ORIGINAL PAPER

# Phytoplankton composition modifies predator-driven life history evolution in *Daphnia*

Matthew R. Walsh · Kimberly J. La Pierre · David M. Post

**Abstract** Organisms experience competing selective pressures, which can obscure the mechanisms driving evolution. *Daphnia ambigua* is found in lakes where a predator, the alewife (*Alosa pseudoharengus*) either does (anadromous) or does not (landlocked) migrate between marine and freshwater. We previously reported an association between alewife variation and life history evolution in *Daphnia*. However, differences in alewife migration indirectly influence phytoplankton composition for *Daphnia*. In 'anadromous lakes', *Daphnia* are present in the spring and experience abundant high-quality green algae. Intense predation by young-of-the-year anadromous alewife quickly eliminates these *Daphnia* populations by early summer. *Daphnia* from 'landlocked lakes' and lakes without alewife ('no alewife lakes') are present during the spring and summer and are more likely to experience high concentrations of sub-optimal cyanobacteria during the summer. To explore links between predation, resources, and prey evolution, we reared third-generation laboratory-born *Daphnia* from all lake types on increasing cyanobacteria concentrations. We observed several significant 'lake type × resource' interactions whereby the differences among lake types depended upon cyanobacteria concentrations. *Daphnia* from anadromous lakes developed faster, were larger at maturation, produced more offspring, and had higher intrinsic rates of increase in the absence of cyanobacteria. Such trends disappeared or reversed as cyanobacteria concentration was increased because *Daphnia* from anadromous lakes were more strongly influenced by the presence of cyanobacteria. Our results argue that alewife migration and phytoplankton composition both play a role in *Daphnia* evolution.

M. R. Walsh · K. J. La Pierre · D. M. Post
Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

M. R. Walsh (✉)
Department of Biology, University of Texas Arlington, Arlington, TX 76019, USA
e-mail: matthew.walsh@uta.edu

🖄 Springer

## Introduction

Organisms typically experience a variety of environmental stressors and thus adaptation is generally viewed as a response to multiple agents of selection (Reznick and Travis 1996). Predator–prey interactions provide a clear illustration of this dynamic. Predators increase the mortality rates of prey, which can select for changes in life history, behavioral, mor- phological, and physiological characteristics (i.e., Lynch 1980; Wellborn 1994; Sparkes 1996; Bronikowski and Arnold 1999; Johnson 2001; Jennions and Telford 2002; Hilton et al. 2002; Ghalambor et al. 2004; Langerhans et al. 2004; Magurran 2005). However, predators also reduce prey densities and, thereby, increase the amount of resources available to surviving individuals (Wootton 1994). Such changes in density and resources can significantly modify resultant evolutionary changes (Reznick et al. 2001, 2004; Walsh and Reznick 2008; terHorst 2010; Lau 2012) and possibly outweigh selection due to predator-induced mortality (Walsh and Reznick 2010, 2011). This work has illustrated the evolutionary interplay between predators, prey, and the *amount* of resources available to prey. Resource bases are not, however, homogeneous in nature and organisms consume an array of food types of differing edibility and nutritional quality. Variation in resource type has fitness consequences as it can influence rates of growth, development, and reproductive output (Twombly et al. 1998; Mayntz et al. 2003; Hassall et al. 2005). Thus, diet type may also have important evolutionary consequences (Hairston et al. 1999; Bochdanovits and de Jong 2003; Hassall et al. 2005; Diamond and Kingsolver 2012). However, our under- standing of the link between resource type and evolution, and how predators may influence this interaction, is limited.

In many lakes, waterfleas (*Daphnia* sp.) are the dominant grazers on phytoplankton and thus strongly influence the abundance and composition of the phytoplankton community. Lakes typically contain a diverse taxonomic array of phytoplankton from high-quality green algae (*Scenedesmus*) (Vijverberg 1989) to grazer-resistant cyanobacteria (i.e., *Anabaena*, *Microcystis*). There are several reasons why cyanobacteria are generally con- sidered to be a sub-optimal food for *Daphnia*. First, cyanobacteria exhibit a colonial or filamentous morphology that can modify the foraging rates of *Daphnia* (DeMott 1989; Gilbert 1990). Filaments interfere with *Daphnia* feeding appendages and can elevate *Daphnia* respiration rates (Porter and McDonough 1984) and decrease assimilation rates (Schindler 1968; Arnold 1971). It also important to highlight that green algae can form colonies in the presence of *Daphnia*, which negatively impact *Daphnia* life history traits (Lürling and Van Donk 1996, 2000). Second, cyanobacteria are nutritionally deficient compared with other phytoplankton species (Von Elert and Wolffrom 2001; Von Elert et al. 2002; Brett et al. 2009). Nutritional shortcomings in cyanobacteria include the absence of essential lipids, such as polyunsaturated fatty acids and sterols (Von Elert et al. 2002; Martin-Creuzberg et al. 2005). Finally, some cyanobacteria can produce intracellular secondary metabolites that may be toxic to *Daphnia* (DeMott et al. 1991; Rohrlack et al. 1999; Ghadouani et al. 2004). These characteristics are important because there is evidence that cyanobacteria have negative consequences for the survival, growth, and reproduction of *Daphnia* (Hairston et al. 1999; Rohrlack et al. 2001, 2003; Lurling 2003; Sarnelle et al.

2010). The negative fitness impacts of cyanobacteria on *Daphnia* can, in turn, exert selection on the life history and physiology of *Daphnia* (Hairston et al. 1999; Sarnelle and Wilson 2005; Blom et al. 2006; Lemaire et al. 2012). As a result, the interplay between populations of *Daphnia*, phytoplankton community composition, and the factors that influence the dynamics of both provide a means to explore the links between diet type and trait evolution.

In North American lakes, the planktivorous fish, the alewife (*Alosa pseudoharengus*), is well known to strongly influence the abundances of zooplankton, including *Daphnia* (Brooks and Dodson 1965; Post et al. 2008). In lakes in Connecticut, human activities (introductions, dam construction) have created two distinct alewife populations (Palkovacs and Post 2008; Palkovacs et al. 2008): (1) lakes with permanent populations of landlocked alewives ('landlocked lakes'), and (2) lakes with anadromous alewives that migrate seasonally between marine and freshwater environments ('anadromous lakes') (Palkovacs et al. 2008). Adult anadromous alewives migrate into lakes to spawn each spring (~March–May), and young-of the-year (YOY) alewives migrate from these lakes each autumn. Anadromous alewives are present in lakes for approximately 5 months per year, while landlocked alewives are present year round. These differences in migratory behavior, in turn, influence the period of time that *Daphnia* are present in the water column. *Daphnia* are very abundant during the spring in lakes with anadromous alewife, but are eliminated by intense YOY alewife predation by early summer and are absent from the water column until populations re-establish from resting eggs the following winter (Post et al. 2008; Walsh and Post 2011). In contrast, in lakes with landlocked alewife, year-round predation maintains a low biomass of *Daphnia* and a community consisting of small zooplankton (Post et al. 2008). *Daphnia* are also found in lakes without alewives ('no alewife lakes') and these lakes further illustrate the strong impact that alewife have on prey populations. Lakes with anadromous, landlocked, and no alewife all contain several species of zooplanktivorous fish (e.g., yellow perch, bluegill, pumpkinseed, etc.) that consume *Daphnia* (Palkovacs and Post 2008; Post et al. 2008). Yet, in lakes without alewife, *Daphnia* are abundant during the spring *and* summer, and attain particularly high densities during the summer (Post et al. 2008).

We recently reported correlations between alewife variation and life history evolution in *Daphnia ambigua* (Walsh and Post 2011, 2012). *Daphnia* from lakes with anadromous alewife grow faster, mature earlier, and produce more offspring (Walsh and Post 2011). This pattern of divergence is best explained by the differences in alewife migration and their associated impacts on *Daphnia* populations. *Daphnia* from lakes with anadromous alewife have a limited opportunity for growth and reproduction in early spring prior to the onset of intense predation by juvenile alewife. Evolution in *Daphnia* appears to thus represent a countergradient response (Conover and Schultz 1995) to this short 'growing' season that is driven as an indirect effect of alewife predation. However, these prior experiments evaluated patterns of life history variation when *Daphnia* was reared on a single species of green algae (*Scenedesmus obliquus*). In many lakes, the taxonomic composition of phytoplankton shifts towards increased amounts of cyanobacteria with increasing water temperatures. For instance, in Connecticut lakes, recent sampling shows that algae from the genus *Chlorophyta* (i.e., green algae) is ~15× more abundant than cyanobacteria in April–May, but this pattern reverses (cyanobacteria is 5× more abundant) in July–August (Fig. 1). Yet, *Daphnia* from lakes with anadromous alewife are only present during the spring and thus have, on average, less exposure to the higher abundances of cyanobacteria observed during the summer (see Walsh and Post 2011, Fig. S1). Previous work has shown that *Daphnia* can adapt to variation in cyanobacteria abundance (Hairston

et al. 1999; Sarnelle and Wilson 2005). Thus, variation in phytoplankton composition may modify patterns of life history evolution between *Daphnia* from lakes with anadromous, landlocked, and no alewife.

Here we explored the influence of diet type on life history traits in *D. ambigua* from lakes with contrasting alewife phenotypes. We reared *Daphnia* from three 'anadromous', three 'landlocked', and three 'no alewife' lakes for three generations in a common laboratory setting. In this experiment, all populations were reared across a gradient of increasing concentrations of cyanobacteria (*Anabaena* sp.) and we assessed patterns of trait variation across this gradient. We adopted this approach from previous evolutionary studies that have shown that organisms can adapt to variation in resource quantity and that examination of interactions between resource treatments and fitness provides an effective means to quantify responses to resource-mediated selection (Falconer and Latyszewski 1952; Mueller and Ayala 1981; Bierbaum et al. 1989; Hillesheim and Stearns 1991, 1992; Walsh and Reznick 2008).

## Materials and methods

### Focal lakes

We evaluated the life history responses of *Daphnia* to an increasing concentration of cyanobacteria using *D. ambigua* from nine lakes in Connecticut. This included three lakes with anadromous alewife (Bride, Dodge, Gorton), three lakes with landlocked alewife (Quonnipaug, Rogers, Long), and three lakes with no alewife (Gardner, Hayward, Black) (see Post et al. 2008 for map of study sites). As mentioned above, no alewife lakes are not fishless as they (as well as anadromous and landlocked lakes) contain several species of non-alewife zooplanktivorous fish such as bluegill (*Lepomis macrochirus*), yellow perch
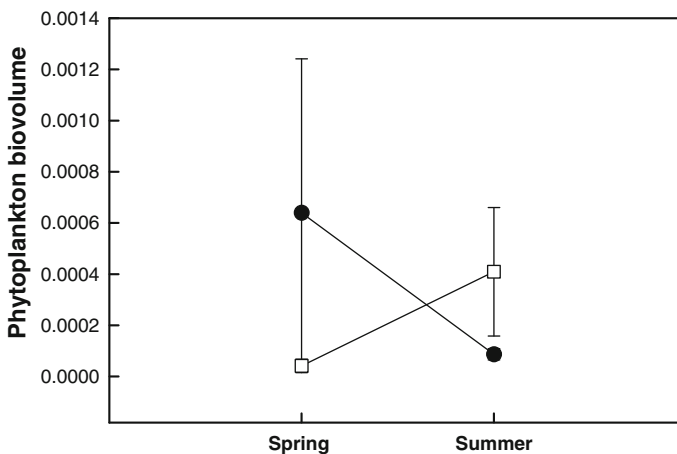


**Fig. 1** Total biovolume of *green algae* (Chlorophyta) and *cyanobacteria* during the *spring* (April–May) and *summer* (July–August) months. Monthly algal samples were taken at 5 lakes (Bride, Dodge, Linsley, Pattaganset, Rogers) in 2005 and 1 lake (Hayward) in 2006. This sampling scheme included 2 anadromous lakes (Bride, Dodge), 2 landlocked lakes (Pattaganset, Rogers), and 2 no alewife lakes (Hayward, Linsley). *Error bars* ±1 SE

(*Perca flavescens*), golden shiners (*Notemigonus crysoleucas*), and pumpkinseed (*Lepomis gibbosus*) (Palkovacs and Post 2008; Post et al. 2008). Previous work has shown that anadromous, landlocked, and no alewife lakes do not differ significantly ($p > 0.05$) in size, depth, productivity, or alewife biomass (for landlocked and anadromous lakes only) (Post et al. 2008; Walsh and Post 2011).

Common garden experiment

Our laboratory populations were established by hatching the resting eggs (ephippia) of *Daphnia* from sediment that was collected in August–September 2009 using an Ekman grab. The one exception was Gorton Pond, where sediment was collected in March 2012. The first laboratory generation consisted of a single post-ephippial female that was reared in a 90 ml jar containing COMBO medium (Kilham et al. 1998) and fed non-limiting supply of high quality algae (species: *S. obliquus*; concentration: $\sim 1.0$ mg C $L^{-1}$ $day^{-1}$). At least 10 clones were established per lake and all clones were reared under common conditions (Photoperiod 14L:10D; 16 °C). Since the resting eggs represent the product of sexual reproduction, we assume that each clone is distinct. Clones were transferred to jars containing new media and algae every other day. For the second laboratory generation, two neonates taken from the second clutch of each clone were reared under the same conditions (i.e. size of container, photoperiod, temperature) as the previous generation.

We quantified life history responses to increasing amounts of cyanobacteria, *Anabaena* sp., using third generation, laboratory reared clones of *D. ambigua* from all focal lakes. This experiment had three resource treatments: (1) 100 % *Scenedesmus*, (2) 80 % *Scenedesmus*, 20 % *Anabaena*, and (3) 50 % *Scenedesmus*, 50 % *Anabaena*. The treatments were created by using regressions between algal cell density and absorbance in a spectrophotometer (at 640 and 750 nm). *Anabaena* was added to the '20 % *Anabaena*' and '50 % *Anabaena*' treatments such that the total concentration of algal cell biovolume was constant across all treatments. The '20 % *Anabaena*' treatment reflects modest concentrations of cyanobacteria that approximate conditions during the spring (and sometimes summer), while the '50 % *Anabaena*' treatment reflects the concentrations of cyanobacteria that are observed commonly during the summer (Fig. 1). Our previous work showed that some of the life history differences between *Daphnia* from anadromous, landlocked, and no alewife lakes depend upon temperature; the differences in development rate were much stronger at 12 °C and were largely absent at 25 °C (Walsh and Post 2011). This experiment used a temperature (16 °C) that is intermediate between spring and summer temperatures, is ecologically relevant for all populations, but should also lead to life history differences under high concentrations of *Scenedesmus*.

The common garden experiment was started by collecting 6 newly-born individuals ($\sim 12$ h old) per clone from the second and subsequent clutches of the second-generation, laboratory reared *Daphnia*. All individuals were initially photographed for quantification of length and area (using ImageJ) and then individually placed into 90 ml containers containing COMBO medium and randomly assigned to one of the food treatments (0 % *Anabaena*, 20 % *Anabaena*, 50 % *Anabaena*). Each clone was replicated $2\times$ per treatment and the experiment consisted of 10 clones per lake (9 lakes × 10 clones per lake × 6 individuals per clone = 540 jars). The experimental conditions were the same as the prior generations.

We measured rates of juvenile growth, age at maturation (defined as the release of the first clutch into the brood chamber), size at maturation, and the number of embryos in clutches 1–3 among lake types. Juvenile growth was measured by photographing all

individuals on day 1 and day 4 and then converting this measurement to growth rate via: [(length on day 4 − length on day 1)]/no. of days. Age at maturation was estimated by monitoring *Daphnia* throughout development. Beginning on the morning of day 3, all *Daphnia* were examined for the release of the first clutch into the brood chamber (i.e., maturation) $3\times$ per day (at approximately at 8.00, 1.00, and 7.00). When the release of the first clutch was confirmed, age at maturation was recorded and each individual was photographed for estimates of size and fecundity (using ImageJ). After maturation, all individuals were examined every day for the production of clutches 2 and 3.

### Intrinsic rate of increase

We combined estimates of age at maturation, clutch size, and interclutch interval to calculate intrinsic rates of increase (r) for each lake at 0, 20, and 50 % *Anabaena* (Gotelli 1998). *r* represents an index of fitness that is a composite of several life history parameters. The data for age at maturation and clutch size were derived from the current study and we used previously published estimates of interclutch interval (see Walsh and Post 2011) (Note that interclutch interval does not differ significantly among lake types). We used data from clutches 1–3 in the current study and from the first two interclutch intervals from our previous work (Walsh and Post 2011). We calculated r as: $r = \ln(R_0)/G$, where $R_0$ is the net reproductive rate (sum of fecundity $\times$ survivorship) and G is generation time (average age of the parents of all offspring produced by a single cohort).

### Statistical analyses

Dependent variables were analyzed with linear mixed models (SAS v.9.1, Sas Institute, Cary, NC) using restricted maximum likelihood estimation (REML). Lake type, resource treatment, and the lake type $\times$ resource interaction were entered as fixed effects. Replicate lake populations nested within lake type and clone nested within lake were entered as random effects. We used between-within subjects degrees of freedom to determine the denominator degrees of freedom and used tests of simple main effects to examine differences among lake types within each resource treatment (Winer 1971). In these tests, we used Bonferroni correction to adjust our *p* values for multiple tests. Since we had three comparisons in these tests of simple main effects, we considered *p* values <0.0167 as 'significant' (*p* value correction: 0.05/3 = 0.0167). A likelihood ratio test was used for tests of significance of the random effects. When random effects were non-significant ($p > 0.05$), they were removed from the model and the data were reanalyzed without them. The data for clutch size were logarithmic transformed to improve homoscedasticity and fits with normality. Differences in intrinsic rates of increase (r) were evaluated using a linear mixed model, with lake type and resource quality entered as fixed effects and lake (nested within lake type) entered as a random effect.

### Missing values and outliers

Pre-reproductive mortality (i.e. from initiation of treatment to maturation) was very low (<1 %). Mortality between maturation and the production of the third clutch was slightly higher, but was similar among lake types [Mortality rates (%): Anadromous = 2.1 %, Landlocked = 5 %, No alewife = 3.7 %]. For the analysis of juvenile growth, we removed extreme values to improve fits with normality. We removed 3–6 extreme values

($>3\times$ the length of box and whisker plot) per lake type. These removals represent a small percentage of the total amount of data ($\sim 2$–$3$ %). The removal of these data also did not alter the trends or significance of the results and it is important to point out that we included all of the data in the figures for completeness.

## Results

Lake type $\times$ resource interactions

There was a significant ($p < 0.05$) interaction between lake type and resource treatment (i.e., 0, 20, 50 % *Anabaena*) for size at maturation, juvenile growth, clutch size (total # of offspring produced in clutches 1–3), and intrinsic rate of increase, but not age at maturation (Tables 1, S1; Figs. 2, 3). For all traits, the differences among lake types depended upon the concentration of *Anabaena*. In general, *Daphnia* from lakes with anadromous alewife grew faster, matured earlier and at a larger size, produced larger clutches of offspring, and had higher rates of populations growth than *Daphnia* from lakes with landlocked or no alewife in the absence of *Anabaena* (Figs. 2, 3). However these differences disappeared or were reversed as the concentration of *Anabaena* increased (Figs. 2, 3). For instance, *Daphnia* from lakes with anadromous alewife produced clutches of offspring that were 20 % larger than *Daphnia* from landlocked or no alewife lakes in the '0 % *Anabaena*' treatment, but minimal differences were observed in the 50 % *Anabaena*' treatment. A similar trend was observed for intrinsic rates of increase. Here, *Daphnia* from anadromous lakes exhibited an intrinsic rate of increase that was $\sim 11$ % higher than *Daphnia* from landlocked or no alewife lakes in the '0 % *Anabaena*' treatment, although small differences were observed as the concentration of *Anabaena* increased (Fig. 3). Reversals of trait variation were observed for size at maturation and juvenile growth; *Daphnia* from no alewife lakes maintained higher growth rates and exhibited larger sizes at maturation than *Daphnia* from the other lake types as the concentration of *Anabaena* increased (Fig. 2).

Tests of simple main effects

We used tests of simple main effects to statistically evaluate the nature of the differences in life history traits as a function of the concentration of *Anabaena*. For age at maturation, clutch size, and intrinsic rate of increase, tests of simple main effects revealed significant ($p < 0.0167$, $p$ value adjusted for multiple comparisons) differences among lake types in the absence of *Anabaena*, but not ($p > 0.0167$) in the treatments that contained high concentrations of *Anabaena* (50 % *Anabaena*) (Table 1). We explored the nature of the significant differences among lake types with post hoc Tukey tests. These tests showed that *Daphnia* from anadromous lakes differed significantly from landlocked lakes for age at maturation and clutch size and from no alewife lakes for clutch size. For size at maturation, significant differences among lake types were found in the 50 % *Anabaena* treatment, but not in the 0 and 20 % *Anabaena* treatments (Table 1). Tests of simple main effects showed that rates of growth did not differ significantly among lake types within any of the *Anabaena* treatments (0, 20, 50 % *Anabaena*) (Table 1).

**Table 1** Analyses of life history variables

| Factor | Num. df | Juvenile growth F (df) | Age at maturation F (df) | Size at maturation F (df) | No. of eggs F (df) | Intrinsic rate of increase F (df) |
|---|---|---|---|---|---|---|
| *Fixed effects* | | | | | | |
| Lake type | 2 | 0.71$^{NS}$ (87) | 5.37** (87) | 2.67$^{+}$ (87) | 2.73$^{+}$ (87) | 4.02$^{+}$ (6) |
| Anabaena | 1 | 5.68** (173) | 9.57*** (173) | 14.2*** (174) | 23.62*** (167) | 24.16*** (12) |
| Laketype × Anabaena | 4 | 2.74* (173) | 1.57$^{NS}$ (173) | 5.11*** (174) | 7.66*** (167) | 4.75* (12) |
| *Random effects* | | Wald Z | Wald Z | Wald Z | Wald Z | Wald Z |
| Lake (Lake type) | 1 | 1.42$^{NS}$ | 1.31$^{NS}$ | 1.42$^{NS}$ | 0.84$^{NS}$ | 1.79* |
| Clone (Lake) | 1 | 4.86*** | 4.34*** | 5.63*** | 5.46*** | – |
| *Simple main effects* | | | | | | |
| Anabaena 0 | 2 | 0.28$^{NS}$ (173) | 7.03$^{\$}$ (173) | 1.21$^{NS}$ (174) | 6.6$^{\$}$ (167) | 7.49$^{\$}$ (12) |
| Anabaena 20 | 2 | 1.72$^{NS}$ (173) | 3.63$^{NS}$ (173) | 3.94$^{NS}$ (174) | 4.45$^{\$}$ (167) | 4.87$^{NS}$ (12) |
| Anabaena 50 | 2 | 1.77$^{NS}$ (173) | 1.6$^{NS}$ (173) | 4.32$^{\$}$ (174) | 0.14$^{NS}$ (167) | 0.2$^{NS}$ (12) |

Linear mixed models were used with lake type and resource treatment entered as fixed effects and lake (nested within lake type) and clone (nested within lake) entered as a random effects. The denominator degrees of freedom are displayed after each F value

$^{+}$ $0.05 < p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $^{NS}$ $p > 0.05$; for tests of simple of main effects $^{\$}$ $p < 0.0167$

## Main effects

### Lake type effects

We observed significant, overall, life history differences among lake types for age at maturation (Table 1; Fig. 2). *Daphnia* from lakes with anadromous alewife matured 6 % earlier than *Daphnia* from landlocked or no alewife lakes.

### Anabaena effects

Increasing concentrations of *Anabaena* influenced the expression of all measured life history traits (Table 1; Fig. 2). *Daphnia* grew slower, developed more slowly, matured at a smaller size, and produced smaller clutches as the concentration of *Anabaena* increased. The negative consequences of *Anabaena* on growth and clutch size were particularly strong; growth rates were 9 % slower and clutch sizes were reduced by 15 % between the 0 and 50 % *Anabaena* treatments.

## Discussion

Our results revealed that life history differences among populations of *D. ambigua* from lakes with contrasting alewife morphs depended strongly on phytoplankton composition. *Daphnia* from lakes with anadromous alewife grew faster, matured earlier and at a larger
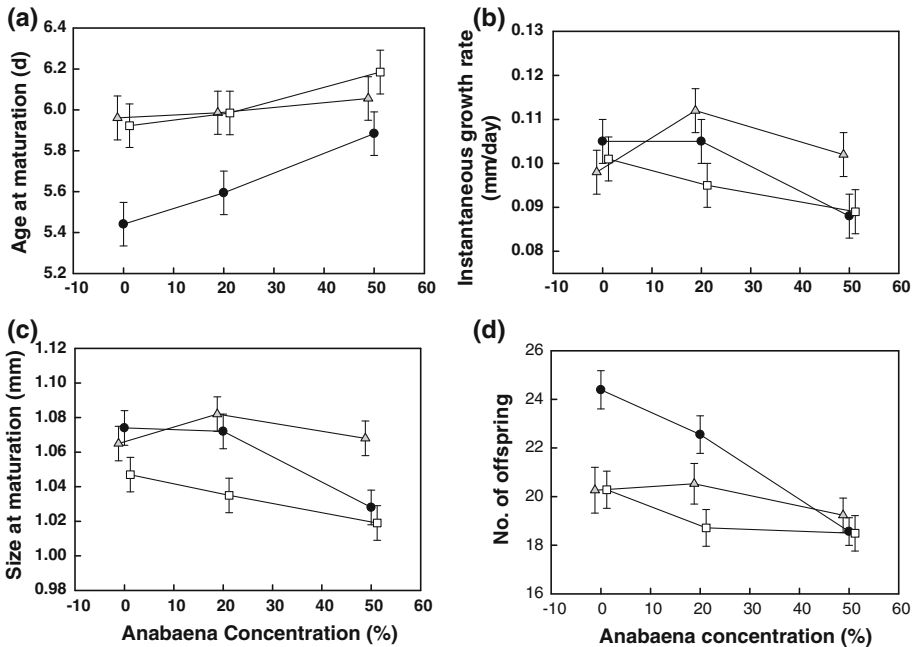
**Fig. 2** Life history differences in age at maturation (**a**), growth rate (**b**), size at maturation (**c**), and clutch size (**d**) among lake types as a function of increasing *Anabaena* concentration. *Closed circles*—anadromous lakes (A), *Open squares*—landlocked lakes (L), *Grey triangles*—no alewife lakes (N). Linear mixed models with lake type, and *Anabaena* concentration (0, 20, or 50 %) entered as fixed effects and lake (nested within lake type) and clone (nested within lake) entered as random effects were used to analyze each variable. Significant ($p < 0.05$) lake type by resource treatment interactions were observed for growth rate, size at maturation, and clutch size. Note that the *symbols* are offset along the x-axis to more clearly display overlapping *error bars*. *Error bars* ±1 SE

size, and produced more offspring than *Daphnia* from lakes with landlocked alewife or no alewife (Figs. 2, 3). These results are in agreement with our previous studies of life history evolution in this system and illustrate an association between variation in fish predator communities and *Daphnia* evolution (Walsh and Post 2011, 2012; see also Walsh et al. 2012). Such trends were only apparent, however, when *Daphnia* was fed green algae (*S. obliquus*) and disappeared or reversed under high (50 %) concentrations of cyanobacteria (*Anabaena*) (Fig. 2). The patterns of variation for age at maturation, clutch size, and intrinsic rates of increase were particularly compelling (Figs. 2, 3). For instance, significant differences for age at maturation and clutch size were observed between *Daphnia* from lakes with anadromous and landlocked alewife in the absence of *Anabaena* (Fig. 2). Yet, these differences were non-significant in the treatments that contained high concentrations of cyanobacteria (Fig. 2). Differences in intrinsic rates of increase also varied as a function of *Anabaena* concentration; *Daphnia* from anadromous lakes exhibited significantly higher intrinsic rates of increase than the other lake types, but only in the treatment that lacked *Anabaena* (Fig. 3). These results beg two questions: (1) how can we interpret the statistical lake type × resource interactions? and (2) what is the mechanism behind the smaller clutches and longer time to maturation with increasing *Anabaena* concentrations in *Daphnia* from anadromous lakes and why does this pattern not occur in lakes with land-locked and no alewife?
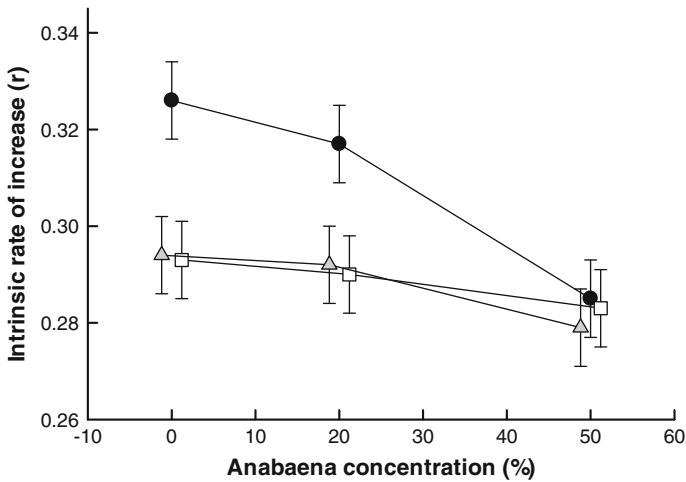
**Fig. 3** Variation in intrinsic rates of increase among lake types as a function of the concentration of *Anabaena*. *Closed circles*—anadromous lakes (*A*), *Open squares*—landlocked lakes (*L*), *Grey triangles*—no alewife lakes (*N*). A significant ($p < 0.05$) lake type by resource treatment interaction was observed. Note that the *symbols* are offset along the x-axis to more clearly display overlapping *error bars*. *Error bars* $\pm 1$ SE

Evolutionary significance of diet type?

Based upon our previous studies, we expected that *Daphnia* from lakes with anadromous alewife would mature earlier and produced larger clutches than *Daphnia* from the other lake types (Walsh and Post 2011). Given the known negative impact of cyanobacteria on *Daphnia* life history traits (Lampert 1987; Hairston et al. 1999; Rohrlack et al. 2001, 2003; Lurling 2003; Sarnelle et al. 2010), we also expected increasing concentrations of *Anabaena* to cause declines in development and reproduction in *Daphnia* (Figs. 2, 3). If the impact of cyanobacteria on *Daphnia* life histories represents a purely ecological interaction, then the responses to increasing *Anabaena* should be parallel among lake types. The significant 'lake type × resource' clearly illustrate non-additive effects of cyanobacteria. These resource-dependent patterns of trait variation and population growth suggest that the life history differences between *Daphnia* from anadromous and landlocked (and no alewife) lakes are not exclusively driven by intraspecific variation in alewife traits. In Connecticut lakes, *Daphnia* from anadromous lakes are only present during the spring, as intense predation by alewife eliminates *Daphnia* from the water column in June each year (Post et al. 2008; see Walsh and Post 2011, Fig. S1). *Daphnia* from lakes with landlocked and no alewife are more common during the summer, although at very different abundances (Post et al. 2008). Green algae are more common in the spring, but this trend reverses in the summer as cyanobacteria become abundant (Fig. 1). Based upon the contrasting responses to increasing concentrations of *Anabaena* (Figs. 2, 3), our results indicate that *Daphnia* from lakes with anadromous alewife have evolved to better exploit the high quality algae that they naturally forage upon, whereas *Daphnia* from lakes with landlocked alewife and no alewife are comparatively better adapted (based upon changes in r across resource treatments) to forage in environments containing cyanobacteria. These results imply a link between the timing and severity of predation, resource type, and life history evolution.

The logic behind the interpretation of our statistical interactions between predation environment and resource type is based upon several disparate research approaches. First, many researchers have performed reciprocal transplant experiments and argued that the presence of interactions between divergent environments is due to evolutionary processes (reviewed: Schluter 2000). Second, the results of laboratory populations that have selectively reared organisms (*Drosophila*, mice, etc.) on contrasting food or density treatments are often context dependent; the differences between divergent selection regimes depend upon population density or controlled levels of food availability in the laboratory (Falconer and Latyszewski 1952; Mueller and Ayala 1981; Bierbaum et al. 1989; Hillesheim and Stearns 1991; Mueller et al. 1991; Hillesheim and Stearns 1992; Barrett et al. 2005). Third, trait variation between natural populations derived from habitats that differ in productivity can depend upon the amount or types of resources that these organisms are fed in the laboratory (Walsh and Reznick 2008; Desmarais and Tessier 1999). Collectively, all of these studies have quantified population × environment interactions and argued that either fitness reversals or reductions in the differences between divergent populations (as in the current study) when exposed to manipulations in the field or laboratory provide evidence for local adaptation.

Our specific notion of a link between algae type and evolution is supported by previous studies on adaptation in *Daphnia* (Hairston et al. 1999; Sarnelle and Wilson 2005, 2010; Blom et al. 2006). Sarnelle and Wilson (2005) compared clones of *Daphnia* from lakes with consistent differences in levels of cyanobacteria and found that clones from lakes with high levels of cyanobacteria have faster rates of growth when fed high levels of cyanobacteria than clones from lakes with lower cyanobacteria. Hairston et al. (1999, 2001) used a resurrection approach to show similar evidence for adaptation to cyanobacteria in *Daphnia galeata* from Lake Constance, Germany. In our study system, the life history traits of *Daphnia* from lakes with anadromous alewife were negatively impacted by the addition of *Anabaena* to a much greater extent than *Daphnia* from landlocked or no alewife lakes (Fig. 2). Interestingly, *Daphnia* from lakes with no alewife largely maintained their rates of growth and development and suffered the smallest declines in fecundity in the face of increasing concentrations of cyanobacteria (Fig. 2). Since *Daphnia* from no alewife lakes attain very high population sizes in the summer (Post et al. 2008), and also since cyanobacteria appear to be more common during the summer (Fig. 1), the relatively flat reaction norms displayed by *Daphnia* from no alewife lakes may indicate that the presence of cyanobacteria is a particularly important selective pressure in these locales. The evolutionary interpretation of our results should be viewed with some caution because we do not know the long-term trends in cyanobacteria abundances in our lakes nor do we know the age of the clones used in this study.

### *Daphnia*-algae mechanisms

In our study, we assessed life history responses in *Daphnia* to simultaneous changes in the concentrations of green algae (decreases) and cyanobacteria (increases). The results of our previous work indicate that the addition of cyanobacteria and not the reduction in the abundance of green algae appears the important driving force behind the contrasting resource-dependent reaction norms. Walsh and Post (2011) evaluated the life history responses of these same populations of *Daphnia* across a threefold gradient in the concentration of *Scenedesmus*. All populations responded to this reduction in food availability in a similar manner and, overall, low concentrations of *Scenedesmus* caused a 5 % decline in clutch size and essentially no change in age at maturation. In the current study, *Daphnia* from anadromous lakes exhibited a 25 % decline in clutch size and an 8 % increase in

maturation time between the '0 % *Anabaena*' and '50 % *Anabaena*' treatments. Thus, a threefold change in the amount of *Scenedesmus* yielded minor life history responses (Walsh and Post 2011), yet the addition of *Anabaena* facilitated much stronger life history changes in this population. Below we consider why the addition of cyanobacteria may differentially impact the expression of life history traits among the populations of *Daphnia*.

As previously stated, cyanobacteria and green algae can differ in morphology (Böing et al. 1998), nutritional quality (Lampert 1987; Demott and Mueller-Navarra 1997; Von Elert and Wolffrom 2001; Von Elert et al. 2002; Brett et al. 2009), and toxicity (DeMott et al. 1991; Rohrlack et al. 1999; Ghadouani et al. 2004). Of these differences, the potential toxic qualities of *Anabaena* represent an unlikely causal mechanism; two previous meta-analyses showed that algal toxicity had no overall influence on zooplankton population growth (and the traits directly associated with population growth) (Wilson et al. 2006; Tillmanns et al. 2008). Several other mechanisms, however, cannot be eliminated. One possibility is that *Daphnia* from lakes with landlocked and no alewife are better able to target and utilize green algae in the presence of cyanobacteria (see Schatz and McCauley 2007; but see DeMott 1986). Nutritional differences may also be particularly important. Research has shown that clones of *Daphnia* that are more efficient at utilizing phosphorus-rich conditions, have low fitness when phosphorus is limiting (Weider et al. 2005). Such an effect could explain the severe decline in fitness observed in *Daphnia* from lakes with anadromous alewife as the concentration of cyanobacteria was increased. Alternatively, it is also possible that *Daphnia* from lakes with landlocked alewife and, especially, *Daphnia* from no alewife lakes are better able to mechanically handle or assimilate this food source (Von Elert et al. 2002). Since we reared all *Daphnia* on *Scenedesmus* for two generations prior to the start of the experiment, we also cannot eliminate divergent maternal effects in this system (Fox and Mousseau 1998). Clearly, elucidating the mechanism for the differing reaction norms requires further investigation.

## Conclusions

The evolutionary consequences of predator-induced mortality on the evolution of prey is well established (i.e., Wellborn 1994; Sparkes 1996; Bronikowski and Arnold 1999; Johnson 2001; Jennions and Telford 2002; Hilton et al. 2002). Here we illustrate an important connection between predation, phytoplankton composition, and the evolution of prey. Intraspecific variation in alewife migratory behavior influences the seasonal dynamics of *Daphnia* and, thereby, the composition of phytoplankton experienced by *Daphnia*. Diet type significantly modifies the influence of alewife variation on *Daphnia* life history evolution as the differences among our focal populations of *Daphnia* depended upon the amount of cyanobacteria in the laboratory (Fig. 2). Such results build upon a growing body of research revealing important interactions between predator-induced mortality and additional ecological variables that ultimately modify selection on prey communities (Walsh and Reznick 2008; terHorst 2010; Lau 2012). The consensus that is emerging from these studies is that resource use plays a significant role in determining resultant patterns of evolutionary change and, more importantly, that a specific consideration of diet is necessary to elucidate these evolutionary changes in a natural setting.

# References

Arnold DE (1971) Ingestion, assimilation, survival, and reproduction of *Daphnia pulex* fed seven species of blue green algae. Limnol Oceanogr 16:906–920

Barrett RDH, MacLean RC, Bell G (2005) Experimental evolution of *Pseudomonas fluorescens* in simple and complex environments. Am Nat 166:470–480

Bierbaum TJ, Mueller LD, Ayala FJ (1989) Density-dependent evolution of life history characteristics in *Drosophila melanogaster*. Evolution 43:382–392

Blom JF, Baumann HI, Codd GA, Juttner F (2006) Sensitivity and adaptation of aquatic organisms to oscillapeptin J and (D-Asp3,(E)-Dhb7) microcystin-RR. Arch Hydrobiol 167:547–559

Bochdanovits Z, de Jong G (2003) Experimental evolution in *Drosophila melanogaster*: interaction of temperature and food quality selection regimes. Evolution 57:1829–1837

Böing W, Wagner A, Voigt H, Deppe T, Benndorf J (1998) Phytoplankton responses to grazing by *Daphnia galeata* in the biomanipulated Bautzen Reservoir. Hydrobiologia 389:101–114

Brett MT, Kainz M, Taipale SJ, Seshan H (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc Natl Acad Sci USA 106:21197–21201

Bronikowski AM, Arnold SJ (1999) The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. Ecology 80:2314–2325

Brooks JL, Dodson SI (1965) Predation, body size, and the composition of plankton. Science 150:28–35

Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol Evol 10:248–252

DeMott WR (1986) The role of taste in food selection by freshwater zooplankton. Oecologia 69:334–340

DeMott WR (1989) The role of competition in zooplankton succession. In: Sommer U (ed) Plankton ecology: succession in plankton communities. Springer, Berlin, pp 195–252

DeMott WR, Zhang QX, Carmichael WW (1991) Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and 3 species of *Daphnia*. Limnol Oceanogr 36:1346–1357

DeMott WR, Mueller-Navarra DC (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshw Biol 38:649–664

Desmarais KH, Tessier AJ (1999) Performance tradeoff across a natural resource gradient. Oecologia 120:137–146

Diamond SE, Kingsolver JG (2012) Host plant adaptation and the evolution of thermal reaction norms. Oecologia 169:353–360

Falconer DS, Latyszewski M (1952) The environment in relation to selection for size in mice. J Genet 51:67–80

Fox CW, Mousseau TA (1998) Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. In: Mousseau TA, Fox CW (eds) Maternal effects as adaptations. Oxford University Press, New York, pp 159–177

Ghadouani A, Pinel-Alloul B, Plath K, Codd GA, Lampert W (2004) Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*. Limnol Oceanogr 49:666–679

Ghalambor CK, Reznick DN, Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). Am Nat 164:38–50

Gilbert JJ (1990) Differential effects of *Anabaena affinis* on cladocerans and rotifers: mechanisms and implications. Ecology 71:1727–1740

Gotelli NJ (1998) A primer of ecology. Sinauer Associates, MA

Hairston NG Jr, Lampert W, Caceres CE, Holtmeier CL, Weider LJ, Gaedke U et al (1999) Rapid evolution revealed by dormant eggs. Nature 401:446

Hairston NG Jr, Holtmeier CL, Lampert W, Weider LJ, Post DM, Fischer JM et al (2001) Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? Evolution 55:2203–2214

Hassall M, Helden A, Goldson A, Grant A (2005) Ecotypic differentiation and phenotypic plasticity in reproductive traits of *Armadillidium vulgare* (Isopoda: Oniscidea). Oecologia 143:51–60

Hillesheim E, Stearns SC (1991) The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypic plasticity in two larval food environments. Evolution 45:1909–1923

Hillesheim E, Stearns SC (1992) Correlated responses in life-history traits to artificial selection for body weight in *Drosophila melanogaster*. Evolution 46:745–752

Hilton C, Walde SJ, Leonard ML (2002) Intense episodic predation by shorebirds may influence life history strategy of an intertidal amphipod. Oikos 99:368–376

Jennions MD, Telford SR (2002) Life history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. Oecologia 132:44–50

Johnson JB (2001) Adaptive life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator induced plasticity. Evolution 55:1486–1491

Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L (1998) COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377:147–159

Lampert W (1987) Laboratory studies on zooplankton–cyanobacteria interactions. NZ J Mar Freshwat Res 21:483–490

Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ (2004) Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305–2318

Lau J (2012) Evolutionary indirect effects of biological invasions. Oecologia 170:171–181

Lemaire V, Brusciotti S, van Gremberghe I, Vyverman W, Vanoverbeke J, De Meester L (2012) Genotype × genotype interactions between the toxic cyanobacterium Microcystis and its grazer, the waterflea *Daphnia*. Evol Appl 5:168–182

Lurling L (2003) *Daphnia* growth on microcystin-producing and microcystin-free Microcystis aeruginosa in different mixtures with the green alga *Scenedesmus obliquus*. Limnol Oceanogr 48:2214–2220

Lürling M, Van Donk E (1996) Zooplankton-induced unicellcolony transformation in *Scenedesmus acutus* and its effect on growth of herbivore *Daphnia*. Oecologia 108:432–437

Lürling M, van Donk E (2000) Grazer–induced colony formation in *Scenedesmus*: are there costs to being colonial? Oikos 88:111–118

Lynch M (1980) The evolution of Cladoceran life histories. Q Rev Biol 55:23–42

Magurran AE (2005) Evolutionary ecology: the Trinidadian guppy. Oxford University Press, Oxford

Martin-Creuzberg D, Wacker A, Von Elert E (2005) Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia 144:362–372

Mayntz D, Toft S, Vollrath F (2003) Effects of prey quality and availability on the life history of a trap-building predator. Oikos 101:631–638

Mueller LD, Ayala FJ (1981) Trade-off between r-selection and K-selection in *Drosophila* populations. Proc Natl Acad Sci USA 78:1303–1305

Mueller LD, Guo P, Ayala FJ (1991) Density-dependent natural selection and trade-offs in life history traits. Science 253:433–435

Palkovacs EP, Post DM (2008) Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feedback to shape predator foraging traits? Evol Ecol Res 10:699–720

Palkovacs EP, Dion KB, Post DM, Cacconne A (2008) Independent evolutionary origins of landlocked alewife populations and rapid parallel evolution of phenotypic traits. Mol Ecol 17:582–597

Porter KG, McDonough R (1984) The energetic cost of response to blue-green algal filaments by cladocerans. Limnol Oceanogr 29:365–369

Post DM, Palkovacs EP, Schielke EG, Dodson SI (2008) Intraspecific variation in a predator affects community structure and cascading trophic interactions. Ecology 89:2019–2032

Reznick D, Travis J (1996) The empirical study of adaptation in natural populations. In: Rose MR, Lauder GV (eds) Adaptation. Academic Press, San Diego, CA, pp 243–290

Reznick DN, Butler MJ IV, Rodd FH (2001) Life history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157:126–140

Reznick DN, Bryant MJ, Roff D, Ghalambor CK, Ghalambor DE (2004) Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095–1099

Rohrlack TK, Henning M, Kohl JG (1999) Mechanisms of the inhibitory effect of the cyanobacterium *Microcystis aeruginosa* on *Daphnia galeata's* ingestion rate. J Plankton Res 21:1489–1500

Rohrlack TK, Dittmann E, Borner T, Christoffersen K (2001) Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp. Appl Environ Microbiol 67:3523–3529

Rohrlack TK, Edvardsen B, Skulberg R, Halstvedt CB, Utkilen HC, Ptacnik R et al (2003) Oligopeptide chemotypes of the toxic freshwater cyanobacterium *Planktothrix* can form subpopulations with dissimilar ecological traits. Limnol Oceanogr 53:1279–1293

Sarnelle O, Wilson AE (2005) Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol Oceanogr 50:1565–1570

Sarnelle O, Gustafsson S, Hansson L (2010) Effects of cyanobacteria on fitness components of the herbivore *Daphnia*. J Plankton Res 32:471–477

Schatz GS, McCauley E (2007) Foraging behavior by *Daphnia* in stoichiometric gradients of food quality. Oecologia 153:1021–1030

Schindler DW (1968) Feeding, assimilation, and respiration rates of *Daphnia magna* under various environmental conditions and their relation to production estimates. J Anim Ecol 37:369–385

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Sparkes TCK (1996) Effects of predation risk on population variation in adult size in a stream-dwelling isopod. Oecologia 106:85–92

terHorst CP (2010) Evolution in response to direct and indirect ecological effects in pitcher plant iquiline communities. Am Nat 176:675–685

Tillmanns AR, Wilson AE, Pick FR, Sarnelle O (2008) Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fundam Appl Limnol 171:285–295

Twombly S, Clancy N, Burns C (1998) Life history consequences of food quality in the freshwater copepod *Boeckella triarticulata*. Ecology 79:1711–1724

Vijverberg J (1989) Culture techniques for studies on the growth, development, and reproduction of copepods and cladocerans under laboratory and in situ conditions. Freshw Biol 21:317–373

Von Elert E, Wolffrom T (2001) Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. Limnol Oceanogr 46:1552–1558

Von Elert E, Martin-Creuzburg D, Le Coz JR (2002) Absence of sterols constrains carbon transfer between cyano-bacteria and a freshwater herbivore (*Daphnia galeata*). Proc R Soc Lond B 270:1209–1214

Walsh MR, Post DM (2011) Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. Proc R Soc Lond B 278:2628–2637

Walsh MR, Post DM (2012) The impact of intraspecific variation in a fish predator on the evolution of phenotypic plasticity and investment in sex in *Daphnia ambigua*. J Evol Biol 25:80–89

Walsh MR, Reznick DN (2008) Interactions between the direct and indirect effects of predators determine life history evolution in a killifish. Proc Natl Acad Sci USA 105:594–599

Walsh MR, Reznick DN (2010) Influence of the indirect effects of guppies on life history evolution in *Rivulus hartii*. Evolution 64:1583–1593

Walsh MR, Reznick DN (2011) Experimentally induced life history evolution in a killifish in response to the introduction of guppies. Evolution 65:1021–1036

Walsh MR, DeLong JP, Hanley TC, Post DM (2012) A cascade of evolutionary change alters consumer resource dynamics and ecosystem function. Proc R Soc Lond B 279:3184–3192

Wellborn GA (1994) Size-biased predation and prey life histories: a comparative study of freshwater amphipod populations. Ecology 75:2104–2117

Weider LJ, Makino W, Acharya K, Glenn KL, Kyle M, Urabe J, Elser JJ (2005) Genotype × environment interactions, stoichiometric food quality effects, and clonal existence in *Daphnia pulex*. Oecologia 143:537547

Wilson AE, Sarnelle O, Tillmanns AR (2006) Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta-analyses of laboratory experiments. Limnol Oceanogr 51:1915–1924

Winer BJ (1971) Statistical principles in experimental design. McGraw-Hill, New York, NY

Wootton JM (1994) The nature and consequences of indirect effects in ecological communities. Am Nat 25:443–466