ORIGINAL ARTICLE

doi:10.1111/evo.13717

SSE SOCIETY for the STUDY of EVOLUTION

EVOLUTION INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

The evolution of eye size in response to increased fish predation in *Daphnia*

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Received November 8, 2018
Accepted February 13, 2019

Variation in eye size is ubiquitous across taxa. Increased eye size is correlated with improved vision and increased fitness via shifts in behavior. Tests of the drivers of eye size evolution have focused on macroevolutionary studies evaluating the importance of light availability. Predator-induced mortality has recently been identified as a potential driver of eye size variation. Here, we tested the influence of increased predation by the fish predator, the alewife (*Alosa pseudoharengus*) on eye size evolution in waterfleas (*Daphnia ambigua*) from lakes in Connecticut. We quantified the relative eye size of *Daphnia* from lakes with and without alewife using wild-caught and third-generation laboratory reared specimens. This includes comparisons between lakes where alewife are present seasonally (anadromous) or permanently (landlocked). Wild-caught specimens did not differ in eye size across all lakes. However, third-generation lab reared *Daphnia* from lakes with alewife, irrespective of the form of alewife predation, exhibited significantly larger eyes than *Daphnia* from lakes without alewife. This genetically based increase in eye size may enhance the ability of *Daphnia* to detect predators. Alternatively, such shifts in eye size may be an indirect response to *Daphnia* aggregating at the bottom of lakes. To test these mechanisms, we collected *Daphnia* as a function of depth and found that eye size differed in *Daphnia* found at the surface versus the bottom of the water column between anadromous alewife and no alewife lakes. However, we found no evidence of *Daphnia* aggregating at the bottom of lakes. Such results indicate that the evolution of a larger eye may be explained by a connection between eyes and enhanced survival. We discuss the cause of the lack of concordance in eye size variation between our phenotypic and genetic specimens and the ultimate drivers of eye size.

KEY WORDS: ***Daphnia ambigua*, eyes, intraspecific variation, predator-prey interactions, sensory evolution, visual ecology.**

The size and structure of eyes vary extensively across taxa (Land and Fernald 1992; Bauer et al.1998; Fernald 2004a,b; Land and Nilsson 2012). Increased eye size is correlated with enhanced aspects of vision (Ritland 1983; Martin 1993; Motani et al. 1999; Møller and Erritzøe 2010; Land and Nilsson 2012; Caves et al. 2017), as well as shifts in foraging, mating behavior, and antipredator responses (Garamszegi et al. 2002; Hall and Ross 2007; Brischoux et al. 2010; Møller and Erritzøe 2010; Liu et al. 2012; McCoy et al. 2015). Given that investment in the eye is energetically costly, and that eye size is connected to fitness, it has long been assumed that variation in ecological factors have the potential to exert selection and drive evolutionary shifts in eye size (Bauer et al. 1998; Garamszegi et al. 2002; Moser et al. 2004; Thomas et al. 2006; Ross and Kirk 2007; Hall 2008; Somanathan et al. 2009; Veilleux and Lewis 2011). Much work has explored the association between light availability and differences in eye size at the macroevolutionary scale (e.g., Moser et al. 2004; Ross and Kirk 2007; Hall 2008; Somanathan 2009; Veilleux and Lewis 2011; Schmitz and Waitwright 2011). Tests of the conditions that drive variation in eye size within a species are far fewer (but see Beston et al. 2017a). We therefore know very little about the process whereby environmental factors induce shifts in eye size (i.e., eye size plasticity; but see Lonnstedt et al. 2013; Brandon and Dudycha 2014) and/or drive evolutionary changes in eye size over time (but see Brandon et al. 2015; Beston et al. 2017a).

Selection due to predation is a potentially important driver of eye size evolution because predators can directly impose selection on eye size via increased mortality (Glazier and Deptola 2011; Brandon et al. 2015; Beston et al. 2017a). For instance, predators often target pigmented characteristics, such as eyes. Such mortality should lead to the evolution of a smaller eye in prey (Zaret and Kerfoot 1975; Lönnstedt et al. 2013; Beston et al. 2017a). Conversely, increased eye size is correlated with enhanced antipredator capabilities (Møller and Erritzøe 2010; Nilsson 2009), suggesting that predators may drive the evolution of a larger eye (see Glazier and Deptola 2011). Predators may also indirectly alter selection on eye size by causing shifts in prey behavior that may, in turn, alter the light environment experienced by prey (i.e., prey hiding in dimmer habitats). Recent studies have shown that increased predation is associated with phenotypic increases in eye size in amphipods (Glazier and Deptola 2011) and genetically based decreases in eye size in killifish on the island of Trinidad (Beston et al. 2017a). Thus, the generality and the manner in which predators impose selection on prey eye size remains unclear.

In Connecticut, waterfleas (*Daphnia ambigua*) are located across a diversity of lakes that vary in composition of the predator community (Brooks and Dodson 1965; Palkovacs and Post 2008; Post et al. 2008). This includes lakes that differ in the presence and duration of predation by the dominant planktivore, the alewife (*Alosa pseudoharengus*). *Daphnia* are found in lakes with: (1) anadromous alewife, (2) landlocked alewife, and (3) no alewife (Post et al. 2008). The weakest predation intensity occurs in lakes without alewife, where *Daphnia* are common in the water column throughout the spring and summer months (Post et al. 2008). Predation intensity is higher in anadromous and landlocked alewife lakes, but in different ways. Landlocked alewife are permanent freshwater residents and have the potential to prey upon *Daphnia* year round. As a result, *Daphnia* are consistently rare in these lakes and predation intensity is high year-round. In lakes with anadromous alewife, adults migrate into lakes from the coastal ocean to spawn in March–April each year. Young-of-the-year (YOY) anadromous alewife then prey upon *Daphnia* during the late spring and summer before exiting lakes for the ocean in autumn. YOY are gape limited until ∼June (Palkovacs and Post 2008; Post et al. 2008). Thus, *Daphnia* are highly abundant in the early spring but are eliminated from the water column by July each year due to intense predation by anadromous alewife (Post et al. 2008). It is important to note, however, that *Daphnia* are exposed to predation by fish in all lake types. Generalist planktivorous fish, including bluegill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*), redbreast sunfish (*Lepomis auritus*), and white perch (*Morone americana*) are present in all lakes (Palkovacs and Post 2008; Post et al. 2008). It has been previously shown that the focal lakes do not differ in potentially confounding environmental factors including size, depth, productivity, or alewife biomass (in landlocked and anadromous lakes) (Post et al. 2009; Walsh and Post 2011).

Variation among the predator communities in lakes in Connecticut is associated with strong evolutionary shifts in the life history traits of *Daphnia* (Walsh and Post 2011, 2012; Walsh et al. 2014; Walsh et al. 2016). This includes differences in trait values (Walsh and Post 2011) and trait plasticity (Walsh and Post 2012; Walsh et al. 2016). In general, *Daphnia* from lakes with anadromous alewife grow faster, mature earlier, and invest more heavily into reproduction than *Daphnia* from lakes with landlocked or no alewife. The life histories of *Daphnia* from lakes with landlocked and no alewife do not differ significantly (Walsh and Post 2011). Thus, the hypothesized driver of life history evolution in *Daphnia* from these lakes is the seasonal nature of alewife predation. That is, the intense seasonal pulse of predation by anadromous alewife that eliminates *Daphnia* from the water column by early summer has selected for a “faster” life history in *Daphnia* in these lakes. More generally, such life-history shifts suggest that variation in our fish predator communities has the clear potential to shape adaptation in *Daphnia*. The extent to which alewives or the form of alewife predation alters selection on *Daphnia* eye size is unknown.

Theory has suggested that temporal stability, such as consistent predation in landlocked alewife lakes or the consistent lack of predation in no alewife lakes, should result in increases in transgenerational plasticity, while temporal variability, such as the pulsed predation events in anadromous alewife lakes, should favor the evolution of within-generation plasticity (Leimar and McNamara 2015; Uller et al. 2015; Kujiper and Hoyle 2015). Walsh et al. (2016) tested the influence of alewives on the evolution of life-history plasticity by rearing *Daphnia* from “anadromous,” “landlocked,” and “no alewife” lakes in the presence and absence of alewife chemical cues in a first experimental generation and tracked life-history responses in a second experimental generation (Walsh et al. 2016). As part of this study, all *Daphnia* were photographed when they attained sexual maturity. In the current study, we used these images to quantify genetic differences in eye size in *Daphnia* from our focal lakes. These archived images allow us to ask two key questions: (1) Do predators induce phenotypic plasticity, including transgenerational plasticity, in eye size? (2) Is variation in alewife predation and/or the form of alewife predation (i.e., anadromous vs. landlocked) associated with evolutionary shifts in eye size? If alewife target *Daphnia* with larger eyes (see Beston et al. 2017a) then we expect that eye size will be smaller in lakes with alewife when compared with *Daphnia* from lakes without alewife. Conversely, if survival (i.e., predator avoidance) increases with eye size, then we expect that the eye size of *Daphnia* from lakes with anadromous and landlocked alewife will be larger than *Daphnia* from no alewife lakes. We then performed follow-up field work where we collected *Daphnia* as a function of depth from lakes with and without alewives to determine if alewife alter the behavior of *Daphnia* and cause *Daphnia* to aggregate at the bottom of lakes where there is less light. This allowed us to test competing predictions that alewives directly alter selection on eye size (due to a connection between eye size and predator avoidance) or indirectly alter selection by altering the behavior and therefore light environment experienced by *Daphnia*.

Materials and Methods

LABORATORY EXPERIMENTS

The details of these experiments were previously published (Walsh et al. 2016) and are briefly described here. Walsh et al. (2016) used clones of *Daphnia ambigua* from three lakes with anadromous alewife (Bridge, Dodge, Gorton), three lakes with landlocked alewife (Amos, Long, Quonnipaug), and three lakes with no alewife (Black, Gardner, Wyassup) (Walsh et al. 2016). The original goal of this work was to test for evolved differences in within-generation and transgenerational plasticity in *Daphnia* from these focal populations. The general approach was to rear all clones in the presence and absence of predator cues in generation 1 and then track life-history responses in generation 2. This experiment included 15 clones per lake except three lakes; 13, 14, and 8 clones were reared from Dodge, Quonnipaug, and Gorton, respectively.

We established laboratory populations of *Daphnia* by hatching resting eggs (ephippia) from lake sediments. Lake sediment was retrieved from the top layers of sediment (approximately < 5 cm deep) using an Ekman grab. The first laboratory generation consisted of a female that hatched from an ephippia. These individuals were reared in a 90 mL jar containing COMBO medium (Kilham et al. 1998) and abundant quantities of algae (species: *Scenedesmus obliquus;* concentration of $\sim$1.0 mg C L^{-1} day^{-1}) (Photoperiod 14L:10D; 13°C). *Daphnia* were transferred to jars containing fresh media and algae every other day throughout the duration of the experiment. To generate the second laboratory generation, we collected two neonates from the second clutch of each clone and these individuals were reared under the same conditions as the previous generation.

Walsh et al. (2016) evaluated patterns of life-history plasticity within- and across-generations using third-generation laboratory reared clones of *Daphnia* from all populations (Walsh et al. 2016). For each clone, we collected six individuals ($<$12 hours old) and assigned each individual to one of the following treatments: (1) predator exposure in generation one, or (2) no predator exposure. The duration of the experiment was two experimental generations. The "predator" treatment included filtered lake water conditioned by alewife (see Walsh et al. 2016). All *Daphnia* received measured quantities of algae (*Scenedesmus obliquus* concentration: 0.8 mg C L^{-1} day^{-1}) and experienced the same temperature (13°C) and photoperiod (L:D 14:10) as the previous generations. In the original experiment, all clones were replicated 3× per treatment (125 clones across all lakes × 3 replicates per treatment × 2 treatments × 2 generations).

When *Daphnia* attained maturation (defined as the release of the first clutch of offspring in the brood chamber) a photo was taken for estimates of size at maturation. Here, we used those photos to quantify *Daphnia* eye size. Body size was measured from the top of the *Daphnia* head to the base of its tail spine. We used ImageJ (Schneider et al. 2012) to measure body size and the diameter of the eye at its widest part for all individuals from generation 1 and 2.

We evaluated our focal populations from Walsh et al. (2016) for differences in predator-induced eye size plasticity using linear mixed models implemented with restricted maximum likelihood estimation (REML) (SPSS v.25). We included lake type (anadromous, landlocked, no alewife), predator treatment (presence, absence), generation, and all interactions among these factors as fixed effects. We tested for differences in absolute eye size, as well as relative eye size by including total body length as a covariate. We evaluated both of these characteristics as absolute eye size is considered a strong approximation for visual capabilities (i.e., more light collection) and eye size corrected for body size (henceforth, relative eye size) accounts for energetic allocation. We included lake (nested within lake type) and clone (nested within lake) as random effects. When random effects were nonsignificant ($P > 0.05$), these terms were removed and the data were reanalyzed without them. These analyses used Sattherwaite approximations as the denominator degrees of freedom. Assumptions of normality and homogeneity of variances were confirmed via inspection of boxplots and residuals. We performed post-hoc Tukey tests following significant main effects.

WILD-CAUGHT *DAPHNIA*

To test the hypothesis that changes in migratory behavior and depth distribution caused by alewife predation might alter selection on eye size of *Daphnia* (see Results), we collected zooplankton across a depth gradient in anadromous alewife and no alewife lakes in May 2018 using a Schindler trap (volume = 12 L). Zooplankton was collected during the day from the morning to early afternoon. We did not sample from landlocked alewife lakes because *Daphnia* are consistently rare due to the continuous presence of alewife. Samples were taken across four depths from two lakes with anadromous alewife (Bride, Dodge) and three no alewife lakes (Wyassup, Gardner, Hayward). Adult anadromous alewife migrate to these lakes in early spring to spawn, thus we sampled in May during a time when *Daphnia* and young-of-the-year (YOY) anadromous alewife are both present. We sampled the water column at approximately the deepest part of each lake and then roughly scaled the four depths across the water column (Wyassup and Bride: 2 m, 4 m, 6 m, 8 m; Dodge: 3.5 m, 7 m, 10.5 m, 14 m; Hayward: 2.5 m, 5 m, 7.5 m, 9.75–10 m; Gardner: 3 m, 6 m, 9 m, 12 m). We completed 10 samples (12 L × 10 samples = 120 L total volume) at each depth across all lakes (except for at Hayward 2.5 m and Wyassup 8 m where we completed 15 samples). All samples were pooled across tows for each depth, respectively. Approximately 30–40 photographs were taken of live, female *Daphnia ambigua* of various developmental stages from the upper-most and bottom-most depths for each lake. We subsequently used photographs to quantify body size and eye diameter at the widest part of the eye in ImageJ (Schneider et al. 2012). All zooplankton samples were then preserved in 70% ethanol to later quantify the density of *Daphnia ambigua* at each depth.

We tested for differences in absolute and relative eye size in *Daphnia* collected at the top and bottom most levels of the water column in anadromous and no alewife lakes. We used linear-mixed models implemented with restricted maximum likelihood estimation (REML) (SPSS v.25). We natural log transformed eye size and total body size to better meet assumptions of normality and homogeneity of variances. We included fixed effects of lake type (anadromous, no alewife) and depth (upper, lower), and an interaction of “lake type by depth,” as well as a random effect of lake nested within lake type. When testing for relative eye size, we included total length as a covariate.

We quantified the density of *Daphnia ambigua* at each lake at each depth to determine the distribution of *Daphnia* in the water column. Samples were split using a plankton splitter down to approximately 200 individuals and then zooplankton were identified and enumerated under a dissection microscope. Using an approach similar to Post et al. (2008), the subsample was identified to genus or species for the first 200 individuals (including immatures and copepod nauplii) and all individuals (except for rotifers) were measured for length (Post et al. 2008). All *Daphnia* were identified to species.

To test for differences in *Daphnia* distribution throughout the water column and between lake types, we counted the number of *Daphnia ambigua* in each subsample of 200 individual zooplankton (see above) for each lake at each depth. We then used linear mixed models. Because Lake Hayward had zero *Daphnia ambigua* in the middle depths, we transformed our count data by adding 1 to all values to scale the data appropriately and remove zeros. We then square root transformed the count data. For our model we entered the number of *Daphnia ambigua* as the dependent variable and included depth (surface, mid-surface, mid-lower, lower), lake type (anadromous, no alewife), and the “depth by lake type” interaction as fixed effects and a random effect of lake nested within lake type. We also incorporated the number of zooplankton in each sample as a covariate because, although each sample was split down to approximately 200 individuals, some samples had either slightly more or slightly less than 200 organisms. Importantly, this covariate was nonsignificant ($P > 0.05$).

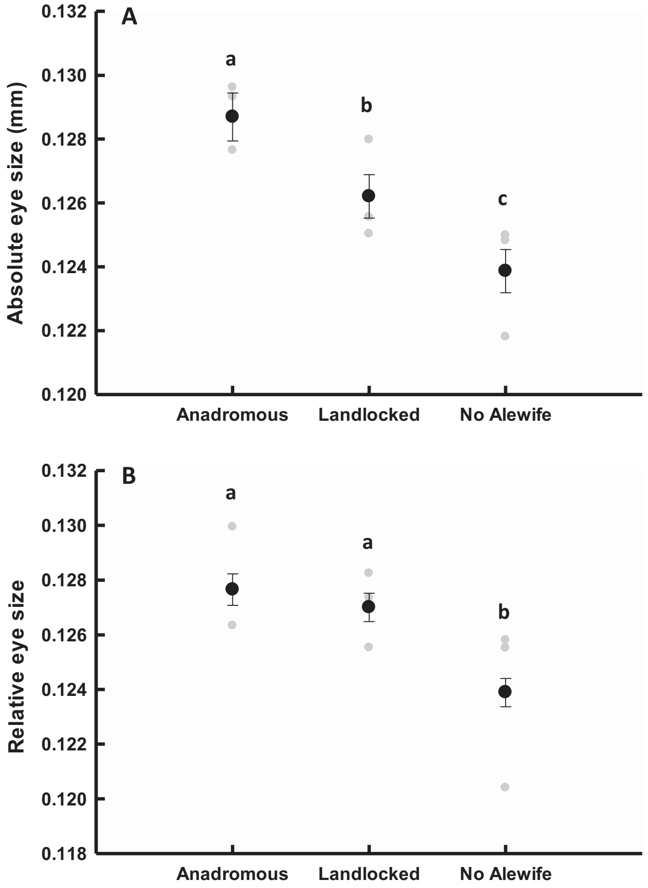

Figure 1. Variation among fish communities is correlated with evolutionary shifts in (A) absolute and (B) relative eye size in *Daphnia*. We observed significant ($P < 0.05$) differences in absolute and relative eye size among *Daphnia* from “anadromous,” “landlocked,” and “no alewife” lakes. The gray circles represent the eye sizes for all replicate lakes. Letters denote significant differences based upon post-hoc tests. Error = ± 1 SE.

Results

EYE SIZE: LABORATORY EXPERIMENTS

Lake type effects

We detected significant differences in absolute and relative eye size among *Daphnia* from lakes with anadromous, landlocked, and no alewife (Table 1; Fig. 1). Post-hoc comparisons revealed that the absolute and relative eye size of *Daphnia* differed significantly ($P < 0.05$) between “anadromous versus no alewife” lakes, as well as for “landlocked versus no alewife” lakes. *Daphnia* from lakes with anadromous and landlocked alewife exhibited

Table 1. Analyses of absolute and relative eye size for laboratory experiments.

		Absolute eye size		Relative eye size	
Effect	df	*F* (denom df)	*P*-value	F (denom df)	*P*-value
Covariates:					
Body size	1	—	—	**899 (1199.8)**	**<0.001**
Fixed effects:					
Lake type	2	**11.4 (123.7)**	**<0.001**	**14.2 (122.1)**	**<0.001**
Predator	1	**9.26 (1127.8)**	**0.002**	3.25 (1126.2)	0.072
Generation	1	**38.89 (1128.5)**	**<0.001**	**14.09 (1130.2)**	**<0.001**
Lake type × predator	2	1.6 (1127.8)	0.2	0.66 (1125)	0.52
Lake type × generation	2	0.71 (1128.7)	0.49	1.44 (1126.5)	0.24
Predator × generation	1	**12.37 (1125.2)**	**<0.001**	**4.17 (1130.9)**	**0.041**
Lake type × predator × generation	2	0.43 (1125.2)	0.74	0.98 (1123)	0.37
Random effects:					
Lake (lake type)	1	0.74	0.46	1.48	0.14
Clone (lake)	1	**4.89**	**<0.001**	**4.92**	**<0.001**

F, *F*-values; ***P***, *P*-values; d.f. numerator degrees of freedom; denom. d.f., denominator degrees of freedom. Significant terms are bolded.

Table 2. Analyses of absolute and relative eye size for wild-caught populations found in the upper and lower depths of the water column.

		Absolute eye size		Relative eye size	
Effect	df	*F* (denom df)	*P*-value	*F* (denom df)	*P*-value
Covariates:					
Body size	1	—	—	**956.43 (382.22)**	**<0.001**
Fixed effects:					
Lake type	1	5.10 (2.99)	0.109	0.02 (3.05)	0.894
Depth	1	**18.48 (381.01)**	**<0.001**	1.16 (380.15)	0.283
Lake type × depth	1	2.97 (381.01)	0.085	**16.96 (380.02)**	**<0.001**
Random effects:					
Lake (lake type)	1	1.11	0.267	1.18	0.237

F, *F*-values; ***P***, *P*-values; d.f., numerator degrees of freedom; denom. d.f., denominator degrees of freedom. Significant terms are bolded.

absolute eye sizes that were approximately 4% and 2% larger than *Daphnia* from lakes without alewife. The observed trends for relative eye size were similar; *Daphnia* from lakes with anadromous and landlocked alewife had a relative eye size that was 3% and 2.5% larger than *Daphnia* from lakes with no alewife, respectively (Fig. 1). The "lake type by generation," "lake type by predator," and "lake type by generation by predator" interactions were all nonsignificant ($P > 0.05$) (Table 1).

Predator by generation interaction

Observed differences in eye size between the predator treatments varied across generations as we observed a significant ($P < 0.05$) "predator × generation" interaction for both absolute and relative eye size (Table 1; Fig. 2). We observed little to no differences in eye size between predator and nonpredator treatments in generation 1 but the absolute and relative eye size of *Daphnia* from the predator treatment was ~4% and ~2% smaller than the nonpredator treatment in generation 2, respectively.

EYE SIZE: WILD-CAUGHT *DAPHNIA*

Lake type by depth interaction

We found no difference in absolute or relative eye size of *Daphnia* between lakes with anadromous and no alewife (Table 2). However, shifts in relative eye size as a function of lake depth differed between lake types. This is because we observed a significant ($P < 0.05$) "lake type by depth" interaction for relative eye size. *Daphnia* at the surface of lakes with anadromous alewife had a relative eye size that was nearly 5% smaller than those found at the bottom of the water column. The opposite pattern was observed in no alewife lakes; *Daphnia* found at the surface of these lakes

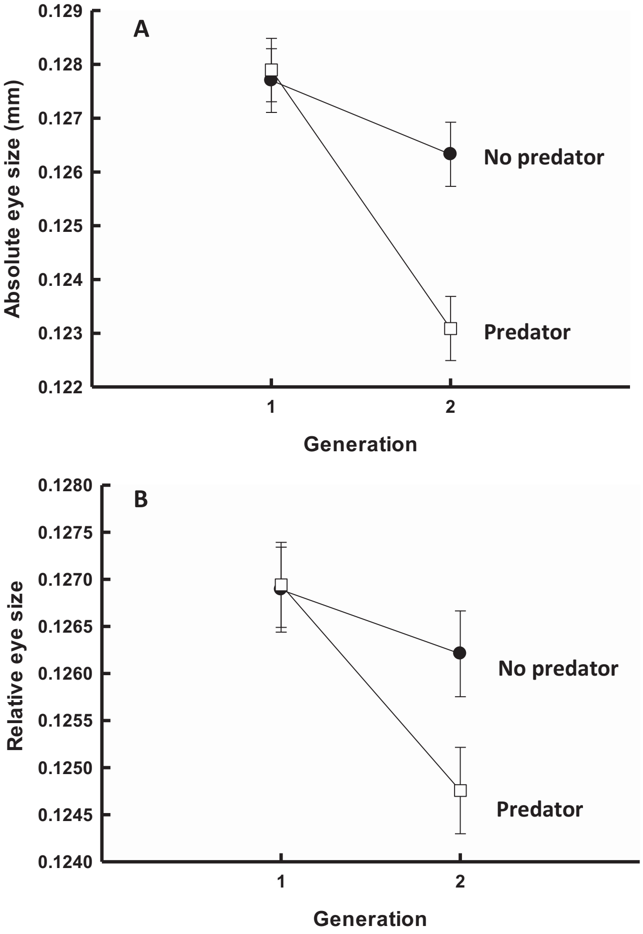

Figure 2. Predator-induced transgenerational plasticity in (A) absolute and (B) relative eye size. "Predator" and "No predator" denote the treatments where *Daphnia* were raised in the presence and absence of alewife predator cues in generation one, respectively. We found a significant ($P < 0.05$) "predator treatment x generation" interaction. Small differences were observed between the predator and nonpredator treatments in generation 1. Maternal exposure to predator cues lead to the expression of a smaller eye size in generation 2. Error = ± 1 SE.

had a relative eye size that was ~3% greater than those found at the bottom of the water column. Because there was a significant "lake type by depth" interaction, we performed tests of simple main effects to further evaluate eye size differences between populations separately for each depth (upper and lower). We used a Bonferroni correction to adjust our *P*-values for multiple comparisons. Because these tests made two comparisons (upper and lower depths), we considered *P*-values < 0.025 as "significant" (*P*-value correction: 0.05/2 = 0.025). Our results showed that differences in relative eye size between *Daphnia* from lakes with anadromous and no alewife were nonsignificant in the upper ($F_{1,3.065} = 0.285$, $P = 0.630$) and lower ($F_{1,3.117} = 1.091$, $P = 0.370$) depths. We also observed a marginally nonsignificant ($0.05 < P < 0.10$) "lake type by depth" interaction for absolute eye size (Fig. 3, Table 2).

Depth effects

We found strong differences in absolute, but not relative, eye size between depths (Fig. 3, Table 2). Absolute eye size in *Daphnia* at the top of the water column was 7% smaller than those found at the bottom of the water column, irrespective of lake type (Fig. 3).

Lake type effects

Wild-caught *Daphnia* from anadromous and no alewife lakes did not differ significantly in absolute or relative eye size (Table 2).

DAPHNIA DENSITY VERSUS DEPTH IN LAKES

Daphnia densities differed by lake type ($F_{1,3.099} = 30.640$, $P < 0.05$). *Daphnia* were approximately seven times more abundant in anadromous lakes when compared to no alewife lakes (Fig. 3), consistent with previous studies completed during the spring season (Post et al. 2008). We found no differences in densities across depths ($F_{3,8.314} = 0.662$, $P = 0.598$), nor did densities vary across depths between lake types ($F_{3,8.252} = 0.681$, $P = 0.588$) or among lakes (Wald $Z = 0.976$, $P = 0.329$).

Discussion

We observed strong genetically based differences in absolute and relative eye size of *Daphnia* from lakes with contrasting fish predator communities (Fig. 1). Our laboratory studies showed that increased predation by alewives, irrespective of alewife phenotype, is associated with the evolution of a larger eye and that the absence of alewife is associated with the evolution of a smaller eye in *Daphnia* (Fig. 1). These trends were repeatable across multiple replicate lakes in our laboratory experiments (Fig. 1). Such results oppose the prediction that visually oriented predators target pigmented characteristics and should therefore drive the evolution of a smaller eye. However, these same experiments also revealed that the direction of predator-induced transgenerational plasticity is opposite to the trajectory of evolution; predators induce a smaller eye in offspring (Fig. 2). Further, wild-caught *Daphnia* from lakes with anadromous and no alewife did not differ in eye size (Fig 3). Taken altogether, these results suggest that the evolutionary ecology of shifts in eye size is more complex than previously thought. Below, we more fully consider (1) why shifts in predation intensity may lead to evolved differences in eye size, (2) why we see strong patterns of divergence in our laboratory experiments, but not in our wild-caught specimens populations, and (3) the role of plasticity in evolutionary shifts in eye size.

There are several explanations for the observed genetically based shifts in eye size between *Daphnia* from lakes with anadromous and landlocked alewife. First, increased eye size has been

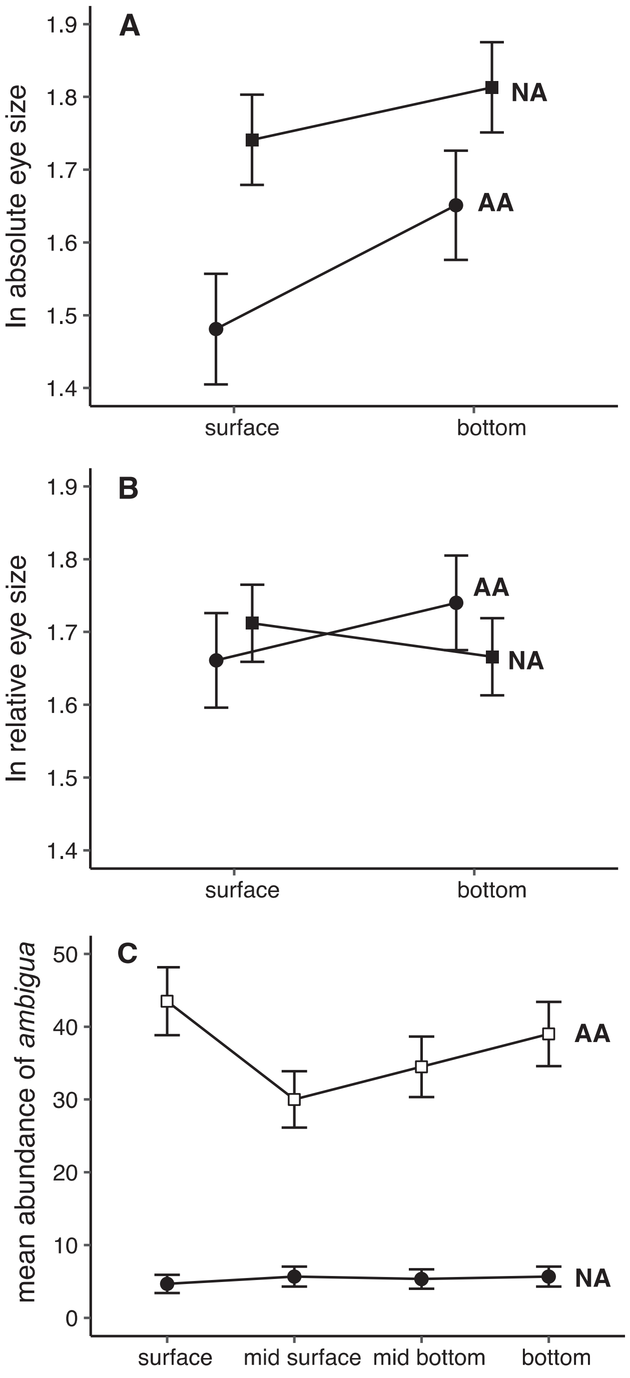

Figure 3. Variation in (A,B) eye size and (C) average densities across depths of wild-caught *Daphnia* populations. We found a moderately nonsignificant ($0.05 < P < 0.10$) and significant ($P < 0.5$) "lake type x depth" interaction for absolute and relative eye size, respectively. In panels A and B, circles represent the mean eye size of *Daphnia* collected from anadromous alewife (AA) lakes and squares represent the mean eye size of *Daphnia* collected from no alewife lakes (NA). In panel C, open squares represent mean abundance of *Daphnia* in anadromous alewife lakes, and closed circles represent mean abundance of *Daphnia* in no alewife lakes. Error bars represent ± 1 SE.

shown to enhance anti-predator capabilities in other organisms (Møller and Erritzøe 2010; Nilsson 2009). It is thus plausible that intense predatory mortality imposed by alewife selects for improved detection and avoidance of fish predators and, in turn, a larger eye size. Second, *Daphnia* commonly respond to the presence of fish predators by migrating vertically to deeper depths during the day to avoid visually oriented fish predators (Boersma et al. 1998; Cousyn et al. 2001). Light availability covaries negatively with depth. If *Daphnia* in lakes with alewife exhibit stronger behavioral responses and spend an increased amount of time in a dimmer environment, then such a response may also select for a larger eye. This is because a larger eye may improve the ability of *Daphnia* to orient themselves and/or forage in dim environments. Our results suggest that indirect selection due to a diminished light environment is not the primary driver of the differences in eye size between alewife and no alewife lakes. There was no evidence that *Daphnia* aggregate at the bottom of lakes to avoid increased predation by alewives (Fig. 3). Instead, *Daphnia* appeared to be distributed sporadically throughout the water column in anadromous lakes and at consistently low densities in no alewife lakes, irrespective of depth. However, we did find contrasting patterns of eye size variation as a function of depth in anadromous versus no alewife lakes; eye size increases with depth in anadromous lakes but decreased with depth in no alewife lakes (Fig. 3). Even though these differences in eye size at the surface and bottom of lakes between lake types were not significant, our results indicate that eye size was slightly larger in *Daphnia* found at the bottom of anadromous alewife lakes when compared to *Daphnia* from the bottom of the water column in no alewife lakes. Therefore, an alternative possibility is that the observed differences in eye size in alewife versus no alewife lakes are driven by predator avoidance behavior; individuals with a larger eye size may be better equipped to see predators and therefore are found deeper in the water column. Indeed, recent work has shown that *Daphnia* have an optomotor response and are therefore able to respond to visual cues, such as a predator (Hathaway and Dudycha 2018). For example, *Daphnia* should be able to see a small bluegill (∼10 cm) from at least 35 centimeters away, allowing for ample time to induce an anti-predator response and sink to a deeper area of the water column to evade the predator (Hathaway and Dudycha 2018). This explanation should be interpreted cautiously as previous work on Blueback herring (*Alosa aestivalis*) has suggested that herring use a "swim and search" behavior whereby they search above their swimming track and swim up to their prey once it is identified. This suggests that prey items are not directly in front of the herring until right before they are consumed (Janssen 1981, 1982). If alewife exhibit similar behaviors, then it is unlikely that *Daphnia* are able to detect their predator early enough to induce an anti-predator response. While we hypothesize that predator avoidance behaviors may explain divergence in eye size of *Daphnia*, tests of eye size as it correlates to predator avoidance and overall visual ability in *Daphnia* are a critical next step.

While there are several explanations as to why evolving a larger eye may be advantageous, there are alternative justifications as to why a smaller eye may be favored in lakes that lack alewives. As stated earlier, we interpret differences in relative eye size as evidence of energetic allocation toward the visual system and absolute eye size as a metric for visual capability. Interestingly, in our laboratory study we observed differences in both absolute and relative eye size, suggesting that both visual ability and allocation towards the sensory system has diverged across lake types (Fig. 1). Eyes are a metabolically expensive organ to develop and maintain (Laughlin 2001; Wong-Riley 2010; Moran et al. 2015). It is therefore possible that in no alewife lakes the benefits of developing a larger eye size do not outweigh its costs. If a larger eye improves *Daphnia* ability to visualize an alewife predator, then perhaps a larger eye is not beneficial in lakes where alewife is absent. We do not know the evolutionary history of these *Daphnia* populations, and therefore cannot make definitive conclusions as to whether *Daphnia* evolve a larger eye size because of the presence of predators or if *Daphnia* evolve a smaller eye size due to the absence of predators.

While our laboratory experiments revealed significant differences in eye size across lakes with and without alewife predators, we failed to observe these differences in our wild-caught surveys (Fig. 3). Our laboratory experiment isolates genetically based differences in eye size, as *Daphnia* were reared over two generations in a common garden, removing both maternal and environmental influences. This suggests that differences in *Daphnia* eye size across lake types are genetically based. But, why did we fail to observe this pattern in our wild-caught populations? There are several explanations for this result. First, wild-caught *Daphnia* are influenced by a multitude of uncontrolled environmental factors that have the potential to influence eye size, such as variation in food, competition, predators (vertebrate and invertebrate), and water clarity. Second, each spring, *Daphnia* populations reestablish from diapausing eggs found in the sediment. The spring is therefore characterized by tremendous genetic variation in the trait values and trait plasticity of *Daphnia*. This is important because we collected *Daphnia* prior to *Daphnia* experiencing significant predatory selection by alewife. Ultimately, these results beg the question, “are alewife actually selecting for differences in eye size in the wild?” To determine if alewife are selecting for shifts in eye size, future work could compare population differences in *Daphnia* eye size before and after YOY alewife begin feeding on *Daphnia ambigua.*

The patterns of transgenerational plasticity revealed in our laboratory experiments provide insight as to why we failed to detect signatures of local adaptation in eye size in our wild-caught samples. In our lab experiments, exposure to predator cues during development did not induce changes in eye size (Fig. 2). That is, there was no evidence for developmental or within-generation plasticity (Walsh et al. 2015). We did, however, detect an influence of maternal predator cue exposure on eye size in the following generation. Parents that were reared in the presence of predator cues in generation one produced offspring with significantly smaller eyes in generation two (Fig. 2). Surprisingly, the direction of the plastic response (smaller eyes) and evolutionary response (larger eyes) are in opposite directions. Assuming *Daphnia* exhibit similar predator-induced transgenerational responses in the wild, then it is not surprising that we failed to observe a difference in eye size in our wild-caught samples. Interestingly, these findings are in the opposite direction of other studies that have compared the directionality of the evolutionary and plastic response. For example, studies on the Trinidadian guppy show that guppies from low predation environments have evolved an offspring size that is larger than guppies from high predation environments (Reznick et al. 1996; Bashey 2006). When female guppies were fed food rations similar to what is experienced in low predation sites (low food availability) versus that of high predation sites (high food availability), female guppies fed the low food treatment produced larger offspring than those fed a higher food level, irrespective of environment of origin (Reznick and Yang 1993; see also Beston et al. 2017a,b). However, these experiments differ from our own in that these studies used (1) different food treatments, rather than differential exposure to predators, and (2) displayed evidence of within generation plasticity, rather than transgenerational plasticity. Of course, we cannot conclusively say which of these explanations, if any, are the reason we observed different patterns in eye size in wild-caught versus laboratory experiments and further investigations are warranted.

Research testing the influence of predator-induced mortality on evolution of eye size is beginning to accumulate (Glazier and Deptola 2011; Brandon et al. 2015; Beston et al. 2017a). Similar to the results of the current study, populations of amphipods (*Gammarus minus*) that cooccur with numerous fish predators exhibited larger eyes than amphipods from freshwater springs with fewer or no predators (Glazier and Deptola 2011). It is unclear if these differences are environmentally or genetically based, but Glazier and Deptola (2011) suggested selection for enhanced detection and avoidance of predation was the driver of the observed differences in eye size in this system (Glazier and Deptola 2011). Beston et al. (2017a) demonstrated that increased predation by fish predators is associated with the evolution of a smaller eye size in a killifish (*Rivulus hartii*) on the island of Trinidad. Such a result is consistent with the hypothesis that predators target larger eyes. It is noteworthy that the association between predators and observed shifts in eye size were in opposite directions in amphipods (Glazier and Deptola 2011) and killifish (Beston et al. 2017a) despite these organisms being found in similar environments (freshwater springs vs freshwater streams). These contrasting results may foreshadow that the trajectory of eye size evolution is dependent upon specific characteristics of the organism and environment, and that selection on eye size may operate differentially depending upon eye type (i.e., camera eye vs a compound eye).

We have previously showed that the form of alewife predation leads to divergence in life history traits of *Daphnia* (Walsh and Post 2011, 2012; Walsh et al. 2014; Walsh et al. 2016). *Daphnia* from lakes with anadromous alewife grow faster, mature earlier, and invest more heavily into reproduction than *Daphnia* from lakes with landlocked alewife (Walsh and Post 2011). *Daphnia* from lakes with anadromous versus landlocked alewife also differ in their sensitivity to alewife predator cues (i.e., phenotypic plasticity) (Walsh and Post 2012; Walsh et al. 2016). These shifts in trait values and trait plasticity are a hypothesized adaptation to differences in the predictability and temporal dynamics of anadromous versus landlocked alewife predation (Walsh and Post 2011, 2012). Interestingly, we did not observe differences in relative eye size between *Daphnia* from lakes with anadromous and landlocked alewife (Fig. 1). Such results argue that the evolution of *Daphnia* eye size is driven by overall increases in alewife predation and is not dependent upon temporal variation in the nature of this mortality.

ALTERNATIVE ECOLOGICAL DRIVERS OF EYE SIZE EVOLUTION

It is important to note that we cannot ultimately eliminate a role for light availability in the evolution of eye size. For instance, it is plausible that eye size covaries with depth in lakes with anadromous alewife because a larger eye enhances foraging and rates of energy intake in low light environments. Or perhaps, overall light availability differs between lakes with and without alewife due to the cascading effects of alewife predation on phytoplankton abundance. For example, *Daphnia* are found at lower abundances during the spring and summer in lakes with landlocked alewife and are found at high abundances in the spring but absent from the water column in the summer in anadromous alewife lakes (Post et al. 2008; Walsh et al. 2012). Lower zooplankton abundances are, in turn, associated with a higher phytoplankton abundance (Post et al. 2008). Though, *Daphnia* were more abundant in lakes with anadromous alewife in our wild-caught samples (Fig. 3) and secchi depth measurements taken during sampling of wild-caught populations did not vary between anadromous alewife and no alewife lakes (Table S1). It should also be noted that previous experiments completed on *Daphnia* sp. under different light conditions showed that bright versus dim environments had little consistent effect on eye size (Brandon and Dudycha 2014), but experimental tests of the importance of predation versus light on the evolution of eyes in this system are now needed.

Other studies have suggested that resource availability and competition intensity may be important players in the evolution of eye size (Brandon and Dudycha 2014). For example, Beston and Walsh (in revision) show that *Rivulus* with a larger eye size in high competition environments grow faster than *Rivulus* with a smaller eye size, suggesting that a larger eye size leads to improved foraging ability. Brandon and Dudycha (2014) show that *Daphnia* reared on low food resources had smaller absolute and relative eye size, pointing to a role for resource limitation in the evolution of sensory systems. However, shifts in competition and resource availability seem an unlikely explanation for our current results. *Daphnia* from anadromous alewife populations experience high densities, and likely intense competition for resources, prior to predation by YOY alewife in the spring (Post et al. 2008), while *Daphnia* from landlocked alewife populations experience consistently low densities and low competition. *Daphnia* from no alewife lakes reach similar densities and likely experience similar levels of competition as anadromous alewife *Daphnia*, but this typically occurs during the late summer (Post et al. 2008). If competition, rather than predation, were the key driver of eye size evolution in this system, then we would expect that eye size should differ between anadromous alewife and landlocked alewife lakes.

CONCLUSIONS

Here, we demonstrated that increased predation by a strong fish predator is associated with evolutionary shifts in the eye size of *Daphnia* (Fig. 1). Follow up tests using wild-caught populations suggest that these differences are not the result of shifts in light availability mediated by changes in *Daphnia* behavior; we instead hypothesize that these differences are a response to selection for enhanced detection of predators. These results build upon recent comparative studies illustrating associations between predators and eye size evolution (Glazier and Deptola 2011; Beston et al. 2017a). Given the idiosyncratic nature of the results of this work, experiments are now needed to better understand how and why predators shape the evolution of eye size in nature.

AUTHOR CONTRIBUTIONS

M.R.W. and J.L.D. conceived the presented idea. M.R.W., D.M.P., and S.M.B. contributed to the design and execution of experiments. S.M.B. and M.R.W. analyzed the data. All authors contributed to the writing of this manuscript.

ACKNOWLEDGMENTS

We thank Kelsey Biles, Nathan Campbell, Ishrat Durdana, Taryn Flink, Julian Holmes, Kaitlyn Howell, Michelle Packer, Jennifer Nguyen, and Mina Wilson for assistance in the laboratory. We thank Andrew MacDonald and Larry Bowman for their assistance in the laboratory and field, and Jared Goos for his insight. S.M.B. was supported by a fellowship from the American Association of University Women (AAUW) during the writing of this manuscript. Comments from editor Keyne Monro and an anonymous reviewer greatly improved the quality of this article. This work was funded by the National Science Foundation DEB-1544356, IOS-1651613 awarded to M.R.W. and DEB-1556848 awarded to D.M.P.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.4dh535d.

CONFLICT OF INTERESTS

The authors have no conflict of interests.

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Associate Editor: K. Monro
Handling Editor: M. R. Servedio

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Secchi depths across lakes and lake types.