimental generations (F0, F1). Each clone was replicated three times for each treatment and generation for a total sample size of 1134 jars (63 clones across all lakes x 3 treatments x 3 replicates per treatment x 2 generations) (AK Clones: E6=6, F4=12; WI Clones: MD=11, MN=10, AL=12 , BM=12).

For this experiment, we quantified size at maturation, age at maturation (defined as the release of the first eggs into the brood chamber), instantaneous juvenile growth rate, and reproductive output (sum of embryos in clutches 1 and 2). All jars were checked daily for maturation and for release of offspring from the brood chamber. Each individual was photographed at the beginning of the experiment on day 1 and then again on day 5 to quantify rates of juvenile growth. Using ImageJ, the Feret’s diameter (also known as maximum caliper) was measured and used to calculate to growth rate via: $[\ln(\text{Feret day 5}) - \ln(\text{Feret on day 1})]/\text{no. of days}$. All individuals were also photographed when they attained maturation to quantify size at maturation. Upon expulsion of the second clutch from the brood chamber, pairs of newly born neonates from each clone were collected to initiate the F₁ experimental generation. These

individuals experienced the same temperature, photoperiod and frequency of food/media replacement as the previous generation.

Statistical analyses

All dependent variables were analyzed with linear mixed models (SPSS v.24) implemented with restricted estimated maximum-likelihood estimation. For each dependent variable, lake history, resource treatment, and generation were entered as fixed effects and clone ID was nested within lake, and lake nested within lake history were entered as a random effects. Lake was nested within lake history was found to be non-significant and was therefore removed from the subsequent analyses. The data for *Daphnia* from lakes from Wisconsin and Alaska were analyzed separately. The presence of normality was evaluated with a Kolmogorov–Smirnov test, and Levene’s test was used to examine the homogeneity of variance if sample sizes were unequal. Data for age at maturation were log-transformed and clutch size was reciprocally transformed to improve fits with normality and homogeneity of variances. Post hoc Tukey-Kramer tests followed significant ($p < 0.05$) treatment effects.

Results

Resource Level effects

Alaska

The linear mixed model revealed no significant ($p < 0.05$) responses to resources levels.

Wisconsin

There was a significant ($p < 0.05$) effect of resource level on the expression of all life history traits (Table 1). Post hoc tests showed that juvenile growth rate was significantly faster in the high food treatments versus the low and medium resource conditions (23% and 14% faster respectively). While the high food treatment differed significantly from the medium and low food treatments, there was no significant difference between the medium and low treatments (Fig. 2e). *Daphnia* also matured significantly earlier in the high food treatments when compared with the low and medium food conditions (13% and 7% earlier at maturation respectively) (Fig. 2c). *Daphnia* in the medium food treatments matured 6% earlier than *Daphnia* in the low resource treatments (Fig. 2c). *Daphnia* from the high food treatments were significantly 6% smaller at maturity than *Daphnia* in the low food treatment. There was a marginally nonsignificant ($p = 0.051$) difference in size at maturation between the high and medium food treatments; *Daphnia* from the high food treatments were 3% smaller than *Daphnia* from the medium food treatments (Fig. 2a). Lastly, females produced significantly larger clutch sizes (17%) in the high food treatment as compared to the low food treatment. There was no significant difference in clutch size between high vs. medium or medium vs. low food treatments.

Lake history effects

Alaska

We observed significant ($p < 0.05$) differences in age and size at maturation between *Daphnia* from high and low productivity lakes (Table 1). In lakes that experienced long-term nutrient additions, *Daphnia* matured 11% later and were 5% larger at maturity as compared to *Daphnia*

from lakes with a history of low productivity (Fig. 2b, d). All other effects based upon lake history were nonsignificant ($p>0.05$).

Wisconsin

In Wisconsin, we observed significant ($p<0.05$) differences between lake types for juvenile growth rate and size at maturation (Table 1). *Daphnia* from highly productive lakes grew 29% faster and were 23% larger at maturation than those from low productivity lakes (Fig. 2a, e). All other differences based on lake history were nonsignificant ($p>0.05$).

Generation effects

Alaska

We observed significant ($p<0.05$) differences in juvenile growth and age at maturation between generations (Table 1). *Daphnia* from the first experimental generation grew 33% faster and exhibited a timing of maturation that was 11% longer than *Daphnia* from generation two (Table 1). All other generation effects were nonsignificant ($p>0.05$).

Wisconsin

We observed significant ($p<0.05$) differences between generations for multiple life history traits. *Daphnia* from the first generation grew 64% faster, matured 14% earlier and produced 13% larger clutches of offspring when compared with generation two (Table 1). There was no significant difference in size between generations ($p>0.05$).

Statistical interactions

Alaska

All statistical interactions were not significant ($p > 0.05$) in this system.

Wisconsin

Multiple statistical interactions were significant ($p < 0.05$) in this system (Table 1, Fig. 3). The lake history by resource treatment interaction was significant ($p < 0.05$) for size at maturation.

Daphnia from both lake types reduced their size in the medium food treatments as compared with the high and low food treatments (Fig. 3a). We also found a significant ($p < 0.05$) resource treatment by generation interaction for juvenile growth rate. For all treatments, there was a reduction in growth rate in the second generation but such declines in growth between generations were strongest in the low food treatments (declines in growth between generation 1 and 2: High: 28%, Medium: 40%, and Low: 49%). Additionally, we observed a significant ($p < 0.05$) lake history by generation interaction for juvenile growth rate and a marginally nonsignificant ($0.05 < p < 0.1$) interaction for clutch size. The growth rates and clutch sizes of *Daphnia* from high and low productivity lakes were reduced in the second generation when compared with the values observed in the first generation. The declines in these trait values between generation one and two were larger in low productivity lakes (growth rate: 31% and 46% for high vs. low productivity lakes respectively; clutch size: 6% and 16%). The interaction between lake history by resource treatment by generation was marginally nonsignificant ($0.05 < p < 0.1$) (Table 2).

Discussion

Our work revealed a strong correlation between increased resource availability and the evolution of a larger body size in *Daphnia* (Fig. 2). This includes the evolution of a larger body size in response to natural (Wisconsin) and experimental (Alaska) increases in lake productivity (Fig. 2). Interestingly, the underlying mechanism that facilitated the evolution of a larger body size differed in each system. In Wisconsin lakes, the evolution of a larger body size in *Daphnia* from lakes with a history of high productivity is associated with the evolution of faster rate of growth. In these lakes, all populations of *Daphnia* matured at the same age irrespective of productivity history. Conversely, in lakes in Alaska, experimental increases in lake productivity has led to the evolution of a larger body size and delayed maturation but no shifts in rates of growth (Fig. 2). Below we consider these results in the context of life history theory as well as the underlying drivers of the trait differences between each system.

The patterns of life history evolution observed in Wisconsin agree with several models that predict that faster rates of growth will evolve in response to increased resource availability (Gadgil and Bossert 1970, Roff 1992, Stearns 1992). These models also predict that increased resources will favor the evolution of earlier maturation. One possibility is that the lack of response in age of maturation observed in the current study may be due to confounding effects of selection mediated by planktivorous fish. Fish predation is known to generally favor the evolution of an earlier age and a smaller size at maturation (Endler 1986). However, studies investigating resource availability in concert with predation have found that food related effects can exceed the direct effects of selective predation, which ultimately results in the evolution of increased body sizes (Skelly and Werner 1990). Furthermore, theory has also shown that rates of

development may be less flexible to changes in resource availability. For instance, Abrams and Rowe (1996) suggested that growth rate and age at maturation are constrained when an organism exhibits flexibility in foraging behavior, which results in size of maturation being free to respond to selection. Finally, the indirect effects of predation can also oppose the direct effects of predation because predators can reduce prey density, which increases per capita resource availability for prey. This again, can ultimately result in increased individual growth rates and increased size (Wootton 1994, Abrams and Rowe 1996). Our results support these ideas, as we found a constrained age of maturation in conjunction with increased growth rates and size at maturation.

In addition to considering the connections between life history theory and the trajectory of evolution, it is also important to consider how eutrophication in lakes in Wisconsin may impact *Daphnia* behavior and potentially the intensity of predation. The surprising trend of the evolution of increased body size in lakes with planktivorous predators may, in part, be explained by shifts in *Daphnia* behavior. *Daphnia*, like most zooplankton, exhibit phototactic foraging behavior known as diel vertical migration (DVM) whereby *Daphnia* spend daylight hours in the dark hypolimnion and rise to the surface at night to feed in warmer, food rich waters (Dodson *et al.* 1997, Gool and Ringelberg 2002, Hays 2003). It is assumed that this behavior is an adaptive response to fish predation as it reduces exposure to visual predators by using the dark depths of lakes as a refuge during the day (Hrbáček *et al.* 1961, Brooks and Dodson 1965, Zaret and Suffern 1976, Lampert 1989). Increased food availability has been shown to alter this pattern of behavior in *Daphnia*. *Daphnia* that have access to increased resources show corresponding increases in lipid reserves, and this in turn, reduces the amount of time needed at the surface to

forage for food as compared with starving *Daphnia*. Well-fed *Daphnia* also exhibit a more extensive photonegative response (Johnsen and Jakobsen 1987, Dini and Carpenter 1992, Gool and Ringelberg 1995, Sekino and Yoshioka 1995, Fiksen and Carlotti 1998, Ringelberg 1999, Sekino and Yamamura 1999). Additionally, when phytoplankton are observed at deeper depths in lakes, zooplankton will remain within these food rich depths (George 1983). Thus, in lakes with increased resource availability, the need to migrate to the surface may be mitigated by food abundance at depth, reducing mortality risk due to predation, and in turn, relaxing fish predator-mediated selection on size at maturation. Similar patterns of reduced foraging effects on body size due to predation have also been found in other systems (see Werner 1991). We propose that the evolution of a larger body size in lakes in Wisconsin in response to increased resource availability is best explained by increased phototactic behavior and declines in rates of predation. Such a hypothesis requires further testing.

An additional surprising aspect of our results is that *Daphnia* from highly productive lakes in Alaska matured later, but grew at the same rate as those in low productivity lakes. It is possible that experimental increases in food resources had little or no effect on the evolution of growth rate in Arctic *Daphnia* as their growth rate may be already maximized due to an extremely short growing season and low temperatures. Research has shown that these factors can exert a strong influence the life history traits of Arctic *Daphnia* (Stross and Kangas 1969, Elser *et al.* 2000). On the other hand, one important difference between the study lakes in Wisconsin and Alaska is that fish predators are largely absent in the lakes in Alaska. As a result, selection on age of maturation is potentially more flexible in lakes that lack fish predators. This is because delaying maturation to reach a larger size would not increase the likelihood of increased

mortality due to fish predation. While we favor differences in the nature of biotic interactions between lakes in Wisconsin and Alaska as the explanation for the contrasting patterns of life history evolution (evolution of faster growth vs. delayed maturation), we cannot rule out species specific differences in allocation patterns.

Evolution of Plasticity

In this study we evaluated the expression of life history traits across a series of resource treatments to test for evolved differences in plasticity between *Daphnia* from lakes that differ in productivity. In contrast to prior research on killifish from streams that differ in food availability (Walsh & Reznick 200, 2010), we observed little evidence for the evolution of resource mediated life history plasticity. The only significant lake type by resource interaction was for size at maturation in *Daphnia* from lakes in Wisconsin (Fig. 3). All other lake by resource interactions were not significant. The evolution of plasticity is dependent upon consistent and predictable variation in an environmental factor (Stearns and Koella 1986). Resources vary spatially and temporally in lakes. Thus, perhaps *Daphnia* experience relatively similar degrees of spatial and/or temporal variation in resources in both high and low productivity lakes, which thereby mitigates the evolution of plasticity in this system.

Conclusions

Daphnia within the *pulex* group are known to be resource integrators. That is, they are able to use different mechanisms to assimilate and store nutrients in excess of what is needed for immediate growth and use nutrient reserves when resource conditions are poor or patchily distributed (Hood and Sterner 2010). Here we show that in lakes in Wisconsin and Alaska,

increases in long term lake productivity resulted in the evolution of a larger size of maturation. Yet, the underlying mechanism that allows for the evolution of genetic shifts in body size differs between the systems (evolution of faster growth vs. delayed maturation). We propose that this contrast in mechanism among regions is related to the nature of the biotic interactions in each system. Our results highlight the difficulty of investigating the evolutionary impacts of resources within natural environments.

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Tables

Table 1. Analyses of life history traits. Linear-mixed models were used with lake history, resource treatment and generation entered as fixed effects. Clone (nested within lake) was entered as a random effect. Non-significant random effects were removed from the model and the data was reanalyzed. The denominator degrees of freedom are displayed after each F-value. Significant ($p < 0.05$) results are displayed in bold.

Fixed Effects	df	Size at Maturity		Age at Maturity		Juvenile Growth		Sum of Clutches (Ave)	
		Alaska	Wisconsin	Alaska	Wisconsin	Alaska	Wisconsin	Alaska	Wisconsin
History	1	6.1* ⁽¹¹⁰⁾	52.6*** ^(47.2)	24.3** ⁽¹¹³⁾	1.6 ^(46.3)	1.8 ⁽²²¹⁾	49.9*** ^(41.6)	1.5 ⁽¹¹²⁾	2.4 ^(48.4)
Resource Treatment	2	0.6 ⁽¹¹⁰⁾	10.1*** ^(510.5)	2.8 ⁽¹¹³⁾	56.6*** ^(516.6)	1.8 ⁽²²¹⁾	20.5*** ⁽⁶⁶⁵⁾	0.4 ⁽¹¹²⁾	12.5*** ^(492.3)
Generation	1	2.2 ⁽¹¹⁰⁾	0.8 ^(511.8)	23.1*** ⁽¹¹³⁾	185.9*** ^(520.2)	10.6*** ⁽²²¹⁾	311.3*** ^(673.5)	1.8 ⁽¹¹²⁾	17.0*** ^(503.3)
History × Resource	2	0.3 ⁽¹¹⁰⁾	3.9* ^(510.5)	0.0 ⁽¹¹³⁾	0.5 ^(516.6)	2.1 ⁽²²¹⁾	0.1 ⁽⁶⁶⁵⁾	0.5 ⁽¹¹²⁾	0.9 ^(492.3)
History × Generation	1	0.0 ⁽¹¹⁰⁾	1.1 ^(511.8)	0.2 ⁽¹¹³⁾	2.1 ^(520.2)	1.0 ⁽²²¹⁾	4.9* ^(673.5)	0.2 ⁽¹¹²⁾	3.3 ^(503.3)
Resource × Generation	2	0.4 ⁽¹¹⁰⁾	0.6 ^(507.9)	0.7 ⁽¹¹³⁾	1.0 ^(511.9)	0.4 ⁽²²¹⁾	5.5** ⁽⁶⁶⁴⁾	0.7 ⁽¹¹²⁾	1.7 ^(487.6)
History × Resource × Generation	2	0.5 ⁽¹¹⁰⁾	2.9 ^(507.9)	2.1 ⁽¹¹³⁾	2.1 ^(511.9)	1.1 ⁽²²¹⁾	0.0 ⁽⁶⁶⁴⁾	0.6 ⁽¹¹²⁾	1.7 ^(487.6)
Random Effects									
Clone(Lake)	1	1.1	3.9***	1.6	3.0**	0.4	2.0*	0.9	2.0*
Lake(History)	1	-	0.7	-	0.4	0.0	0.7	-	0.6

F (D,df)

+0.05 < p < 0.07.

*p < 0.05.

**p < 0.01.

***p < 0.001.

Table 2. Least-square means (SE) for resource treatment, generation and lake history. Values in parentheses are $\pm 1.0S.E.$

		LOW		MEDIUM		HIGH	
		G1	G2	G1	G2	G1	G2
WISCONSIN							
Size at Maturity							
LP	Allequash	1.89(0.03)	1.80(0.06)	1.82(0.03)	1.88(0.05)	1.78(0.03)	1.80(0.03)
	Big Muskellunge	2.00(0.03)	1.88(0.06)	1.97(0.03)	1.97(0.05)	1.88(0.03)	1.82(0.04)
HP	Mendota	2.23(0.04)	2.23(0.06)	2.17(0.04)	2.15(0.07)	2.11(0.04)	2.14(0.05)
	Monona	2.22(0.05)	2.30(0.13)	2.13(0.05)	1.96(0.10)	2.11(0.05)	2.11(0.07)
Age at Maturity							
LP	Allequash	18.50(1.17)	28.14(2.34)	14.45(1.15)	24.21(1.66)	12.17(1.03)	18.79(1.17)
	Big Muskellunge	20.36(1.17)	30.33(2.24)	15.78(1.12)	28.53(1.74)	13.46(1.17)	19.12(1.35)
HP	Mendota	19.03(1.36)	25.46(2.20)	19.33(1.32)	24.36(2.40)	13.34(1.34)	21.04(1.56)
	Monona	21.12(1.59)	40.67(4.59)	18.07(1.53)	26.33(3.25)	15.30(1.45)	20.93(2.13)
Juvenile Growth							
LP	Allequash	0.10(0.01)	0.05(0.01)	0.10(0.01)	0.06(0.01)	0.11(0.01)	0.08(0.01)
	Big Muskellunge	0.10(0.01)	0.03(0.01)	0.10(0.01)	0.05(0.01)	0.10(0.01)	0.06(0.01)
HP	Mendota	0.11(0.01)	0.08(0.01)	0.12(0.01)	0.09(0.01)	0.12(0.01)	0.10(0.01)
	Monona	0.12(0.01)	0.05(0.01)	0.11(0.01)	0.06(0.01)	0.12(0.01)	0.09(0.01)
Sum of Eggs							
LP	Allequash	5.46(0.57)	5.13(1.06)	7.00(0.56)	4.14(0.80)	7.11(0.50)	6.27(0.59)
	Big Muskellunge	6.19(0.63)	4.30(1.13)	9.29(0.61)	5.40(0.92)	8.21(0.62)	7.08(0.71)
HP	Mendota	7.30(0.77)	6.53(1.15)	7.06(0.76)	7.40(1.41)	8.82(0.77)	9.13(0.91)
	Monona	6.59(0.81)	3.67(2.20)	6.83(0.78)	3.75(1.91)	8.41(0.73)	7.36(1.02)
ALASKA							
Size at Maturity							
LP	Fog 4	2.53(0.03)	2.57(0.05)	2.54(0.03)	2.53(0.08)	2.41(0.04)	2.61(0.10)
HP	E6	2.68(0.07)	2.71(0.11)	2.55(0.07)	2.65(0.10)	2.62(0.06)	2.69(0.13)
Age at Maturity							
LP	Fog 4	22.55(1.31)	31.71(2.32)	20.88(1.53)	25.00(3.54)	20.67(1.58)	30.00(4.33)
HP	E6	36.71(2.47)	40.40(4.13)	24.27(2.39)	41.50(3.77)	28.94(2.31)	42.50(4.62)
Juvenile Growth							
LP	Fog 4	0.06(0.01)	0.04(0.01)	0.06(0.01)	0.07(0.01)	0.07(0.01)	0.05(0.01)
HP	E6	0.05(0.01)	0.04(0.01)	0.06(0.01)	0.03(0.01)	0.07(0.01)	0.05(0.01)
Sum of Eggs							
LP	Fog 4	4.68(0.46)	4.00(0.89)	4.13(0.54)	6.00(1.26)	3.60(0.56)	5.00(1.54)
HP	E6	3.86(0.61)	3.60(1.01)	4.00(0.59)	3.50(0.92)	3.88(0.57)	5.25(1.13)

Figures

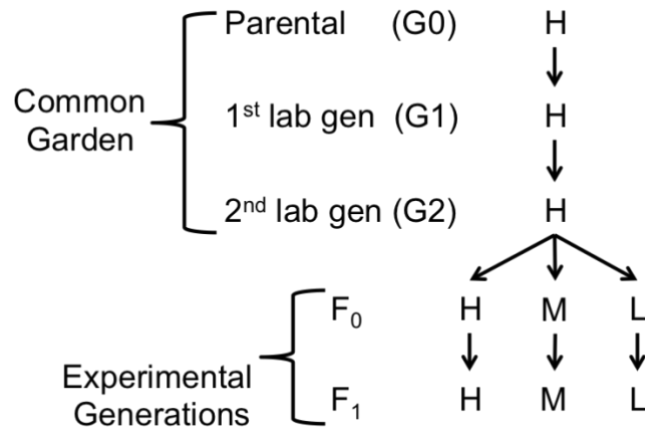


Figure 1. Experimental design for evaluating the effects of productivity. “L” – Low Food Treatment (0.2 mg C L⁻¹ day⁻¹). “M” – Medium Food Treatment (0.5 mg C L⁻¹ day⁻¹). “H” – High Food Treatment (1.0 mg C L⁻¹ day⁻¹).

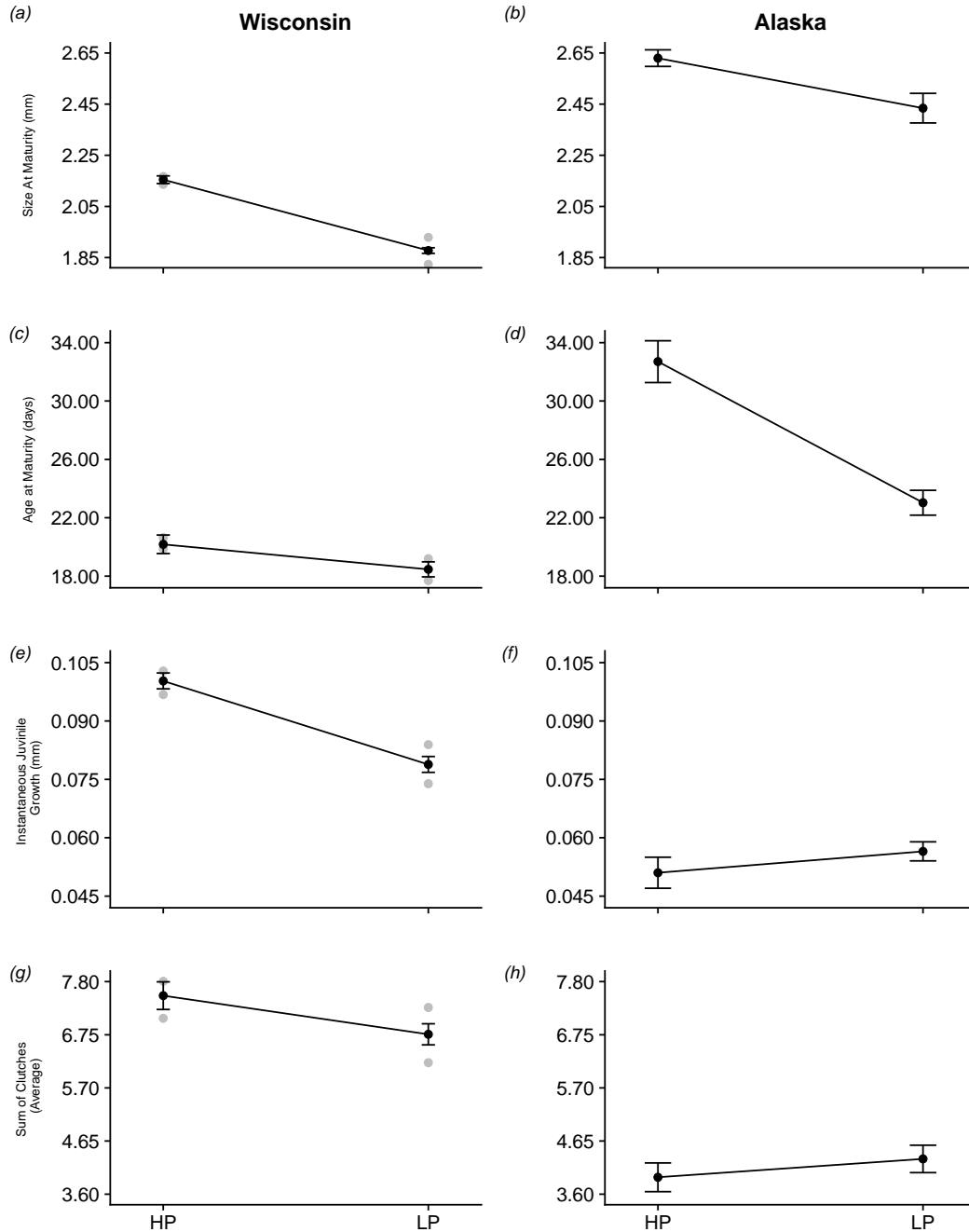


Figure 2. *Lake History Effects.* Variation in *Daphnia* life history traits between High-Productivity (HP) lakes and Low-Productivity lakes (LP) in a natural system (Wisconsin) and an experimental system (Alaska). (a,b) size at maturity, (c,d) age at maturity, (e,f) instantaneous juvenile growth, (g,h) sum of clutches (average). For all panels *black circles* represent population means, for panels (a,c,e,g) *grey circles* indicate lake means. We observed significant ($p < 0.05$) differences between HP and LP lake populations in the natural system (WI) for size at maturity, and instantaneous juvenile (a,e), and in the experimental system (AK) for size at maturity, and age at maturity (b,d).

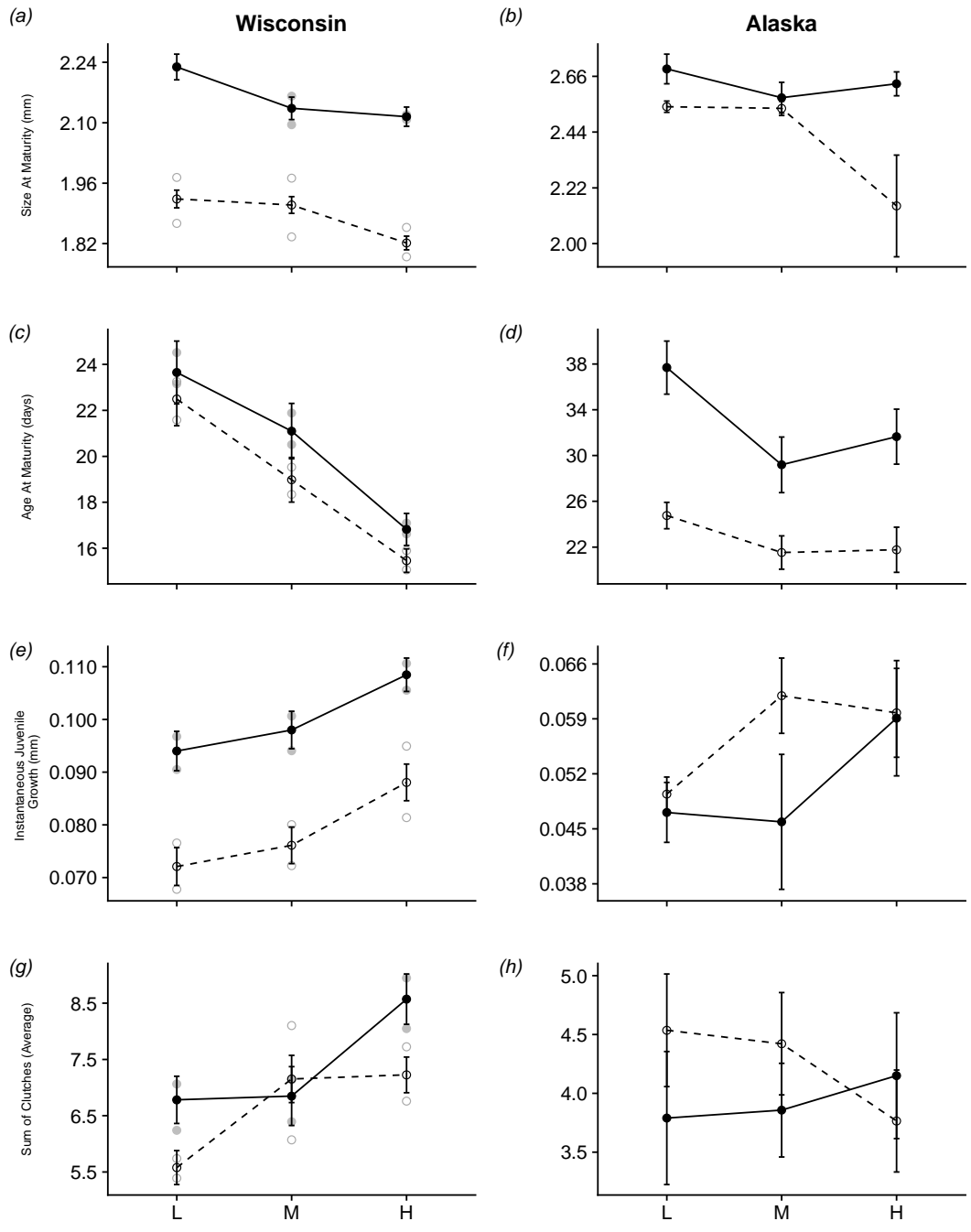


Figure 3. Food Treatment by Lake History Effects. Variation in *Daphnia* life history traits to an interaction between food treatment and lake history. Natural system (WI) and experimental system (AK). (a,b) size at maturity, (c,d) age at maturity, (e,f) instantaneous juvenile growth, (g,h) sum of clutches (average). For all panels: Black circles represent population means, for panels (a,c,e,g,i), grey circles represent lake means. Open circles/dashed lines - Low Productivity (LP) lakes, closed circles/solid lines - High Productivity (HP) lakes. We observed significant ($p < 0.05$) food treatment by lake history interactions in the natural system (WI) for size of maturation (a).

Chapter 3

Evolutionary insights from endemic predation: Linking *Daphnia* life history evolution to stickleback phenotypes in Alaskan lake ecosystems

Michelle Packer¹ and Matthew R. Walsh¹

¹Department of Biology, 501 S. Nedderman Drive, University of Texas at Arlington, Arlington, TX 76019 USA

Abstract

Variation among species has long been acknowledged to influence the environment. Studies have shown that phenotypic variation can influence ecological structure and function as well as community composition. This phenotypic variation may also impose selection and drive patterns of evolutionary change. Understanding the impacts of intraspecific variation is crucial for predicting reciprocal interactions between ecological and evolutionary forces. We tested the influence of divergent forms of three-spine stickleback (*Gasterosteus aculeatus*) predation on the evolution of life history traits and life history plasticity in *Daphnia* from lakes in Alaska. We compared the traits of *Daphnia* from lakes that have trophically divergent forms of stickleback (benthic vs. limnetic) as well as lakes that had no stickleback. We found that *Daphnia* from benthic and no stickleback lakes matured earlier and at a smaller size than *Daphnia* from lakes with limnetic stickleback. These divergent life history responses are likely due to covariation between stickleback morphotype and lake size. Our results highlight the importance of including intraspecific variation when evaluating ecological effects of predation and the potential cascading impacts on prey populations.

Introduction

It is well known that predation can facilitate a multitude of predator-specific changes within prey populations (Relyea 2001, Wilson *et al.* 2005, Stuart-Fox *et al.* 2008, Frommen *et al.* 2011).

These responses range from the environmental induction of inducible defenses and shifts in life history traits to evolutionary changes in multiple aspects of prey phenotypes (Hebert and Grewe 1985, Reznick *et al.* 1990, Fisk *et al.* 2007). Furthermore, differences in predation can have important correlated ecological impacts. For instance, changes in the frequency or duration of predation have been shown to influence the structure and dynamics of prey communities (Post *et al.* 2008, Harmon *et al.* 2009, Palkovacs and Post 2009, Walsh and Post 2011, Walsh *et al.* 2012). Recent empirical evidence suggests that evolutionary divergence in phenotypic and population-level traits of a predator can also significantly affect ecosystem structure and function (Harmon *et al.* 2009, Bassar *et al.* 2010, Howeth *et al.* 2013, des Roches *et al.* 2013, Brodersen *et al.* 2015).

For many species of fish, morphological diversification and the evolution of discrete trophic polymorphisms are associated with variation in resource availability. Such divergence is often driven by individuals feeding within distinct habitats and is related to prey capture and/or consumption (McPhail 1984, Robinson and Wilson 1994, Schluter 2000). This leads to within species specialization in characteristics such as feeding behavior, niche use, cranial-facial and body morphology, and in some populations, can ultimately lead to speciation (Bentzen and McPhail 1984, Schluter 1993, Day and McPhail 1996, McKinnon and Rundle 2002, Vines and Schluter 2005, Hendry *et al.* 2009). One such example of resource mediated phenotypic divergence are populations of stickleback in the United States and Canada. Stickleback are

widely distributed within the northern hemisphere, and are often found in isolated populations in stream, lake, and marine habitats (Colosimo *et al.* 2005). As a result, stickleback have become a widely accepted model system for ecological speciation due to the presence of repeated parallel evolution observed between populations (Schluter and McPhail 1992, McPhail 1993, Albert and Schluter 2004, Colosimo *et al.* 2005, Barrett *et al.* 2008). Many studies have quantified evolutionary differences in behavior, life history, dietary preference, morphology, and habitat use in populations of stickleback that inhabit contrasting environments (Anker 1974, Day *et al.* 1994, Day and McPhail 1996, Walker 1997, Baker *et al.* 2005, Hosoki *et al.* 2019). However, few studies have explored the pathway from evolutionary divergence in stickleback to selection on the traits of their prey.

In the Cook's Inlet Area of Alaska, several lakes within two separate lake basins contain divergent populations of 'benthic' and 'limnetic' three-spine stickleback (*Gasterosteus aculeatus*) (Willacker *et al.* 2010). Benthic morphotypes are defined as fish that feed in the littoral zone and have derived specialized characters such as deep bodies and heads, with few and short gill rakers which aid in the consumption of macroinvertebrates (Bentzen and McPhail 1984, McPhail 1984, Schluter 1993, Robinson and Wilson 1994, Uchii *et al.* 2007). The deep body is associated with increased maneuverability and decreased turning radius, attributes which provide an advantage when catching macroinvertebrates in a structurally complex environment. The deep head increases suction feeding performance, and in turn efficiency, when foraging for large prey that are immersed in the benthos, or clinging to rocks or submerged vegetation. The shape and size of gill rakers help with the passing of debris after suction feeding in the benthos (Harmon *et al.* 2009, Hosoki *et al.* 2019). The foraging modality of limnetic ecotypes favors several

morphological traits such as the evolution of larger eyes, a more fusiform body shape with a long slender head and mouth, and longer and more numerous gill rakers which increase foraging efficiency on zooplankton. Limnetic morphotypes primarily reside in open water habitats and mainly feed on zooplankton (Bentzen and McPhail 1984, Lavin and McPhail 1986). This variation in prey selectivity and foraging habitat use may promote divergent predatory selection on stickleback's prey.

Many of these lakes in Alaska that contain contrasting populations of stickleback also contain populations of *Daphnia rosea*. *Daphnia* are primarily pelagic and are rarely found in shallow environments when deeper refugia are available. *Daphnia* are a dominant grazer on phytoplankton and provide a key link to the transfer of nutrients across trophic levels (Carpenter *et al.* 1987, 1992, Elser *et al.* 1988). *Daphnia*'s small size, ease of culture, and rapid generation time (producing clutches of offspring every couple of days) make them extremely useful for experimental studies. Additionally, *Daphnia* exhibit well known patterns of phenotypic plasticity when they are exposed to fish predator cues; *Daphnia* generally mature faster and increase investment in reproduction in the presence of fish predators (Stibor 1992, Weider and Pijanowska 1993, Macháček 1995, Riessen 1999, Ślusarczyk 1999). These shifts in life history traits in *Daphnia* are directly linked to increases in population growth, and are thus presumed to be adaptive (Riessen 1999).

Here, we quantified predator-induced plasticity (within and across generations) and shifts in life history traits in *Daphnia rosea* in response to trophically divergent forms of stickleback predation. Our goal was to determine if contrasting forms of stickleback predation is associated

with divergent selection on *Daphnia* life history traits and plasticity. We collected *Daphnia* from lakes that contained either ecotype of stickleback and subsequently quantified patterns of life history divergence and life history plasticity by rearing 3rd generation lab born *Daphnia* in the presence and absence of fish predator cues. We focused on life history traits because they are intricately connected to rates of population growth and are therefore a strong proxy for fitness. We predicted that *Daphnia* from lakes with limnetic forms of stickleback would experience increased contact rates with stickleback predators, and ultimately increased predatory selection as compared to *Daphnia* from lakes with benthic stickleback. We therefore expected that *Daphnia* from 'limnetic' lakes would mature earlier, at a smaller size, and exhibit increased reproductive effort when compared with *Daphnia* from 'benthic' lakes (Tollrian and Harvell 1999, Walsh and Post 2012, Walsh *et al.* 2016). If the contrasting stickleback ecotypes exert divergent selection on phenotypic plasticity, we predicted that *Daphnia* from limnetic lakes would exhibit stronger responses to the presence of kairomones (Reger *et al.* 2018). Additionally, we compared these results to the traits of *Daphnia* from lakes which do not contain stickleback (No stickleback - NS) to examine trends which may be attributable to stickleback predation in general.

Materials and Methods

Lake Systems and Focal Lakes

Daphnia rosea were collected from lakes located within two lake basins in the Cook's Inlet Area of Alaska, the Matanuska-Susitna (Mat-Su, *MS*) and Kenai Peninsula Lake Basins (Kenai, *K*). It is important to note that in 2003 a comparison of these lake basins concluded that while there were significant differences between basins, 98% of the variance could be explained by the

differences in non-nanosized silicon, which does not have developmental effects on *Daphnia* sp., and there were no significant differences in zooplankton biomass (Jones *et al.* 2003). Lakes were chosen based on the presence of divergent stickleback ecotypes (Willacker *et al.* 2010). Lake sampling was prioritized by magnitude of divergence along the benthic/limnetic axis in stickleback populations, with the lakes containing the most extreme ecotypes ranked first. Six lakes with benthic stickleback (Tern (K), Corcoran (MS), Walby (MS), Watson (K), Finger (MS), Jean (K)), five lakes with limnetic stickleback (South Rolly (MS), Spirit (K), Long (MS), Wik (K), Milo (MS)), and 10 lakes with no stickleback [G (K), Leisure pond (K), Leisure lake (K), Rancho (K), Hope (K), Loon (K), Crystal (K), CC (K), Fred's (K), Warfle (K)] were sampled. *Daphnia rosea* were identified and collected from Watson, Walby, Finger, Tern, Spirit, Wik, Long, Warfle and Loon lakes. However, only clones from three benthic (Watson, Walby, Finger), one limnetic (Spirit) and one no stickleback (Loon) populations survived transport and common garden rearing in a laboratory environment. As described above, the main difference in predator communities between lakes is the divergent form of stickleback present. Importantly, these lakes do not differ in macroinvertebrate predator communities, as *Daphnia* can also respond to predation by other zooplankton and larvae (Hebert and Grewe 1985, Spitze 1991, Weiss *et al.* 2018).

Experimental protocols

We tested *Daphnia* for divergence in life history traits and plasticity, as well as transgenerational responses to predator cues by rearing clones in a common garden environment. Adult females were identified and isolated in the field, then shipped back to the University of Texas at Arlington. Individuals were slowly transitioned to COMBO medium. All generations of *Daphnia*

were reared in COMBO, under controlled temperature ($16 \pm 1^\circ\text{C}$) and light (12:12 light-dark cycle) conditions (Lynch *et al.* 1986) and fed a non-limiting supply of *Scenedesmus obliquus* (concentration: 1.0 mg carbon (C) L⁻¹ day⁻¹) (Kilham *et al.* 1998). All clones were reared in a common garden setting for two generations before initiating the experiments. Three adult females were taken from each clone line to establish the parental generation (G₀) and reared individually in a 90 ml jar. All subsequent generations (G₁, G₂, G₃, G₄) were taken from the second clutch of the previous generation and consisted of neonates less than 12 hrs old. For the first laboratory generation (G₁), two neonates were taken from a G₀ mother, and reared together in a new 90 ml jar, with each clone having 3 replicates. The second laboratory generation (G₂) was taken from the second clutch of G₁, the mothers discarded, and two neonates were reared together in the original jar, such that there were three replicates of G₂ generation with two individuals in each jar, for each clone. *Daphnia* were transferred to fresh media and algae every other day. Starting on day 4, all *Daphnia* were checked daily for the maturity and production of the first and second clutch.

We evaluated patterns of within- and across-generation plasticity, and divergence in life history traits starting with the third generation (G₃) lab reared clones for all lakes. Six neonates (less than 12-h old) were collected from each clone (i.e. multiple females were available to produce the experimental treatments) and placed into separate jars to make three replicates of each treatment. Each pair was randomly assigned to one of two treatments: (i) predator exposure in the first generation (G₃= P, G₄ = PN) and (ii) no predator exposure (G₃ = N, G₄ = NN). The experiment was run for two experimental generations (G₃, G₄) (Fig.1). The second experimental generation (G₄) was not exposed to the presence of predators because previous work has shown

that across-generation responses are similar in magnitude for continual exposure to predator cues for multiple generations and for *Daphnia* that are only reared in the presence of the cue for the first generation (Walsh *et al.* 2015, 2016). Second generation individuals in the predator treatment trial were exposed to predator cues during embryonic development and very early life-stages. Our predator treatment included the addition of filtered tank water which was conditioned by redbreast sunfish (*Lepomis auratus*) (see *Kairomone collection*). Each clone was replicated three times for each treatment and generation for a total sample size of 444 jars (37 clones across lakes x 2 treatments x 3 replicates per treatment x 2 generations) (Clones: Watson = 3, Walby = 10, Finger = 10, Spirit = 10, Loon = 4).

For this experiment we quantified size at maturation, age at maturation (defined as the release of the first eggs into the brood chamber) and the size of the first three clutches of offspring. We defined average clutch size per clone as the average number of eggs in each clutch, found by summing the number of eggs in clutches 1-3 and dividing by 3. Total average reproductive output is the total number of eggs produced over three clutches. All jars were checked daily for maturation and for release of offspring from the brood chamber. Upon maturation, age at maturation and clutch size were recorded and each individual was photographed for estimates of size (using ImageJ). After maturation, jars were checked daily for the production of clutches. Upon release of the second clutch from the brood chamber, pairs of newly born neonates from each clone were collected to initiate the second experimental generation (G4). The G4 generation was placed in a new jar containing fresh media and algae, and experienced the same temperature, photo-period and frequency of food/media replacement as the previous generations.

Kairomone collection

We generated fish kairomones by collecting COMBO medium from a tank containing two redbreast sunfish (*Lepomis auratus*) (~ 10 cm in total length) in 7 L of COMBO. Sunfish were used as our predator species for all lakes except for Loon (for which stickleback were used with the same methodology as below). Previous research has shown that *Daphnia* respond similarly to the presence of fish kairomones regardless of the species of fish predator, with minor variations in effect size (Stibor 1992, de Meester and Weider 1999, Elert and Pohnert 2000, Weber 2003, Castro *et al.* 2007, Walsh and Post 2012, Walsh *et al.* 2015, 2016, Hintz and Relyea 2017, Packer and Walsh 2017). Fish were fed > 200 *Daphnia rosea* the morning of media collection as injured *Daphnia* have shown to emit chemical cues that magnify the magnitude of the phenotypic response to predation (Laforsch *et al.* 2006, Pestana *et al.* 2013). Predator conditioned COMBO was collected from the tanks 1 hour after feeding and filtered using membrane filters (47 mm diameter, 0.45 μm , followed by 47 mm diameter, 0.2 μm , Millipore Corporation). The chemical kairomones were then transferred to sterile 50 ml falcon tubes and stored at -20°C for 1 week prior to the start of the experiment. The concentration of kairomones in the predator treatments equaled 0.0069 fish L⁻¹.

Statistical analyses

We performed two separate analyses. The first analysis tested for variation between benthic, limnetic and no stickleback lakes. The second tested for a general effect of stickleback predation by comparing the traits of *Daphnia* between lakes that did or did not have stickleback irrespective of stickleback morphology. Variation in dependent variables was analyzed initially with linear mixed models (SPSS v.24), implementing restricted estimatable maximum-likelihood

estimation (REML). For each variable, lake type (benthic, limnetic, no stickleback), predator treatment (presence, absence), generation and all interactions among factors were entered as fixed effects and clonal ID was nested within lake as a random effect. Unexplained variation among clones from each lake was controlled by treating clone (nested within lake) as a random effect. We characterized the presence of transgenerational plasticity (TGP) as a significant interaction between predator treatment and generation. Additionally, we tested for local adaptation in TGP by evaluating the third-order interaction between lake type, predator cue, and generation. We initially evaluated the significance and explanatory power of interactions between all fixed effects and nested random terms. For the general effects of stickleback predation analysis, clone (nested within lake) was significant for the size of maturation. For all other variables in both analyses, the significance of the fixed effects did not depend on the presence or removal of clone (nested within lake), and the random term was removed from the model. Following the removal of the random effect, we reran the analysis as a general linear model. When significant lake-type effects were detected for the univariate analysis, we evaluated the differences among lake types with post hoc Fisher's LSD procedure. LSD is known to preserve the experimentwise type I error rate at the nominal level of significance when the number of treatment groups is three (Hayter 2012, Howell 2013). Such is the case in our experiment. Clones from Watson lake were underrepresented due to mortality, however, significance of fixed effects did not depend on the presence or removal of this data, so the lake was included. Data for size at maturation was reciprocal root-transformed, while all reproductive output data (Clutch 1-3 sizes, average clutch size and total reproductive output) was square root transformed to improve fits with normality and homogeneity of variances.

Intrinsic rate of increase

We combined our average age at maturation and clutch size with average interclutch interval to calculate intrinsic rates (r) for each clone from each lake (Gotelli 1998). We calculated r as $r = \ln(R_0)/G$, where R_0 is the net reproductive rate (sum of fecundity x survivorship) and G is generation time (average age of the parents of all offspring produced by a single cohort). We evaluated differences in r using a linear-mixed model with lake type, predator treatment, and generation as fixed effects, and clone (nested within lake) entered as a random effect.

Results

Benthic vs. Limnetic stickleback

Lake Type Effects

We observed significant ($p < 0.05$) differences in age and size at maturation between *Daphnia* from lakes with benthic, limnetic, and no stickleback (Table 1). Post-hoc tests showed significant differences between ‘limnetic’ lakes and the other focal lake types. *Daphnia* from lakes with limnetic stickleback matured 15% (1.9d) later and were 9% (0.13 mm) larger than *Daphnia* from benthic lakes, and 19% (2.3d) later and 9% (0.12mm) larger than *Daphnia* from no stickleback lakes. No significant differences in size or age at maturation were found between *Daphnia* from no stickleback and benthic lakes (Fig. 2A, C). Differences in average clutch size and total reproductive output were also significant ($p < 0.05$) between focal lakes (Table 1). Post-hoc tests revealed that the reproductive outputs of *Daphnia* from benthic and limnetic lakes differed significantly ($p < 0.05$) from *Daphnia* from no stickleback lakes. *Daphnia* from lakes with benthic morphotypes produced 25% (2.15) fewer eggs with a 17% smaller average clutch size, while *Daphnia* from limnetic lakes produced 14% (1.25) fewer eggs with a clutch size that was 13%

smaller than *Daphnia* from lakes with no stickleback (Fig. 2B, D). The differences in clutch size and total reproductive effort between *Daphnia* from benthic and limnetic lakes were not significant. The observed variation in interclutch interval was not significant. We observed a marginally nonsignificant ($F_{2,24} = 3.1, p=0.062$) difference in the intrinsic rates of increase between lakes. Post-hoc tests revealed that *Daphnia* from lakes with benthic stickleback exhibited a significantly ($p < 0.05$) lower intrinsic rate of increase (approx. 6%) than those from lakes with limnetic stickleback. There was no difference in intrinsic rates of increase between no stickleback versus ‘benthic’ and ‘limnetic’ lakes. (Fig. 2F).

Predator Cue Effects

Exposure to predator cues significantly ($p < 0.05$) influenced intrinsic rates of increase (r) (Table 1). *Daphnia* from predator treatments exhibited an intrinsic rate of increase that was 5% higher when compared to *Daphnia* that were not reared in the absence of predator cues (Fig. 5). The influence of predator cues on the expression of all other traits was not significant (Table 1).

Generation Effects

We observed significant ($p < 0.05$) differences between generations for all measured traits with the exception of interclutch interval (Table 1). *Daphnia* from generation 1 matured 14% (1.75 d) earlier and were 4% (0.06mm) larger at maturation. *Daphnia* from generation 1 also produced an average clutch size that was 58% (1.10 eggs) larger than the clutch size observed in generation 2 (mostly attributable to a significant result of a 37% larger second clutch) resulting in a 43% increase in reproductive output when compared with *Daphnia* from generation 2.

Statistical Interactions

We observed a marginally nonsignificant ($F_{2,234} = 2.6, p=0.076$) interaction between lake type and predator treatment for age of maturation (Table 1). *Daphnia* from lakes with limnetic versus benthic (and no) stickleback exhibited contrasting responses to predator cues. *Daphnia* from no stickleback and ‘benthic’ lakes matured 18% (2.4d) and 10% (1.4d) earlier when reared in the presence of predator cues, while *Daphnia* from ‘limnetic’ lakes matured 9.4% (1.3d) later when exposed to predator cues (Fig. 4A). We also observed a significant ($p<0.05$) lake type \times predator interaction for intrinsic rates of increase (Table 1). In the absence of predator cues, *Daphnia* from lakes with benthic stickleback had an intrinsic rate of increase that was 11% lower than *Daphnia* from lakes with limnetic forms. However, in the presence of predator cues, these differences disappeared (*Daphnia* from benthic stickleback lakes exhibited 5% higher intrinsic rate of increase between predator and no predator treatments, while the intrinsic rate of increase for *Daphnia* from lakes with limnetic stickleback declined by 5% between predator and no predator treatments). The result is that the values for r for benthic and limnetic populations were similar in the presence of predator cues (Fig. 4F). This interaction was also influenced by a large increase in r in *Daphnia* from no stickleback lakes (r increased by ~20% between predator and no predator treatments).

Presence/Absence of Stickleback

Lake Type Effects

We performed a complementary analysis to examine shifts in trait values and plasticity based strictly on the presence/absence of stickleback. Differences in the age or size at maturation and interclutch interval were not significant ($p>0.05$) (Table 2; Fig. 3A, C, E). We observed

significant differences ($p < 0.05$) in reproductive output between *Daphnia* from lakes with and without stickleback (Table 2). *Daphnia* from lakes with stickleback had 15% smaller clutches (average number of eggs in each clutch) and a 21% lower average reproductive output (total number of eggs produced over three clutches) than those from lakes with no stickleback (Fig. 3B, D). There was no significant difference ($p > 0.05$) in the intrinsic rates of increase between lakes with and without stickleback (Table 2; Fig. 3F).

Predator Cue Effects

We found a significant ($p < 0.05$) effect of predator cue on intrinsic rates of increase (Table 2). *Daphnia* exposed to predator cue had an 11% higher intrinsic rate of increase as compared to *Daphnia* that were not exposed to predator cues (Fig. 5). All other effects due to predator cues were not significant.

Interactions

The interaction between predator cue and lake type (presence/absence of stickleback) for intrinsic rates of increase was significant (Table 2). Differences in r in the absence of predator cues between lakes with and without stickleback were small. In the absence of predation cue, *Daphnia* from lakes with stickleback exhibited a 3% higher r as compared to populations with no stickleback. In the presence of predator cues, *Daphnia* from lakes with stickleback then exhibited an 11% higher intrinsic rate of increase as compared to *Daphnia* from lakes with no stickleback (Fig. 4F).

Discussion

Daphnia from Benthic vs. Limnetic Stickleback Lakes

Our results show that the presence of distinct trophic morphotypes of stickleback is associated with life history divergence in populations of *Daphnia rosea*. *Daphnia* from lakes with benthic stickleback matured earlier and at a smaller size than *Daphnia* from lakes with limnetic stickleback (Fig. 4). Such a pattern of life history evolution opposes our *a priori* expectations – we expected that *Daphnia* in lakes with limnetic stickleback would experience increased contact rates and higher predatory mortality as compared to *Daphnia* that co-occur with benthic stickleback. Life history theory predicts that increased rates of adult extrinsic mortality should favor the evolution of earlier maturation, a smaller size at maturation, and increased reproductive effort (Gadgil and Bossert 1970, Law 1979, Michod 1979, Gardmark and Dieckmann 2006). Fish are visually oriented predators and we therefore expected stickleback to target larger, adult *Daphnia*. Prior studies of life history evolution have shown that increased fish predation is correlated with a trajectory of life history evolution in *Daphnia* that matches the expectations from theory (Walsh and Post 2012). Thus, our results beg the question: why are benthic stickleback associated with the evolution of a faster life history? Below we further discuss the differences in trait values between the focal lakes, consider why we observed the specific patterns of life history evolution in this study, and examine the evidence for differences in phenotypic plasticity.

Contrary to our expectations, *Daphnia* from ‘benthic’ populations displayed the expected adaptive pattern of life history evolution in response to fish predatory pressure (earlier maturation at a smaller size) (Fig. 4). Furthermore, it is also important to note that the trait values

are similar for age and size at maturation between *Daphnia* from benthic and no stickleback lakes, yet *Daphnia* from lakes 'limnetic' stickleback differ from both lakes with benthic and no stickleback (Fig. 4). Thus, the life history strategy of *Daphnia* from limnetic lakes is unique when compared to the other focal lakes. In contrast with theory, we did not detect any difference in reproductive traits between 'benthic' and 'limnetic' populations as both populations exhibited a significantly lower fecundity when compared to *Daphnia* from lakes with no sticklebacks (Fig. 2B, D). In our study, reduction in size and age at maturation were not associated with increases in reproductive output and did not lead to higher overall fitness for *Daphnia* from lakes with benthic stickleback (Fig. 2F). Small, non-significant differences in reproductive traits resulted in benthic populations exhibiting a lower overall intrinsic rate of increase than 'limnetic' and no stickleback populations (Fig. 2B, D, E, F). It is unclear why we observed shifts in developmental traits but not reproductive traits. Such contradictory patterns of life history evolution require further investigation.

Driver of divergence between benthic and limnetic lakes.

The presence of limnetic and benthic stickleback covary with lake size. 'Limnetic' lakes are larger and deeper than 'benthic' lakes. Therefore, our focal lakes provide divergent spatial heterogeneity in habitat depth. *Daphnia* from lakes with limnetic stickleback have an increased opportunity to mitigate the risk of predation from visually oriented fish by moving into the deeper waters of the limnetic zone where it is colder, darker, and nutrient poor [i.e., they exhibit diel vertical migration (DVM)] (Dawidowicz and Loose 1992, Loose and Dawidowicz 1994, Dodson *et al.* 1997, Gool and Ringelberg 2002). This movement potentially alleviates exposure to predation during daylight hours when rates of predation are presumably the most intense. In

our study, *Daphnia* clones coexisting with limnetic stickleback were from a lake which was 4.5 times deeper than the deepest lake with benthic populations of stickleback (Table 3). This increase in depth provides mid-water refuge areas for larger-bodied plankton populations. This is relevant because *Daphnia* from limnetic lakes matured later at a larger size. While limnetic ecotypes of stickleback are more efficient when foraging on planktonic prey, our unexpected results foreshadow that *Daphnia* may experience weaker mortality rates in lakes with limnetic stickleback. We propose that *Daphnia* in these lakes are able to use the deep hypolimnion as a refugia to escape predation, thereby reducing selection due to fish predators (Wright and Shapiro 1990). Such results suggest that DVM may provide a buffer against predatory selection, and that this behavioral response imparts higher fitness benefits than changes in life history traits when fish predation is constant, but refugia is available.

Conversely, the lakes in our study which support benthic stickleback are shallower, lack significant spatial heterogeneity and generally provide one homogenous habitat for *Daphnia*. Bathymetric maps show that there is an increase in depth in the center of the lakes that we sampled, but none are deeper than 5.5m. Thermoclines are absent and light penetrates into the benthos, eliminating a dark daytime refuge for zooplankton. Consequently, *Daphnia* in these lakes are exposed to a more constant selective pressure as they cannot avoid predation via diel vertical migration. While benthic stickleback exhibit reduced foraging efficiency on zooplankton as compared to limnetic forms, they do still consume zooplankton. Stomach contents indicate that planktonic prey can represent up to 50% of their diet (Larson 1976, Bentzen and McPhail 1984, Day *et al.* 1994). We therefore propose that reduced depth yields increased predatory mortality in lakes with benthic stickleback. Such predictions are supported by an analysis of the

catch per unit effort (CPUE) between lakes. The CPUE was 39% higher in lakes with benthic vs. limnetic stickleback, and the largest lake containing a benthic population (Finger) supported more than three-times the population of an equivalently sized lake with a limnetic population of stickleback (Spirit) (Table 3). Thus, predation appears to be particularly intense in lakes with benthic stickleback. Furthermore, *Daphnia* from lakes with benthic and no stickleback exhibited a similar age and size at maturation. This is relevant because the no stickleback lake used in this study was also shallow and representative of a typical 'benthic' lake. It appears that the covariation between stickleback morphotype and lake size is the best explanation for the observed patterns of life history evolution.

Evolution of phenotypic plasticity?

Evidence for evolved differences in phenotypic plasticity between lakes with limnetic and benthic stickleback is limited. This is because all predator cue by lake type interactions were not significant except for the significant interaction of intrinsic rate of increase. For this latter result, the interaction is largely driven by a strong response to predator cues exhibited by *Daphnia* from no stickleback lakes. One potential signal for adaptive shifts in plasticity between benthic and limnetic lakes is the marginally nonsignificant predator cue by lake type interaction for age at maturation. Interestingly, when *Daphnia* from benthic lakes were exposed to predator cues, they exhibited the typical 'adaptive' response to fish predation. They matured faster in the presence versus absence of predator cues which resulted in a significant positive change in the intrinsic rate of increase. In contrast, *Daphnia* from lakes with limnetic stickleback responded to the presence of predator cues by delaying maturation (Fig. 4A, B, D, E). One plausible interpretation is that *Daphnia* from benthic lakes have evolved to respond adaptively to the presence of fish

predators. The patterns of plasticity in these populations ultimately resulted in a positive impact on intrinsic rates of increase. Conversely, *Daphnia* from limnetic stickleback lakes seem to lack similar adaptive responses. They matured later at a larger size, and when exposed to predation cue, reduced reproductive output which resulted in a decline in fitness.

The lack of widespread evidence for the evolution of plasticity is theoretically not surprising. Stickleback from the lakes in our study are landlocked and non-migratory. Therefore, there is no temporal heterogeneity in the presence or absence of stickleback since they are present year-round. This is relevant because theory predicts that the evolution of plasticity is highly dependent on spatial or temporal heterogeneity in a change in environmental conditions (Levins 1968, Scheiner 1993, Alpert and Simms 2002, Lind and Johansson 2007) A variable but predictable environment favors plasticity, while stability or the lack of a predictable environmental signal favors genetic control on the expression of traits (Levins 1968, Scheiner 1993, Crispo *et al.* 2010, Walsh and Post 2012). In the lakes of interest, there is little to no seasonal variation in the intensity or frequency of predation on *Daphnia* populations when they are present in the water column.

Stickleback vs. No Stickleback as compared to Individual Lake Types

In addition to comparisons between *Daphnia* from benthic and limnetic lake types, we examined our data for the impact of general stickleback predation on *Daphnia* populations. Specifically, we wanted to ask if our results would differ when we did not account for morphological variation within the predator population. When life history trait responses for each population (benthic, limnetic, no stickleback) were compared, reproductive output and average clutch size of benthic

and limnetic populations differed from no stickleback populations (Fig. 2B, D). These results were maintained when we evaluated the trends based upon the presence-absence of stickleback, as both benthic and limnetic populations exhibited a reduced fecundity. When we compared each population independently for age and size of maturation, *Daphnia* from limnetic stickleback lakes differed significantly from those from lakes with no stickleback present (Fig. 2A, C). However, the variance detected in these traits within the limnetic population disappeared when the data was analyzed to address a simpler hypothesis only involving differences between *Daphnia* from lakes with and without stickleback. This analysis revealed no significant differences in age or size at maturation (Fig 3A, C). The age and size of maturation of *Daphnia* from benthic stickleback lakes were very similar to those found in the absence of stickleback, causing a loss of resolution in detecting population level differences. Similarly, comparisons of intrinsic rates of increase were not significant between lakes with and without stickleback (Fig. 3F). In contrast, the analysis that included stickleback morphotype revealed a divergence in fitness between *Daphnia* in benthic and limnetic stickleback lakes (Fig. 2F).

Conclusions

The results of this experiment revealed complex interactions between habitat specific predators and their prey. Simpler hypotheses that only consider presence vs. absence of sticklebacks without consideration of the trophic morphological variation may not be sufficient to explain the impact on prey trait evolution. Phenotypic diversity is hypothesized to be at the core of ongoing reciprocal interactions between ecological and evolutionary forces in natural environments (eco-evolutionary feedbacks) (Fussmann *et al.* 2007, Post and Palkovacs 2009, Schoener 2011, Reznick 2013, Matthews *et al.* 2014, Hendry 2016, des Roches *et al.* 2018). There is increasing

evidence that phenotypic variation can influence population dynamics, community composition and ecosystem processes (Crutsinger *et al.* 2006, Whitham *et al.* 2006, Post *et al.* 2008, Harmon *et al.* 2009, Palkovacs and Post 2009, Bassar *et al.* 2010, Duffy 2010, Schreiber *et al.* 2011, Turcotte *et al.* 2011, Vasseur *et al.* 2011, Urban 2013, El-Sabaawi *et al.* 2015, Rudman *et al.* 2015, des Roches *et al.* 2018). Predation by divergent phenotypes has been shown to have direct effects on prey community dynamics (Post *et al.* 2008). Yet, only a handful of studies have investigated the potential cascading effects that intraspecific variation can have on the selective landscape, and how it can alter evolutionary outcomes for prey (Walsh and Post 2011, 2012, Walsh *et al.* 2012, 2016). Our study advances such research and provides insight into how predator ecotypes such as those represented by benthic and limnetic stickleback, have cascading effects on life history trait evolution in prey.

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Tables

Table 1. Results of linear univariate ANOVA model analyses of life history traits in *Daphnia* from benthic and limnetic stickleback lakes. Generation, predator treatment and lake type were entered as fixed effects. Clone (nested within lake) was entered as a random effect but was non-significant for all variables and removed from the model, with the exception of intrinsic rate of increase (r). For the (r), clone(lake) was significant and results are from a linear mixed model analysis, and for this variable, df equals the denominator degrees of freedom. Values for the clone term are Wald Z statistics from a likelihood ratio test.

Fixed Effects	df	Size at Maturation	Age at Maturation	Clutch 1	Clutch 2	Clutch 3	Average Interclutch Interval	Average Clutch Size	Tot Repro Output	Intrinsic rate of increase (r)
Generation	1	1.1*** (202)	5.7* (224)	9.5** (226)	11.3*** (216)	4.9* (176)	0.2(189)	16.5*** (226)	11.1*** (226)	0.2 (200)
Stickleback Lake Type	2	21.1***(202)	4.8** (224)	0.9 (226)	2.3 (216)	3.1* (176)	0.1(189)	4.0* (226)	4.8** (226)	3.1† (24)
Predator Treatment	1	0.1 (202)	1.2 (224)	0.1 (226)	0.0 (216)	0.2 (176)	0.2(189)	0.1 (226)	0.6 (226)	252.5*** (200)
Gen. × Lake Type	2	1.8 (202)	3.4* (224)	4.2* (226)	0.6 (216)	2.4† (176)	0.1(189)	3.7* (226)	1.9 (226)	0.3 (200)
Gen. × Pred.	1	0.2 (202)	0.0 (224)	1.1 (226)	2.0 (216)	0.4 (176)	0.3(189)	0.8 (226)	0.4 (226)	0.3 (200)
Lake Type × Pred.	2	0.9 (202)	2.6† (224)	0.2 (226)	0.8 (216)	1.4 (176)	0.1(189)	0.8 (226)	1.5 (226)	446.4*** (200)
Gen. × Lake Type × Pred.	2	1.2 (202)	1.7 (224)	0.4 (226)	0.1 (216)	0.6 (176)	0.4(189)	0.2 (226)	0.1 (226)	0.6 (200)
Random Effects										
Clone (Lake)		1.9	1.6	1.1	-	0.8	0.9	-	0.2	3.4***

F (df)

† 0.05 < p < 0.1

* p < 0.05

** p < 0.01.

*** p ≤ 0.001.

Table 2. Analysis of life history traits in *Daphnia* populations based on presence/absence of stickleback. A univariate ANOVA model was used with generation, predator treatment and presence/absence of stickleback entered as fixed effects. Clone (nested within lake) was entered as a random effect but was non-significant for all variables and removed from the model, with the exception of intrinsic rate of increase (r). For Size at Maturation, and (r), clone(lake) was significant and results are from a linear mixed model analysis, and for this variable, df equals the denominator degrees of freedom. Values for the clone term are Wald Z statistics from a likelihood ratio test.

Fixed Effects	df	Size at Maturation	Age at Maturation	Clutch 1	Clutch 2	Clutch 3	Average Interclutch Interval	Average Clutch Size	Tot Repro Output	Intrinsic rate of increase (r)
Generation	1	0.1 (185)	1.8 (228)	2.3 (230)	5.3* (220)	1.5 (180)	0.1 (193)	5.6* (230)	4.4* (230)	0.0 (205)
Predator Treatment	1	0.0 (178)	1.7 (228)	0.3 (230)	0.3 (220)	0.8 (180)	0.1 (193)	0.2 (230)	0.6 (230)	358.2*** (205)
Presence/Absence Stickleback	1	0.3 (18)	1.86 (228)	1.6 (230)	3.8† (220)	5.4* (180)	0.3 (193)	8.2** (230)	8.2** (230)	1.1(25)
Gen. × Pred.	1	0.0 (178)	0.1 (228)	0.6 (230)	1.7 (220)	0.2 (180)	0.2 (193)	0.9 (230)	0.5 (230)	0.5 (205)
Gen. × Pres./Abs. SB	1	3.0 (185)	1.9 (228)	7.1 ** (230)	1.0 (220)	2.5 (180)	0.0 (193)	2.6* (230)	1.6 (230)	0.0 (230)
Pred. × Pres./Abs. SB	1	0.1 (178)	1.4 (228)	0.2 (230)	0.3 (220)	1.9 (180)	0.01 (193)	0.3 (230)	0.7 (230)	167.1*** (205)
Gen. × Pred. × Pres./Abs. SB	1	0.3 (178)	0.0 (228)	0.0 (230)	0.2 (220)	1.1 (180)	0.0 (193)	0.3 (230)	0.1 (230)	0.5(230)
Clone (Lake)		2.3*	1.6	1.0	-	0.8	0.7	-	0.2	3.5***

F (df)

† 0.05 < p < 0.1

* p < 0.05

** p < 0.01.

*** p ≤ 0.001.

Table 3. Lake data including lake name, location of lake basin, ecotype of stickleback found within lake, maximum depth, surface area and the catch per unit effort (CPUE) for stickleback for each lake.

Lake	Lake Basin	Stickleback Morphotype	Max Depth (m)	Lake Size (surface acres)	CPUE (Fish per trap soak hour)
Finger	Mat-Su	Benthic	4.72	362	987
Spirit	Kenai	Limnetic	21.03	340	304
Watson	Kenai	Benthic	4.27	58	75
Walby	Mat-Su	Benthic	5.49	54	211
Loon	Kenai	No Stickleback	2.89	108	0

Table 4. Least-square means (SE) for lake, treatment and generation from benthic vs. limnetic analysis. Benthic (B), Limnetic (L), No Stickleback (NS). Values in parentheses are ± 1.0 S.E.

	NON-PREDATOR		PREDATOR	
	G1	G2	G1	G2
Average Size at Maturity				
B	1.37(0.02)	1.35(0.03)	1.38(0.02)	1.27(0.05)
L	1.47(0.03)	1.44(0.03)	1.48(0.02)	1.45(0.03)
NS	1.32(0.05)	1.36(0.06)	1.31(0.05)	1.36(0.02)
Average Age at Maturity				
B	12.17(0.73)	14.55 (1.07)	12.05(0.77)	11.89(1.10)
L	12.50(0.88)	15.38(1.17)	12.50(0.85)	18.00(1.17)
NS	13.40(1.47)	13.50(1.90)	11.18(1.55)	11.00(1.90)
Average Clutch Size				
B	2.91(0.21)	1.96(0.29)	3.10(0.21)	2.08(0.30)
L	3.28(.024)	2.24(0.32)	3.23(0.23)	1.77(0.32)
NS	2.72(0.40)	3.03(0.52)	3.33(0.43)	2.97(0.52)
Total Reproductive Output				
B	7.10(0.57)	5.40(0.84)	7.72(0.61)	5.89(0.87)
L	9.39(0.70)	6.53(0.92)	8.65(0.66)	5.13(0.92)
NS	7.95(1.16)	8.08(1.50)	10.00(1.23)	8.67(1.50)
Average Interclutch Interval				
B	5.10(0.12)	5.02(0.18)	5.08(0.13)	5.06(0.18)
L	5.04(0.14)	5.12(0.18)	5.21(0.15)	4.96(0.19)
NS	4.93(0.23)	4.94(0.36)	5.10(0.24)	4.96(0.29)
Average Intrinsic Rates of Increase (r)				
B	0.12(0.00)	0.12(0.00)	0.12(0.00)	0.12(0.00)
L	0.13(0.00)	0.13(0.00)	0.12(0.00)	0.12(0.00)
NS	0.12(0.00)	0.12(0.00)	0.14(0.00)	0.14(0.00)

Figures

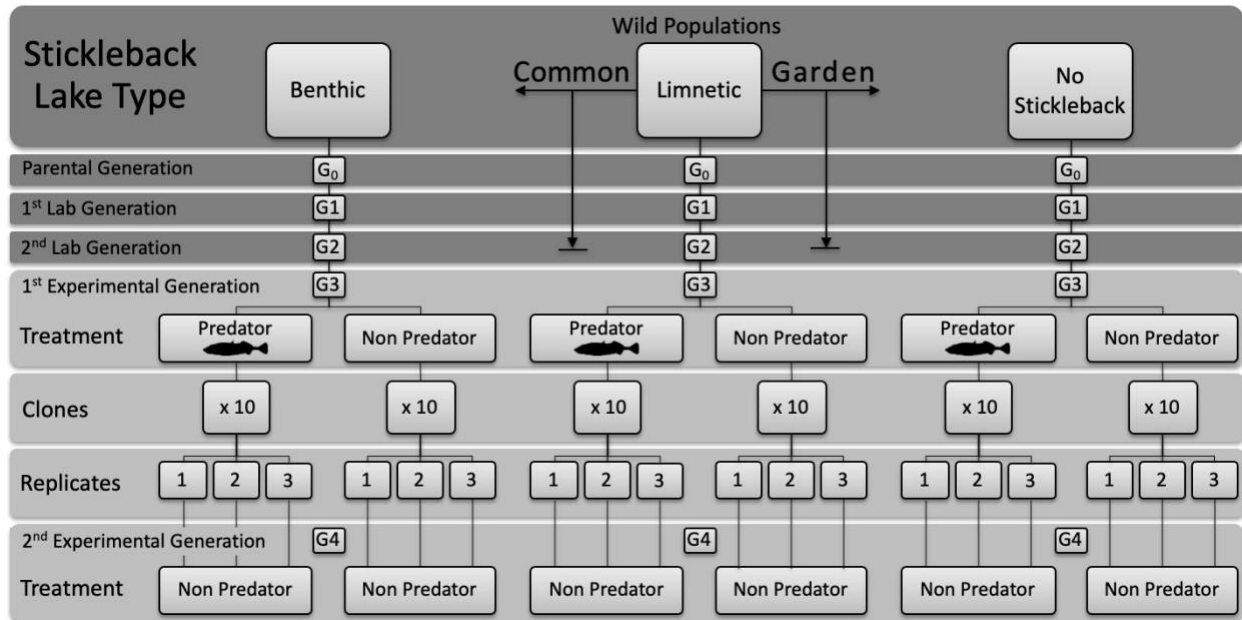


Figure 1. Experimental design for evaluating effects of predator cue exposure on life history traits.

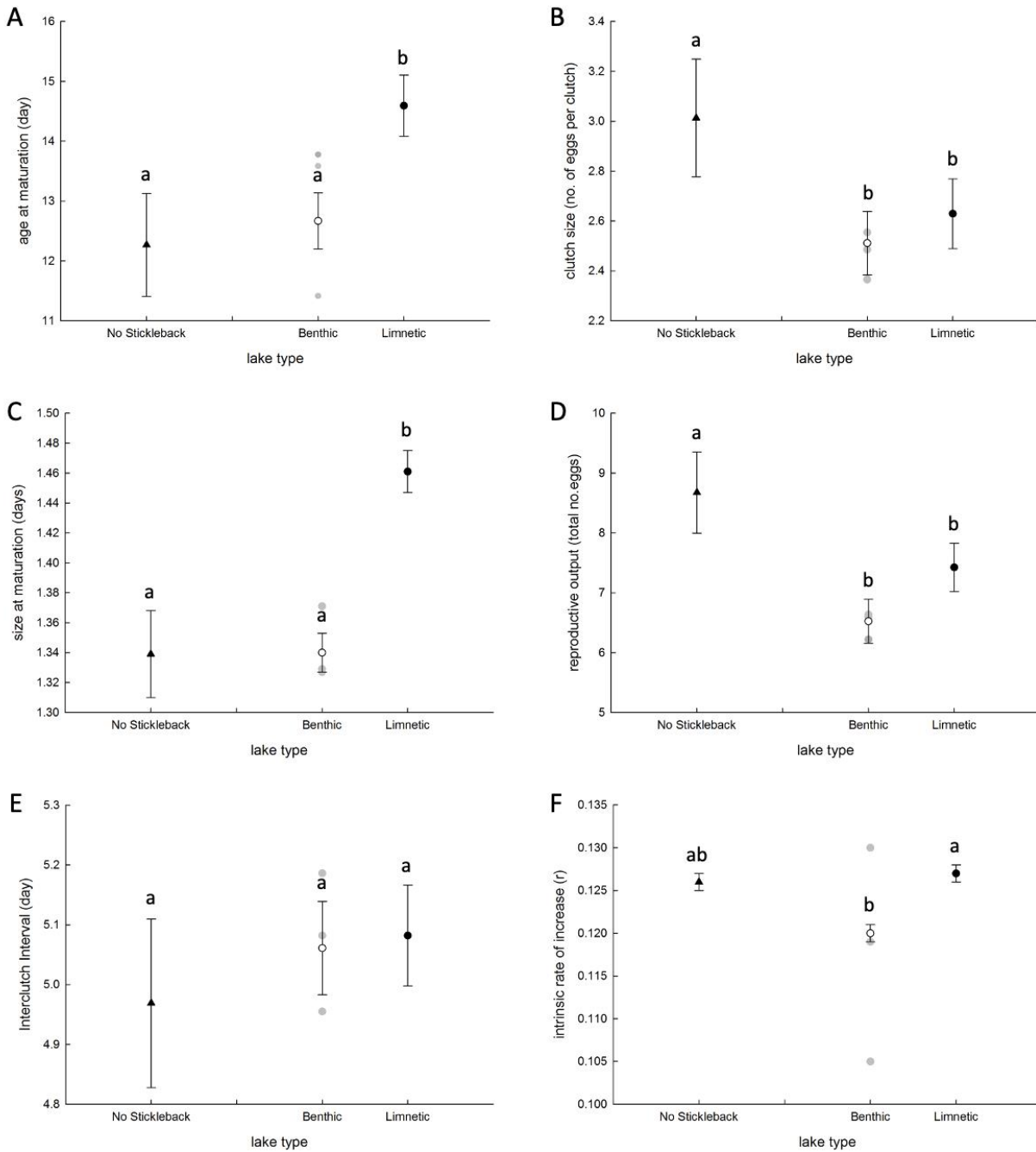


Figure 2. Life history trait response differences for benthic, limnetic and no stickleback *Daphnia* populations. (A) Age at maturation, (B) clutch size - average number of eggs in each clutch, (C) size at maturation, (D) total reproductive output - average total number of eggs produced over three clutches, (E) interclutch interval, (F) intrinsic rate of increase. Letters (a, b, etc.) denote significant ($p < 0.05$) differences found between each lake type stemming from post-hoc LSD tests. Grey circles = lake means. Error bars = ± 1 SE.

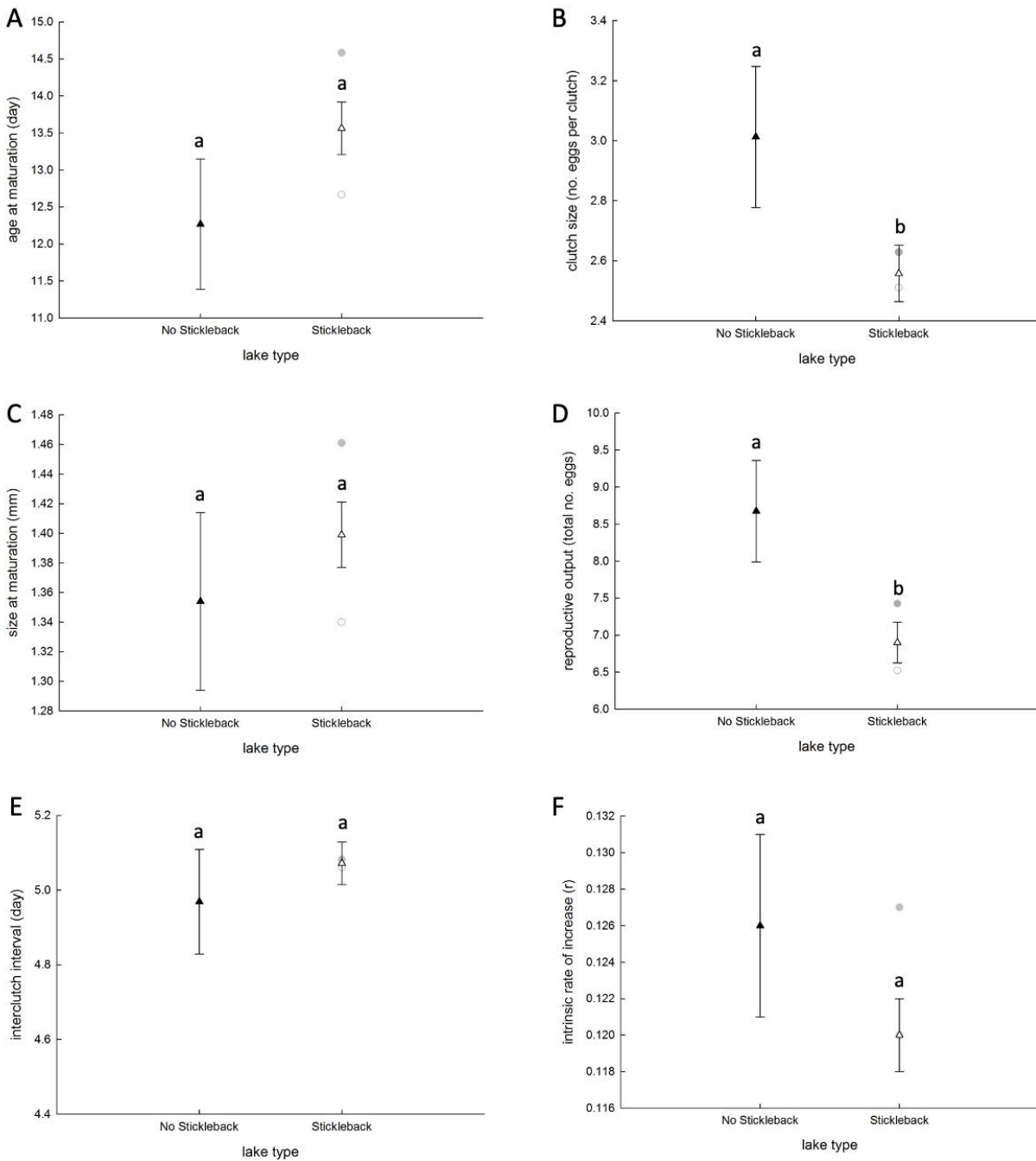


Figure 3. Complimentary analysis examining life history differences between lakes with and without stickleback populations not accounting for morphological variation in stickleback. (A) age at maturation, (B) clutch size - average number of eggs in each clutch, (C) size at maturation, (D) total reproductive output - average total number of eggs produced over three clutches, (E) interclutch interval, (F) intrinsic rate of increase. Letters (a, b, etc.) denote significant ($p < 0.05$) differences found between each lake type from post-hoc LSD tests. Open grey circles = benthic, filled grey circles = limnetic. Error bars = ± 1 SE.

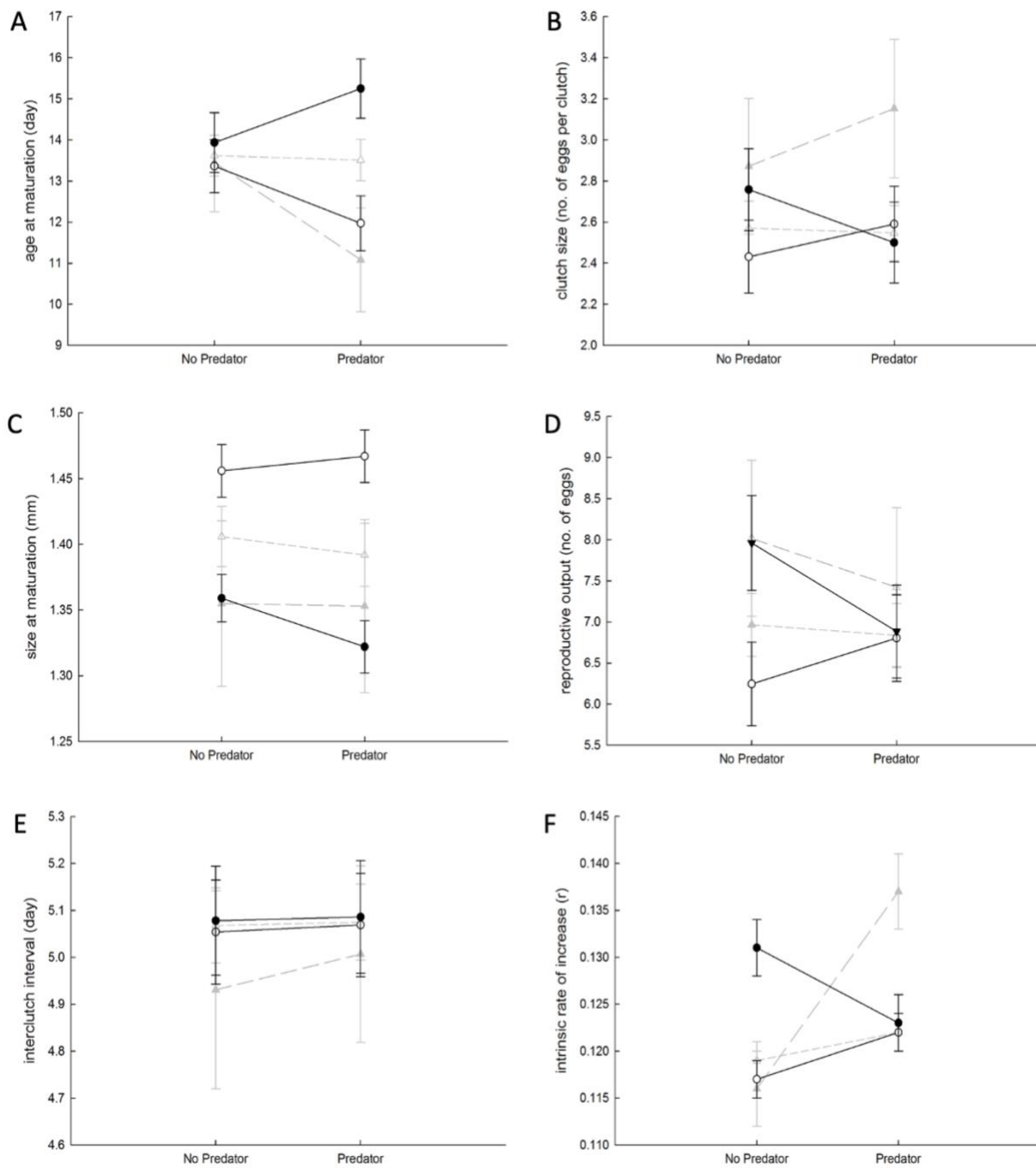


Figure 4. Lake type by predator interactions in life history traits. Closed black circles = limnetic, open black circles = benthic. Complimentary presence/absence of stickleback analysis represented with open grey triangles = stickleback, closed grey triangles = no stickleback. Error bars = ± 1 SE.

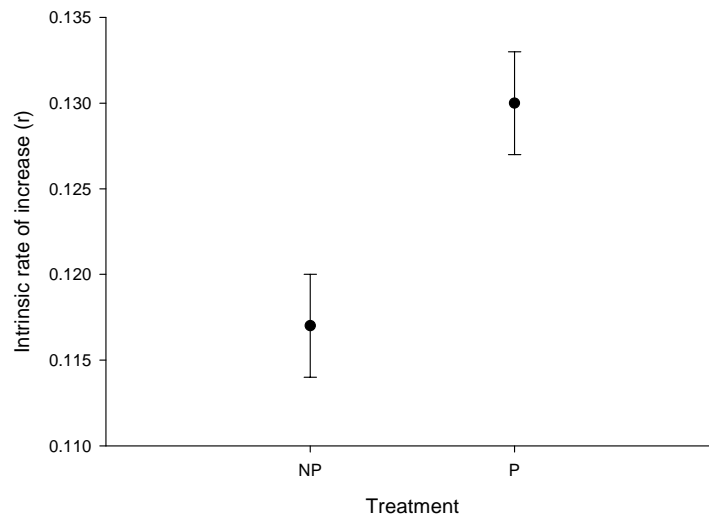


Figure 5. Predator cue effects on the intrinsic rate of increase. Error bars = ± 1 SE.

Chapter 4

Phenotypic plasticity accurately predicts the pattern of evolutionary divergence among experimental populations of water fleas

Michelle Packer¹ and Matthew R. Walsh¹

¹Department of Biology, 501 S. Nedderman Dr., The University of Texas at Arlington, Arlington, TX 76010, USA

Abstract

The role that environmentally-induced phenotypic plasticity plays in adaptive evolution has been debated for decades. Does plasticity shield genotypes from selection following a novel shift in the environment and, in turn, impede adaptation? Or, does phenotypic plasticity accelerate the rate at which populations attain new fitness peaks and thereby facilitate adaptation? Answers to these questions remain unresolved because experimental evaluations of the connection between plasticity and adaptation are rare. We tested the link between phenotypic plasticity and the rate and trajectory of evolutionary divergence using the interplay between water fleas (*Daphnia pulicaria*) and their fish predators. We created genetically diverse experimental populations of *Daphnia* based upon three patterns of predator-induced plasticity: (1) ‘adaptive’ responses to predator cues that are positively associated with population growth, (2) ‘maladaptive’ plasticity that is negatively linked to rates of population growth, and (3) populations that are not plastic (‘no response’). These populations were allowed to grow for a period of time in mesocosms and were then subjected to periodic bouts of predation as we tracked changes in trait values and trait plasticity. We found that ancestral patterns of plasticity predicted the direction of divergence between populations characterized by adaptive versus maladaptive forms of plasticity. *Daphnia* from the maladaptive populations exhibited delayed timing of maturation and were larger in size

than *Daphnia* from the adaptive treatments. However, we did not observe differences in rate of divergence over time. Our results provide new insights into the connection between plasticity and adaptation, and questions the extent to which plasticity influences rates of evolutionary change.

Introduction

It has long been recognized that organisms exhibit the capacity to modify the expression of traits in response to a change in environmental conditions (West-Eberhard 2003, Crispo 2007, Ghalambor *et al.* 2007, Pfennig *et al.* 2010, Schlichting and Wund 2014, Hendry 2015, Levis and Pfennig 2016). Such ‘phenotypic plasticity’ has been documented in response to many environmental stimuli in taxa spanning the tree of life (West-Eberhard 2003). Despite widespread acceptance for the adaptive significance of phenotypic plasticity, the role that plasticity plays in evolutionary processes has been debated for more than a century (Baldwin 1896, Waddington 1942, West-Eberhard 2003, Crispo 2007, Ghalambor *et al.* 2007, Schlichting and Wund 2014, Levis and Pfennig 2016). On one hand, plasticity may shield an individual from environmentally-driven selection and thereby constrain the evolutionary processes (Levin 1988, Huey and Kingsolver 1993, Linhart and Grant 1996). Conversely, some work has speculated that the ability to modify traits during development may allow for plasticity to facilitate, and thereby promote, adaptive evolution [the ‘Plasticity-First Hypothesis’] (West-Eberhard 2003, Badyaev 2005, Pigliucci 2006, Crispo 2007, Ghalambor *et al.* 2007, Moczek *et al.* 2011, Schwander and Leimar 2011, Schlichting and Wund 2014, Levis and Pfennig 2016, 2020, Levis *et al.* 2018). Despite extensive interest in the connection between plasticity and adaptation there remains little consensus regarding the extent to which plasticity promotes or impedes evolution.

Daphnia are a ubiquitous feature of freshwater habitats with clearly defined roles as grazers on phytoplankton, transferring nutrients up the trophic web (Carpenter *et al.* 1992) and they exhibit many characteristics that make them experimentally tractable for laboratory study (easy to culture, short generation time, multiple quantifiable traits). Patterns of plasticity have been documented in *Daphnia sp.* for numerous stimuli (predators, temperature, food quantity and quality) across a wide array of traits (life history, behavior, physiology, morphology) (Stibor 1992, Riessen 1999, Walsh *et al.* 2015, Stoks *et al.* 2016). Research has shown that shifts in environmental conditions can impose selection and drive evolutionary changes, including shifts in plasticity, in *Daphnia* populations (Hairston *et al.* 1999, Cousyn *et al.* 2001, Walsh and Post 2011, 2012, Stoks *et al.* 2016, Walsh *et al.* 2016, Miner *et al.* 2012).

Here, we tested the connection between phenotypic plasticity and adaptation via experimental evolution in a laboratory setting. We first assessed patterns of predator-induced plasticity from a genetically diverse assemblage of clones of *Daphnia pulex* from lakes in Wisconsin. We defined three general patterns of phenotypic plasticity based on trait responses to fish predator cues: (i) 'Adaptive' responses that increased rates of population growth (faster development) and, in turn, fitness, (ii) 'Maladaptive' responses, associated with decreased rates of population growth, (iii) and clones that exhibited 'no response' to predator cues. We then initiated mesocosm experiments which were seeded with *Daphnia* from each of the three plasticity groups. After a period of initial population growth, all mesocosms were exposed to predation by small planktivorous fish. During these experiments, shifts in trait values and trait plasticity were tracked over time. We also assessed plasticity across multiple generations to determine if selection by fish predators alters patterns of transgenerational plasticity (Walsh *et al.* 2015,

2016). We specifically addressed two questions: First, does the form of plasticity influence the rate of adaptation? Second, does the ancestral direction of plasticity accurately predict the trajectory of evolution? If adaptive phenotypic plasticity facilitates evolutionary change, then we expect that *Daphnia* from the 'adaptive' treatments will exhibit rapid shifts in trait values and/or trait plasticity over time. Conversely, if maladaptive responses that move *Daphnia* further from a fitness peak ultimately result in stronger selection (Ghalambor *et al.* 2015) then we expect that faster rates of evolution will be observed in *Daphnia* from the maladaptive treatments.

Materials and Methods

Experimental protocol

Plasticity assay

Common garden experiment.

The aim of this first experiment was to characterize the plastic responses of approximately 100 clones *Daphnia pulicaria* to fish predator cues and to quantify the pattern and magnitude of the responses. To establish our laboratory populations, 32 clones of *D. pulicaria* were collected in May 2016 via plankton tows (80 μ m mesh net) from lakes (Mendota, Monona and Allequash) located in Wisconsin, USA (Figure 1). Live adult females were isolated (hereafter referred to as 'clones') and transported to laboratory facilities at UTA. All individuals were cultured at 15°C, photoperiod 12 L : 12 D, slowly acclimated to COMBO media (Kilham *et al.* 1998) and fed ample quantities of green algae (*Scenedesmus obliquus*; concentration: approx. 1.0 mg C l⁻¹ d⁻¹). Clones were reared under common garden conditions for multiple generations before initiating common garden rearing for the experiments. For the first laboratory generation, we collected six newly born (less than 12 h old) individuals from the second clutch of each parental clone and

divided these individuals equally between three 90 ml jars containing COMBO medium (Kilham *et al.* 1998) and algae (concentration: 1.0 mg C l⁻¹ d⁻¹). These individuals were transferred to fresh media and algae every other day and were reared under the same conditions as the parents. We hatched an additional 64 clones of *D. pulicaria* from sediment samples that were collected using an Ekman grab from each lake. For each hatched clone, the first laboratory generation consisted of a single post-ephippial female that was reared individually in a 90 ml jar in the same conditions as our live-caught populations. The second laboratory generation was established by collecting replicate sets of two newly born neonates from the second clutch of each clone for both the live caught and hatched populations. These individuals experienced the same conditions (temperature, photoperiod, food quantities) and frequency of food/media replenishment as the previous generation. Over the course of common garden rearing, we successfully cultured 64 clones of *Daphnia pulicaria* to be entered into the experiment.

We evaluated the effects of predator cues on the expression of life history traits of *D. pulicaria* beginning with third generation laboratory-born individuals. This experiment ran for a single generation. To begin the experiment, we collected twelve newly born individuals (less than 12-h old) per clone and placed pairs into six 90 ml jars containing COMBO media. Each pair was randomly assigned to one of two treatments: predator (P) and no predator (N). The (P) treatment received conditioned media containing fish kairomones every other day throughout the entire experiment (see *Kairomone collection* below). The (N) treatment received no fish chemical cues. Each treatment was replicated 3 × per clone ($n = 64 \text{ clones} \times 3 \text{ replicates} \times 2 \text{ treatments} = 384 \text{ jars} \times 2 \text{ females per jar} = 768 \text{ individuals}$). All other experimental conditions were the same as described above (temperature = 15°C, photoperiod = 12 L : 12 D). All

individuals were fed specified, non-limiting quantities of *S. obliquus* (1.0 mg C l⁻¹ d⁻¹) and were transferred to fresh media and algae (and kairomones where appropriate) every other day.

We quantified size and age at maturation (defined as the release of the first eggs into the brood chamber), juvenile growth and the size of the first three clutches of offspring for average reproductive output. Average clutch size per clone is defined as the average number of eggs in each clutch, found by summing the number of eggs in clutches 1-3 and dividing by 3. Total average reproductive output is the total number of eggs produced over three clutches. Less than 1% of clones did not produce all three clutches, and these clones were excluded from reproductive calculations. All jars were checked daily for maturation and for the release of offspring from the brood chamber. Upon maturation, age at maturation and clutch size were recorded and each individual was photographed for estimates of size (using ImageJ) (Abràmoff *et al.* 2004). After maturation, jars were checked daily for the production of clutches 2 and 3. Juvenile growth was measured by photographing all individuals on day 1 and day 5. Using ImageJ, the Feret's diameter (also known as maximum caliper) was measured and used to calculate to growth rate via: $[\ln(\text{Feret day 5}) - \ln(\text{Feret on day 1})]/\text{no. of days}$.

Kairomone collection

Kairomones (steriochemicals) have long been known to transmit information in aquatic communities. Specifically, kairomones from predators have been shown to induce plasticity in their prey (Stibor 1992, Weider and Pijanowska 1993, Macháček 1995). Fish predator kairomones were collected from tanks which contained COMBO media that was conditioned by the presence of sunfish (*Lepomis sp.*) (~50 mm in total length) in 7L of COMBO. All media was

changed twenty-four hours prior to kairomone collection. Sunfish were fed >200 *Daphnia pulicaria* on the same morning as kairomone collection. Since injured *Daphnia* emit chemical cues that contribute to the magnitude of the phenotypic response to predation (Laforsch *et al.* 2006, Pestana *et al.* 2013), it is likely that our predator-conditioned water contained both kairomones and *Daphnia* alarm cues. All media was filtered using a membrane filter progression (47mm diameter, 1.2 μm , 0.45 μm , 0.2 μm mesh). This filtering helps to prevent the fish-conditioned media from providing supplemental nutrients (Brendleberger 1991). The chemical kairomones were then transferred to sterile 50 ml falcon tubes and stored at -20°C for 1 week prior to the start of the experiment. The concentration of kairomones that was used in this experiment was 0.0029 fish/l.

Statistical Analysis

We evaluated variation in all dependent variables using standardized data. We performed analyses that examined shifts in trait values and trait plasticity over time. We also performed a complementary analyses that examined the magnitude of shifts in trait values irrespective of the direction at each time point by examining the absolute values of standardized traits. Variation in age and size at maturation, total average reproductive output (clutch size), and juvenile growth rate were analyzed using a multivariate general linear model (SPSS v. 24). Treatment (predator cue) and clone were entered as fixed effects, and jar density was entered as a covariate. Data for age at maturation were reciprocally transformed and total reproductive output was natural log transformed to improve fit with normality and homogeneity of variances. Only females that produced all three clutches of offspring were included in total reproductive output analyses. We entered clone as a fixed effect because we were specifically interested in identifying clonal

variation in plasticity and clonal patterns of plasticity are consistent and 'fixed' when not sexually reproducing (Rohlf and Sokal 1995, Bolker *et al.* 2009). Following a significant MANOVA, each trait was analyzed using a univariate general linear model with treatment and clone as fixed effects, and density as a covariate.

Principal component analysis

We also used a principal component analysis to visualize patterns of trait covariation. We specifically wanted to evaluate the degree of overlap in trait values among clones in the absence of predator cues. As a result, patterns of covariation for age and size at maturation, juvenile growth and total reproductive output were evaluated for *Daphnia* reared in the absence of predator cues. Here we used a PCA based upon a correlation matrix with a direct oblimin rotation with Kaiser normalization (Gorsuch 1983).

Pre-experimental trials

Part 1 - Fish predation trials.

To determine the proper temporal length of predation events for our experimental study, three mesocosms identical to our experimental design tanks were used to evaluate the intensity of sunfish predation on *Daphnia* population abundances. Random groups of *Daphnia pulicaria* clones from our Wisconsin lakes were added to 9L tanks filled with 8L of COMBO medium (Kilham *et al.* 1998). *Daphnia* were fed non-limiting quantities of *Scenedesmus obliquus* (1.0 mg C l⁻¹ d⁻¹) every other day. Populations were allowed to grow for 20 days. Initial density prior to introduction of fish and the density after each predation event was assessed by taking three 250 ml random samples from each tank and counting the individuals present in each sample. The

average per 250 ml was multiplied by 4 to calculate *Daphnia* per liter for each tank. Initially, one 50 mm sunfish chosen at random from a population of 12 sunfish was introduced into each tank to evaluate the average duration of active feeding. Fish were visually observed from a distance of six feet as to not disturb feeding behavior. Fish were not fed 24 hours before predation trials. Our goal was to replicate strong predatory selection events but retain sufficient population sizes for reproduction and survival. These trials indicated that predations events that occur every 3-4 days are sufficiently frequent to cause declines in the abundances of *Daphnia* but not too frequent to eliminate the experimental populations.

Part 2 - Tank density trials.

To assess the appropriate number of *Daphnia* needed for our experimental mesocosms and to evaluate population growth rate, carrying capacity and the incidence of sexual reproduction in the absence of a fish predator, two mesocosms identical to our experimental design tanks (9L tanks filled with 8L of COMBO medium) were seeded with two starting populations of adult female *Daphnia pulicaria* clones from our Wisconsin lakes. Mesocosm 1 had a beginning population of 2.5 *Daphnia* per liter (20 individuals), while mesocosm 2 started with 2 *Daphnia* per liter (40 adult females). Three random samples of 250 ml were pulled from each mesocosm over a six-week period to assess population density. *Daphnia* were fed non-limiting quantities of *Scenedesmus obliquus* (1.0 mg C l⁻¹ d⁻¹) every other day. On week 4, quantity of algae was increased to (2.0 mg C l⁻¹ d⁻¹) every other day.

Mesocosm experiment

Clone selection

Clones were defined as exhibiting one of three forms of plasticity ('adaptive' (A), 'mal-adaptive' (M) or 'no response' (N)) based on results from the plasticity assay (see *plasticity assay* results below). We defined groups by rank ordering percent change in age of maturation means, as this variable exhibited a strong, significant ($p < 0.006$) clone by treatment interaction when *Daphnia* were exposed to predation cues. Percent change was calculated for each clone by subtracting average age of maturation from the predator cue treatment from the average age of maturation from the no predator treatment, and dividing the outcome by the no predator treatment mean. Clones which reduced their age of maturation in response to fish predator cues (-3% to -16%) were considered adaptive, while clones that increased age of maturation (7% to 31%) were grouped as maladaptive. Clones with minimal to no responses (-2% to 2%) were classified as no response. Fourteen clones from each plasticity group were selected for the mesocosm study based on strength of response and survival of the clonal lineage. All clones were reared outside of the mesocosms in a common garden environment for two generations using the same procedures as described above. Second generation lab reared females were reared to maturation and produced at least 1 clutch but not more than 3 clutches of offspring before entering the experimental mesocosms.

Mesocosm set-up

Mesocosms were designed to simulate fish predator exposure conditions, such that *Daphnia* populations would be exposed to constant fish kairomone cues and undergo direct selection by a fish predator (Brown *et al.* 1970, Stibor 1992, Cousyn *et al.* 2001, Chakri *et al.* 2010, Walsh and

Post 2011). A mesh flow-through barrier was permanently inserted into each tank to divide *Daphnia* populations from the fish predator. The mesh size was sufficiently small such that *Daphnia* offspring could not cross the barrier and remained on the “*Daphnia*” side of the tank (Figure 2). *Daphnia* density was recorded weekly over the course of the experiment by random sampling each mesocosm, pulling four 250 ml (1L total) aliquots from the *Daphnia* side of the tank and counting the number of *Daphnia* present. To assess the way *Daphnia* evolved over the course of the experiment (33 days), subsamples of *Daphnia* from each mesocosm were taken every fifth day. All subsamples consisted of 9 adult females pulled randomly from each tank (9 mesocosms x 9 individuals per mesocosm = 81 individuals per subsample). A total of 6 subsamples were taken over the total study (81 individuals per subsample x 6 temporal subsamples; n = 486). Subsampling populations over time allowed us track shifts in traits and trait plasticity as the experiment progressed (*experimental overview see* Figure 3).

Experimental mesocosms were set up in a replicated, blocked design, such that there were 3 replicate tanks for each plasticity category (adaptive, maladaptive and no response) for 9 total mesocosms. Each 9L glass fish tank mesocosm received forty-two adult females comprised of 3 adult females per each of the 14 clonal lines selected from the original plasticity assays. Each plasticity category tank received the same clones, and the same number of individuals from each clone (i.e. each adaptive tank received the same 14 clones, with three adult females per clone). Tanks were filled with 8L of COMBO medium for consistency with common garden rearing and successive experimental conditions. Air stones were added to improve oxygenation and water flow on the fish side of the barrier. *Daphnia* were introduced to the mesocosms prior to the introduction of fish and allowed to grow and reproduce for 7 days (approximately 1-2

clutches of offspring) in the absence of the non-lethal threat of predators (and thereby predator cues). During this time, *Daphnia* were fed non-limiting quantities of *Scenedesmus obliquus* (2.0 mg C l⁻¹ d⁻¹) every other day according to pre-trial data and not subjected to direct fish predation events (Figure 4).

On day 8, prior to any exposure to fish or direct predation events, the first subsamples were taken from each mesocosm. The same day, equally sized sunfish were added to the “fish” side of each tank (Figure 2). A single fish resided in the mesocosm for the duration of the experiment. Over the course of the experiment, controlled predation events were conducted by moving sunfish over the barrier wall, allowing them to prey on *Daphnia* populations for 10-minute intervals. On the first day of fish inclusion (day 8), *Daphnia* populations were subjected to the first direct predation event. Between day 8 and day 13, two predation events were carried out to reduce population levels within the tanks. A single direct predation event then occurred between all other subsamples (Figure 4).

Common garden experiment

For each subsample, 9 individual adult females (hereafter referred to as “clones”) were selected at random and transferred from each mesocosm (9 females x 9 mesocosms = 81 clones) to a common environment (Figure 3). Seven clones were entered into the experiment, while the other two clones were reared as back up samples in the event of mortality. For each clone, the first laboratory generation consisted of a single female transferred from the mesocosm and reared individually in a 90 ml jar in the same conditions as the plasticity assay described above. The second laboratory generation was established by collecting six newly born (less than 12 hrs old)

individuals from the second clutch of the first laboratory generation clone and dividing these individuals equally between three 90 ml jars. We evaluated the effects of predator cues on the plasticity in life history traits of *Daphnia* from each mesocosm beginning with third generation laboratory-reared individuals. This experiment ran for two generations. Eight neonates (less than 12-hrs old) were collected from the second clutch of second-generation females and placed into separate jars in pairs to make two replicates of each treatment. Each pair was randomly assigned to one of two treatments: (i) predator exposure in the first generation (P, PN) or (ii) no predator exposure in either generation (N, NN) (Figure 5). Our predator treatment included the addition of sunfish predator conditioned media. We evaluated trait plasticity during the first experimental generation in *Daphnia* from all sampling events. We evaluated transgenerational responses in a second experimental generation in *Daphnia* from a subset of the sampling events (day 8, 18, and 28 of the experiment). The second experimental generation was not exposed to the presence of predators because previous work has shown that across-generation responses are similar in magnitude for both; continual exposure to predator cues for multiple generations, and for *Daphnia* that are only reared in the presence of cue for the first generation (Walsh *et al.* 2015, 2016). Second generation individuals in the predator treatment trial were exposed to predator cues during embryonic development and very early life-stages. Each treatment was replicated 2× per clone (Gen 1: $n = 9 \text{ mesocosms} \times 6 \text{ subsamples} \times 7 \text{ clones} \times 2 \text{ replicates} \times 2 \text{ treatments} \times 2 \text{ females per jar} = 3024$, Gen 2: $n = 6 \text{ mesocosms} \times 3 \text{ subsamples} \times 7 \text{ clones} \times 2 \text{ replicates} \times 2 \text{ treatments} \times 2 \text{ females per jar} = 1008$). The experimental conditions were the same as described in the plasticity assay (temperature = 15°C, photoperiod = 12 L : 12 D). Beginning on day 3, *Daphnia* were checked daily to for maturation (defined as the release of the first clutch into the brood chamber). Upon maturation, clones were photographed for estimates of size (using

ImageJ) and number of eggs were counted. Thereafter, all individuals were monitored for the production of clutches 2 and 3. Upon release of the second clutch from the brood chamber, pairs of newly born neonates from each clone were collected to initiate the second experimental generation.

Statistical analysis

We evaluated variation in all dependent variables using standardized data. We performed analyses that examined shifts in trait values and trait plasticity and also performed complementary analyses that examined the magnitude of shifts in trait values irrespective of the direction at each time point by examining the absolute standardized trait values. Variation in age and size at maturation, interclutch interval and total average reproductive output (clutch size) were each analyzed using a repeated measures linear mixed model (SPSS v. 24), implementing restricted estimatable maximum-likelihood estimation (REML). Compound symmetry was used for repeated covariance type as there was constant variance and covariance between measurement times. Generation, predator treatment (presence or absence), plasticity category (adaptive, no response or maladaptive), subsample (time), and all interactions among factors were entered as fixed effects. Clonal ID was nested within tank and tank nested within plasticity category were entered as random effects. We characterized the presence of transgenerational plasticity (TGP) as a significant interaction between predator treatment and generation. Additionally, we tested for local adaptation in TGP by evaluating the third-order interaction between plasticity category, predator treatment, and generation. We initially evaluated the significance and explanatory power of interactions between all fixed effects and nested random terms. Clone (nested within tank) was significant for the size of maturation. For all other

variables, the significance of the fixed effects did not depend on the presence or removal of clone (nested within tank) or tank (nested within plasticity category), and the random terms were removed from the model. When significant time or plasticity category effects were detected in the analysis, we evaluated the differences using a Bonferroni adjustment for multiple comparisons. Total reproductive output was log transformed to improve fits with normality and homogeneity of variances.

Results

Plasticity assay

We first analyzed the data stemming from the plasticity assays that were performed to determine the clones that formed the basis of our selection experiments.

Statistical Interactions

We observed a significant ($p < 0.05$) clone by treatment interaction for age at maturation (this term explained 29% of the variance) (Table 1). It is important to note that age at maturation has the strongest influence on population growth. For this reason, we used plasticity in age at maturation to identify three forms of plasticity (adapted, maladapted or no response). The full analyses for all variables are displayed in Table 1.

Clone Identification

Clones were identified categorically by assessing the directional average difference in plasticity in age at maturity between predator and non-predator treatments (plasticity in age at maturation = trait value in predator treatment - trait value in non-predator treatment). Clones in the 'adaptive'

group had negative plasticity values (i.e., faster development in the presence of predators), clones in the 'maladaptive' group had positive plasticity values, while clones in the 'no response' group exhibited plasticity values near 0. We identified clones for the selection experiment that: (1) yielded the largest separation among groups, (2) contained the largest responses in the adaptive and maladaptive groups, and (3) minimized the % change in the no-response group. The adaptive group displayed an average decrease in the average age of maturity of 9.1% (with a range of -3 to -16%) in response to exposure to predator cues. The no-response clones displayed an average change in age at maturation of -0.34% (range -2-2%), while the maladaptive plasticity group included clones that exhibited an average increase in the timing of maturation of 14% (range 7-31%). These clones represent the strongest plastic responses in either direction for age at maturity when exposed to predator cues (see Fig. 6).

The main goal of the selection experiment was to determine if plastic responses to predator cues influence the trajectory and tempo of evolution. It was therefore important that we included *Daphnia* clones in the contrasting plasticity treatments that exhibited similar overall trait values in the absence of predator cues. The four life history variables were analyzed using a principal component analysis (PCA) to evaluate patterns of covariation in the absence of predator cues (Figure 7). Bartlett's test of sphericity was significant, and our KMO value was 0.55. The analysis yielded two components with Eigenvalues > 1 , which explained a total of 81% of the variance (Figure 8, Table 2). The first component explained 49.2% of the variance. The second component explained 31.9% of the variance (Table 2). Variation in PC1 is largely driven by a strong positive influence of size at maturation and reproductive output (Table 2). Juvenile growth loads strongly on PC2 (Table 2). While more than 80% of variance explained by the

components, there is no clear pattern of response between categorized plasticity groups (adaptive, no response, and maladaptive). There is extensive overlap between groups, which suggests that there are no overall life history differences in the absence of predator cues (Figure 7).

Pre-experimental trials

We performed preliminary predation trials to determine the duration and frequency of the predation events that were used in the selection experiment. We also used these trials to determine the patterns of *Daphnia* growth in mesocosms in the lab.

Part 1 - Fish predation trials

Repeated predation trials indicated that sunfish ceased to forage on *Daphnia* after an average of 45 minutes, however the most intense feeding took place during the first 10 minutes of each trial. The effects of 10-minute predation events on *Daphnia population* density was then assessed over a six-week period. On average, population density was decreased by 15% after each predation event, and after 6 weeks, the tanks showed an average decline from peak density of 80% (Figure 9).

Part 2 - Tank density trials

The population densities of *Daphnia* attained a peak population size after 11-14 days of growth. Overall population size was not dependent on the initial starting number of *Daphnia*. During the fourth week of the trials, population densities started to decline (Figure 10). Starting on the fourth week, algal concentrations were increased to (2.0 mg C l⁻¹ d⁻¹) every other day, and

populations rebounded to a higher density. We therefore used 2.0 mg C l⁻¹ d⁻¹ as a food concentration in the selection experiment. Initial starting populations of 40 adult female *Daphnia* were chosen to start the mesocosm tanks, as this number would allow for inclusion of clonal replicates (3 individuals per clone line x 14 clones) per plasticity category. Mating and sexually produced eggs were observed in the populations of both tanks.

Experimental evolution in mesocosms

Tank Densities

The population dynamics in each tank were comparable throughout the experiment as we observed similar rates of decline across all plasticity types (Figure 11). The predation events also had a similar influence on the average abundances of *Daphnia* across all mesocosms (Figure 11).

Shifts in trait values and trait plasticity

We began to quantify and track shifts in trait values and trait plasticity starting on day 8 of the experiment (prior to the first predation event). Below we focus on the results that relate to the core hypotheses tested as part of this study.

Time x plasticity category interactions

The key result stemming from this experiment is that the shifts in traits values over the course of the experiment varied among the plasticity treatments (adaptive, maladaptive, or no response). That is, we observed significant time by plasticity interactions for all for all measured traits.

The age at maturation of *Daphnia* from the adaptive and maladaptive plasticity treatments increased over the course of the experiment (Figure 12a). However, this increase in the timing of maturation was largest in *Daphnia* from the maladaptive treatment. Shifts in the no response groups were more variable. Here, age at maturation increased between day 8 and 13, but then declined. The differences in age at maturation among the treatment groups were not significant ($p>0.05$) between day 8 and day 23 (third week). By day 23, all three categories exhibited significantly different ages at maturation. *Daphnia* from the maladaptive treatment group exhibited an age at maturation that was significantly larger than *Daphnia* from the adaptive and no response treatments (Figure 12a). These differences were then maintained for the remainder of the experiment. Over the course of the experiment, clones from the adaptive and maladaptive group exhibited an 8.4% and 10% increase in the timing of maturation, respectively (trait value on day 33 vs. day 8). By the end of the experiment (day 33), shifts in age at maturation in the no response groups were not significant (between day 33 and day 8) (Figure 12a).

Size at maturation in the maladaptive group was largely consistent over time except for a decline in size of 5.6% in the last sampling event. *Daphnia* from the adaptive treatments exhibited a significant decline in their size at maturation over the course of the experiment. Starting on day 13, *Daphnia* from the adaptive treatments exhibited a decline in size at maturation of 7.6% as compared to their size at maturation on day 8, by day 23 showed a 7.74% decrease, and on day 33 showed an overall 11.78% decrease in size as compared to day 8 (Figure 12b). The differences in size at maturation between the adaptive and maladaptive groupings were largely significant over the course of the experiment after day 8; *Daphnia* from the maladaptive

treatments were larger than *Daphnia* from the adaptive plasticity group (Figure 12b). Shifts in the no response group largely paralleled the trends observed in the adaptive group. Size at maturation declined by 15.6% over the course of the experiment in the no response group.

In general, *Daphnia* from the adaptive and maladaptive group exhibited an increase in interclutch interval over the course of the experiment (Figure 12c). These increases were larger in *Daphnia* from the maladaptive group as the interclutch interval was significantly longer in *Daphnia* from the maladaptive vs. adaptive group on day 28 and 33. Overall, *Daphnia* from the adaptive and maladaptive groups exhibited an increase in interclutch interval of 5% and 11% between day 8 and 33, respectively. Shifts in interclutch interval in the no response group were more variable. Interclutch interval increased between day 8 and 13 but then declined thereafter. *Daphnia* from the no response group displayed a significantly lower interclutch (3.5%) interval between day 33 and 8 (Figure 12c).

Shifts in reproductive output (clutch size) between *Daphnia* from the adaptive and maladaptive categories were similar (Figure 12d). *Daphnia* in both treatments exhibited significant declines in clutch size over time. These declines were larger in the adaptive group on day 28, but such differences were reversed by the end of the experiment (Figure 12d). For this trait, the significant statistical interaction between time and plasticity was largely driven by a more variable temporal pattern in the no response groups (clutch size declined between day 8 and 18 but then increased thereafter).

Shifts in the absolute magnitude of standardized trait values over time

We also performed analyses that compared the absolute magnitude of the trait response at each sampling event. These analyses measure the magnitude of the response irrespective of directionality. These analyses revealed that on day 8, prior to exposure to predation or predator cues, there were no significant differences between our plasticity groups for any trait (Figure 12a - d, upper panels). For age at maturation, there were no significant differences in the magnitude of change until day 33 (Figure 12a, upper panel). *Daphnia* from the adaptive group showed a significantly lower magnitude of trait change as compared to maladapted and no response clones. Starting as early as day 13, we observed significant difference in the size of maturation, although the magnitude of these shifts varied among the groups over time (Figure 12b, upper panel). We did not detect difference in the magnitude of changes between plasticity groups for interclutch interval until day 28 of the experiment. Here, *Daphnia* from the adaptive group displayed significantly lower responses (in terms of the change in interclutch interval) than both the maladaptive and no response treatments (Figure 12c, upper panel). For total reproductive output, we only detected a significant difference between the plasticity groups on day 18 (no response clones exhibited a higher magnitude of change than both maladapted and adapted clones) (Figure 12d, upper panel).

Time x plasticity x predator treatment interactions

All statistical interactions between time period, the plasticity groups, and exposure to predator cues in the lab were not significant ($p > 0.05$) (Table 3).

Generation x plasticity x predator treatment interactions

The interactions between generation, plasticity group and predator cue exposure were not significant ($p > 0.05$) (Table 3).

Generation x time x plasticity x predator treatment interactions

All interactions between generation, time period, plasticity group, and predator cue were not significant ($p > 0.05$) (Table 3).

Generation x plasticity category interactions

We observed significant ($p < 0.05$) interactions between generation and plasticity category for size at maturation, interclutch interval and total reproductive output (Table 3). The underlying patterns responsible for these interactions varied. For size at maturation, *Daphnia* from the adaptive group matured at a smaller size than *Daphnia* from the maladaptive group in generation 1, but such differences disappeared in generation 2 (Figure 13b). The opposite pattern was observed for interclutch interval. Small differences in trait values were observed in generation 1, but *Daphnia* from the adaptive group exhibited an interclutch interval that was lower than *Daphnia* from the maladaptive group in generation 2 (Figure 13c). Trends for reproductive output were reversed between generations; *Daphnia* from the adaptive group produced more offspring than the maladaptive group in generation 1 but such trends were reversed in generation 2 (Figure 13d).

Generation x time x plasticity category interaction

Significant ($p < 0.05$) interactions were found between generation, time and plasticity category for size at maturation and total reproductive output (Table 3). For size at maturation, clones from the adaptive group were initially (day 8) smaller at maturation in generation 2 versus 1 (4.9% smaller). By day 18, *Daphnia* from adaptive group were larger at maturation (by 4.4%) in generation 2 versus 1, but by day 28, there were no significant differences found between generations (Figure 14b). Comparatively, *Daphnia* from the maladaptive group were initially smaller at maturation in generation 2 (on day 8 by 4.3%), showed no difference between generations on day 18, and *Daphnia* pulled on day 28 were 5.7% smaller than generation 2 when compared with the size of *Daphnia* from generation 1 (Figure 14f). Interestingly, there was no significant difference in the size at maturity between plasticity categories in the second generation (Figure 14b, f). Responses for total reproductive output in *Daphnia* from both plasticity categories on day 8 showed no difference between generations (Fig. 14d, h). However, on day 18, clones from the adaptive group exhibited a reproductive output that was 11.4% higher in generation 2. In comparison, maladapted clones showed no significant difference between generations for this subsample. On day 28, *Daphnia* from both plasticity categories significantly ($p < 0.05$) reduced total reproductive output in the second generation (A: 4.1%, M 5.7%) (Fig. 14d, h).

Time x plasticity x predator treatment interactions

All statistical interactions between time period, the plasticity groups, and exposure to predator cues in the lab were not significant ($p > 0.05$) (Table 3).

Generation x plasticity x predator treatment interactions

The interactions between generation, plasticity group and predator cue exposure were not significant ($p > 0.05$) (Table 3).

Generation x time x plasticity x predator treatment interactions

All interactions between generation, time period, plasticity group, and predator cue were not significant ($p > 0.05$) (Table 3).

Discussion

Our results revealed rapid evolutionary shifts in trait values, but not trait plasticity, in response to fish predation in experimental mesocosms (Fig. 12). *Daphnia* that were characterized as exhibiting ‘adaptive’ versus ‘maladaptive’ phenotypic plasticity diverged strongly in age and size at maturation (Fig. 12). In general, *Daphnia* from the maladaptive treatment group exhibited a slower rate of development and were larger at maturation than *Daphnia* from the adaptive (and no response) treatments. The ancestral population of the maladaptive group responded to predator cues by delaying maturation in favor of a larger size. As a result, the patterns of ancestral plasticity accurately predicted the patterns of divergence observed in the experimental populations. However, there was little evidence that the experimental plasticity groupings differed in the rate of divergence over time. That is, the shifts in trait divergence were similar in magnitude of change over the course of the experiment for adaptive and maladaptive groups. Below we elaborate on these results.

The extent to which adaptive versus maladaptive plasticity accelerates the rate of evolution has been the source of much debate (Ghalambor *et al.* 2007). There is some growing evidence that maladaptive plasticity speeds up the rate of adaptation. In a particularly noteworthy study, Ghalambor *et al.* (2015) evaluated changes in gene expression in guppies that were transplanted from sites with predators to localities that lacked predators. This study showed that patterns of plasticity that aligned with the direction of evolutionary divergence constrained adaptation. That is, adaptive plasticity impeded evolution. On the other hand, 'maladaptive' patterns of plasticity (responses opposite to the evolutionary response) resulted in rapid changes in gene expression (see also (Ho and Zhang 2018)). In the current study, we generally did not observe differences in the rate in which experimental populations of *Daphnia* that exhibited adaptive versus maladaptive plasticity diverged over time (Fig. 15). That is, *Daphnia* from the contrasting plasticity grouping diverged at similar rates from the starting trait values. This is despite *Daphnia* being constantly exposed to predators, which presumably induced the patterns of ancestral plasticity. It is possible that our experimental evolution experiment was shorter than what was necessary to detect plasticity induced divergence since we did begin to see the magnitude of maladaptive plasticity exceed adaptive plasticity toward the last few weeks in the study. It is also possible that the strength of the plastic response degraded over time as *Daphnia* became desensitized to the continual presence of predators. Or that the strength of selection imposed by fish predation in relatively small mesocosms swamps any of the potential fitness benefits of plasticity.

While we did not detect divergence in the strength of plasticity as our populations evolved, our results demonstrated that the direction of divergence in the experimental

populations proceeded in the expected direction based on plasticity. These findings support a growing body of work which makes it increasingly clear that phenotypic plasticity can accurately predict the trajectory of adaptation (Scoville and Pfrender 2010, Casasa and Moczek 2018). Radersma *et al.* (Radersma *et al.* 2020) recently showed that ancestral patterns of phenotypic plasticity frequently predict patterns of divergence among locally adapted populations. Levis & Pfennig (Levis *et al.* 2018) showed that the direction of diet-induced morphological plasticity (i.e., production of a carnivorous morph of tadpole) predicts trait evolution between ancestral and derived species of spadefoot toads. Schaum & Collins (Schaum and Collins 2014) used laboratory selection to show that ancestral patterns of plasticity in algae (in response to exposure to CO₂) accurately predicted the magnitude of evolution but not the direction of evolution. Our study represents one of the only experimental approaches to test the connection between plasticity and adaptation and provides further evidence for a link between ancestral plasticity and trait divergence.

There are several reasons why plasticity may be intertwined with evolutionary processes. One is that that ancestral plasticity may enhance fitness in a novel environmental and such plasticity is then refined by selection over time. There is growing evidence for such genetic accommodation (or genetic assimilation) (Scoville and Pfrender 2010, Corl *et al.* 2018, Levis *et al.* 2018, Wang and Althoff 2019). For example, Corl *et al.* (Corl *et al.* 2018) compared populations of lizards that are located on or off a lava flow that formed >22000 years ago. They found that ancestral plasticity in pigmentation likely facilitated initial survival and such plasticity was then modified by natural selection. Noble *et al.* (Noble *et al.* 2019) provide an alternative perspective on why plasticity can be connected to evolution. They showed that ancestral

plasticity is typically aligned with the main axis of the genetic architecture that harbors significant additive genetic variance. In other words, when phenotypic plasticity directionally aligns with the axis of maximum additive genetic variation in a novel environment, the highest quantitative genetic evolvability can be achieved (Noble *et al.* 2019, Payne and Wagner 2019). However, this axis of the genetic variance-covariance matrix can bias and constrain evolutionary responses due to limited genetic variation or strong genetic correlations present within the genetic architecture (McGlothlin *et al.* 2018, Uller *et al.* 2018). This means that plasticity can appear to ‘lead’ evolutionary responses, but that evolution actually proceeds due to constraints associated with genetic architecture, and these constraints bias divergence in the direction of an axis which has maximum additive genetic variation and plasticity alignment. In the current study, there is little evidence for genetic accommodation as we did not observe shifts in plasticity in response to the fish predation events. This could indicate that instead, the connection between ancestral plasticity and trait divergence is due to an alignment between plasticity and the main axis of additive trait variances. Such a notion requires further testing.

Conclusions

Plastic responses to novel environments have long been assumed to contribute to the evolutionary process. However, the exact mechanisms by which phenotypic plasticity helps or hinders adaptation is still unclear. Here, we did not find evidence to support theories of genetic accommodation. Nor did we find that different forms of plasticity (adaptive, maladaptive) alter the rate of adaptation. We instead found that evolutionary divergence followed the direction of ancestral plasticity. Our findings are some of the first experimental based evidence demonstrating a link between ancestral plasticity and trait divergence. Assessing the role of

plasticity in adaptive diversification requires further investigation to determine the correlations between environmentally induced phenotypic plasticity and genetic constraints within the genetic architecture.

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Tables

Table 1. Analysis for life history traits from the common garden plasticity assay. General linear models were used with clone and treatment as fixed factors, and the number of females in each jar (jar density) as a covariate. When the covariate was nonsignificant ($p>0.05$), it was removed from the model, and the data were reanalyzed.

Effect	Age at		Size at		Reproductive		Juvenile	
	Trait	maturity, F	Trait	Maturity, F	Trait	Output, F	Trait	Growth, F
Clone	63	11.364 ^{***}	63	11.310 ^{***}	61	6.987 ^{***}	62	6.971 ^{***}
Treatment	1	0.462	1	5.206 [*]	1	0.065	1	0.600
Treatment x Clone	63	1.60 ^{**}	63	1.135	60	1.150	61	1.271
Density (<i>Covariate</i>)	1	17.457 ^{***}	1	1.823	1	20.717 ^{***}	1	0.145
RSS (df)		0.017 (247)		2.769 (246)		6.44 (225)		0.065 (238)

Bold entries represent significant terms; RSS(df), residual sums of squares (degrees of freedom);

NS, not significant ($P > 0.1$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Loading values from pattern matrix of principal component analysis using direct oblimin rotation with Kaiser normalization. Communalities for each variable and percent of total variance for each component.

	PC1	PC2	Communality
Size at Maturity	0.919	-0.069	0.829
Total Reproductive			
Output	0.896	0.067	0.83
Age at Maturity	-0.194	0.092	0.757
Juvenile Growth	0.31	0.767	0.826
Eigenvalue	1.967	1.274	
% of Total Variance	49.187	31.855	
Total Variance		81.04%	

Table 3. Analysis for life history traits. All traits were analyzed using a repeated measures general linear models. Generation, time, plasticity category, and treatment were entered as fixed effects. When random variables were nonsignificant ($p>0.05$), they were removed from the model and the data were reanalyzed.

Fixed Effects	df	Age at Maturation	Size at Maturation	Average Interclutch Interval	Tot Repro Output
		F (ddf)	F (ddf)	F (ddf)	F (ddf)
Generation	1	27.665^{***} (854.81)	7.956^{**} (919.593)	0.273 (787.879)	0.079 (816.799)
Time	1	7.015^{***} (1295.92)	41.338^{***} (1312.557)	20.457^{***} (1276.452)	44.489^{***} (1291.551)
Plasticity Category (A M, N)	5	11.515^{***} (373.74)	24.033^{***} (389.547)	10.172^{***} (362.655)	0.399 (375.233)
Predator Treatment	2	0.889 (619.24)	1.24 (667.132)	7.015^{**} (578.335)	17.922^{***} (599.846)
Generation × Time	2	6.947^{***} (1280.04)	10.995^{***} (1301.014)	3.714[*] (1265.02)	17.594^{***} (1277.023)
Generation × Plasticity	1	0.167 (854.81)	11.155^{***} (933.489)	4.337[*] (787.879)	10.764^{***} (816.799)
Generation × Treatment	1	0.03 (854.81)	0.391 (928.787)	0.001 (787.879)	1.213 (816.799)
Time × Plasticity	10	3.238^{***} (1290.95)	5.49^{***} (1309.771)	3.63^{***} (1270.623)	3.213^{***} (1286.389)
Time × Treatment	5	1.535 (1295.92)	1.025 (1317.273)	2.314[*] (1276.452)	1.14 (1291.551)
Plasticity × Treatment	2	0.387 (373.74)	0.054 (377.965)	0.632 (362.655)	0.604 (375.233)
Generation × Time × Plasticity	2	0.465 (1280.04)	4.315^{***} (1300.808)	0.803 (1265.02)	3.007[*] (1277.023)
Generation × Time × Treatment	1	1.131 (1280.04)	0.015 (1301.211)	0.712 (1265.02)	0.449 (1277.023)
Generation × Plasticity × Treatment		0.194 (854.81)	0.02 (928.267)	0.7 (787.879)	1.478 (816.799)
Time × Plasticity × Treatment	10	0.321(1290.95)	0.805 (1312.714)	0.596 (1270.623)	0.641 (1286.389)
Gen. × Time × Plasticity × Treatment	2	0.723 (1280.04)	0.69 (1300.912)	0.805 (1265.02)	1.534 (1277.023)
Clone (Tank) *Wald Z (SE)		-	2.022[*] (0.000)	-	-

* $p \leq 0.05$

** $p \leq 0.01$.

*** $p \leq 0.001$.

Figures



Figure 1. Location of lakes in Wisconsin. *Daphnia* were sampled from two separate watersheds.



Figure 2. Experimental mesocosm flow through barrier set up. *Daphnia* were exposed to non-lethal threat of predation by sunfish (left side of figure) over the course of the selection experiment. The experimental *Daphnia* populations were located on the right-hand side of the image.

Experimental Evolution Design

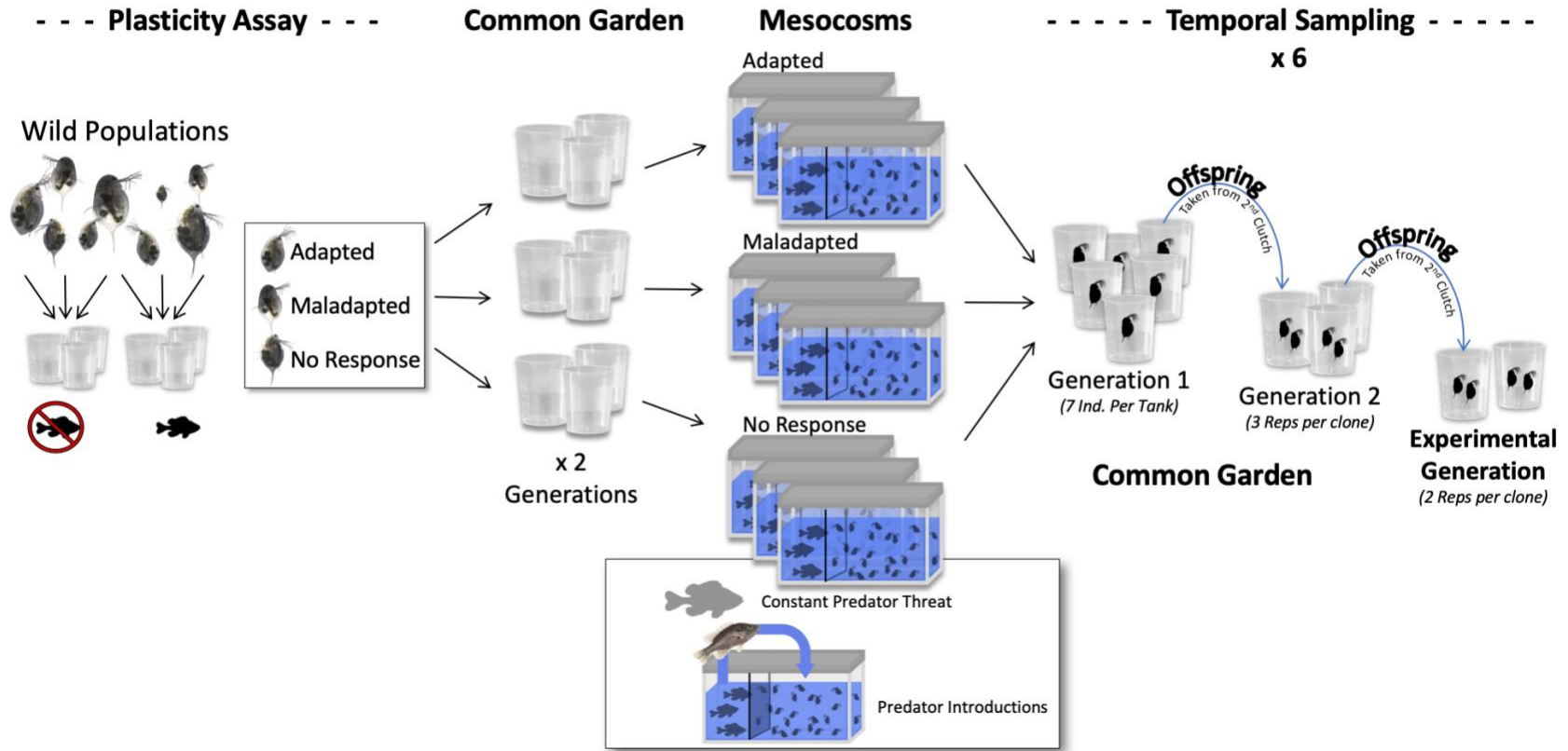


Figure 3. Overall experimental design. Initial plasticity assay, with assignments of plasticity categories, common garden rearing for entry into mesocosms, temporal subsampling and common garden experiment for exploration of changes in plasticity and life history traits over time.

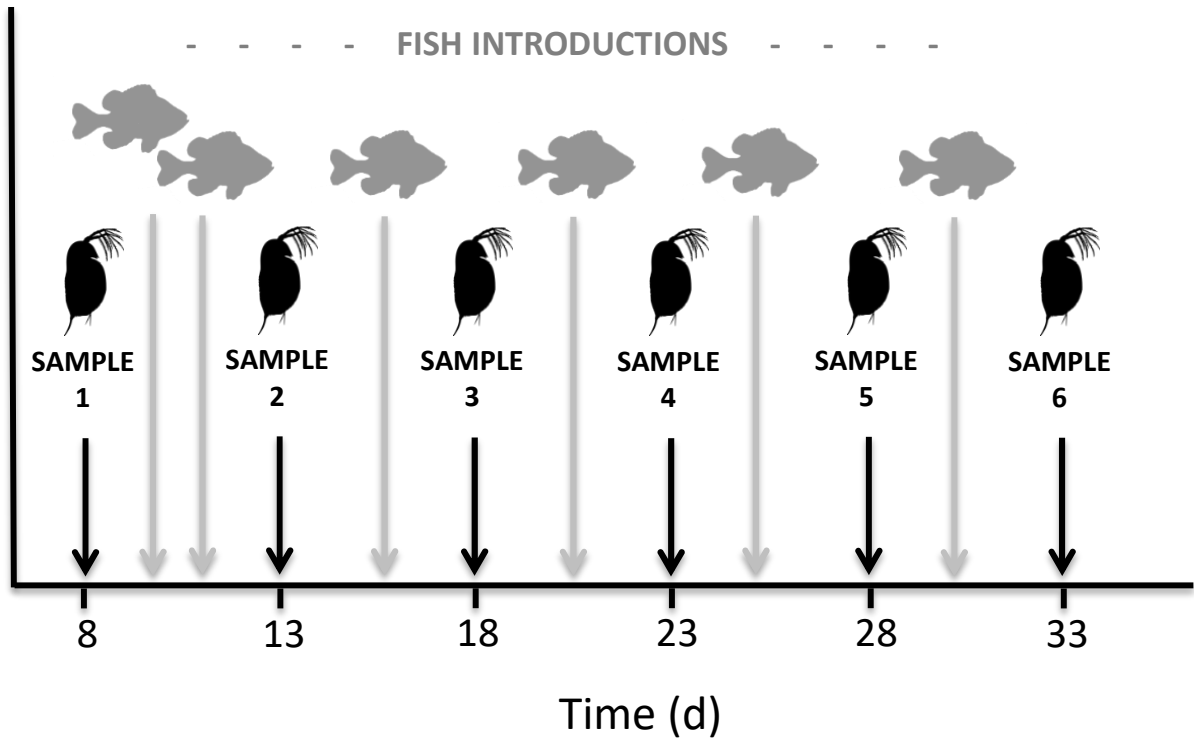


Figure 4. Mesocosm predator introduction and sampling schedule. Grey arrows indicate predator introductions into the *Daphnia* side of the mesocosms. Black arrows indicate the *Daphnia* sampling events to quantify trait values and trait plasticity.

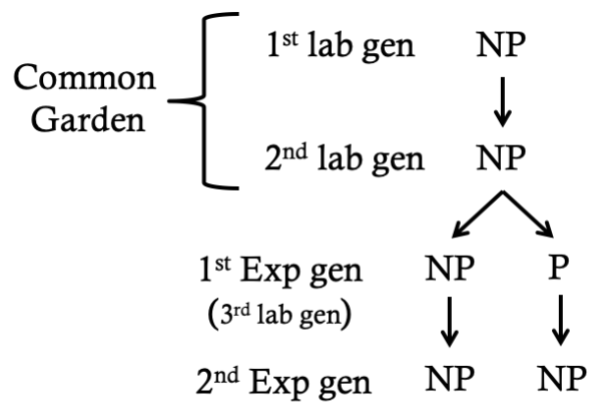


Figure 5. Common garden experimental design. NP = No Predator treatment, P = Predator treatment.

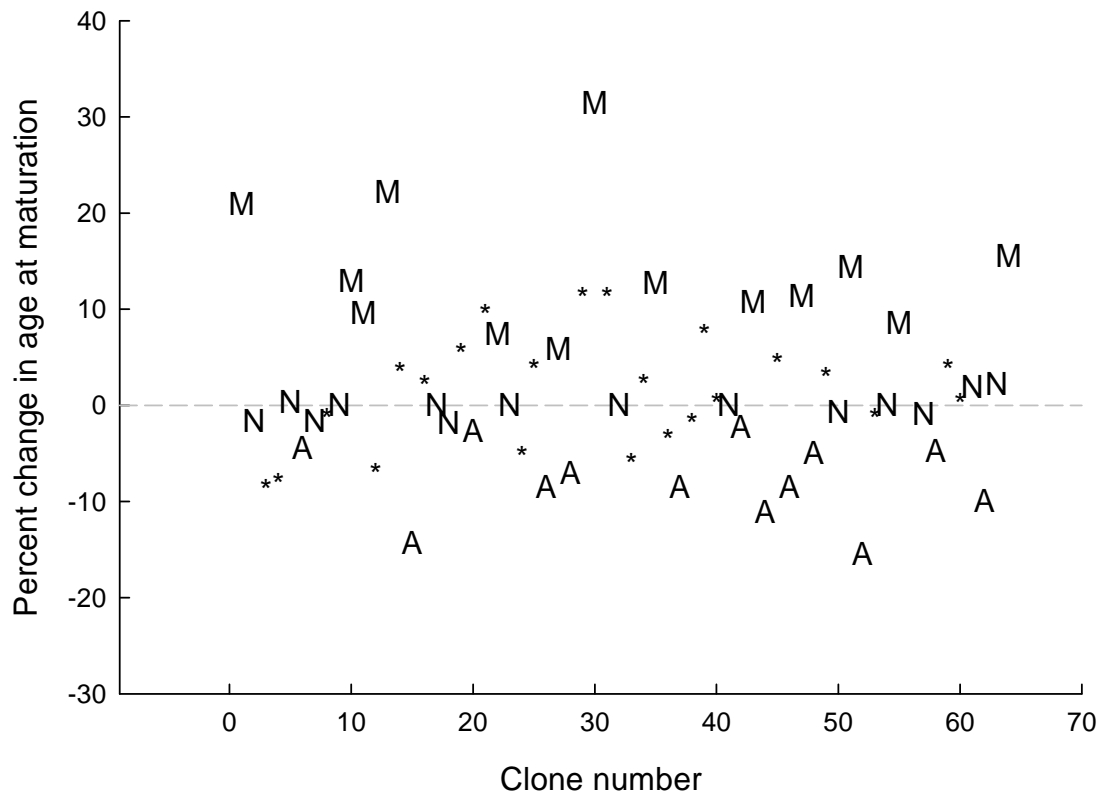


Figure 6. Average percent change in age at maturation results from plasticity assay. Letters indicate final plasticity category assignments for each clone. A = adapted, M = maladapted, N = no plasticity, * = unassigned/unused clones.

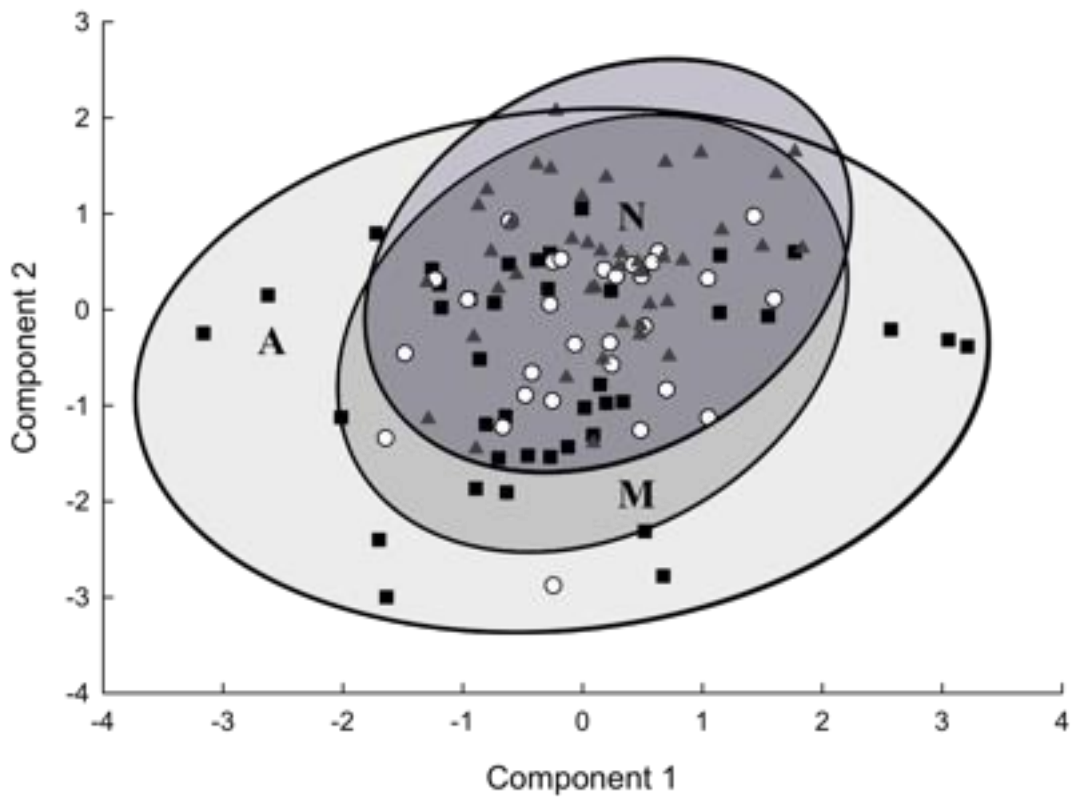


Figure 7. Plots of the first two components from a principal component analysis using a direct oblimin rotation. Grey triangles are clones assigned to the no response group (N), black squares represent the adaptive group (A), and open circles, the maladaptive group (M). Each data point represents a unique clonal lineage. Loading values are given in Table 2.

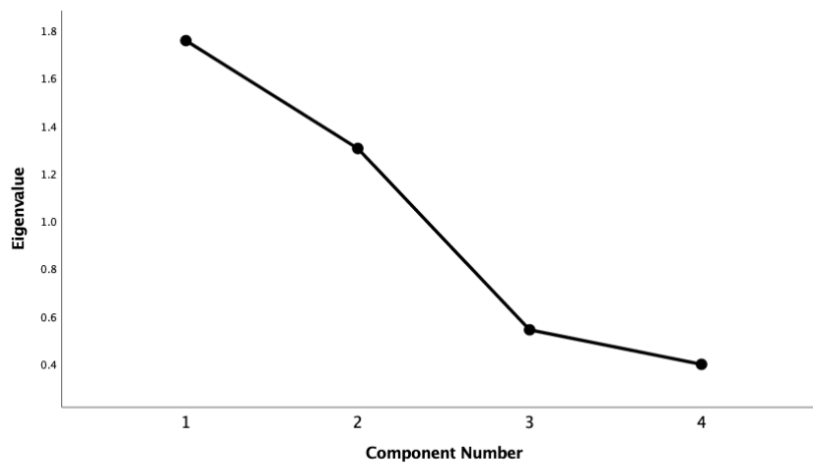


Figure 8. Scree plot from principal component analysis with elbow at the third component

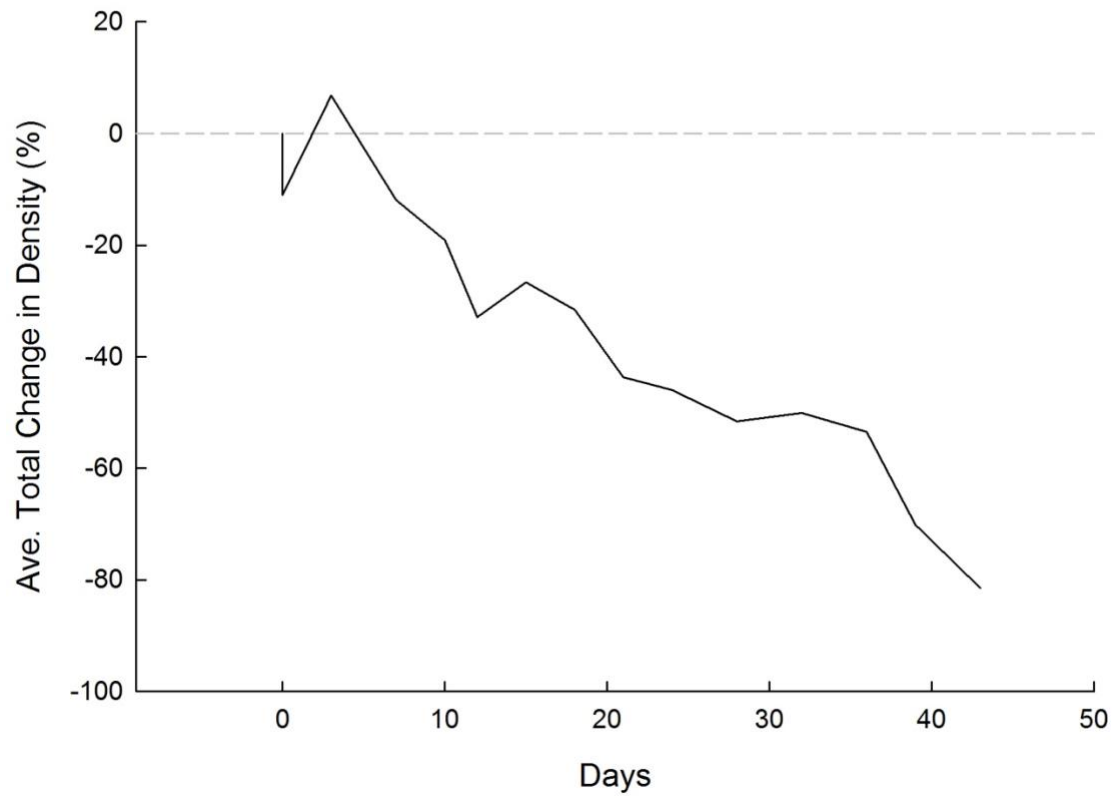


Figure 9. *Predation Pre-Trial.* Average effect of predation by sunfish *sp.* on *Daphnia* population density over a six-week period. Dates on x-axis are dates of fish predator introductions and density measurements.

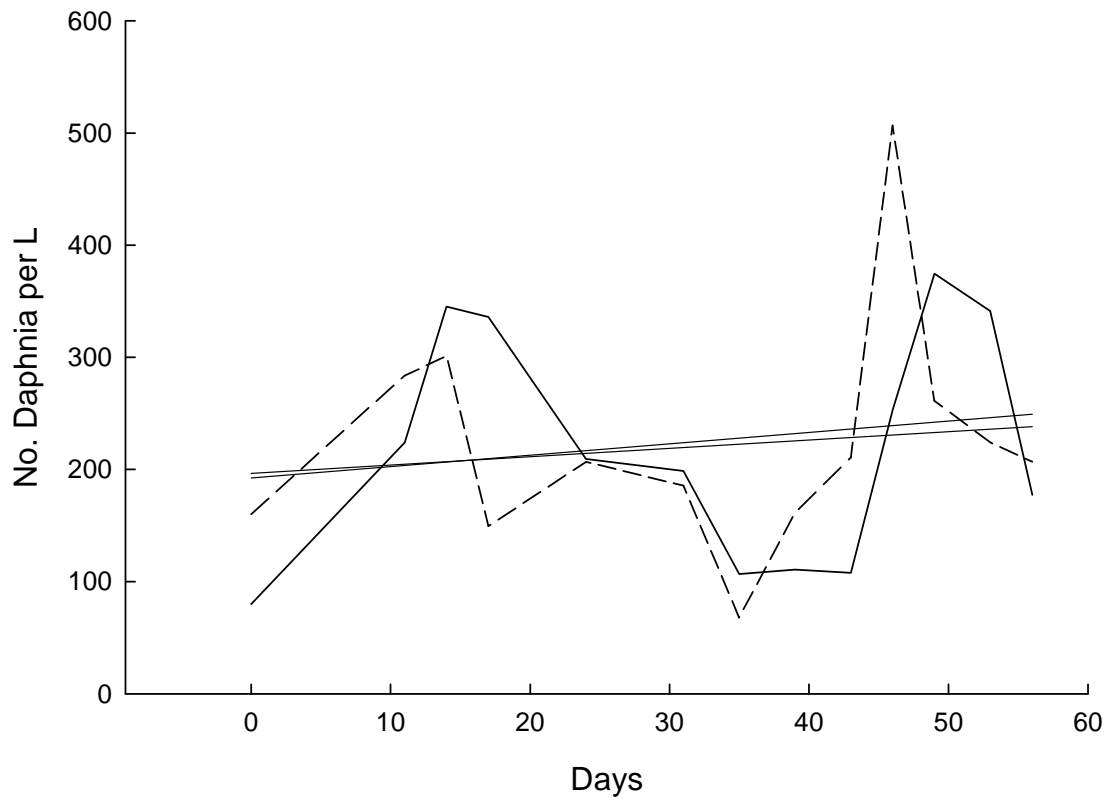


Figure 10. Population Growth Pre-Trial. Average growth rate of *Daphnia* population over a six-week period. Black line = starting population of 2.5 *Daphnia* l-1, dashed black line = starting population of 5 *Daphnia* l-1. Dashed grey reference line indicates increase in food density to 2.0 mg C l-1 d-1.

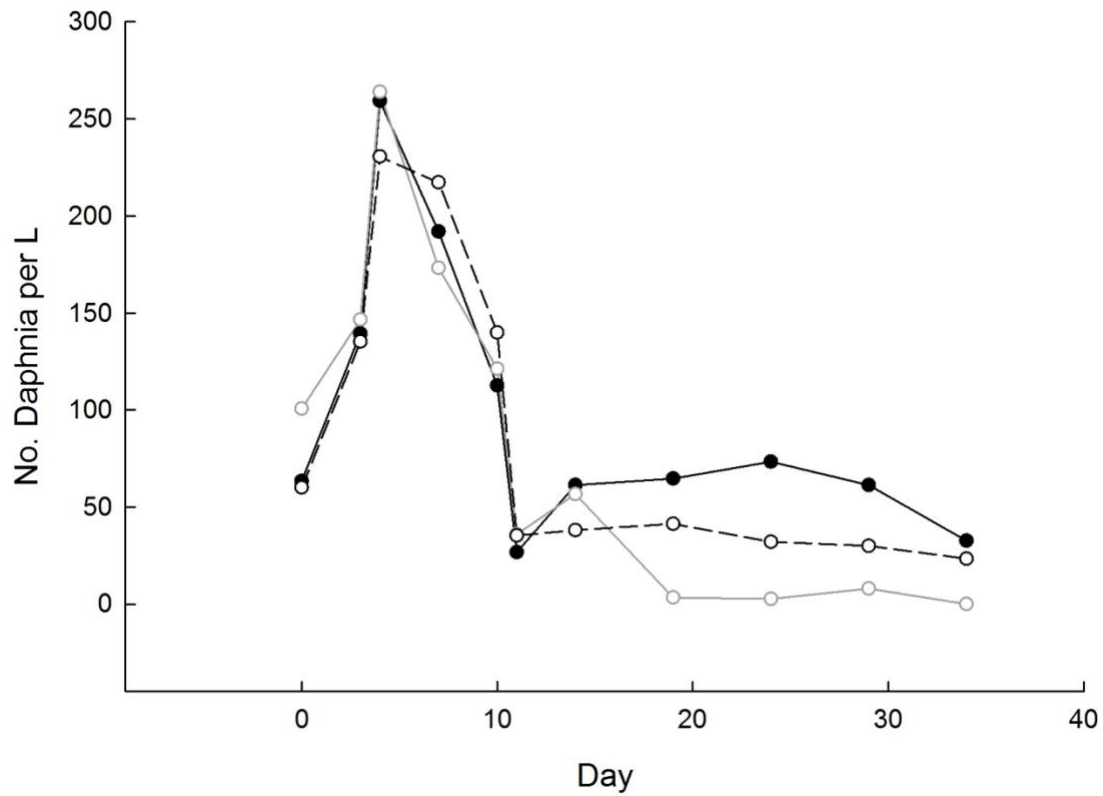


Figure 11. *Daphnia* population dynamics over the course of the selection experiment. Average mesocosm tank densities in *Daphnia* per liter. Black circles, solid black line = adaptive clones; grey open circles, solid grey line = maladaptive clones; black open circles, dashed black line = no response clones.

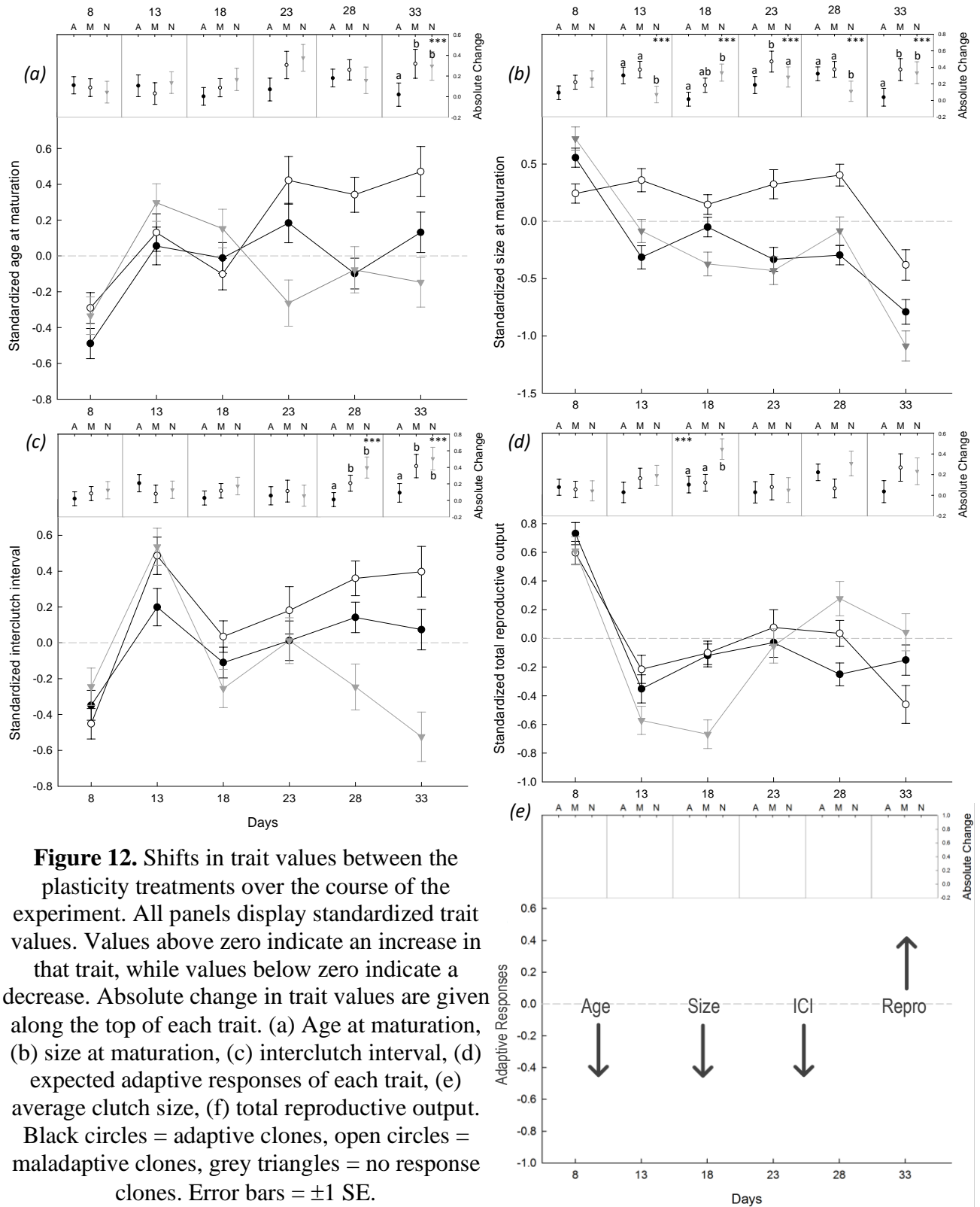


Figure 12. Shifts in trait values between the plasticity treatments over the course of the experiment. All panels display standardized trait values. Values above zero indicate an increase in that trait, while values below zero indicate a decrease. Absolute change in trait values are given along the top of each trait. (a) Age at maturation, (b) size at maturation, (c) interclutch interval, (d) expected adaptive responses of each trait, (e) average clutch size, (f) total reproductive output. Black circles = adaptive clones, open circles = maladaptive clones, grey triangles = no response clones. Error bars = ± 1 SE.

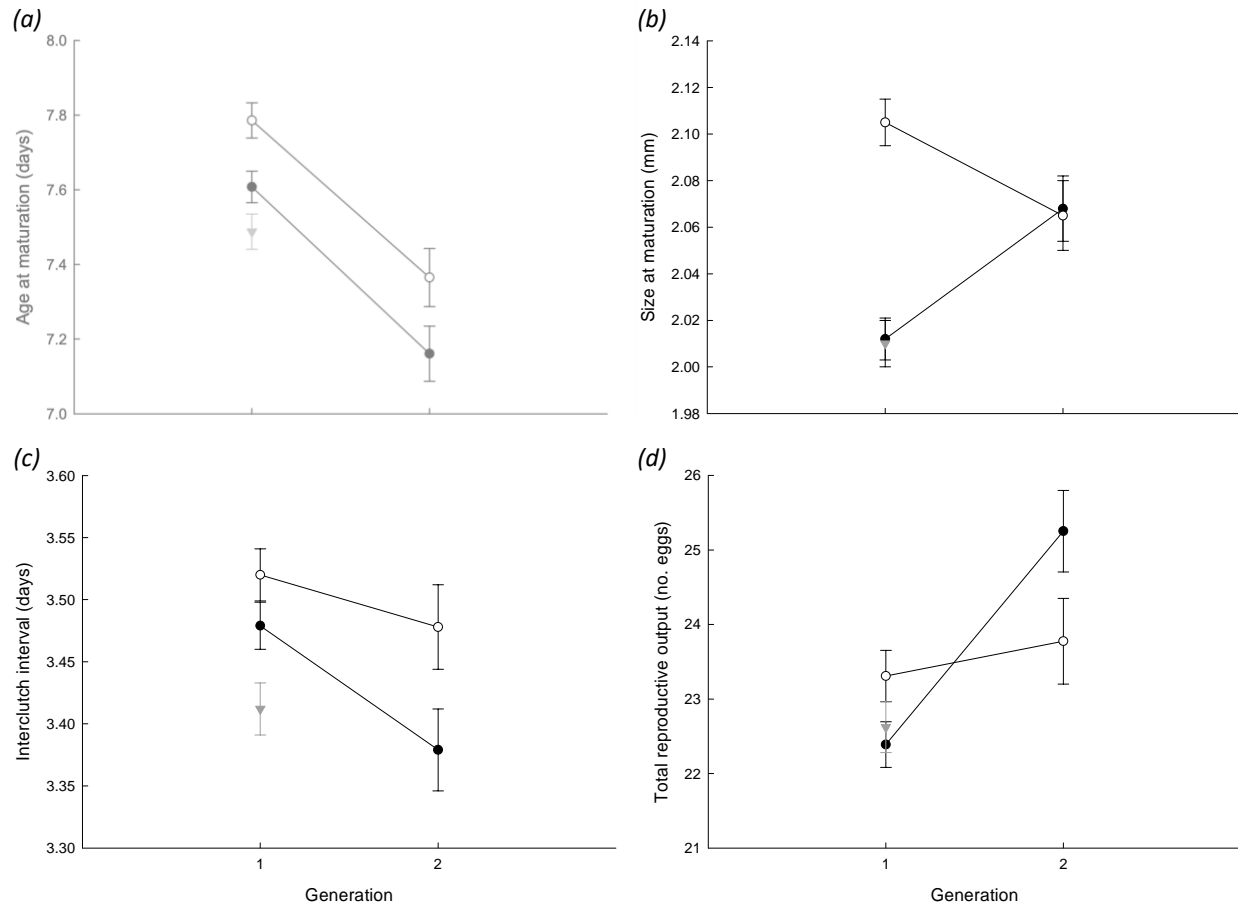


Figure 13. Differences in transgenerational responses between the plasticity groups. . Black circles = adaptive clones, open circles = maladaptive clones, grey triangles = no response clones. No response clones were not evaluated in the second generation. Error bars = ± 1 SE.

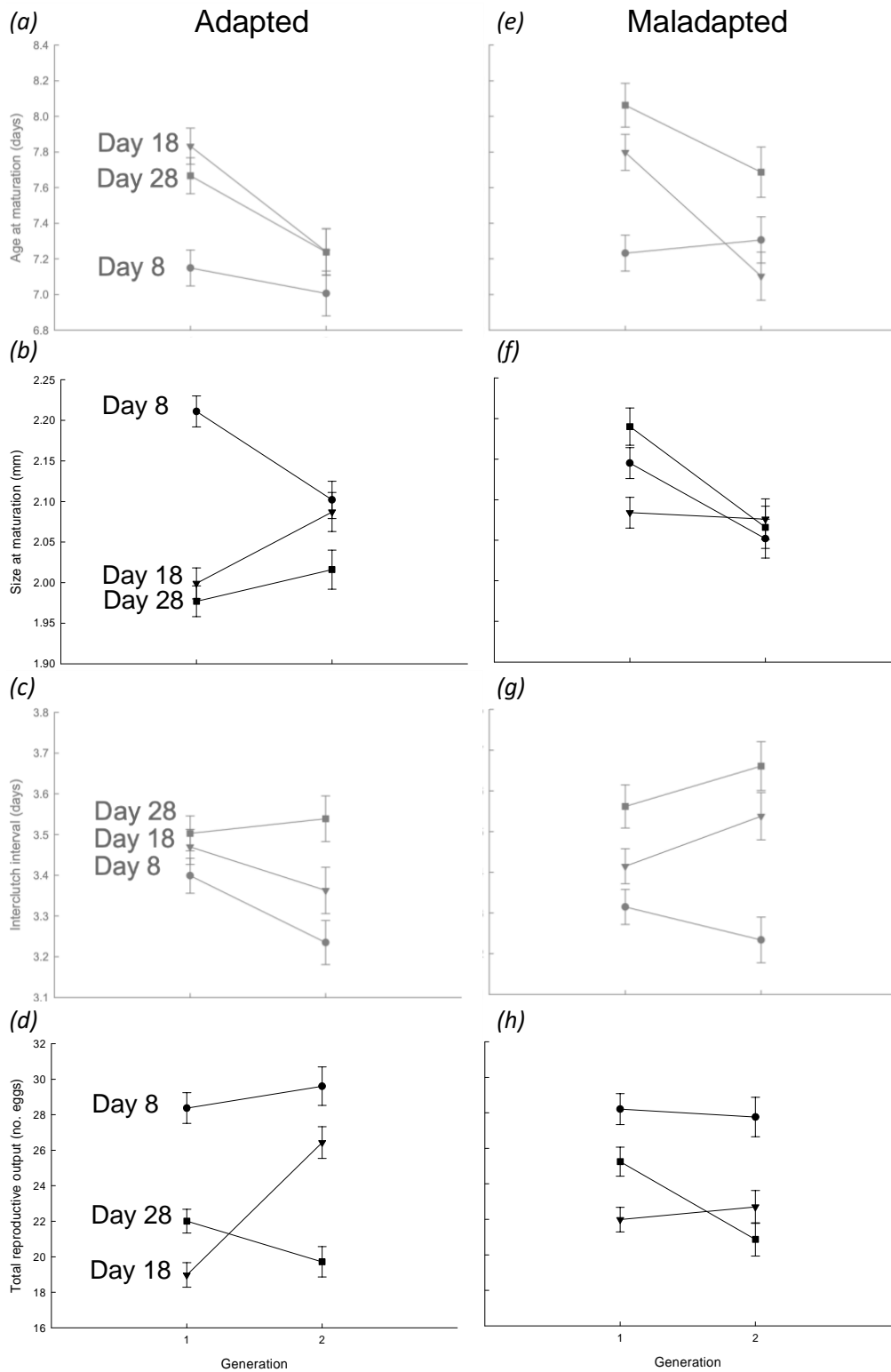


Figure 14. Shifts in transgenerational response between plasticity groups over the course of the experiment. Error bars = ± 1 SE.

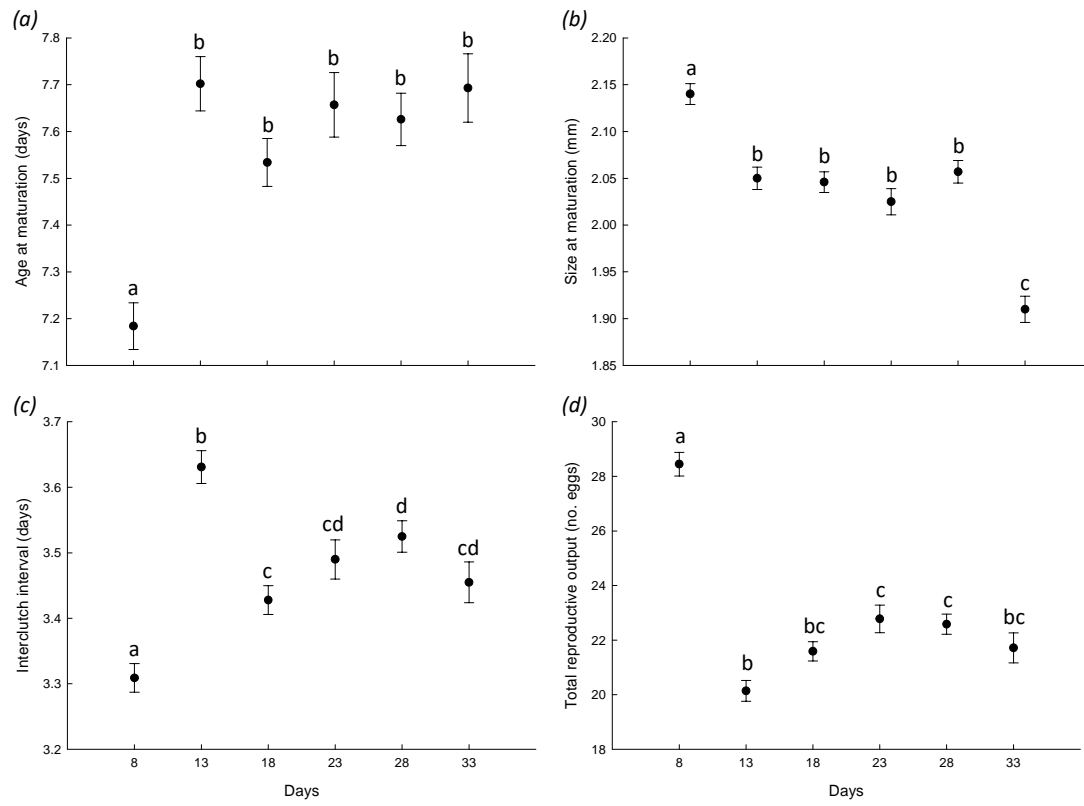


Figure 15. Life history trait changes over the course of the experiment. Error bars = ± 1 SE.