THE EVOLUTION OF COMPLEXITY: TESTS OF THE ECOLOGICAL DRIVERS OF EYE SIZE EVOLUTION IN NATURE

by

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DEDICATION

For my grandfather, Robert Ehrlich

ABSTRACT

THE EVOLUTION OF COMPLEXITY: TESTS OF THE ECOLOGICAL DRIVERS OF EYE SIZE EVOLUTION IN NATURE

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Organisms exhibit extensive variation in eye size and structure across the animal kingdom. The long standing hypothesis is that divergent ecological selective pressures drive and maintain this variation. While multiple studies have explored variation in eye size across species, far fewer studies have explored how ecological factors shape the evolution of eye size within species. Additionally, the vast majority of studies to date have evaluated light availability as a key selective force in the evolution of the eye while far fewer studies have addressed the role of alternative ecological selective pressures, such as predation and competition. My dissertation examines (1) how variation in ecological pressures, specifically predation and competition, can drive evolutionary shifts in eye size within species, and (2) the repeatability of these patterns across systems and organisms. First, I explore how ecological factors, including predation, competition, resource availability, and light availability influence the evolution of eye size in natural populations of Trinidadian killifish *Rivulus hartii*. In my second chapter, I evaluate the fitness correlates of shifts in eye size in killifish from high predation and high competition environments to determine the relationship between eye size, survival, and growth. In my third chapter, I test the mechanistic basis of patterns observed between eye size and growth rate in

small scale mesocosm experiments. Finally, I use *Daphnia ambigua* from lakes in Connecticut that differ in predation intensity to determine the repeatability of ecologically driven shifts in eye size evolution.

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CHAPTER 1: INTRODUCTION

"To suppose that the eye… could have been formed by natural selection, seems, I freely confess, absurd to the highest degree." – Charles Darwin, *The Origin of Species by Means of Natural Selection,* pg. 155

Understanding the evolution of complex organs is a long-standing goal in evolutionary biology (Darwin 1859; Land and Fernald 1992; Nilsson and Pelger 1994; Reznick et al. 2002). In particular, the intricate structure of the eye is frequently presented as an example that challenges our understanding of evolution by natural selection (Darwin 1859; Goldsmith 1990; Nilsson and Pelger 1994). Eyes have evolved independently multiple times and vary tremendously in both size and structure across taxa (Land and Fernald 1992; Bauer et al. 1998; Fernald 2004a,b; Lisney and Collin 2007; Land and Nilsson 2012; Caves et al. 2017). The long-standing assumption has been that observed differences in eye size are the result of divergent patterns of ecologically driven selection (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; Brischoux et al. 2010; Møller and Erritzøe 2010; Veileux and Lewis 2011). Eyes are an indispensable organ for a vast majority of organisms and are nearly ubiquitous in vertebrate species. Increases in eye size are correlated with improved vision (Motani et al. 1999; Kiltie 2001; Thomas et al. 2006; Ross and Kirk 2007; Hall 2008; Moller and Erritzoe 2010; Land and Nilsson 2012; Caves et al. 2017). Additionally, shifts in eye size and visual capabilities have been associated with shifts in mating, foraging, and predator avoidance behavior (Huber et al. 1997; Garamszegi et al. 2002; Thomas et al. 2006; Somanathan et al. 2009; Hall and Ross 2007; Liu et al. 2012; Brandon et al. 2015; McCoy et al. 2015). Light availability has long been viewed as an important selective force on the evolution of eyes across macroevolutionary timescales (Hiller-Adams and Case 1988; Werner 1969; Jones et al. 1992). However, it is becoming increasingly clear that eye size can vary within species (Beston et al. 2017; Beston et al. in press; Brandon et al. 2015; Glazier and Deptola 2011; Abecia et al. 2018) and that this variation may be driven by an array of ecological forces. Such variation provides the raw materials for contrasting ecological conditions to drive evolutionary shifts in eye size and structure. Yet tests for microevolutionary shifts in eye size have been conspicuously absent. Within this introductory chapter I (1) outline the link between eye size and visual aptitude, (2) broadly discuss ecological drivers of eye size evolution, (3) provide empirical evidence of a lack of within species studies on eye size, and (4) present two natural systems that have become key players in understanding the evolution of eye size in nature.

The link between eye size and visual aptitude

There now exists a large body of literature that has established a clear link between eye size and visual acuity (Kiltie 2001; Veilleux and Kirk 2014; Caves et al. 2017). Visual acuity (sometimes described as resolution) is the ability of an organism to resolve spatial detail. Larger eyes have been associated with greater acuity largely due to the strong relationship between eye diameter and focal length (Hughes 1977; Kirschfield 1976). A key factor that dictates an organism’s visual acuity is the angular width of the area viewed by each photoreceptor in the eye. The angular width of a photoreceptor is equal to the diameter of the photoreceptor divided by the focal length of the eye. Thus, increases in focal length can result in smaller angular widths leading to greater acuity. On the other hand, when photoreceptors have a smaller angular width they can only collect light from a smaller angular area which can result in decreased visual sensitivity (Land and Nilsson 2012). However, it is possible to mediate this trade-off between sensitivity and acuity by increasing total eye size (and therefore the focal length of the eye). An increase in total eye size allows for acuity or sensitivity to increase without the other decreasing. Thus, evolutionary shifts in eye size should be dictated by behaviors and ecological conditions that enhance fitness and are strongly related to visual acuity and sensitivity.

Ecological drivers of the visual system

It is well established that an animal's eye is shaped by its environment. Multiple studies have identified both biotic and abiotic features of the environment that relate to eye size. These include studies that examine the biotic and abiotic factors that shape the eye. For example, multiple studies have addressed the direct and indirect consequences of light availability on eye size across habitats (e.g. depth gradients in aquatic environments) and as it relates to activity patterns (diurnality versus nocturnality), as well as turbidity and eutrophication in aquatic environments (Caves et al. 2017; Bartels et al. 2016; Dugas and Franssen 2012). Other studies have focused on how predators are related to shifts in eye size within prey (Beston et al. 2017; Beston et al. in press; Glazier and Deptola 2011). From a qualitative standpoint, it appears that the vast majority of literature on the ecological correlates of shifts in eye size focuses on factors both directly and indirectly related to light.

Database compilation. To determine what percentage of studies focus on different ecological variables as they relate to eye size, I completed a literature search in Web of Science at the beginning of March 2019 using the search term "eye size" . A total of 710 entries were returned and I identified studies that focused on the role of ecological variables in shifts in eye size both across and within species. I did not include book chapters or presentation abstracts. In order to be included in the database, studies had to measure some aspect of eye size (i.e., eye diameter, lens diameter, focal length, etc.). This resulted in a database of 104 papers published between 1985-2018. I then categorized studies by the ecological variable in question. This resulted in five broad categories: diet, activity pattern, habitat, light, or predation. Some studies encompassed more than one category. Studies within the diet category asked questions about eye size as it related to different food levels, food consumed, trophic levels, and foraging technique (e.g. sessile versus mobile prey). Activity pattern reflected studies that compared eye size of diurnal, nocturnal, and crepuscular individuals or species and/or the time at which certain activities were initiated. Studies within the habitat category focused on animal location within a given ecosystem (e.g. arboreal versus ground dwelling), different depths for aquatic organism (e.g. littoral versus pelagic), as well as temperature and other habitat characteristics such as eutrophication or turbidity in aquatic ecosystems. Light studies evaluated shifts in eye size as it related to light levels. This category also included studies that compared cave dwelling to non-cave dwelling organisms. Finally, the predation category encompassed studies that evaluated eye size in prey as it related to predators.

Results. Of these studies, 30% evaluated eye size as it related to activity pattern (Fig. 1), while another third of these studies focused on variation in habitat (Fig. 1). It is important to note that of the studies that were categorized as related to "habitat", most of these studies evaluated eye size as it related to depth in the water column. If we consider all categorizes that directly and indirectly (activity pattern, habitat categories) assess light as it relates to eye size, then well over two thirds of the current literature on eye size focuses on light as a key factor in eye size. Studies that address alternative factors, such as diet, 15.4%, and predation, 5.4%, are notably fewer .

Studies on eye size within and across species

The vast majority of studies to date have evaluated patterns in eye size across species, while notably fewer have focused on how ecological factors can shape patterns within species. Using the same database compiled above, I categorized studies into two groups: (1) those that made comparisons of eye size between populations (or subjected individuals of the same species to different experimental treatments) or (2) those that made comparisons across species. The number of studies that evaluate eye size across species is nearly double that of studies that focus on shifts in eye size within species (Fig. 2). While there has been an overall increase in studies that focus on eye size over the past four decades (Fig. 2), studies evaluating shifts in eye size within species are still notably less than those that focus on this variation cross species (Fig. 2).

I also quantified the number of articles published on specific taxon as it relates to eye size. I categorized studies into those that evaluated eye size in arthropods (insects and crustaceans), reptiles, fish, mammals, and birds. The majority of studies on eye size have been completed in arthropods and fishes (Fig. 3). This is not surprising as there is a rich history of visual ecologists specifically focusing on vision in fishes and crustaceans (Warrant and Locket 2004; Moeller and Case 1995; Warrant 2000; Denton et al. 1972).

Study systems

As noted above, the majority of studies on eye size have been completed on fishes and arthropods (Fig. 3), but research that evaluates trends of eye size evolution within species are lacking (Fig. 2), as are studies that explore how predation can shape the evolution of prey eye size (Fig. 1). In my dissertation I focus on two aquatic systems, Trinidadian streams and lakes in

Connecticut, that naturally vary in predator communities across environmental gradients to understand how predation and other ecological factors can shape the evolution of eye size within species.

Killifish in Trinidadian streams. Streams on the island of Trinidad present the opportunity to test the ecological drivers of evolutionary change in a natural setting. *Rivulus hartii* (Fig. 4A) are widespread across stream communities on the island of Trinidad, as well as the neighboring island of Tobago (pers. comm. D. Phillip). *Rivulus* are ubiquitous across riverine habitats on the island due to their impressive dispersal capabilities and are found across a diversity of fish communities that vary in predation intensity (Gilliam et al. 1993; Fraser et al. 1999; Furness and Reznick 2014); (1) 'high predation' sites where *Rivulus* are preyed upon by large, piscivorous fish (i.e. *Crenichichla frenata* and *Hoplias malabaricus*), (2) 'low predation' sites where juvenile *Rivulus* are subject to predation by guppies until they attain a size which exceeds the gape of the guppy predator, and (3) '*Rivulus*-only' sites where *Rivulus* are the only fish species present. Waterfalls truncate these sites into discrete communities preventing the movement of predators from lowland, high predation sites into the headwater *Rivulus*-only tributaries. These communities are tens of meters from one another, share similar physical habitat, and do not differ significantly in water quality variables, such as temperature and dissolved oxygen (Walsh and Reznick 2009). Importantly, *Rivulus* are found at their lowest densities in sites with large predators because they experience higher mortality rates in high predation versus *Rivulus*-only sites (Walsh and Reznick 2008). Similarly, *Rivulus* are three times less abundant in low predation localities than *Rvulus*-only sites because guppies elevate rates of larval mortality (Fraser and Lamphere 2013; Furness and Reznick 2014). Previous work has revealed that this increase in mortality is associated with evolved differences in life history traits between sites that differ in the presence and absence of predators (Walsh and Reznick 2008, Walsh and Reznick 2010).

Rivulus are egg layers and reproduce via external fertilization (Fraser and Gilliam 1992; Gilliam et al. 1993). *Rivulus* are thought to reproduce year round in the field (Fraser and Gilliam 1992), but appear to be most reproductively active towards the end of the wet season on the island (Beston and Walsh, pers. obs.). In the laboratory, fish can lay eggs on a daily basis (Beston, pers. obs.). At time of hatching, *Rivulus* fry are 6 mm (Fig. 4B) and mature at approximately 30-35 mm. Mature male *Rivulus* are easily identifiable due to the development of white bars on the top and bottom edges of their caudal fin (Fig. 4C), while females typically display a more pronounced eye spot than males.

Daphnia from Connecticut lakes. The waterflea, *Daphnia ambigua,* is found across a diversity of lakes in Connecticut that differ in predator community (Brooks and Dodson 1965; Palkovacs and Post 2008; Post et al. 2008). Notably, these lakes differ in the presence and duration of the dominant plankton predator, the alewife (*Alosa pseudoharengus)*. We categorize these lakes as (1) anadromous alewife, (2) landlocked alewife, and (3) no alewife lakes (Post et al. 2008). Anadromous alewife lakes are home to alewife between early spring, when adult alewife enter the lake from the ocean to spawn, and late summer when all alewife leave the lake and return to the ocean until next spring. In landlocked alewife lakes, alewife are permanent freshwater residents. Alewife from both lakes represent a strong predator of *Daphnia.* However, predation by alewife in anadromous alewife lakes is highest during late spring, while predation by alewife in landlocked lakes is intense year round. *Daphnia* are consistently rare in these lakes as a result. In anadromous alewife lakes, alewife migrate to the lakes from the ocean between March and April. Young-of-the-year (YOY) alewife then prey upon *Daphnia* intensely during the late spring and summer before leaving the lakes in autumn. YOY are gape limited until ~June but engage in such intense predation that most *Daphnia* are depleted from the water column in these lakes by the end of June, early July (Post et al. 2008). While these lakes differ in alewife presence and duration of predation, these lakes do not differ in fish community composition or potentially confounding environmental variables such as size, depth, productivity or alewife biomass (in landlocked and andromous alewife lakes) (Post et al. 2008; Walsh and Post 2011).

Daphnia are an ideal organism in which to study evolutionary change as they are easy to culture in laboratory, have a short generation time, and have many quantifiable traits, including eye size. Multiple studies have explored how various ecological factors, such as predation, temperature, diet, and light, influence the evolution and plasticity of a variety of traits (Goos et al. 2018; Whittington and Walsh 2015; Brandon and Dudycha 2014), including life history (Walsh and Post 2011; Walsh et al. 2015, 2016), behavior (De Meester and Weider 1999) , and more recently eye size (Brandon and Dudycha 2014; Brandon et al. 2015; Beston et al. in press).

Overview of dissertation goals

As stated earlier, much work has evaluated the connections between various ecological factors and eye size, but the majority of this research has focused on patterns across species and has largely centered on light availability and activity patterns. Far fewer studies have examined how eye size varies within species and what the consequences of this variation are. Thus, my dissertation evaluates how ecological selective pressures, specifically predation and competition, can influence the evolution of eye size within species and determines the repeatability of these within species patterns across species and systems. In my first chapter, I use wild-caught specimens and common garden reared Trinidadian killifish, *Rivulus hartii*, to make connections between eye size, predation intensity, and competition. I also evaluate potential confounding ecological factors, specifically light and resource availability. In my second chapter, I use mark-recapture experiments and determine the link between between eye size, survival, and growth in high predation and *Rivulus*-only sites. I then use small scale mesocosm experiments to test for the underlying mechanism of eye size evolution in *Rivulus*. Finally, I use both laboratory and wild-caught *Daphnia ambigua* from lakes in Connecticut that differ in their exposure to predation to determine if patterns observed in killifish are repeatable across organisms and systems.

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FIGURES

Figure 1. Published studies on eye size evolution and ecological variables by category. A literature search on "eye size" in Web of Science revealed that of the 104 papers that evaluate eye size as it related to ecological factors. 19.2% of these studies evaluated eye size as it related to diet, 30% asked questions of eye size as it related to activity pattern, 30% explored eye size and habitat, 15.4% explored the relationship between eye size and different light levels, and 5.4% evaluated the link between prey eye size and predation pressure.

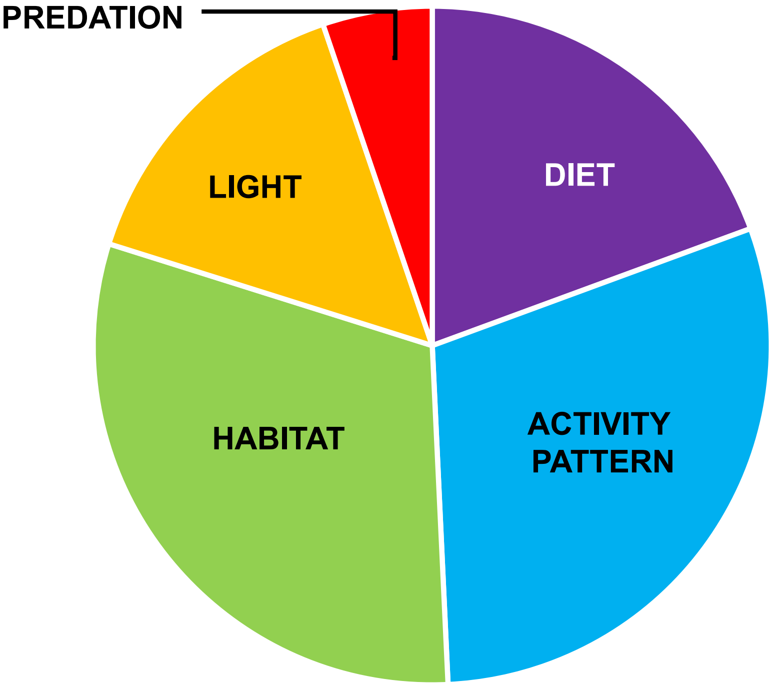


Figure 2. Comparison of studies published on eye size and ecological factors within and across species. Between 1985 and 2018 over 100 studies have investigated eye size as it related to ecological variables. Studies across species (indicated with the blue line) nearly double that of studies within species (indicated with the red line).

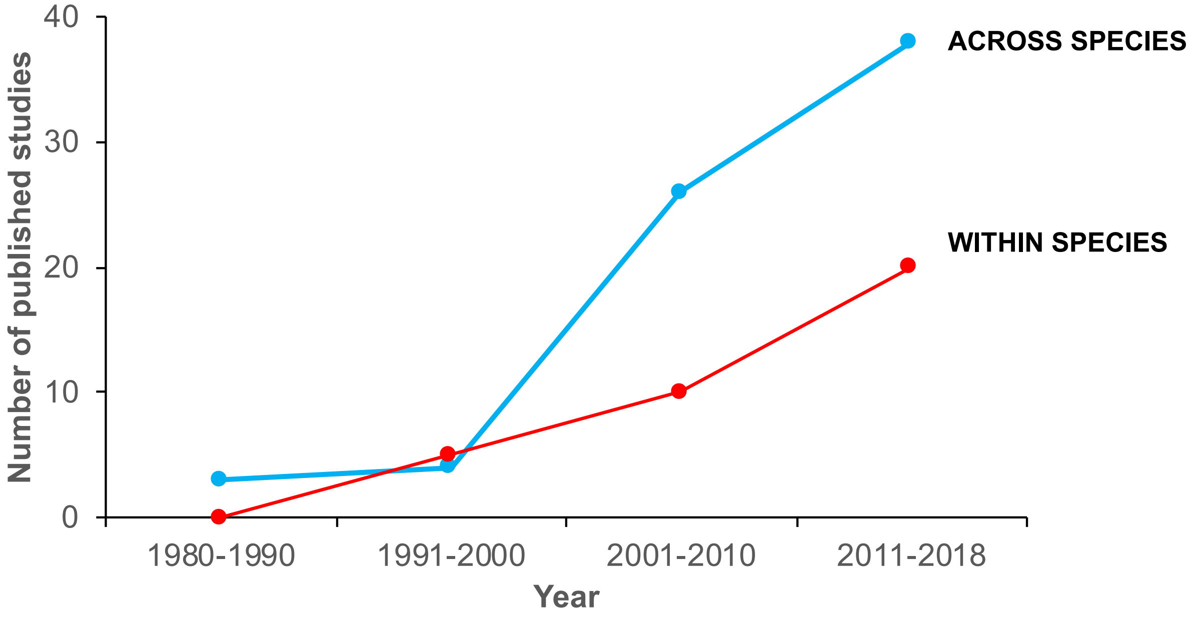


Figure 3. Number of studies completed on eye size of a given taxon. The vast majority of studies on eye size have been completed in fishes (37%) and arthropods (28%). Studies on mammals make up 13% of studies and on birds 16%. Only 6% of studies on eye size have been completed in reptiles.

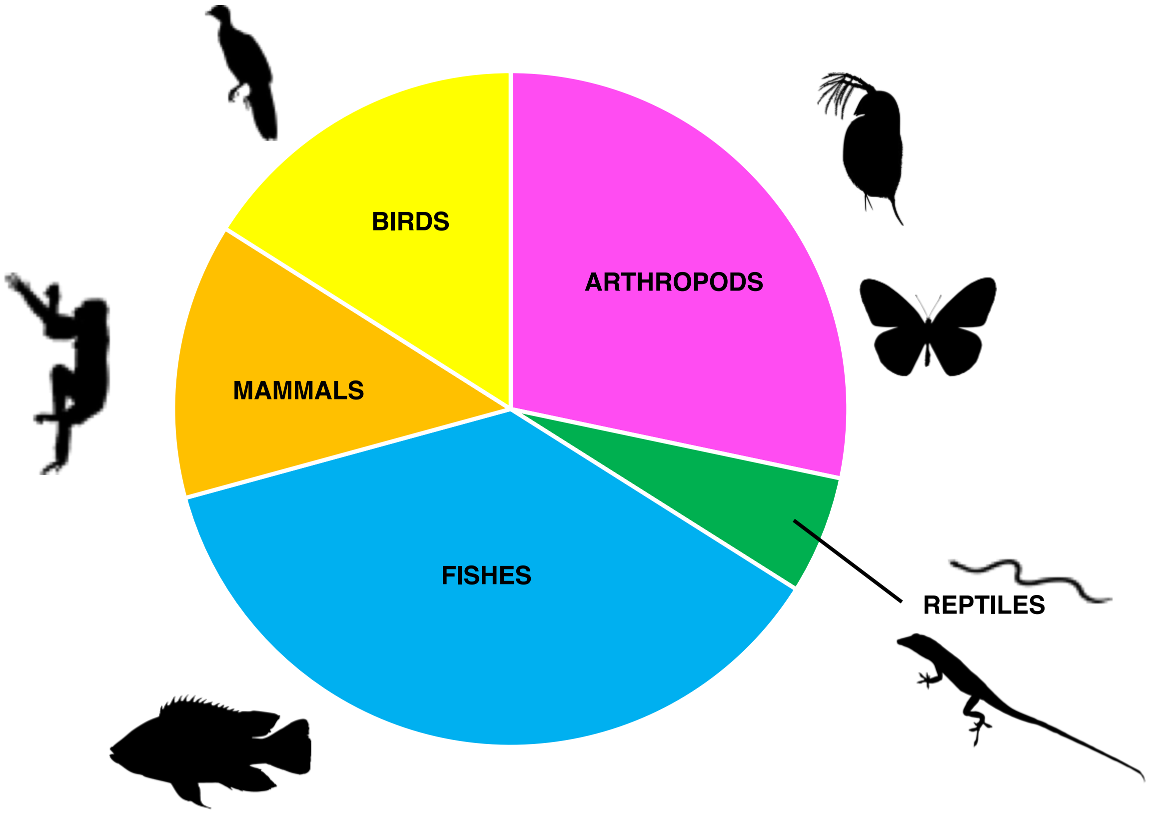
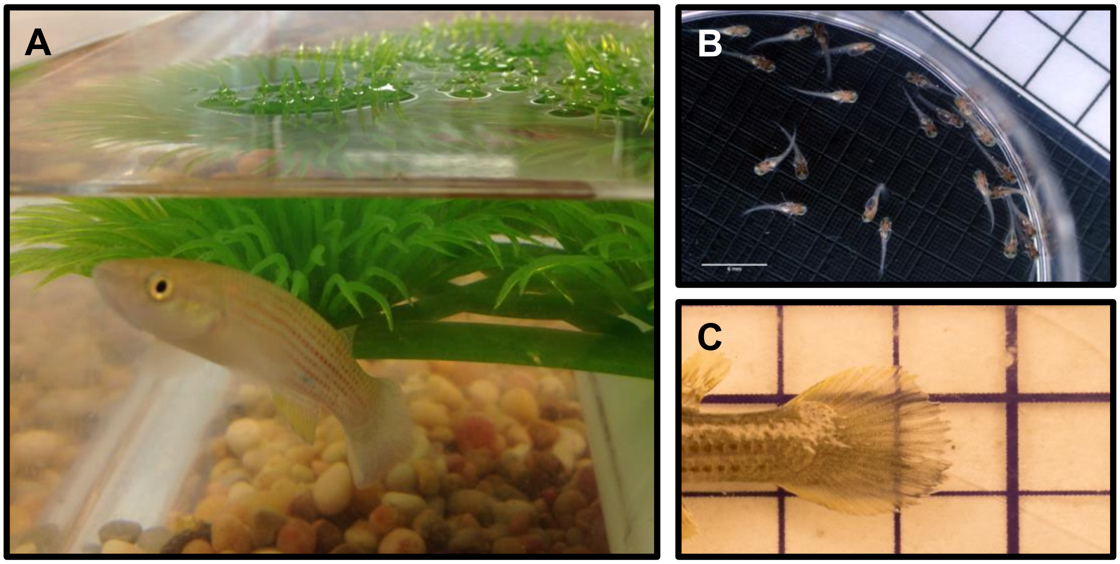


Figure 4. *Rivulus hartii.* (A) Adult *Rivulus* in the lab. (B) *Rivulus* at time of hatching (photograph from Furness and Reznick 2014). (C) Caudal fin of a mature male *Rivulus.*



CHAPTER 2:

THE EVOLUTION OF VERTERBRATE EYE SIZE ACROSS AN ENVIRONMENTAL GRADIENT: PHENOTYPE DOES NOT PREDICT GENOTYPE IN A TRINIDADIAN KILLIFISH[§]

Shannon M. Beston[1], Elijah Wostl[1], and Matthew R. Walsh[1]

Beston, S.M., E. Wostl, and M.R. Walsh (2017) The evolution of vertebrate eye size across an environmental gradient: phenotype does not predict genotype in a Trinidadian killifish. *Evolution*, 71: 2037-2049.

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ABSTRACT

Vertebrates exhibit substantial variation in eye size. Eye size correlates positively with visual capacity and behaviors that enhance fitness, such as predator avoidance. This foreshadows a connection between predation and eye size evolution. Yet, the conditions that favor evolutionary shifts in eye size, besides the well-known role for light availability, are unclear. We tested the influence of predation on the evolution of eye size in Trinidadian killifish, *Rivulus hartii*. *Rivulus* are located across a series of communities where they coexist with visually oriented piscivores ("high predation" sites), and no predators ("*Rivulus*-only" sites). Wild-caught *Rivulus* from high predation sites generally exhibited a smaller relative eye size than communities that lack predators. Yet, such differences were inconsistent across rivers. Second-generation common garden reared fish revealed repeatable decreases in eye size in *Rivulus* from high predation sites. We performed additional experiments that tested the importance of light and resources on eye size evolution. Sites that differ in light or resource availability did not differ in eye size. Our results argue that differences in predator-induced mortality underlie genetically-based shifts in vertebrate eye size. We discuss the drivers of eye size evolution in light of the nonparallel trends between the phenotypic and common garden results.

INTRODUCTION

It is well known that eye size varies tremendously across taxa (Land and Fernald 1992; Bauer et al. 1998; Fernald 2004a, b; Lisney and Collin 2007; Land and Nilsson 2012). The long-standing assumption is that observed differences in vertebrate eye size are the result of divergent patterns of ecologically driven selection (Bauer et al. 1998; Garamszegi et al. 2002; Fernald 2004b; Moser et al. 2004; Thomas et al. 2006; Ross and Kirk 2007; Hall 2008; Somanathan et al. 2009; Moller and Erritzoe 2010; Veilleux and Lewis 2011). This is because eyes are an indispensable organ for the vast majority of vertebrates. In vertebrates, increases in eye size are associated with improved vision (Walls 1942; Hughes 1977; Ritland 1982; Martin 1993; Motani et al. 1999; Møller and Erritzoe 2010; Land and Nilsson 2012; Caves et al. 2017) and shifts in mating, foraging, and predator behavior (Huber et al. 1997; Garamszegi et al. 2002; Thomas et al. 2006; Hall and Ross 2007; Brischoux et al. 2010; Møller and Erritzoe 2010; Liu et al. 2012; McCoy et al. 2015). Light availability has long been viewed as an important selective force on the evolution of eyes and is associated with both increased and decreased eye size across taxa (Von Salvini-Plawen and Mayr 1977; Hiller-Adams and Case 1985; Fernald 2004a, b; Moser et al. 2004; Ross and Kirk 2007; Hall 2008; Tobler et al. 2008; Somanathan et al. 2009; Schmitz and Wainwright 2011; Veilleux and Lewis 2011; Hall et al. 2012; Martinez-Ortega et al. 2014). However, recent work has begun to explore variation in eye size within species (Glazier and Deptola 2011; Pearce and Dunbar 2012; Brandon et al. 2015). This variation provides the raw materials for contrasting ecological conditions to favor evolutionary shifts in eye size and structure, but tests for microevolutionary shifts in eye size are conspicuously absent (but see Brandon et al. 2015).

Predator-induced mortality has been identified as an ecological selective pressure that has the potential to shape the evolution of vertebrate eye size (Brandon et al. 2015). Research has shown that increases in eye size are correlated with enhanced predator avoidance capability (Nilsson 2009; Møller and Erritzoe 2010). This relationship between predators, eye size, and prey behavior leads to the prediction that increased predation should favor the evolution of larger eyes (Møller and Erritzoe 2010; Glazier and Deptola 2011). Conversely, work has also shown that predators target heavily pigmented eyes and may therefore select for the evolution of a smaller eye (Zaret and Kerfoot 1975; Lönnstedt et al. 2013). Despite the known importance of visual capabilities in the face of predation (Freund and Olmstead 2000; Kelley and Magurran 2003; Møller and Erritzoe 2010; Smolka et al. 2011; Hettyey et al. 2012), and other visually driven behaviors that are both directly and indirectly related to fitness (Dobberfuhl et al. 2005; Thomas et al. 2006; Hall 2008; Liu et al. 2012), the extent to which predators exert selection and drive the evolution of eye size is unclear.

Fish communities on the island of Trinidad present an opportunity to test for selection on eye size across an environmental gradient. The Trinidadian killifish, *Rivulus hartii*, is found across a diversity of communities that vary in predation intensity (Gilliam et al. 1993; Fraser et al. 1999; Walsh and Reznick 2008, 2009; Walsh et al. 2011; Furness and Reznick 2014). This includes: (1) “high predation” sites where *Rivulus* are preyed upon by large, piscivorous fish (i.e., *Crenicichla frenata* and *Hoplias malabaricus*), (2) “low predation” locations where adult guppies, *Poecilia reticulata,* prey upon juvenile *Rivulus*, and (3) *Rivulus*-only sites where *Rivulus* are the only species present. These communities are located tens of meters from one another, are similar in structure of the physical habitat, and do not differ significantly in water quality variables, such as temperature and dissolved oxygen (Walsh and Reznick 2009). More importantly, these sites differ in mortality rates. *Rivulus* are found at their lowest densities in sites with large predators because they experience higher mortality rates in high predation versus *Rivulus*-only sites (Walsh and Reznick 2008). Similarly, *Rivulus* are 3 × less abundant in low predation localities than *Rivulus*-only sites because guppies elevate rates of larval mortality (Fraser and Lamphere 2013; Furness and Reznick 2014).

Increased rates of predation are correlated with shifts in other ecological variables that have the potential to exert selection on eye size. High predation environments exhibit a more open canopy and increased light availability than upstream low predation and *Rivulus*-only sites (Grether et al. 2001; Reznick et al. 2001). *Rivulus* in high predation sites also experience increased growth rates when compared to *Rivulus* from *Rivulus*-only locations (Walsh and Reznick 2008, 2009). This increased rate of individual growth likely reflects increased food availability due to the lower abundances of *Rivulus* found in these sites (Walsh and Reznick 2008, 2009). Similarly, *Rivulus* from low predation localities exhibit increased growth rates and experience increased per-capita food availability as adults (Walsh and Reznick 2009, 2010, 2011; Walsh et al. 2011; Fraser and Lamphere 2013). Increased light availability is commonly associated with declines in eye size (Hiller-Adams and Case 1985; Garamszegi et al. 2002; Thomas et al. 2006; Lisney and Collin 2007; Ross and Kirk 2007; Hall 2008; Veilleux and Lewis 2011; Pearce and Dunbar 2012), while higher resource availability is correlated with the production of a larger relative eye size (Brandon and Dudycha 2014). The "direct" (i.e., increased mortality) and "indirect" (i.e., increased food availability) consequences of predation intensity in the Trinidad system have been linked to shifts in life history traits, behavior, and even complex traits such as brain size (Gilliam and Fraser 2001; Walsh and Reznick 2008, 2010, 2011; Walsh et al. 2016; see also Beston et al. 2017). These known connections between predation and the evolution of other components of fitness provide a means to test the role of predation, and the ecological correlates of predation, on eye size evolution.

Here, we evaluated the ecological drivers of eye size evolution in *Rivulus* from high predation, low predation, and *Rivulus*- only sites. Our overarching goal was to test the influence of increased predation on eye size evolution in *Rivulus.* We evaluated our focal populations for differences in relative eye size (i.e., eye size corrected for body size) to determine how increased predation exerts selection on relative eye size. We collected wild-caught *Rivulus* from high predation, low predation, and *Rivulus*-only sites across multiple rivers to test for phenotypic differences in eye size. Because patterns of brain size evolution differ between the sexes in this system (Walsh et al. 2016), and also because brain size and eye size can covary (Garamszegi et al. 2002), we specifically evaluated the "population by sex" interaction to determine if eye size evolves in parallel between the sexes. We also used second generation common garden reared specimens from previously completed work (Walsh and Reznick 2008, 2010, 2011) to determine if there are genetically based differences in eye size among these populations. This prior work reared populations of *Rivulus* under multiple food levels that match known differences in growth to explore the importance of predator mediated increases in resource availability on the trajectory of evolution (see Walsh and Reznick 2008, 2010, 2011). If increased eye size enhances antipredator responses, then we expect that increased predation in high predation environments will favor the evolution of a larger eye size in *Rivulus.* Conversely, if visually oriented predators target larger pigmented eyes, high predation *Rivulus* will exhibit a smaller eye size than *Rivulus* from *Rivulus*-only sites. If the known differences in resources between these sites modify selection on eye size, then we predict that the differences in eye size between high predation and *Rivulus*-only sites will depend upon the controlled levels of food in the laboratory (i.e., significant "population by food" interaction) (Walsh and Reznick 2008).

We then performed complementary comparisons and experiments to test for the potential influence of resource and light availability on eye size evolution. Because predation by guppies is limited to the smallest size-classes of *Rivulus,* and also because *Rivulus* experience per capita increases in food in low predation sites (Walsh et al. 2011; Fraser and Lamphere 2013) comparisons between low predation and *Rivulus*-only sites allow us to isolate the role that resource availability plays in evolutionary shifts in eye size in the absence of predators that can consume adult *Rivulus.* We thus compared wild-caught and second generation common garden reared *Rivulus* from low predation and *Rivulus*-only sites for differences in eye size. Finally, we tested the link between variation in light availability on selection on eye size. The canopy in two *Rivulus*-only sites was thinned in 2007 to experimentally increase light availability and, in turn, resource availability, to test the role of these factors on trait evolution (Grether et al. 2001). We evaluated shifts in *Rivulus* eye size 8–9 years following the experimental thinning of canopy cover in these streams to determine if increased light availability is associated with phenotypic shifts in the eye size of *Rivulus.*

MATERIALS AND METHODS

Wild-caught specimens

We collected *Rivulus* from four rivers (Arima, Aripo, El Cedro, Guanapo) across three populations (high predation, low predation, *Rivulus*-only) to test the influence of predator community on the evolution of *Rivulus* eye size. We expect that *Rivulus* from *Rivulus*-only sites will have smaller eyes than those from high predation sites if a larger eye size is advantageous in the avoidance of predators. We predict the converse if predators selectively prey upon *Rivulus* with larger eyes. Approximately, 30 males and 30 females were collected from each location during May– June 2016. Upon collection, fish were immediately euthanized with MS-222, preserved in 10% formalin, and then stored in 70% ethanol until they were photographed (for assessments of eye size) and measured for total length. Sexually mature male *Rivulus* exhibit white bars on the top and bottom edge of the caudal fin at around 29 mm total length, making them easily distinguishable from females. Therefore, all fish less than 30 mm total length were considered to be juveniles (Walsh and Reznick 2008, 2010; Walsh et al. 2011) and were not included in analyses.

Common-garden experiments

We evaluated second generation common garden reared specimens to determine the extent to which phenotypic differences in eye size among *Rivulus* from high predation, low predation, and *Rivulus*-only sites are genetically based. A series of experiments were performed from 2005 to 2008 that compared the life history traits of *Rivulus* from high predation, low predation, and *Rivulus*-only sites (Walsh and Reznick 2008, 2010, 2011). The experimental methodology is previously published (Walsh and Reznick 2008, 2010, 2011) and is summarized here. The experiments comparing high predation versus *Rivulus*-only and low predation versus *Rivulus*-only sites used different rivers and were completed at separate times. *Rivulus* were collected from *Rivulus*-only and high predation populations from the Arima and Guanapo rivers in July 2005 (i.e., two high predation and two *Rivulus*-only sites) and low predation and *Rivulus*-only sites in the Aripo, Guanapo, and Quare rivers in January 2007 (i.e., three low predation and three *Rivulus*-only sites). Wild-caught females and males were used to establish laboratory stocks (20 to 25 males and females from each locality). To generate the first common garden generation, females and males from the same locality (i.e., same river and same population) were randomly paired and placed in 9-L aquaria with artificial spawning substrate. Eggs from each pairing were collected and incubated in Petri dishes. Once hatched, eight to 12 larvae were reared in 9-L aquaria and fed a diet of liver paste and brine shrimp *nauplii* ad libitum.

The second common garden generation was established using mature females from each lineage from the first generation paired with mature males from a different lineage, but same locality. Offspring from six to eight (high predation/*Rivulus*-only) or eight to 12 (low predation/*Rivulus*-only) unique pairings per population were used. All offspring from each pairing were reared in 9-L tanks and fed ad libitum. After 20 days, eight fish from each pairing were selected at random and each placed in 9-L tanks. Each aquaria lacked gravel, but included a clay pot and artificial spawning substrate. Fish were randomly allocated to two food treatments: (1) a high food treatment or a (2) low food treatment. The high food diet mimicked growth in high predation and low predation localities respectively, while the low food treatment sustained growth rates similar to that observed in *Rivulus*-only locations (Walsh and Reznick 2008; Walsh et al. 2011). Fish were fed appropriate portions of liver paste in the morning and brine shrimp *nauplii* to sustain these divergent rates of growth. Males were reared until maturation and then euthanized and preserved in 5% formalin. All fish were preserved until being photographed and weighed starting in 2014.

Canopy manipulations

We quantified differences in eye size between populations of *Rivulus* from sites where the canopy cover was experimentally thinned to increase light availability versus sites with intact canopy cover. If light availability is an important factor in the plasticity or evolution of eye size, then we expect that eye size should differ significantly between sites with thinned versus intact canopies. The canopy of two *Rivulus*-only sites was thinned to match the light availability typically observed in high predation environments (Grether et al. 2001; Reznick et al. 2001) in July 2007 and July 2008. These “thinned” treatments were paired with two nearby *Rivulus*-only control streams that retained an intact canopy. The thinning of the canopy at the Upper Lalaja site took place in 2007 and is paired with the Lower Lalaja site as its control. The thinning of the canopy in the Taylor River occurred in 2008 and is paired with the Caigual River as its control. In June 2016, approximately 60 *Rivulus* were collected from each site (Caigual, Taylor, Lower Lalaja, and Upper Lalaja), anaesthetized, sexed, photographed, and returned to their site of collection. Eye diameter and total length were measured using ImageJ (Schneider et al. 2012) as described below.

Photography and measurements

All wild caught and common garden fish were photographed on their side using a Canon PowerShot ELPH180 or Nikon CoolPix S610 camera. *Rivulus* collected from locations with canopy manipulations were photographed using a Canon EOS 7D Mark II. We measured the diameter of the eye cavity at the widest part for each photograph. Our assessments of eye size for the common garden fish were limited to males because we did not have archived photographs for females.

Statistical design and analyses

Wild-caught specimens. To determine if relative eye size varied across our focal populations (high predation, low predation, *Rivulus*-only), we used linear mixed models implemented with restricted maximum likelihood (REML) (SPSS version 23). Our model included population (high predation, low predation, *Rivulus*-only), sex, and the "population by sex" interaction as fixed effects and a random effect of population nested within river (Arima, Aripo, El Cedro, Guanapo) to account for heterogeneity in the predator communities among rivers (Table S1). We followed up this analysis with tests of simple main effects to explore the significant "population by sex" interaction (see Results section). For all analyses, absolute eye size was entered as the dependent variable with total length included as a continuous variable and we statistically tested for differences in eye size at the center of this covariate. We ln-transformed eye size and total length.

Common-garden fish. The data for the high-predation versus *Rivulus*-only experiments and low predation versus *Rivulus*-only experiments were analyzed separately because they were performed at different times using different populations. To determine if the differences we observed in our wild-caught specimens were genetic in origin, we evaluated variation in relative eye size from second generation common garden reared specimens between high predation and *Rivulus*-only sites and low predation and *Rivulus*-only sites, respectively. Similar to the analyses of our wild-caught data, we used linear mixed models with fixed effects of population (high predation, *Rivulus*- only), food (high, low), and a "population by food" interaction. We included a random effect of "population by food" nested within river (high predation vs. *Rivulus*-only: Arima, Guanapo; low predation vs. *Rivulus*-only: Aripo, Guanapo, Quare) (Table S1). We included total length as a continuous covariate. For this model, data were not transformed as this provided the best fit for this model. We used the same model structure to evaluate differences in eye size between low predation and *Rivulus*-only common garden specimens, but included ln-transformed eye size and ln-transformed total length as a covariate for these analyses.

Canopy manipulations. These analyses for eye size comparisons between thinned and intact canopies closely follow the procedures described above. We included site (Caigual, Taylor, Lower Lalaja, Upper Lalaja) nested within canopy treatment (thinned, intact) as a random effect. Sex (male, female), canopy, and a "sex by canopy" interaction were entered as fixed effects (Table S1). We included total length as a covariate. Eye diameter and total length were ln-transformed.

RESULTS

Wild-caught specimens

Differences in relative eye size across populations were dependent upon sex, as well as river of origin (Table 1; Fig. 1; Table S1). Female *Rivulus* from high predation sites had a mean eye size that was 7% and 12% smaller than female *Rivulus* in low predation and *Rivulus*-only sites, respectively (Fig. 1). These differences were reduced in males; the eye size of male *Rivulus* from high predation sites was 6–7% smaller than male *Rivulus* from low predation and *Rivulus*-

only locales (Fig. 1). Because there was a significant “sex by population” interaction, we performed tests of simple main effects to further evaluate eye size differences among populations separately for each sex. We used a Bonferroni correction to adjust our *p*-values for multiple comparisons. Because these tests made two comparisons (males and females), we considered *p*-values <0.025 as “significant” (*p*-value correction: 0.05/2 = 0.025). Our results showed that differences in eye size among high predation, low predation, and *Rivulus*-only sites were marginally nonsignificant in females ($F_{2,\,9.091} = 3.589$; $P = 0.071$) and nonsignificant in males ($F_{2,\,8.948 = 1.065}$; $P = 0.385$). A Sidak post hoc test of main effects revealed that differences in *Rivulus* eye size between high predation and *Rivulus*-only sites is marginally nonsignificant in female *Rivulus* ($P = 0.074$), but not males ($P = 0.476$).

Observed differences in eye size between *Rivulus* from high predation, low predation, and *Rivulus*-only sites varied across rivers as the population (river) term was significant (Table 1; Fig. 1; Table S1). In the Arima and Aripo rivers, eye size was approximately 34% and 13% smaller in high predation sites when compared to *Rivulus*-only sites (Fig. 1). In the El Cedro and Guanapo rivers, eye size differed little among *Rivulus* from high predation, low predation, and *Rivulus*-only sites (Fig. 1).

Common-garden specimens

Eye size differed significantly between *Rivulus* from high-predation and *Rivulus*-only sites (Table 1, Fig. 2; Table S1). Eye size was 4% smaller in fish from high predation sites when compared to those from *Rivulus*-only localities (Fig. 2). These differences in eye size between high predation and *Rivulus*-only sites were consistent across rivers [population × food (river) = *p*

> 0.05]; relative eye size was 3.5% and 5% smaller in high predation versus *Rivulus*-only sites in the Arima and Guanapo rivers, respectively. In these same experiments, declines in food availability had a marginally nonsignificant influence on eye size (Table 1; Fig. 2). Eye size was 2–3% larger under low food levels when compared to fish that were fed high food levels (Fig. 2). In our low predation versus *Rivulus*-only experiments, differences in relative eye size between populations, food treatments, and rivers were not significant ($p > 0.05$) (Table 1; Fig. 2).

Canopy manipulations

Rivulus eye size did not differ significantly ($p > 0.05$) between sites with intact versus thinned canopies, nor between the sexes (Table 1; Fig. 3). Additionally, we did not observe any differences in eye size across sites (Table 1; Fig. 3).

DISCUSSION

Our results show that increased rates of mortality due to the presence of large, visually oriented predators is correlated with phenotypic declines in the eye size of *Rivulus* (Fig. 1). Yet, such shifts varied across rivers (Fig. 1). Wild-caught *Rivulus* from the Arima and Aripo rivers from high predation sites exhibit significantly smaller eyes than *Rivulus* from sites that lack predators (Table 1, Fig. 1). There were minimal differences in eye size among high predation, low predation, and *Rivulus*-only sites in the El Cedro and Guanapo rivers (eye size was slightly larger in high predation sites than in *Rivulus*-only sites in these rivers). It is important to note, however, that second generation common garden reared fish from two high predation sites displayed consistently smaller eyes than corresponding *Rivulus*-only sites (Fig. 2). Because these differences in eye size were maintained following two generations of common garden rearing, this suggests that this variation in eye size is genetic in origin, but this does not explain why we fail to see a consistent pattern in eye size at the phenotypic level. Our results beg two central questions: (1) what influences phenotypic variation in eye size? And (2) what are the ultimate (i.e., evolutionary) drivers of eye size evolution?

Ecological correlates of phenotypic variation in eye size

Averaged across all rivers, phenotypic differences in eye size are inconsistent with the prediction that increased predation selects for a larger eye due to the positive relationship between eye size and predator avoidance behavior (Møller and Erritzoe 2010; Glazier and Deptola 2011). The evolution of a smaller eye in high predation environments is instead better explained as a directional response to increased predator-induced mortality targeted at large, highly pigmented eyes (Zaret and Kerfoot 1975; Lonnstedt et al. 2013). However, increased predation is also associated with differences in other ecological factors that may influence eye size (Grether et al. 2001; Reznick et al. 2001; El-Sabaawi et al. 2012; Walsh and Renzick 2008, 2009). In particular, increased predation in high predation sites is correlated with increased light and resource availability (Grether et al. 2001; Reznick et al. 2001). It has been hypothesized that increases in eye size (and therefore visual acuity) may increase rates of energy intake by improving an organism's ability to identify optimal microhabitats for foraging (Brandon et al. 2015). *Rivulus* from sites that lack predators are often found swimming in open water and are not restricted to stream margins as in high predation environments (Beston, pers. obs.). As a result, *Rivulus* from *Rivulus*-only sites share increased opportunities for foraging when compared to high predation sites. Additionally, *Rivulus* from *Rivulus*-only sites are subject to lower levels of food availability (Walsh and Reznick 2008) and selection for maximal conversion of resources into somatic tissue is strong in these sites (Walsh and Reznick 2010). It is currently unclear as to why females exhibit a greater divergence in eye size between high predation and *Rivulus*-only sites when compared to males. Perhaps selection on eye size is stronger in females than males, as efficient energy acquisition is likely to be especially important in maintaining high reproductive efforts. In comparison, male reproductive investment is significantly less than females. Overall, we hypothesize that larger eyes are specifically favored in *Rivulus*- only locales due to the fitness benefits associated with increased foraging efficiency in environments characterized by lower light and food levels, and that this might be especially important in females.

Observed phenotypic differences in eye size among the focal *Rivulus* communities varied across rivers (Fig. 1). High predation fish exhibited smaller eyes in two of four focal rivers (Fig. 1). Such nonparallel results foreshadow that there are confounding factors that influence phenotypic variation in eye size that are not captured by the simple, categorical classification into discrete predator communities. That is, while “high predation” and “*Rivulus*-only” categories do indeed accurately reflect the presence and absence of predators in these sites, these discrete categories do not take into account the heterogeneous nature of predation intensity and the ecological correlates of predation.

As described above, high predation sites exhibit increased light and food availability (Grether et al. 2001; Reznick et al. 2001). Yet, the magnitude of differences in light between high predation and *Rivulus*-only sites varies across rivers (El-Sabaawi et al. 2012). In particular, sites within the Guanapo watershed exhibit much smaller differences in light availability and resources when compared with other streams (El-Sabaawi et al. 2012). For example, canopy cover differs by only 5% between high predation and *Rivulus*-only sites in the Guanapo river, but the canopy is 27% and 19% more open in high predation sites in the Arima and Aripo rivers when compared to their respective *Rivulus*-only localities (see Table 1 in El-Sabaawi et al. 2012). Similarly, the abundance of macroinvertebrates, a key component of the diet of *Rivulus* (Fraser et al. 1999), is 40% greater in high predation versus *Rivulus*-only communities in the Guanapo river, but 148% and 173% higher in high predation sites in the Arima and Aripo rivers, respectively (see Table 1 in El-Sabaawi et al. 2012). Although we do not have data on light or resource availability for the El Cedro river, it is important to note that the El Cedro is located within the Guanapo watershed and may therefore exhibit similar characteristics. Given that we expect that declines in light and resources select for a larger eye size, the lack of variation in resources and light availability in high predation versus *Rivulus*-only sites in the Guanapo river (and possibly the El Cedro river), when compared to the larger differences between these sites in the Arima and Aripo rivers, is a plausible explanation for the lack of consistent divergence in eye size at the phenotypic level in our wild-caught specimens.

High predation sites are home to multiple species of fish, including *H. malabaricus, Aequidens pulcher, Rhamdia quelen, P. reticulata, Synbranchus marmoratus,* and *C. frenata* (Furness and Reznick 2014; Gilliam et al. 1993). However, predator abundance and diversity can vary within and across high predation sites; sites at a lowland elevation in the main stem of the river typically have a greater number of species than those upstream (Gilliam et al. 1993). For example, the highly piscivorous *C. frenata* are found in the lowest area of the Guanapo watershed, but do not appear further upstream (Gilliam et al. 1993). Fish abundance and diversity also vary as a function of stream size (i.e., stream order), as the greatest species diversity is observed in the main stem of large rivers and smallest diversity in headwater tributaries (Evans and Noble 1979; Beecher et al. 1988; Rahel and Hubert 1991; Gorman and Karr 1978; Angermeier and Karr 1983). Importantly, the width of the high predation zone of the El Cedro river is smaller than the other rivers (Reznick and Endler 1982) and we typically observe far fewer predators in the El Cedro high predation site (Beston and Walsh, pers. obs.). It is thus plausible that predator-mediated selection is weaker in the El Cedro river. Given that *Rivulus* from high predation and *Rivulus*-only sites in the Guanapo river differ strongly in life history traits (Walsh and Reznick 2008, 2009), weaker predatory selection seems unlikely to explain the lack of eye size divergence in this river.

Ultimate drivers of eye size evolution

Patterns of divergence in eye size among wild-caught *Rivulus* from high predation and *Rivulus*-only sites were nonparallel (Fig. 1). However, second generation common garden reared male *Rivulus* revealed repeatable, genetically based shifts in eye size in response to predation (Fig. 2). *Rivulus* from high predation sites in the Guanapo and Arima rivers exhibited significantly smaller eyes than the corresponding *Rivulus*-only sites (Fig. 2). Therefore, *Rivulus* from the Guanapo river did not differ in eye size at the phenotypic level (Fig. 1), but exhibited strong differences in eye size between high predation and *Rivulus*-only sites following two generations of common garden rearing (Fig. 2). As described above, the overall differences in eye size for the wild-caught fish were larger in females than males, but the common garden comparisons are for males only. It is therefore likely that the data from our common garden experiments represents a conservative estimate for eye size divergence as we lacked data for females. In general, it appears that environmental influences on the phenotypic expression of eye size mask an underlying genetic difference between high predation and *Rivulus*-only sites. But, what is the driver of the genetically based differences in eye size between high predation and *Rivulus*-only sites?

The difference in eye size between high predation and *Rivulus*-only sites is consistent with one of our a priori hypotheses. That is, predators target pigmented structures, such as the eyes, and ultimately favor the evolution of smaller eyes. Yet, the confounding effects of increased light and resource availability that covaries with increased predation could also potentially exert selection on eye size. In low predation sites, *Rivulus* are subjected to predation by guppies as juveniles and are therefore found at lower densities than *Rivulus*-only sites (Walsh et al. 2011; Fraser and Lamphere 2013). If increased food availability is the main driver of eye size variation, then we expected that eye size of *Rivulus* in low predation sites would be similar to that of *Rivulus* in high predation sites. We observed small phenotypic differences in eye size between low predation and *Rivulus*-only sites in the field (Fig. 1). But, such small differences were not maintained after two generations of common garden rearing and are thus not likely genetic in origin (Fig. 2). This lack of divergence argues that resources are not the main driver of evolved differences in eye size between high predation and *Rivulus*-only sites.

In addition to resources, increased light availability is commonly associated with declines in eye size across species (Hiller- Adams and Case 1985; Garamszegi et al. 2002; Thomas et al. 2006; Lisney and Collin 2007; Ross and Kirk 2007; Hall 2008; Veilleux and Lewis 2011; Pearce and Dunbar 2012). To evaluate the influence of light availability on eye size, we compared experimental sites where the canopy was thinned in 2007 and 2008 versus those where the canopy remained intact. These complementary comparisons between thinned and intact canopies found no evidence of divergence in eye size between sites that vary in light levels to date (Fig. 3). This suggests that light is a weak driver of selection on eye size in *Rivulus,* although these canopies have only differed in light levels for approximately 10 years and the tempo of eye size evolution in *Rivulus* is unknown. Brandon and Dudycha (2014) evaluated patterns of plasticity in the eye size of *Daphnia* in response to manipulations of resource and light availability. They demonstrated that variation in resource levels induced stronger shifts in eye size when compared with the effects due to variation in light availability and that increases in light resulted in the production of *larger* eyes in *Daphnia* (Brandon and Dudycha 2014). Such results, in conjunction with trends revealed in the current study, suggest that while light availability and resources may have an environmental influence on eye size, it is unlikely that these factors are the main drivers of eye size evolution in this system.

In general, studies on intraspecific variation in eye size and structure are accumulating (Zaret and Kerfoot 1975; Brown et al. 2004; Fuller et al. 2004; Glazier and Deptola 2011; Pearce and Dunbar 2012; Lönnstedt et al. 2013; Brandon et al. 2015). Brandon et al. (2015) showed that eye size is under selection in a natural population of *Daphnia* and also that changes in eye size have clear fitness consequences; small increases (1%) in the eye diameter of *Daphnia obtusa* increase reproductive outputs by 20% (Brandon et al. 2015). Research on bluefin killifish explored plasticity in opsin expression in fish under different light conditions (Fuller et al. 2004), while work by Pearce and Dunbar (2012) identified lateral variation in light as a potentially important driver of orbital variation in humans. Research has also considered connections between predators and eye size variation (Brown et al. 2004; Glazier and Deptola 2011;

Lönnstedt et al. 2013). For example, Lönnstedt et al. (2013) showed that both eyes and eye spots are plastic in Ambon damselfish; eye spot increased while eye size decreased when juvenile damselfish were exposed to predators (Lönnstedt et al. 2013). Glazier and Deptola (2011) showed that *Gammarus minus* from ponds with fish predators had larger eyes than crustaceans in ponds that lacked predators (Glazier and Deptola 2011). These latter studies in particular foreshadow that the evolution of eye size is likely to be molded by contrasting predation regimes. The results of the present study build upon this body of work by revealing inconsistent patterns of eye size variation at the phenotypic level between communities that differ in predation intensity, but display consistent, repeatable differences at the genetic level. The totality of this evidence argues that predation is the driver of eye size evolution. However, the nonparallel phenotypic patterns in our wild-caught populations open up the possibility for resources and light to play a role in the evolution of eye size in *Rivulus*.

Resource-driven plasticity in eye size

Previous work in Trinidad has indicated that fish respond to variation in resource availability by adaptively altering the expression of traits (Bashey 2006; Walsh and Reznick 2008). For example, *Rivulus* and guppies respond to declines in resources by producing larger eggs and/or offspring. The production of larger eggs/offspring is potentially advantageous in low-food treatments because a larger egg and offspring size can facilitate increased larval survival (Walsh et al. 2006). In the current study, *Rivulus* fed low levels of food exhibited larger eyes when compared to those fed the high food treatment (Fig. 2) irrespective of population (although such trends were marginally nonsignificant; Table 1). These responses to resources are potentially adaptive as increases in eye size increase visual capabilities (Caves et al. 2017) and thereby may increase rates of energy acquisition via improved foraging. In our previous work on life history evolution (Walsh and Reznick 2008), we found that the life history traits of *Rivulus* from high predation sites were more sensitive to variation in food levels than *Rivulus* from *Rivulus*-only sites. For example, high predation fish increased the size of their eggs under low food conditions, but fish from *Rivulus*-only sites produced relatively large eggs irrespective of food level (Walsh and Reznick 2008). These "population by food" interactions were detected for most life history traits and we interpreted these divergent reaction norms as being driven by the known differences in resources between these sites (Reznick et al. 2001). In the current study, all "population by food" interactions were not significant (Table 1). Though, it is important to note that the differences in eye size between high predation and *Rivulus*-only sites were stronger under low food than when fed high food levels (Fig. 2). Increased eye size in response to declines in resource levels, as well as qualitative evidence for contrasting responses to low food between high predation and *Rivulus*-only sites suggests that resources also have the potential to shape the evolution of eye size.

Coordinated evolution of eye size and brain size

Research has shown that there is a strong covariation between eye size and brain size across taxa (Garamszegi et al. 2002). We previously evaluated *Rivulus* for evolved differences in brain size among high predation, low predation, and *Rivulus*-only sites (Walsh et al. 2016; Beston et al. 2017). The question that naturally arises is: Did we observe parallel trajectories of evolution for brain and eye size? Similar to the current study, *Rivulus* from low predation and *Rivulus*-only sites did not differ in brain size (Beston et al. 2017). *Rivulus* from high predation sites also exhibited significantly smaller brains than fish from *Rivulus*-only sites (Walsh et al.

2016). However, such differences in brain size were observed in males, but not females. In the current study, eye size was smaller in high predation sites in males *and* females. These differences may indicate that the ultimate drivers of the evolutionary shifts in brain and eye size are distinct. For instance, we hypothesize that the differences in eye size between high predation and *Rivulus*-only sites are due to increased predation that targets fish with larger eyes. We conversely hypothesize that the shifts in brain size are due to known differences in learning and behavior between sites with and without predators as such differences are often stronger in males than females.

However, it is possible that shifts in brain and eye may reflect selection on individual components of the sensory system. This is because selection may be operating on specific areas of the brain to enhance visual processing capabilities. For example, a larger eye in *Rivulus* from *Rivulus*-only sites may correspond with a larger visual cortex in the brain of *Rivulus* to accommodate the visual input of the surrounding environment. Recent work in natural populations of the electric fish, *Brachyhypopomus occidentalis*, showed that the presence of predators can decrease cell proliferation in certain areas of the brain (Dunlap et al. 2016). It is unclear as to how the presence of predators influences neurogenesis in visual areas of the brain and, in turn, how this relates to overall eye size. Therefore, it is likely that these patterns are the result of more specialized, underlying mechanisms associated with specific regions of the brain. The manner in which predator-driven selection operates on brain and eye size in nature, as well as the corresponding structures of the eye and brain requires further testing.

CONCLUSIONS

Here, we identified a link between predation pressure and the evolution of eye size in Trinidadian killifish. Increases in predator- induced mortality are associated with evolutionary declines in prey eye size. However, a lack of parallel responses to predation across wild-caught populations presents a multitude of new questions in relation to how selection operates on eye size in natural systems. We hypothesize that small eyes are favored in high predation environments due to visually mediated selection by predators and that larger eyes may be beneficial in sites that lack predators due to high densities, low food availability, and the benefits associated with optimal foraging. But, high predation sites differ in other variables, such as resources and light availability, which may be of importance in eye size evolution. This taken along with nonparallel patterns of eye size in wild-caught populations, as well as *Rivulus'* plastic response to shifts in resources, suggests that predator induced mortality may not act in isolation, but in concert with other ecological variables. Studies are now needed to test these proposed mechanisms of selection, as well as to understand how various ecological factors contribute to the plasticity and evolution of eye size.

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TABLES

Table 1. Analyses of relative eye size for wild-caught and common garden *Rivulus*.

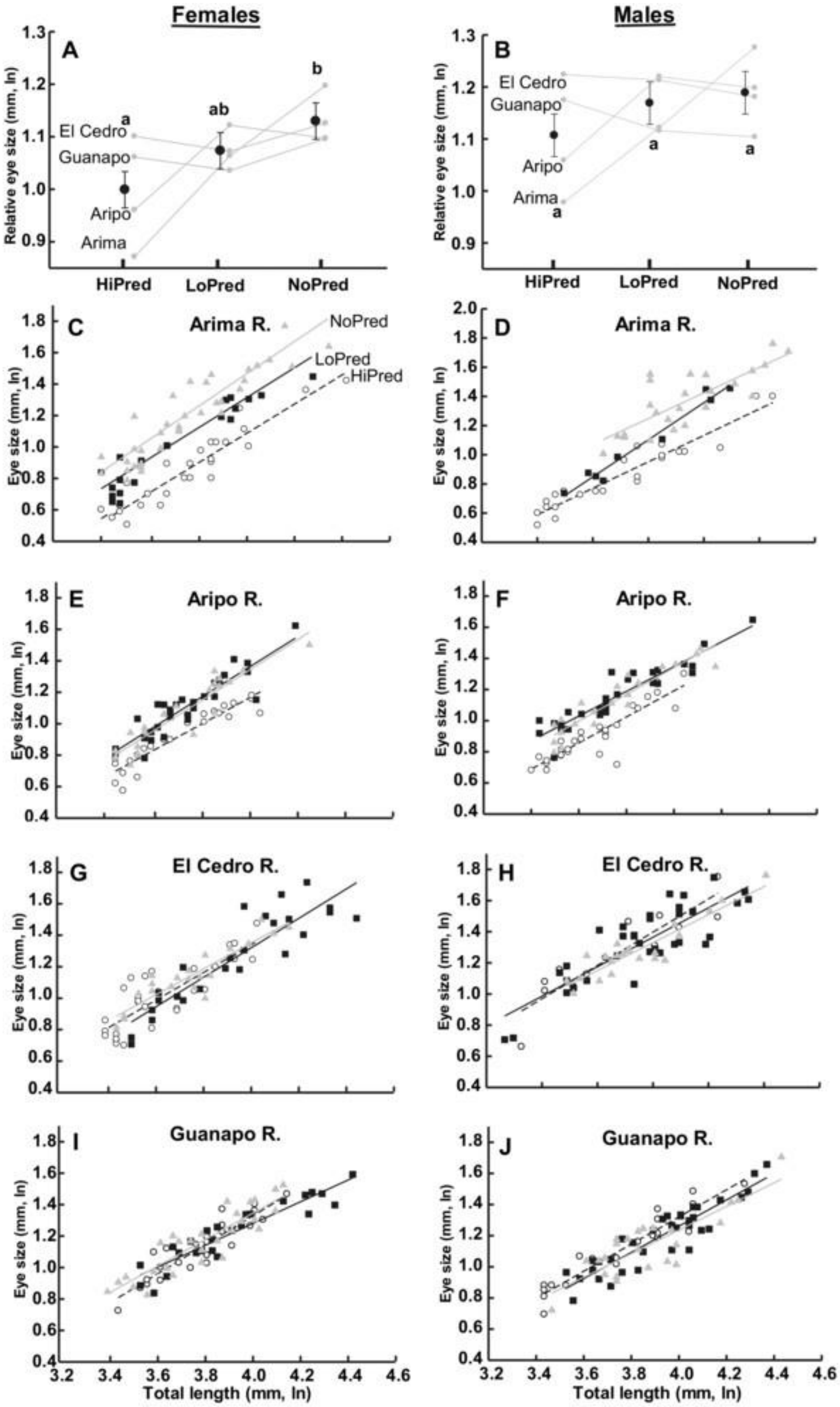
	Wild-caught fish			*HiPred/RivOnly common garden experiment*			*LoPred/RivOnly common garden experiment*			*Canopy manipulations*		
	Trait, df	F(ddf)	*P*	Trait, df	F(ddf)	*P*	Trait, df	F(ddf)	*P*	Trait, df	F(ddf)	*P*
Covariate												
length (mm, ln*)	1	2661.87(591.04)	**<0.001**	1	81.89(33.91)	**<0.001**	1	87.66(97.45)	**<0.001**	1	2750.14(179.83)	**<0.001**
Fixed effects												
population	2	2.16(9.02)	0.172	1	7.28(8.77)	**0.025**	1	0.001(5.79)	0.983	---	---	---
sex	1	2.69(589.03)	0.101	---	---	---	---	---	---	1	0.64(179.49)	0.426
food	---	---	---	1	4.92(4.97)	0.078	1	1.74(7.43)	0.226	---	---	---
canopy	---	---	---	---	---	---	---	---	---	1	0.17(1.96)	0.718
population x sex	2	4.00(589.34)	**0.019**	---	---	---	---	---	---	---	---	---
population x food	---	---	---	1	1.32(4.13)	0.313	1	1.00(5.81)	0.357	---	---	---
canopy x sex	---	---	---	---	---	---	---	---	---	1	0.07(179.64)	0.792
Random effects		Wald Z			Wald Z			Wald Z			Wald Z	
population(river)	1	2.05(1)	**0.04**	---	---	---	---	---	---	---	---	---
population x food(river)	---	---	---	1	0.52(1)	0.603	1	0.89(1)	0.374	---	---	---
site(canopy)	---	---	---	---	---	---	---	---	---	1	0.49(1)	0.626

F, F-values; df, numerator degrees of freedom; ddf, denominator degrees of freedom. Significant terms are indicated in bold. Denominator degrees of freedom use a Satterwaite approximation.

*Data for high predation versus *Rivulus*-only analyses are not ln-transformed.

FIGURES

Figure 1. Variation in eye size between predator communities for wild-caught *Rivulus*. Estimated marginal means for differences in (A) female and (B) male eye size among high predation (HiPred), low predation (LoPred), and *Rivulus*-only (NoPred) sites. Closed circles – average eye size for each fish community, gray circles – average values for each replicate river. All values are the estimated marginal means stemming from the linear mixed models described in the Materials and Methods section. Regressions between total length (mm, ln) and absolute eye size (mm, ln) in wild-caught specimens from high predation (open circles, dashed regression line), low predation (closed squares, solid black regression line), and *Rivulus*-only sites (gray triangles, gray regression line) for: (C) females from the Arima R., (D) males from the Arima R., (E) females from the Aripo R., (F) males from the Aripo R., (G) females from the El Cedro R., (H) males from the El Cedro R., (I) females from the Guanapo R., and (J) males from the Guanapo R. We observed marginally nonsignificant ($0.05 < P < 0.1$) differences in female eye size. Differences in male eye were no significant ($P > 0.05$). Differences in female and male eye size among the predator communities varied across rivers (i.e., significant population [river] term). Error bars = ± 1 S.E.



Females
Males
A
B
Relative eye size (mm, ln)
1.3
1.2
1.1
1.0
0.9
El Cedro
Guanapo
Aripo
Arima
a
ab
b
a
a
a
HiPred
LoPred
NoPred
C
Arima R.
NoPred
LoPred
HiPred
D
Arima R.
E
Aripo R.
F
Aripo R.
G
El Cedro R.
H
El Cedro R.
I
Guanapo R.
J
Guanapo R.
Eye size (mm, ln)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
Total length (mm, ln)

Figure 2. Common garden differences in eye size. (A) Eye size differences between high predation (HiPred; open circles; open gray circles are replicate river means) and *Rivulus*-only (NoPred; gray triangles; open gray triangles are replicate river means) sites. (B) Eye size variation between low predation (LoPred; closed squares; gray squares are replicate river means) and *Rivulus*-only (NoPred; gray triangles; open grey triangles are replicate river means) sites. HiFood, high food levels; LoFood, low food levels. Regressions between total length (mm) and absolute eye size (mm) in common garden specimens for high predation (open circles, dashed regression line) and *Rivulus*-only sites (gray triangles, gray regression line) reared under (C) high food and (E) low food levels. Regressions between total length (mm) and absolute eye size (mm) in common garden specimens for low predation (closed squares, solid regression line) and *Rivulus*-only sites (gray triangles, gray regression line) reared under (D) high food and (F) low food levels. We observed significant ($p < 0.05$) differences in eye size between high predation and *Rivulus*-only sites. Eye size differences between low predation and *Rivulus*-only sites were not significant ($P > 0.05$). Error bars = ± 1 SE.

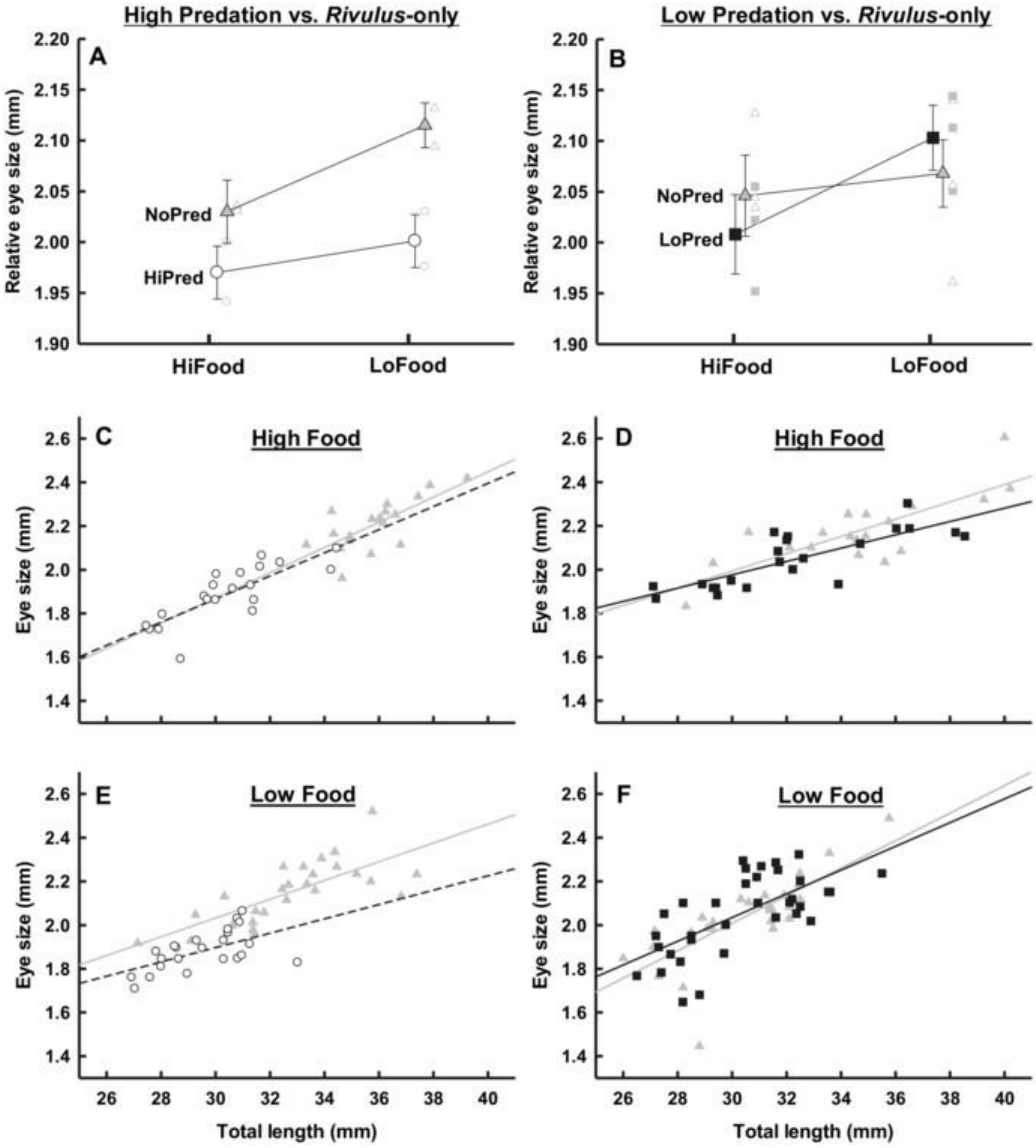
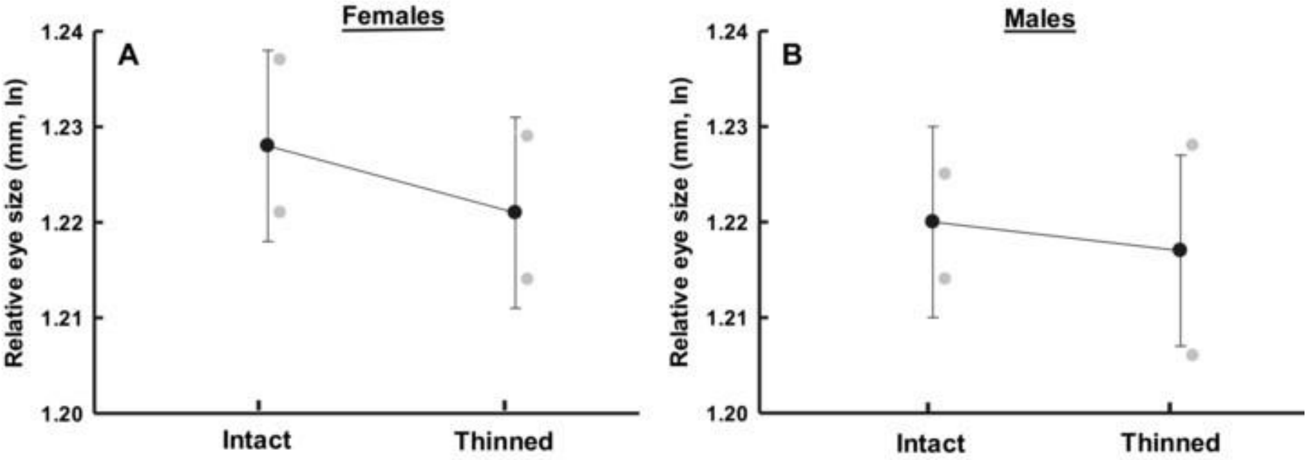

High Predation vs. *Rivulus*-only
Low Predation vs. *Rivulus*-only
A
B
Relative eye size (mm)
2.20
2.15
2.10
2.05
2.00
1.95
1.90
NoPred
HiPred
LoPred
HiFood
LoFood
C
D
High Food
E
F
Low Food
Eye size (mm)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
26
28
30
32
34
36
38
40
Total length (mm)

Figure 3. Eye size differences between *Rivulus* from sites with intact and thinned canopies. (A) Female eye size, (B) Male eye size. “Intact” – canopy has not been manipulated, “Thinned” – sites with reduced canopy cover. Closed circles – overall mean per canopy treatment, gray circles – mean value per replicate. Eye size did not differ significantly ($P > 0.05$) between sites with thinned versus and intact canopy. Error bars = ± 1 S.E.



SUPPORTING INFORMATION

Table S1. The effect of predator community and experimental canopy thinning on eye size in wild-caught and common garden specimens from linear mixed effect models.

	Estimate	*SE*	*Df*	*T*	*z*	*P-value*
Wild-caught specimens						
Ln Length	0.9	0.02	591.04	51.59	---	<0.001
High predation	-0.08	0.05	9.8	-1.51	---	0.162
Low predation	-0.02	0.05	9.67	-0.30	---	0.772
Female	0.02	0.01	588.43	1.31	---	0.189
High predation x female	-0.05	0.02	588.92	-2.67	---	0.008
Low predation x female	-0.04	0.02	589.42	-2.15	---	0.032
Population (River)	0.01	0.003	1.00	---	2.05	0.040
HP vs. RO common garden						
Length	0.05	0.01	33.91	9.05	---	<0.001
High predation	-0.11	0.04	4.83	-3.21	---	0.025
High food	-0.08	0.04	4.97	-2.31	---	0.070
High predation x high food	0.05	0.05	4.13	1.15	---	0.313
Population x food (River)	0.0004	0.0008	1.00	---	0.52	0.603
LP vs. RO common garden						
Ln Length	0.68	0.07	97.45	9.36	---	<0.001
Rivulus-only	-0.02	0.02	5.03	-0.81	---	0.456
High food	-0.04	0.03	5.57	-1.68	---	0.148
Rivulus-only x high food	0.04	0.04	5.81	1.00	---	0.357
Population x food (River)	0.0005	0.0005	1.00	---	0.89	0.374
Canopy manipulations						
Ln Length	0.76	0.01	179.83	52.44	---	<0.001
Intact canopy	0.003	0.01	4.23	0.19	---	0.859
Female	0.004	0.01	178.98	0.37	---	0.715
Intact canopy x female	0.004	0.02	179.64	0.26	---	0.792
Site (canopy)	0.00006	0.0001	1.00	---	0.49	0.626

CHAPTER 3:

NATURAL SELECTION FAVORS THE EVOLUTION OF A LARGER EYE IN RESPONSE TO INCREASED COMPETITION IN NATURAL POPULATIONS OF A VERTEBRATE[§]

Shannon M. Beston[1] and Matthew R. Walsh[1]

Beston, S.M., and M.R. Walsh. In press. Natural selection favors the evolution of a larger eye in response to increased competition in natural populations of a vertebrate. *Functional Ecology*

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ABSTRACT

1. Eye size varies notably across taxa. Much work suggests that this variation is driven by contrasting ecological selective pressures. However, evaluations of the relationship between ecological factors and shifts in eye size have largely occurred at the macroevolutionary scale. Experimental tests in nature are conspicuously absent.

2. Trinidadian killifish, *Rivulus hartii*, are found across fish communities that differ in predation intensity. We recently showed that increased predation is associated with the evolution of a smaller eye. Here, we test how divergent predatory regimes alter the trajectory of eye size evolution using comparative mark-recapture experiments in multiple streams.

3. We found that increases in eye size are associated with enhanced survival, irrespective of predation intensity. More importantly, eye size is associated with enhanced growth in communities that lack predators, while this trend is absent when predators are present.

4. Such results argue that increased competition for food in sites that lack predators is the key driver of eye size evolution.

INTRODUCTION

Eye size varies markedly across vertebrate species (Caves, Sutton, & Johnsen, 2017; Howland, Merola, & Basarab, 2004; Land & Nilsson, 2012; Thomas, Székely, Powell, & Cuthill, 2006). Increases in eye size are correlated with shifts in vision (Caves et al., 2017; Land & Nilsson, 2012). The visual system acts as an important intermediary between the organism and its environment. Thus, visual capability, and in turn eye size, contribute to a variety of activities, such as habitat identification, foraging, and recognition of conspecifics, mates, and predators (Cronin, Johnsen, Marshall, & Warrant, 2014; Land & Nilsson, 2012; MacIver, Schmitz, Mugan, Murphey, & Mobley, 2017). These connections between eye size, vision, and fitness implies that ecologically-mediated natural selection should be an important driver of eye size evolution.

There are several reasons why the environment is likely to impose selection on eye size. This is largely because there are multiple pathways between eye size and visual performance (Kiltie, 2001; Land & Nilsson, 2012; Motani, Rothschild, & Wahl Jr, 1999; Walls, 1942). Larger eyes are associated with a greater aperture diameter and increased focal length. An important component of acuity is the angular width of the area viewed by an individual photoreceptor, calculated as the diameter of the photoreceptor divided by focal length (Land & Nilsson, 2012). Thus, greater focal lengths lead to smaller angular widths and, in turn, enhanced acuity. Previous work has indeed shown that increases in eye size are associated with enhanced visual acuity (Caves et al., 2017; Kiltie, 2001) that is likely mediated by longer focal lengths (Kiltie, 2001). However, this relationship between eye size and acuity depends on other components of the eye. For example, larger eyes can also lead to a greater pupil diameter and enhanced visual sensitivity, both of which can impact the ability of an organism to detect light (Land & Nilsson, 2012). But, there are trade-offs between acuity and sensitivity. While increases in focal length lead to smaller angular widths resulting in greater acuity, when photoreceptors have a smaller angular width they also collect light from a smaller angular area, leading to reduced sensitivity (Land and Nilsson 2012). A way to mediate this trade-off between acuity and sensitivity is to increase total eye size (and therefore, focal length). By increasing total eye size, acuity or sensitivity can increase without decreasing the other. Evolutionary shifts in eye size should therefore be associated with behaviors and ecological conditions that rely upon variation in visual acuity and sensitivity.

Indeed, studies have shown that ecological factors such as habitat complexity, foraging, diel activity, and predation are associated with evolved differences in eye size (Banks, Sprague, Schmoll, Parnell, & Love, 2015; Beston, Wostl, & Walsh, 2017; Brandon, James, & Dudycha, 2015; Caves et al., 2017; Glazier & Deptola, 2011; Hammerschlag et al., 2018; Thomas et al., 2006, 2002; Veilleux & Kirk, 2014; Zaret & Kerfoot, 1975). Although such studies provide information on the relationship between eye size and the environment, the vast majority of this work has compared eye size across species (but see Beston et al., 2017; Brandon et al., 2015; Lönnstedt, McCormick, & Chivers, 2013). Despite years of research, very little work has experimentally tested how natural selection shapes the evolution of complex traits, such as the vertebrate eye.

Populations of Trinidadian killifish, *Rivulus hartii*, allow us to test the connection between divergent ecological conditions and the evolution of vertebrate eye size. *Rivulus* are found across a diversity of fish communities on the island of Trinidad that differ in the presence and absence of predatory fish (Fraser, Gilliam, MacGowan, Arcaro, & Guillozet, 1999; Gilliam, Fraser, & Alkins-Koo, 1993). *Rivulus* are found in: (1) ‘high predation’ (HP) sites where they are subject to intense predation by large piscivorous fish, such as *Crenicichla frenata* and *Hoplias malabaricus*, and (2) ‘*Rivulus*-only’ sites where *Rivulus* are the sole fish species present. These communities share a similar physical habitat and do not differ in water quality variables (Walsh & Reznick, 2009). However, these sites do differ in both mortality rates and densities. *Rivulus* in high predation sites experience increased mortality via predation (Walsh & Reznick, 2008), leading to lower densities when compared to *Rivulus*-only localities (Fraser & Lamphere, 2013; Furness & Reznick, 2014). As a result, *Rivulus* experience acute competition for resources in *Rivulus*-only sites.

We recently tested the influence of increased predation on the evolution of eye size in *Rivulus* (Beston, Wostl, et al., 2017). We found that *Rivulus* in high predation sites displayed a significantly smaller relative eye size when compared to *Rivulus* from sites without predators. These differences in eye size were maintained following two generations of common garden rearing (Beston, Wostl, et al., 2017), suggesting that these differences are genetic in origin. However, increased predation in high predation sites is also correlated with increased resources and light availability (Reznick, Butler IV, & Rodd, 2001). For instance, high predation environments tend to have a more open canopy than *Rivulus*-only sites (Grether, Millie, Bryant, Reznick, & Mayea, 2001; Reznick et al., 2001) and resources are higher due to the indirect consequences of increased mortality (Reznick et al., 2001; Walsh & Reznick, 2008). We further evaluated the extent to which covarying shifts in light and resources explain the observed divergence in eye size between high predation and *Rivulus*-only sites using complementary comparisons. This approach showed that the presence (or absence) of predators, and not differences in light or food availability, best explain these evolved differences in eye size (Beston, Wostl, et al., 2017).

While this previous work shows a clear relationship between predation, competition, and evolved differences in eye size, these results tell us nothing about how predators are associated with the evolution of a smaller eye or how increased levels of competition are associated with the evolution of a larger eye. Nor, does this tell us anything regarding how selection operates on this trait in nature. Here, we used mark-recapture experiments to evaluate the fitness correlates of shifts in eye size between communities that differ in the presence and absence of predators. This allowed us to test two competing hypotheses for the evolution of eye size in this system. The first is that *Rivulus* have evolved smaller eyes in high predation sites because predators target pigmented characteristics, such as the eyes (Zaret & Kerfoot, 1975). If larger eyes make *Rivulus* more conspicuous to predators, then we predict that survival will covary negatively with eye size in high predation sites. An alternative possibility is that increased eye size enhances fitness in the absence of predators when competition for food and microhabitats is intense. If this is the case, then we expect that rates of survival and/or growth should covary positively with eye size in *Rivulus* from *Rivulus*-only sites.

MATERIALS AND METHODS

Mark-recapture

We executed a mark-recapture study in the Arima and Aripo rivers (access points provided by request) to evaluate the relationship between apparent survival, growth rate, and eye size in high predation and *Rivulus*-only sites in January 2017. *Rivulus* were collected from roughly 18:00 to 22:00 each night with dip nets. We sampled from clearly defined pools across sites (approximately 9 to 11 pools in *Rivulus*-only sites and 12 to 13 pools in high predation sites). All fish were processed the morning after capture. Each *Rivulus* was measured for total length, weight, and a photograph was taken of the fish's left side using Canon Powershot SX530 HS or Canon Powershot ELPH180 cameras. Eye size was later quantified from these photographs using ImageJ freeware (Schneider, Rasband, & Eliceiri, 2012) by measuring the diameter of the eye cavity at its widest part. Fish were then sexed by identifying the presence or absence of white bars at the top and bottom of the caudal fin; sexually mature male *Rivulus* display white bars making them noticeably different from females. Previous work in wild-caught populations of *Rivulus* has indicated that 35 mm is the approximate divide between mature and juvenile *Rivulus* (see Furness & Reznick, 2014). Thus, we excluded all individuals below 35 mm in our analyses to ensure that we are evaluating patterns of selection on adult traits. Once each fish was measured for total length and photographs were taken, each fish was given a unique marker with an elastic polymer that fluoresces under UV light (Northwest Marine Technology Incorporated, Shaw Island, Washington, USA). A unique two-dot code was generated from six different body positions and four different colors (see Fraser et al., 1999; Gilliam & Fraser, 2001; Walsh, Fraser, Bassar, & Reznick, 2011). Once marked, *Rivulus* were immediately returned to their initial site of capture and were recaptured every 11 days throughout the 22-day period. At each recapture event, all marked *Rivulus* were again measured for total length and weight and any unmarked *Rivulus* were measured for length and weight, were sexed, and given an individual mark. We resampled all populations two times for a total of three sampling events at each site (2 streams x 2 populations x 3 sampling events = 12 dates of collection).

Survival

To determine if predators selectively prey upon *Rivulus* with a larger relative eye size, we measured apparent survival using Cormack-Jolly-Serber (CJS) models implemented in PROGRAM MARK (Lebreton, Burnham, Clobert, & Anderson, 1992). CJS models are basic mark-recapture models that use maximum likelihood estimation of survival and recapture parameters (Lebreton et al. 1992). CJS models consider two possible outcomes when marked fish are not recaptured: that the fish died between recapture events (Φ, apparent survival), or that the fish was not recaptured (*p,* recapture probability). The parameters estimated from our models were therefore apparent survival and recapture probability. To estimate survival and recapture probability, we used the approach of comparing complex models with simpler models using $QAIC_C$ (Quasi-likelihood adjusted Akaike's Information Criteria; Anderson, Burnham, & White, 1994). QAIC is the most commonly used approach to assess the fit of models from capture-recapture studies and is a model selection criteria that corrects for overdispersion in data based upon notions of quasi-likelihood and variance inflation (Hurvich & Tsai, 1995; Wedderburn, 1974). Generally, models with a lower $QAIC_C$ are thought to be better models. Differences between models are considered to be strong when there is a $\Delta QAIC_C$ that is greater than 7 (Burnham & Anderson, 2001). A model with a $\Delta QAIC_C$ between 2 and 7 is thought to be moderately different than the model being compared to, while a $\Delta QAIC_C$ less than 2 indicates that the models being compared have equivalent support (Burnham & Anderson, 2001).

As a first step in model selection, we evaluated the dispersion in our data set. Over dispersion takes place when some individuals are more likely to be captured than others, violating the basic assumptions of the CJS model. Even slight over-dispersion can influence model selection and thus it is considered good practice to correct for this using a correction factor, c-hat, prior to analyzing the data (Lebreton et al., 1992). We used a parametric bootstrap goodness-of-fit (GOF) test executed in PROGRAM MARK with Φ and *p* parameterized with stream (Arima, Aripo), population (high predation, *Rivulus*-only), and sex (male, female). This GOF test estimates c-hat, a variance inflation factor. A model with a perfect fit will have a c-hat of 1. C-hat is estimated by using the data from the model being evaluated for goodness of fit; the parameter estimates (survival and recapture) for the specified model are used to simulate the data. The simulated data meets the exact assumptions of the model such that there is no over-dispersion. Values from the bootstrap simulation can then be compared to our actual values. C-hat is used as a correction factor when applied to all candidate models to generate corrected QAIC ($QAIC_c$) values for model selection. We did not include the individual covariate of relative eye size in the GOF model as there is currently no *good* method for testing the fit of a CJS model that incorporates individual covariates (Cooch & White, 2010). Thus, the recommended approach is to run GOF testing on the most general model without the individual covariates and then use the c-hat value generated from this to correct for all subsequent models used in model selection, even those that include individual covariates (Cooch & White, 2010). We calculated c-hat from the most general model (stream, population, sex) after 1000 simulations. We divided the observed deviation by the mean bootstrapped deviation (18.5166/16.802) to calculate c-hat. This value indicated minor over-dispersion (c-hat = 1.10), as a value of 1 indicates a perfect model and values with corrections less than 3 are considered acceptable (Cooch & White, 2010).

We estimated recapture probability and apparent survival using a two-step approach that has been previously applied to *Rivulus* (Furness & Reznick, 2014; Walsh et al., 2011) and guppies (Bryant & Reznick, 2004; Gordon et al., 2009) in this system. Because we have only two recapture events, we ignored time effects in our models because we cannot independently estimate survival and recapture probabilities for the second recapture event (Lebreton et al., 1992). Only fish that were caught during the initial capture event were included in these analyses as at least two recapture events are needed in order to calculate recapture probability. Relative eye size (i.e., eye size corrected for body size) was calculated for these fish by outputting standardized residuals from a general linear model with population (high predation, *Rivulus*-only), river (Arima, Aripo), sex (male, female), and all interactions entered as fixed effects and absolute eye size entered as the dependent variable. We included total length (mm) as a continuous covariate. We used a natural log transformation on both eye size and total length to better meet assumptions of normality and homogeneity of variances. Residuals that were greater than ± 3 were considered outliers and excluded from analyses (1 Arima RO male and 1 Aripo HP female; n = 386). We first estimated recapture probabilities from our candidate model set by comparing $QAIC_c$ values among models. Survival was parameterized with stream, population, sex, and a 'population x relative eye size' interaction to determine the best model for recapture probability (Table S1). Results indicated that the best model for recapture probability was a model that incorporated no effects (Table S1). To estimate apparent survival, we then constrained all models in the candidate model set using the best fit model for recapture probability (a recapture model that included no effects). We compared more complex models with simpler models to determine the importance of eye size in survival between populations of *Rivulus*.

Growth rate

We calculated instantaneous growth rate as follows: $[(\ln(\text{wet weight}_{\text{recap}} - \ln(\text{wet weight}_{\text{initial}}))/\text{day}_{\text{recap}} - \text{day}_{\text{initial}})]$. All fish that were recaptured at least once were included in the analyses of growth rate (n=327). We generated standardized residuals of relative eye size using a model that included absolute eye size as the dependent variable, stream, population, sex, and all interactions entered as fixed effects, and fish total length entered as a covariate). Residuals ± 3 were considered outliers. We had one outlier from the Arima RO which was removed from the analyses (n=326). We calculated individual growth rates from the longest time period between capture events. For example, if an individual were captured during the initial marking event and again during the first recapture and second recapture, we would calculate instantaneous growth rate by using values from the initial marking episode and the second recapture event (see Furness & Reznick, 2014; Walsh et al., 2011).

We then used linear mixed models implemented with restricted maximum likelihood (REML) to determine if the relationship between instantaneous growth rate and eye size varied between populations. Our model included a dependent variable of instantaneous growth rate, a covariate of relative eye size, and fixed effects of population, sex, and the ‘population x relative eye size’ interaction. We also included a random effect of population nested within river to account for heterogeneity between predator communities (Table S2). We were specifically interested in the ‘population x relative eye size interaction,’ to determine if growth rate differs as a function of eye size between populations.

Estimates of predator perception of killifish eyes

We explored the extent to which the killifish eye is visible to one of its common predators, *Hoplias malabaricus*. Previous work by Caves and colleagues examined the connection between eye size and visual acuity across over 80 species of ray-finned fish and found that eye size is a strong predictor of acuity (Caves et al., 2017). Because visual acuity has never been assessed in *Hoplias malabaricus*, we used the slope and intercept from this previously published phylogenetic least squares regression of eye size versus acuity across species (see Table S2 in Caves et al. 2017) to estimate the acuity of *Hoplias malabaricus*. We used a photograph of *H. malabaricus* (see Fig. S1) from a stream in Trinidad to measure eye diameter in ImageJ (Schneider et al., 2012) and then calculated the predicted acuity for the given eye diameter using the following equation: $y = 0.806x + 0.341$, where y equals acuity (cpd) for a given eye diameter, x (see Table S2 in Caves et al. 2017). Because Caves et al. 2017 also reports the 95% C.I. for this regression, we were able to examine the predicted upper and lower thresholds of acuity in *H. malabaricus* (upper 95% C.I.: $y= 0.97x + 0.47$; lower 95% C.I. = $y=0.63x + 0.21$). Eye size of *H. malabaricus* from our photograph was 5.00 mm. Because the above regression was based upon values of eye size and acuity that were log-transformed (E. Caves, pers. correspondence), we log transformed our measurement of eye diameter and when calculating acuity (and subsequently inverse log transformed this to obtain values of acuity in cpd).

Using these values, we then numerically determined the distance that shifts in eye size could be detected. Endler (1978) presents an equation that can be used to determine patterns viewed at a given distance, d, for animals with a given acuity angle, ϕ (Endler, 1978). The visual acuity angle, ϕ, is the smallest angle that the eye is able to resolve. That is, ϕ is the angle at which two objects in an observer's visual field are just able to be distinguished as separate. Endler applies this equation to color patterns and crypsis, but here we apply this equation to determine the distance that the eye is distinguishable from its background. The 'effective disc of acuity', or the distance at which color patches (in this case, the pigmented eye) of a given size are distinguishable, is based on the species' acuity angle and the distance that prey are observed. The diameter of color patches distinguishable by an animal is $l = 2d\tan(\phi/2)$ (see Fig. 4 in Endler 1978), where d is the distance from the eye of the viewer to the object. Objects smaller than size l will blend together, while objects larger than size l will be distinguishable. We use the above equation and set l equal to the average eye size of wild-caught *Rivulus* from HP and RO sites using all fish from our mark-recapture experiments (HP = 2.81 mm, RO = 3.09 mm) and solve for d, the distance at which the given eye size is distinguishable for *H. malabaricus.*

In order to use the above equation, we had to determine acuity angle ϕ based upon *H. malabaricus'* predicted acuity. Spatial frequency is equal to $1/(2\Delta\phi)$, where $\Delta\phi$ represents the inter-receptor angle at the nodal point of the eye. We use the inter-receptor angle as our angle of acuity for the above calculation as this represents the fineness at which an image is sampled (Land and Nilsson 2012).

RESULTS

We marked 110 and 90 fish from the Arima high predation and Aripo high predation sites and 189 and 131 fish from the Arima *Rivulus*-only and Aripo *Rivulus*-only sites, respectively (Table S3). We recaptured approximately 47-55% and 69-71% marked fish in the high predation and *Rivulus*-only sites, respectively (Table S3). *Rivulus* from high predation and *Rivulus*-only sites displayed similar allometries between eye size and body size (Fig. 1). The average total length of *Rivulus* from a high-predation site was approximately 48 mm and the average absolute eye size for a fish of this size in a high predation site was 2.79 mm. In *Rivulus*-only sites the absolute eye size of a fish of this size was 3.11 mm; approximately 10% larger than that of a high predation fish of equal body length (Fig. 1).

Survival

Overall, estimates of apparent survival were on average 15% greater in *Rivulus*-only sites when compared to high predation sites (Table 1). Our recapture probabilities ranged from 53-71% in our high predation sites and from 68-80% in *Rivulus*-only sites. Estimates of apparent survival suggest that males have a lower probability of survival when compared to females, irrespective of population and survival was slightly greater in populations from the Aripo river when compared to the Arima river (Table 1).

Because we were interested in the role of relative eye size in survival of *Rivulus* between high predation and *Rivulus*-only sites, we compared models of survival that included a ‘population x relative eye size’ interaction with more simple models that did not include this interaction (Table 2). Our results show that the best fitting model is the model that incorporates eye size as a covariate, but does not include the ‘population by relative eye size’ interaction (Table 2). This model is considered equivalent in fit to the full model because the $\Delta QAIC_C$ between the full model and reduced model is negligible (~2), indicating that the interaction between population and relative eye size does not explain a significant amount of variation in apparent survival (Table 2).

However, our results do show that relative eye size is important in apparent survival, irrespective of population (Table 2, Fig. 2). Models that incorporated eye size were a substantially better fit to the data than those that did not incorporate eye size, as models that display a difference in $QAIC_C$ between 2 and 7 are interpreted as being moderately supported. This is largely because increases in eye size are associated with increased survivorship irrespective of predator community (Fig. 2).

Growth rate

We found that the relationship between relative eye size and growth varied between high predation and *Rivulus*-only sites. That is, we detected a significant 'population x relative eye size' interaction ($F_{1, 319.110} = 6.653$, $p < 0.01$; Fig. 3). In *Rivulus*-only sites, the relationship between relative eye size and growth was positive, but such a relationship between eye size and growth was absent in high predation sites (the relationship between these two factors was slightly negative). We performed follow-up linear regressions to evaluate the magnitude and significance of these trends within each population. The regression between eye size and growth was significant in *Rivulus*-only sites ($F_{1,223} = 5.220$, $p = 0.023$; Fig. 3). In high predation sites, the relationship between relative eye size and instantaneous growth rate was not significant ($F_{1,101} = 1.817$, $p = 0.181$; Fig. 3). We also performed regressions on each 'stream x population' and found that although non-significant, the relationships between relative eye size and growth rate were consistently negative in high predation sites (Arima HP: $F_{1,51} = 1.681$, $\beta = -0.180$, $p =$

0.201; Aripo HP: $F_{1,49} = 0.251$, $\beta = -0.072$, $p = 0.618$) and consistently positive in *Rivulus*-only sites (Arima RO: $F_{1,129} = 3.728$, $\beta = 0.168$, $p = 0.056$; Aripo RO: $F_{1,93} = 1.616$, $\beta = 0.131$, $p = 0.207$) (Fig. 3). We found no significant relationship between sex ($F_{1,319.3} = 0.069$, $p = 0.792$), population ($F_{1,1.548} = 12.345$, $p = 0.103$), or relative eye size ($F_{1,319.1} = 0.455$, $p = 0.501$) and instantaneous growth rate. Nor did we find a significant effect of population(stream) (Wald Z = 0.795, $p = 0.426$).

Predator perception of killifish eyes

From the best-fit line of a PGLS regression of eye size versus acuity, *H. malabaricus* is predicted to have an acuity of 8.03 cpd (95% C.I. = 4.47, 14.07 cpd) and an inter-receptor angle of 0.062 degrees (95% C.I. = 0.036, 0.119). Applying these values, *H. malabaricus* can distinguish an eye size of 2.81 mm from approximately 2.60 m (95% CI = 1.35, 4.47) away and an eye size of 3.09 mm from approximately 2.86 m (95% CI = 1.49, 4.92) away.

DISCUSSION

Our results show that increases in relative eye size are associated with enhanced survival, irrespective of the presence or absence of predators (Fig. 2, Table 2). That is, individuals with a larger relative eye size experience increased survival in both high predation and *Rivulus*-only localities (Fig. 2). Given these parallel trends across fish communities, the connection between eyes and survival do not appear to explain the locally adapted differences in eye size in *Rivulus* (Beston, Wostl, et al., 2017). However, we observed divergent relationships between eye size and rates of growth between high predation and *Rivulus*-only sites. Specifically, we observed a positive association between relative eye size and growth in *Rivulus*-only sites, but no relationship between relative eye size and growth rate in high predation sites. Such results provide experimental evidence that a connection between eye size and foraging capabilities underlie the evolution of eye size. Below, we further discuss how selection operates on eye size in this system.

As previously described, we recently showed that *Rivulus* from high predation sites have evolved a smaller relative eye size when compared to those from *Rivulus*-only sites (Beston et al., 2017). Such trends are consistent with the 'visual predation hypothesis', which suggests that pigmented characteristics, such as the eye, make prey more susceptible to predators (Lönnstedt et al., 2013; Zaret & Kerfoot, 1975). Our current results show that *Rivulus* eyes are distinguishable to a predator from two to three meters away. Previous work on predator striking distance on guppy prey indicates that *Crenicichla frenata*, another predator of guppies and *Rivulus,* initiates striking from 0.4 meters away (Endler, 1991). Assuming *H. malabaricus* initiates striking from a similar distance, these results suggest that eye size could potentially make prey more conspicuous to their predators as the eye is distinguishable to the wolf fish at a distance greater than its potential striking distance. However, it seems unlikely that predators are able to detect small differences in eye size as the average eye size of a *Rivulus* in an HP site versus that of the typical larger eye size of an RO *Rivulus* only differ by tenths of a meter. Direct tests of this are needed. Regardless, the results from our mark-recapture analyses found that individuals with a larger relative eye size experience increased survival in both high predation and *Rivulus*-only sites. Such results do not support the visual predation hypothesis. Thus, contrasting rates of predator-induced mortality do not appear to explain the evolved shifts in eye size in this system.

In addition to a connection between eye size and survival, increases in eye size may potentially increase fitness via improved identification of optimal microhabitats leading to enhanced foraging (Banks et al., 2015; Bauer & Kredler, 1993; Brandon et al., 2015; Garamszegi; Lisney et al., 2013; Martin, 1998; C. J. Moran, Ward, & Gibb, 2018; Thomas et al., 2006; Veilleux & Kirk, 2014). Individuals in *Rivulus*-only sites experience high densities and intense competition for resources in the absence of predators (Walsh & Reznick, 2008). We thus predicted that a larger eye size may enhanced fitness via an improved foraging ability for *Rivulus* in *Rivulus*-only populations. Our results support this prediction, as we found that both growth and survival increase as a function of eye size in sites that lack predators, but not in high predation sites (Fig. 2, Fig. 3). Interestingly, work on populations of guppies in the Trinidad system has found that guppies from low predation sites consume comparatively lower quality food than guppies from high predation sites (Zandonà et al., 2011). In addition to this, authors found that guppies from high predation environments selectively consume invertebrates that have a higher nutritional value, while guppies from low predation environments feed on invertebrates indiscriminately (Zandonà et al., 2011). If we assume *Rivulus* follow a similar pattern as guppies, this suggests that *Rivulus* in *Rivulus*-only sites are subject to strong selective pressures for optimal foraging and, in turn, a larger relative eye size, as they not only experience low food availability, but also low food quality.

Beyond experiencing divergent patterns of resource-mediated selection, *Rivulus* also differ in their habitat use and foraging behavior; such differences may have implications for how selection operates on eye size. The diet of *Rivulus* is primarily composed of aquatic dipteran larvae, ants, and winged insects (Fraser et al., 1999) as *Rivulus* actively forage on terrestrial prey that fall into the stream or aerially prey upon these terrestrial resources (Seghers, 1978; Seghers, 1973). Such a foraging strategy should rely heavily on eye size. This is important because it was recently proposed that proximity of refugia may play an important role in the evolution of visual acuity and eye size in butterflyfishes (Hodge et al., 2018). Species of butterflyfish that are considered benthic hunters have a large relative eye size when compared to butterflyfish species that are obligate grazers (Hodge et al., 2018). Benthic hunters need to actively hunt for their prey, while obligate grazers are found close to their coral refugia while feeding (Hodge et al., 2018). Therefore, selection on eye size in this system may also be influenced by the demands to forage across a wider range of habitats. *Rivulus* in *Rivulus*-only sites utilize all available habitat (stream margins, open water), whereas *Rivulus* from high predation sites may need to engage in less active foraging as they are found at lower densities and are found in stream margins where there is often an abundance of terrestrial plant matter overhanging the stream (Beston, pers. obs). Indeed, studies have shown that animals that rely upon visually mediated hunting have enhanced acuity when compared to more passive foragers (Veilleux & Kirk, 2014), which likely reflects the need of greater resolution in capturing mobile prey. In partial support of this hypothesis, research has shown that ambush predators have vertically elongated pupils (Banks et al., 2015), suggesting that foraging mode may play a critical role in not just eye shape, but eye structure.

One surprising aspect of our results is that a larger eye increases survival, but not growth, in high predation sites. There are two explanations, which are not necessarily mutually exclusive, that might explain these contrasting patterns. The first is that a larger eye may enhance detection of predators. Research has shown a positive relationship between eye size and predation pressure across species (Glazier & Deptola, 2011; Hammerschlag et al., 2018). For instance, recent work showed that several species of prey fish from reefs with depleted shark populations exhibited a significantly smaller eye when compared to reefs with robust populations of sharks (Hammerschlag et al., 2018). Furthermore, eastern cottontails with a larger eye size were also slightly better at detecting a predator model from farther distances (Smith & Litvaitis, 1999). It is therefore possible that *Rivulus* with larger eyes are better at detecting predators and display enhanced vigilance in high predation sites. Studies across species have indeed shown that increases in predation can lead to enhanced vigilance (Artiss and Martin, 1995; West, Letnic, Blumstein, & Moseby, 2018) and some work has even indicated that individuals can alter their vigilance depending upon the visibility of the predator (Whittingham, Butler, Quinn, & Cresswell, 2004). Such a relationship between eye size and predator detection ability could then explain why individuals with a larger relative eye size exhibited increased survival in high predation populations.

A second explanation for the survival patterns that we observed in high predation sites relates to the indirect effects of predation on the intensity of intraspecific competition. Increased rates of predation in high predation sites are associated with indirect increases in food availability for *Rivulus* (Walsh & Reznick, 2008); *Rivulus* grow 2x faster in high predation than in *Rivulus*-only sites and such differences are likely environmentally based. In the current study, increases in eye size did not enhance growth in high predation sites (Fig. 3). It is therefore plausible that higher resource levels minimize the benefits associated with a larger eye (due to better foraging) or that selection on eye size is relaxed in these populations as competition declines in high predation sites. The relationship between eye size and predator detection, as well as foraging behavior requires further testing.

It is noteworthy that we observed a positive relationship between survival in both high predation and *Rivulus*-only sites. We hypothesize that eye size and survival increase positively in both sites because competition, and not predation, is the key driver of eye size in *Rivulus.* If competition is the key driver of eye size evolution than we would expect to see selection for a larger relative eye size in *Rivulus*-only sites and weak selection on eye size in high predation sites because there is less competition. Eyes are energetically expensive organs (Laughlin, 2001; Moran, Softley, & Warrant, 2015), and we know that there is strong selection for maximal conversion of resources into somatic tissue in *Rivulus*-only sites (Walsh & Reznick, 2010) while high predation *Rivulus* instead invest heavily in reproduction (Walsh & Reznick, 2008). Larger eyes may enhance survival in high predation sites due to the improved detection of predators, but it is possible that the observed phenotypic divergence in this trait (Beston, Wostl, et al., 2017) is a reflection of higher food levels minimizing the need to invest in such a costly organ.

Although we argue that a link between eye size and foraging explains the evolutionary shifts in eye size in this system, we feel compelled to highlight that there are other mechanistic explanations between eyes and behavior that may help explain the observed variation in eye size in *Rivulus*. This is because much work has shown that the sensory system dictates what an organism discerns and processes from its surroundings, and therefore, visual capabilities and behaviors are tightly linked (Caves, Brandley, & Johnsen, 2018; Hall & Ross, 2007; Zeil, 1983). For example, studies in mammals have shown that acuity is greater in mammals that engage in diurnal activity (Veilleux and Kirk, 2014). This is because adaptations that improve light sensitivity usually trade-off with acuity (Hughes, 1977; Land & Nilsson, 2012; Walls, 1942).

Thus, animals that engage in nocturnal activity likely experience greater benefits of visual adaptations related to improved visual sensitivity at low light levels, while acuity is likely favored in diurnal species. *Rivulus* in high predation sites are rarely seen foraging during the day, while *Rivulus* from *Rivulus*-only sites display crepuscular behaviors (Beston, pers. obs.). Assuming differences in relative eye size are related to shifts in sensitivity, it is possible that differences in eye size between high predation and *Rivulus*-only sites are linked to variations in diel foraging activities.

Eye size and growth rate as a plastic response

Organisms typically respond to declines in resource levels by altering the expression of a variety of traits (Allen, Buckley, & Marshall, 2007; Bashey, 2006; Beston, Broyles, & Walsh, 2017; Beston, Wostl, et al., 2017; Brandon & Dudycha, 2014; Walsh & Reznick, 2008). For example, *Rivulus* and guppies produce larger eggs and offspring when reared under low food conditions (Bashey, 2006; Walsh & Reznick, 2008). We recently showed that the eye size of *Rivulus* is significantly larger when they are fed low food levels in the lab (Beston, Wostl, et al., 2017). We interpreted these responses as potentially being adaptive if eye size enhances foraging ability under low resource conditions. Given that *Rivulus* experience lower food levels in *Rivulus*-only sites, and that eye size and growth covary positively in *Rivulus*-only sites, the results of the current study further support the interpretation that plasticity in eye size is adaptive in *Rivulus.*

CONCLUSIONS

Here, we evaluated the fitness correlates of evolved differences in eye size in natural populations of Trinidadian killifish that differ in the presence and absence of predators. A larger relative eye size is associated with increased survival irrespective of the presence or absence of predators. We observed a positive relationship between relative eye size and growth rate in *Rivulus* from *Rivulus*-only sites, but found no relationship between growth and eye size in high predation sites. *Rivulus* from *Rivulus*-only sites are subject to intense competition due to high densities and low food availability. We therefore hypothesize that larger eyes lead to enhanced survival and growth in *Rivulus*-only sites as larger eyes may be beneficial to foraging in these populations. More mechanistic studies, as well as estimates and behavioral tests of visual performance, are now needed to test how competition can select for shifts in eye size in nature and what the functional implications of slightly larger eyes are.

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TABLES

Table 1. Apparent survival (Φ) and recapture probability (*p*) estimates (± 1 standard error) from the mark recapture study. Estimates reflect the best fitting model for survival and the second best fitting model for recapture probability (see Table S1), as the best fit model for recapture probability included no effects.

	Males		Females	
	Φ	*p*	Φ	*p*
Arima River				
High predation	0.62 (0.08)	0.68 (0.07)	0.72 (0.07)	0.71 (0.06)
Rivulus-only	0.78 (0.05)	0.80 (0.06)	0.84 (0.05)	0.70 (0.06)
Aripo River				
High predation	0.76 (0.08)	0.53 (0.11)	0.84 (0.05)	0.70 (0.05)
Rivulus-only	0.87 (0.05)	0.68 (0.07)	0.91 (0.04)	0.70 (0.06)

Table 2. Results from model selection on apparent survival. All models were constrained by a recapture model with no effects. We found that models with the 'population x eye size' interaction were equivalent ($\Delta QAIC_C < 2$) to models that did not include the interaction but included eye size as a covariate. However, we did find moderately strong support ($5 < \Delta QAIC_C < 7$) for eye size in survivorship irrespective of predator community.

Model effects (Φ)	No. of parameters	$QAIC_C$	$\Delta QAIC_C$	$QAIC_C$ weight	$QAIC_C$ deviance
Stream, sex, population, eye	6	923.1522	0	0.69918	911.0108
Stream, sex, population*eye	7	925.1973	2.0451	0.25148	911.0085
Stream, sex, population	5	928.4705	5.3183	0.04895	918.3697
No effects	2	938.1122	14.9600	0.00039	934.0921

FIGURES

Figure 1. The relationship between total length (mm) and absolute eye size (mm) of *Rivulus* from high predation (HP) and *Rivulus*-only (RO) sites. Closed gray circles and represent HP *Rivulus* and open circles represent RO *Rivulus*.

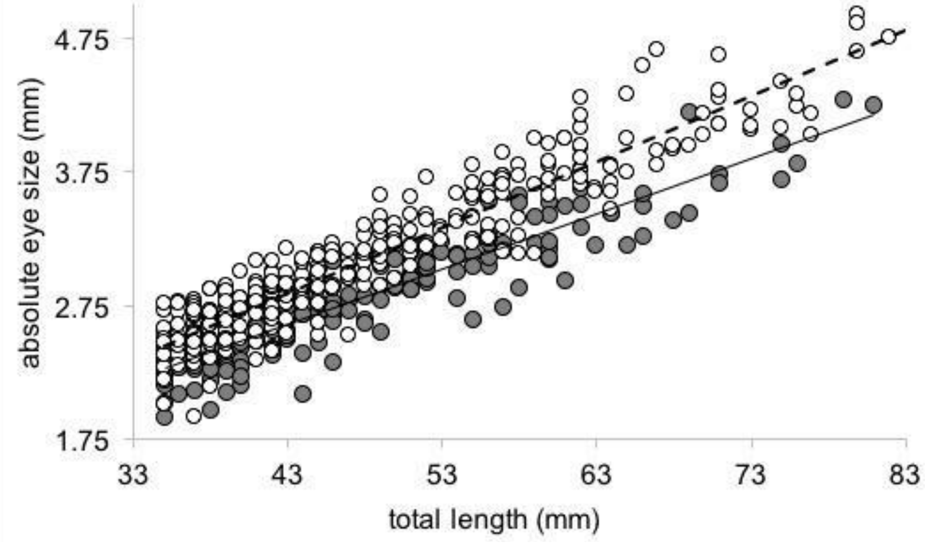


Figure 2. Survivorship predictions as a function of eye size in *Rivulus* from (A) Arima high predation (HP), (B) Arima *Rivulus*-only (RO), (C) Aripo high predation, and (D) Aripo *Rivulus*-only sites. Values outputted from a CJS model that incorporated stream, population, and relative eye size. Solid lines represent mean values, while dashed lines represent the upper and lower limits of the 95% confidence interval, respectively.

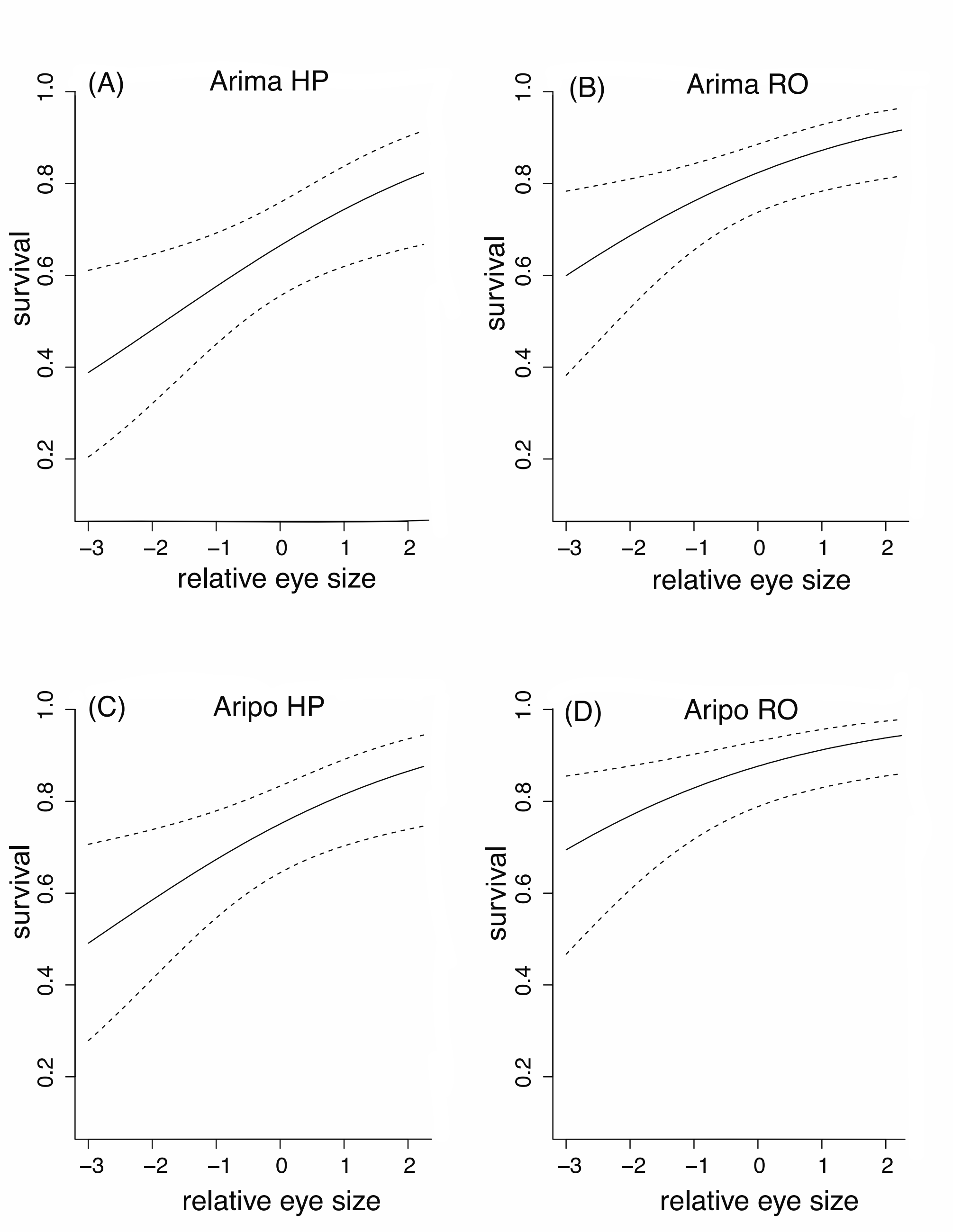
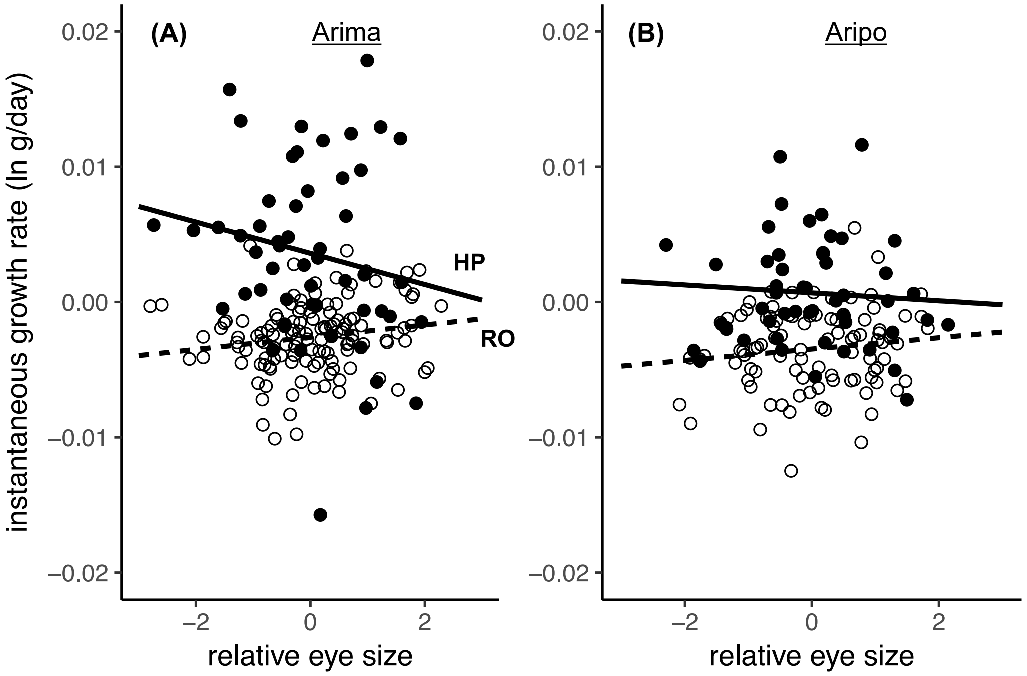


Figure 3. Relative eye size versus instantaneous growth rate in high predation and *Rivulus*-only populations in the (A) Arima and (B) Aripo rivers. Closed circles and solid lines represent individuals from high predation sites, while open circles and dashed lines represent those from *Rivulus*-only sites.



SUPPORTING INFORMATION

Figure S1. Photograph of *Hoplias malabaricus* used to measure eye diameter.

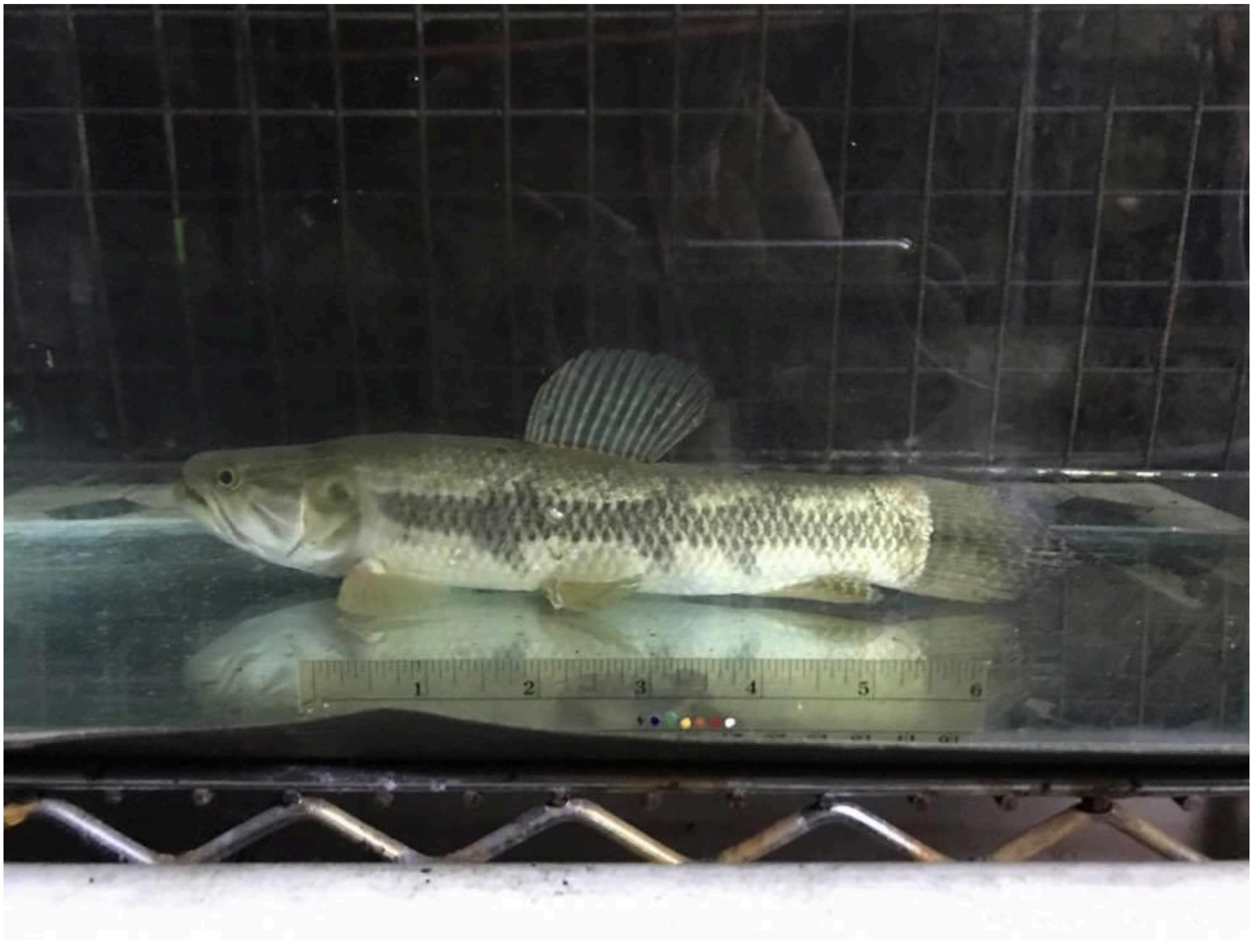


Table S1. Results from model selection for recapture probability (*p*). All models had a full model for survival (Φ). The best fitting model was the model with no effects.

Model effects (*p*)	No. of parameters	QAIC$_C$	ΔQAIC$_C$	QAIC$_C$ weight	QAIC$_C$ deviance
No effects	7	925.1973	0	0.79049	911.0085
Stream, population, sex	10	928.8619	3.6646	0.12651	908.4891
Stream, population, sex, eye	11	930.7176	5.5203	0.05002	908.2694
Stream , sex, population*eye	12	931.5514	6.3541	0.03297	907.0208

Table S2. Parameter estimates from the linear mixed model evaluating the interaction between relative eye size and instantaneous growth rate in *Rivulus* from high predation and *Rivulus*-only sites.

	Estimate	***SE***	***df***	***t***	***z***	***p***
Population	0.005189	0.001477	1.548	3.513	---	0.103
Sex	-0.000111	0.000423	319.279	-0.264	---	0.792
Relative eye size	0.000440	0.000266	319.116	1.656	---	0.099
Population x Relative eye size	-0.001192	0.000462	319.110	-2.579	---	0.010
Population(stream)	1.97×10^{-6}	2.48×10^{-6}	1.00	---	0.795	0.426

Table S3. Mark-recapture data and sample sizes. ‘Marked’ columns include the number of newly marked individuals for each capture/recapture event. ‘Recaptured’ columns dictate the number of previously marked individuals that were captured during the event. ‘Total marked’ includes all individuals marked and ‘total recapture’ includes the number of individuals that were recaptured at least once.

	Initital capture		**1st recapture**		**2nd recapture**		**Total marked**	**Total recaptured**
Stream & population	marked	recaptured	marked	recaptured	marked	recaptured		
Arima:								
high predation	74	---	36	33	---	35	110	52
Rivulus-only	134	---	55	82	---	99	189	131
Aripo:								
high predation	74	---	16	31	---	38	90	50
Rivulus-only	106	---	25	69	---	65	131	94

CHAPTER 4:

COMPETITION AS A MECHANISM FOR EVOLUTIONARY SHIFTS IN EYE SIZE IN *RIVULUS HARTII*

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ABSTRACT

It is now well established that shifts in eye size reflect an organism's environment. Extensive variation in eye size and structure across species is thought to be the result of divergent ecological selective pressures. While much work has evaluated these patterns on the macroevolutionary scale, a growing body of literature has explored the role of ecological variables in shifts in eye size within species. However, the vast majority of this work is correlative. Trinidadian killifish (*Rivulus hartii*) are found in sites with abundant predators ("high predation") and upstream sites where they are the only fish present ("*Rivulus*-only"). *Rivulus* experience lower mortality in *Rivulus*-only sites and, in turn, are found at very high densities and experience intense competition. We recently showed that declines in predation are associated with the evolution of a larger eye. We subsequently evaluated the fitness correlates of shifts in eye size via mark-recapture experiments in natural streams. *Rivulus* experience increased survival as eye size increases, irrespective of predation regime. However, *Rivulus* from *Rivulus*-only sites display a strong positive relationship between eye size and growth rate, while *Rivulus* from high predation sites do not. We thus hypothesized that intense competition favors the evolution of a larger eye size in this system. Here, we used mesocosm experiments where we exposed *Rivulus* from high predation and *Rivulus*-only sites to high competition and low competition environments to test the link between eye size and fitness (i.e. growth). We found that *Rivulus* from *Rivulus*-only sites, but not high predation fish, displayed a strong, positive relationship between eye size and growth rate in high density treatments, but not in low density treatments. These findings argue that competition is a potential mechanism underlying divergent patterns of eye size evolution in Trinidadian killifish.

INTRODUCTION

Variation in eye size across species is extensive (Bauer et al. 1998; Fernald 2004 a,b; Land and Fernald 1992; Land and Nilsson 2012; Caves et al. 2017). The readily accepted hypothesis for this variation is that environmental factors drive and maintain evolutionary shifts in eye size. Increases in eye size are associated with enhanced visual performance (Caves et al. 2017; Land and Nilsson 2012; Martin 1993; Møller and Erritzøe 2010; Motani et al. 1999). A large body of work has shown that ecological factors are correlated with evolutionary divergence in eye size (Veilleux and Lewis 2011; Beston et al. 2017; Beston et al., in press; Garamszegi et al. 2002; Thomas et al. 2006; Moser et al. 2004). While the majority of this research has assessed this question on the macroevolutionary scale, recent studies have begun to address how ecological selective pressures may act on eye size within species (see Beston et al. 2017; Beston et al, in press; Brandon et al. 2015).

Multiple studies have made connections between eye size and vision (Caves et al. 2017; Kiltie 2001) and how these traits interface with ecological factors such as foraging, diel activity, competition, predation, and light availability (Caves et al. 2017; Brischoux et al. 2010; Garamszegi et al. 2002; Hall and Ross 2007; Liu et al. 2012; McCoy et al. 2015; Møller and Erritzøe 2010; Beston et al. 2017; Beston and Walsh, in press; Beston et al., in press). Interestingly, many patterns that have been observed at the macroevolutionary scale are not replicated in studies at the intraspecific level. For example, a study on mammal eye shape and size showed that across species, mammals found in low light habitats had relatively larger corneas than those found in more open habitats (Veilleux and Lewis 2011), while studies completed on Trinidadian killifish *Rivulus hartii* found no effect of light on evolutionary shifts in eye size (Beston et al. 2017). Further, many studies that have linked shifts in eye size with ecological factors within species have found contrasting patterns. For example, Beston et al. 2017 showed that increased predation is associated with the evolution of a smaller eye in populations of *Rivulus,* while studies completed on *Daphnia ambigua* show that increased predation is correlated with the evolution of a larger eye size (Beston et al., in press). Other studies on *Gammarus minus* have shown a similar positive relationship between eye size and predation (though this study does not show a genetic basis for this relationship) (Glazier and Deptola 2011). Taken altogether, these findings suggest that the relationship between eye size and ecological variables is more complex than previously thought and species level shifts in eye size may be highly context specific. To greater understand these seemingly idiosyncratic patterns, empirical tests that elucidate the underlying mechanisms of variation in eye size are needed.

Populations of Trinidadian killifish (*Rivulus hartii*) have lent themselves to understanding how divergent ecological selective pressures can shape the evolution of eye size at the intraspecific level. *Rivulus* are ubiquitous on the island of Trinidad and are found across a diversity of sites that differ in ecological factors, most notably predation intensity (Fraser et al. 1999; Gilliam et al. 1993). *Rivulus* are found in (1) 'high predation' (HP) sites where they are exposed to intense predatory selection by piscivorous fish, such as *Crenichichla frenata* and *Hoplias malabaricus,* as well as (2) '*Rivulus*-only' (RO) sites where *Rivulus* are the only fish species present. These sites not only differ in predator induced mortality (Walsh and Reznick 2008), but also differ in densities. Because *Rivulus* in high-predation sites experience higher levels of mortality these fish are found at lower densities when compared to *Rivulus*-only sites

(Walsh and Reznick 2008; Fraser and Lamphere 2013; Furness and Reznick 2014). As a result, *Rivulus* from high predation sites experience notably weaker competition for resources when compared to *Rivulus* in *Rivulus*-only sites.

We recently showed that ecological divergence in rates of predation are associated with evolved differences in eye size in *Rivulus* (Beston et al. 2017). *Rivulus* from *Rivulus*-only sites have a significantly larger relative eye size (eye size corrected for body size) when compared to those fish from high predation sites. Such differences in eye size are likely genetically-based as these patterns were maintained following two generations of common garden rearing (Beston et al. 2017). We then performed mark-recapture experiments to determine the fitness correlates of eye size in nature. We found that irrespective of predation intensity, a larger eye is associated with enhanced survival (Beston and Walsh, in press). However, a larger relative eye size was positively associated with faster rates of individual growth in *Rivulus* from *Rivulus*-only sites (Beston and Walsh, in press). That is, *Rivulus* from *Rivulus*-only sites with a larger relative eye size grew faster than those with a smaller relative eye size. We thus hypothesized that increased competition, rather than increased predation is the key driver of eye size evolution in this system and that a smaller eye size in high predation sites has resulted from relaxed selection due to lower levels of competition in these populations.

Here, we test these hypotheses using three mesocosm experiments to determine the mechanistic basis of competition as a selective force in the evolution of eye size in *Rivulus.* In our first experiment, we tested if competition (i.e., increased density) influences the connection between relative eye size and growth in *Rivulus*-only sites. We predicted that *Rivulus* with relatively larger eyes exposed to high densities would be better competitors and would thus grow more than *Rivulus* with smaller eyes. In our second experiment, we used experimental manipulations of eye size to determine if shifts in eye size alter fitness irrespective of variation in density. We hypothesized that larger eyed *Rivulus* would outperform smaller eyed *Rivulus* irrespective of density manipulations. In our final experiment, we tested the connection between eye size, density, and growth between high predation and *Rivulus*-only sites. We expected to find greater benefits of a larger eye size under high density treatments and that this effect would be stronger in *Rivulus* from *Rivulus*-only sites.

MATERIALS AND METHODS

To determine the role of competition as it relates to shifts in eye size, three competition experiments were carried out in Trinidad in May 2017 and November-December 2018. All experiments used horticulture pots (40 cm x 53 cm). Each pot contained gravel substrate, stream water, PVC piping and artificial habitat. For all experiments, photographs were taken to measure eye size. Eye size was measured using ImageJ (Schneider et al. 2012) by measuring the diameter of the eye cavity at its widest part. Fish were identified to sex by the presence or absence of white bars at the top and bottom of the caudal fin. Sexually mature male *Rivulus* have white bars, while females do not. All photographs were taken of the fish's left side using Canon Powershot SX530 HS, Canon Powershot ELPH180, or Canon EOS 7D Mark II cameras.

Competition experiment 1 – Competition & eye size in RO sites

To determine if competition influences the relationship between eye size and growth, *Rivulus* were collected from the Aripo RO site in May 2017. Following collection, each fish was measured for length, weight, and given a unique marking using elastomer (Northwest Marine Incorporated, Shaw Island, Walshington, USA). For high density treatments 5 fish were placed in each pot and for the low density treatment 3 fish. Sex ratios were such that there was only 1 male in each pot; high competition treatments had 4 females and 1 male, while low competition treatments had 2 females and 1 male. Each density treatment was replicated 12 times (2 densities x 12 replicates = 24 pots). Several mesocosms were lost due to a landslide that took place (1 low treatment and 3 high treatment pots) prior to the experiment being completed. In addition to this, 6 fish died or escaped during the course of the experiment. These fish were replaced with a fish of a similar size and same sex to keep densities consistent throughout the experiment, but were not included in analyses. One fish did not have a usable photograph and was therefore not included in analyses. Each pot was fed 16 mg of Tetramin Tropical Flake food daily for 28 days. Fish were weighed at the end of the experiments and were euthanized with an overdose of MS-222 and preserved in 10% formalin. Photographs were taken of each fish for measurements of eye size in February 2019.

Competition experiment 2 – Small scale shifts in eye size in RO Rivulus

To determine if shifts in eye size influence the fitness of *Rivulus* irrespective of differences in density, we performed a short-term competition experiment in the horticulture pots for 14 days in December 2018. We collected a total of 248 fish from the Arima RO population over two nights using dip nets, as well as during the day using a sein. We assigned each fish a unique number, measured the fish for total length, and took a photograph of each fish for eye size. We only included fish between 35-50 mm total length (TL). We then outputted residuals from a regression of eye size and body size to obtain measurements of relative eye size. From these residuals we selected fish with the smallest eyes (-1.3 SD from the mean) and largest eyes (+1.3 S.D. from the mean). Sex ratios within each pot were skewed such that there were slightly more females than males in each pot (high density: 3 females, 2 males; low density: 2 females, 1 male) as males tend to engage in male-male and male-female aggression, especially when food is limited (Beston, pers. obs). Each treatment (large eyes, small eyes) was replicated two times for each density treatment (high density, low density) (2 eye treatments x 2 density treatments x 2 replicates = 8 pots total). Each pot was fed 16 mg of Tetramin Tropical flakes daily. Seven fish either jumped or died during the duration of this experiment and were replaced with fish of a similar size and sex and when possible from the appropriate end of the distribution for large eyed versus small eyed fish.

Competition experiment 3 – Eye size, competition, and growth in HP & RO Rivulus

To test the relationship between eye size, density, and growth between high predation and *Rivulus*-only fish, we performed small scale mesocosm experiments over a period of 21 days in November-December 2018. 24 fish were collected from high predation and *Rivulus*-only sites in the Aripo and Arima Rivers during the evening with dip nets (n=96). Fish were then transported back to the field station, and processed the next morning. Fish were measured for length, weight, and given a unique marking and a photograph was taken for measurements of eye size. Fish were kept in small aquaria (~ 2L) for ~24 hours prior to being placed in horticulture pots. Prior to being placed in each pot, fish were assigned to either a high or low density treatment. High density treatments had 5 fish, while low density treatments had 3 fish per pot. Sex ratios were the same as described in competition experiment 2. Each treatment (high density, low density) was replicated 3 times for each combination of stream (Arima, Aripo) and population (high predation, *Rivulus*-only) (2 treatments x 2 streams x 2 populations x 3 replicates = 24 pots) for a total of 24 mesocosms. Each pot was fed 16 mg of Tetramin Tropical flakes daily for 21 days. On day 22, fish were removed from the pots and measured for weight.

Throughout our experiment, we had a total of 19 fish either escape or die. In these cases, we replaced the dead or missing fish as soon as we noticed the fish had died or jumped out of the mesocosm. Fish were replaced with a fish of a similar size and the same sex to keep the competition treatment consistent throughout the trial. These replacement fish were not included in analyses. Mesocosms were checked for missing fish approximately every 1 to 2 days.

Statistical analyses

Competition experiment 1. Measurements of eye size and total length were natural log transformed. To obtain measurements of relative eye size, we outputted standardized residuals from a linear model with a dependent variable of absolute eye size, a fixed effect of sex, and a covariate of total length. We then used a general linear model with a dependent variable of absolute growth, fixed effects of density, sex, a covariate of relative eye size and an interaction of density x relative eye size. Absolute growth was calculated as $\ln(\text{weight}_{\text{end}}) - \ln(\text{weight}_{\text{start}})$. We also explored the relationship between relative eye size and absolute growth for high and low density treatments using simple linear regression.

Competition experiment 2. We used general linear models to determine if differences in eye size within *Rivulus*-only populations under divergent density conditions influenced growth. Our models included a dependent variable of absolute growth and fixed effects of eye treatment (large, small), density (high, low), sex and an interaction of 'eye treatment x density'.

Competition experiment 3. We used a linear model to determine if the relationship between eye size, growth, and competition differs between high predation and *Rivulus*-only fish. We natural log transformed measurements of absolute eye size and total length. First, to obtain measurements of relative eye size, we outputted standardized residuals from a full factorial linear model with a dependent variable of absolute eye size and effects of stream, population, sex, and a covariate of total length. To determine if growth was influenced by eye size under different competition treatments, we used a general linear model with a dependent variable of absolute growth. We included fixed effects of population, treatment, and sex, a random effect of stream, a covariate of relative eye size, and 'population by treatment', 'population by eye size', 'treatment by eye size', and 'population by treatment by eye size' interactions. In addition to using linear models, we also explored the relationship between relative eye size and instantaneous growth rate by using simple linear regression for each combination of treatment and population pooled across stream.

RESULTS

Competition experiment 1

There was no significant effect of sex, density, or 'density x eye size' on growth (Table 1). In line with previous studies, we found a strong and significant ($p < 0.01$) effect of relative eye size on instantaneous growth rate (Table 1). Linear regression of low density treatments and high density treatments, respectively, revealed a significant and positive relationship between relative eye size and instantaneous growth rate in high densities treatments ($F_{1,40} = 4.485$, $p < 0.05$), but not in low density treatments ($F_{1,29} = 1.722$, $p = 0.200$) (Fig. 1).

Competition experiment 2

Density, eye size, and sex did not significantly influence growth of *Rivulus*-only fish (Table 2). In high density treatments, large eyed *Rivulus* grew slightly less than that of small eyed *Rivulus* (Fig. 2). In low density treatments, large and small eyed *Rivulus* grew nearly equal amounts (Fig. 2).

Competition experiment 3

Absolute growth different significantly ($p < 0.05$) between populations (Table 3; Fig 3). Irrespective of density treatment, *Rivulus* from RO populations grew approximately 0.041 g, while those from high predation sites lost approximately 0.017 g over the course of the experiment. In line with previous studies, we found a moderately significant effect of eye size on growth (Table 3). However, the 'population x eye size' interaction was not significant (Table 3). There were no differences in growth between density treatments or the sexes, nor did we find significant effects ($p > 0.05$) of 'eye size x treatment', 'population x treatment x eye size', or 'population x treatment' (Table 3).

DISCUSSION

Understanding how divergent ecological factors shape the evolution of eye size in nature is an important step in furthering our knowledge of how natural selection operates on complex traits. Much work has begun to evaluate the role of ecological factors in the evolution of eye size within species (Brandon et al. 2015; Beston et al. 2017; Beston et al., in press), but studies identifying underlying mechanisms that shape eye size evolution are lacking. Here, we tested potential mechanisms to explain divergent patterns of ecologically driven natural selection on eye size in Trinidadian killifish using multiple mesocosm experiments. Our results show that *Rivulus* in *Rivulus*-only populations exposed to high levels of competition have a strong positive relationship between growth and eye size, but *Rivulus* exposed to lower levels of competition do not display the same relationship (Fig. 1). We found no relationship between eye size and growth in *Rivulus* from high predation sites exposed to different levels of competition (Fig. 3, Table 3), nor did we find any evidence of small-scale shifts in eye size influencing growth rate in *Rivulus* exposed to divergent levels of competition (Fig. 2). We discuss these results in the context of the literature and the Trinidad system.

Beston and Walsh (in revision) showed that larger eye size was positively associated with greater growth rate in *Rivulus*-only sites in natural streams. In our first controlled experiment, we found that when *Rivulus* from a *Rivulus*-only site were exposed to divergent competition treatments (i.e. high competition, low competition), that *Rivulus* exposed to greater levels of competition had a stronger relationship between eye size and growth than those exposed to lower levels of competition (Fig. 1). This suggests that *Rivulus* with a larger eye size are better competitors under high competition conditions than those with a smaller eye size. However, we did not find a significant 'eye size x density' interaction. Because we found no significant interaction, this suggests that the relationship between eye size and growth may only be marginally influenced by competition within *Rivulus*-only populations. This is further supported when we consider the results of our second competition experiment where we experimentally manipulated eye size to determine if shifts in eye size alter fitness irrespective of variation in density. Results from this experiment showed that individuals with slightly larger eyes appear to have no advantage over those with slightly smaller eyes within *Rivulus*-only sites (Fig. 2). However, we cannot rule out that additional factors may be at play here. A previous study on foraging in shorebirds found that birds that foraged at night had a larger eye size than those that foraged during the day (Thomas et al. 2006). *Rivulus* are active during the day and at night in *Rivulus*-only populations. It is therefore possible that there are differences in foraging that relate to timing of foraging within each site; that is, some *Rivulus* forage during the day and other at night and these fish may differ in eye size, as well as foraging and competitive ability as a result. However, this is purely speculative and further exploration of this is needed.

Our previous work has shown that *Rivulus* from high predation environments have evolved a smaller relative eye size when compared to those from *Rivulus*-only sites (Beston et al. 2017). We have also shown that increases in eye size are associated with enhanced survival irrespective of predation intensity (Beston and Walsh, in press). We hypothesized that these evolved differences in eye size were the result of divergent conditions of competition and relaxed selection on eye size in high predation environments. *Rivulus* in high predation zones experience little competition for food due to their low densities (Furness and Reznick 2014; Fraser et al. 1995). When evaluating the relationship between eye size, density, and growth between high predation and *Rivulus*-only sites (experiment 3), we expected to find greater benefits (i.e., growth) of a larger eye size under high density treatments and that this effect would be stronger in *Rivulus* from *Rivulus*-only sites. However, we found no significant interaction of ‘eye size x treatment x density' (Table 3) and thus no evidence of relaxed selection on eye size in high predation *Rivulus.*

Rivulus from high predation sites exposed to high competition and low competition treatments did not differ in their absolute growth and overall, *Rivulus* from high predation zones grew less than *Rivulus* from *Rivulus*-only sites (Fig. 3). This is curious as in the wild *Rivulus* from HP sites grow faster than RO *Rivulus*. In fact, most studies that have tracked shifts in growth rate of *Rivulus* have shown that fish in these sites will on average lose weight (Walsh et al. 2011; Beston and Walsh, in press). It is possible that *Rivulus* from high predation sites experience such low levels of competition in nature that the food treatment used in this experiment is lower than what *Rivulus* typically consume or that *Rivulus* are adapted to even lower densities in HP sites than what was provided throughout our experiment. The latter is likely true as previous work completed by Fraser et al. (1995) found that *Rivulus* density per 100m ranged from approximately 3-6 *Rivulus* in high predation zones and 72 to 215 *Rivulus* in no predator zones. Considering our low density treatment had 3 *Rivulus* in a pot that has a base diameter of 40 cm, *Rivulus* from HP sites were experiencing a substantially higher density than what they experience in nature, even in our 'low density' treatments. An alternative explanation for differences in growth between populations is that this could be evidence of a compensatory response in RO *Rivulus. Rivulus* in RO sites are severely food limited. Compensatory growth is characterized by rapid increases in growth during favorable conditions that follow periods of starvation or food limitation (Ali et al. 2003). In fish, periods of starvation alter storage reserves, specifically lipids (Ali et al. 2003). Thus, this may be a physiological response of RO *Rivulus* to a steady food resource that is not normally experienced in the wild.

One puzzling component of our results is that our findings in competition experiment 1 and competition experiment 3 are contradictory. We fail to see the same patterns we observe in competition experiment 3 in our first competition experiment in *Rivulus*-only fish. In our first experiment, we find a positive and significant relationship between eye size and growth rate in high competition treatments in *Rivulus*-only fish (Fig. 1). In our third experiment, we fail to see this same pattern in eye size and growth rate in high density, *Rivulus*-only treatments. There are a few potential explanations for this. The first is that our third mesocosm experiment may not have had the necessary power to reveal the patterns we see in our first experiment. In our third experiment our design only allowed for 12 *Rivulus*-only pots with 6 pots subjected to high and low competition treatments, respectively. Our first experiment, however, nearly doubled this replication. Additionally, our third experiment had far more death than what was experienced in our first experiment. This may largely have to do with the density of males used in our first experiment versus our third experiment. In our third experiment, 1-2 males were placed in all pots, while in the first experiment only 1 male was placed in each pot. Male *Rivulus* are known to engage in both male-male and male-female aggression, especially when competing for food. Thus, it may have been better to limit the number of males used in our experiment. This may also explain why we found unexpected patterns of growth in our third experiment: high predation *Rivulus* lost weight throughout the experiment irrespective of treatment, when compared to *Rivulus*-only fish.

An alternative explanation of our findings may be that divergence in eye size is not related to competition and is instead a byproduct of differences in growth rate between high predation and *Rivulus*-only fish (Beston and Walsh, in press; Furness and Reznick 2014). However, lab based experiments on *Rivulus* have shown that differences in growth rate that are observed in the field are not genetically based (Walsh and Reznick 2008), while differences in eye size are consistent between common garden reared fish and those in the field (Beston et al. 2017). In high predation environments, *Rivulus* grow faster, mature earlier, and are a smaller size when they reach maturation when compared to *Rivulus* from *Rivulus*-only sites (Walsh and Reznick 2008). It is thus possible that rather than investing in an energetically expensive organ, such as the eye, that *Rivulus* in these populations invest primarily in reproduction as these fish experience higher mortality rates and therefore likely have a shorter lifespan than those in *Rivulus*-only sites. Further tests are now needed to evaluate differences in visual aptitude between high predation and *Rivulus*-only fish, as well as tests that connect eye size and vision to foraging behaviors.

CONCLUSIONS

Here we present a potential mechanism for evolved differences in eye size in populations of Trinidadian killifish. We found evidence that suggests that increases in eye size are associated with a competitive advantage in the high density and high competition environments of *Rivulus*-only sites. Future work should test this mechanism using foraging trials and should also further explore how timing (i.e., diurnal versus nocturnal) of foraging may influence eye size.

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TABLES

Table 1. Analyses of absolute growth for competition experiment 1. F, F-values, df = numerator degrees of freedom. Significant terms are bolded.

Effect	df	Absolute growth F	p-value
Covariate:			
eye size	1	**5.608**	**0.021**
Fixed effects:			
sex	1	0.422	0.518
density	1	0.415	0.521
density x eye size	1	0.17	0.681
error	66		

Table 2. Analyses of absolute growth for competition experiment 2. F, F-values, df = numerator degrees of freedom.

Effect	df	Absolute growth F	p-value
Fixed effects:			
density	1	0.75	0.397
eye	1	0.239	0.631
sex	1	0.29	0.596
density x eye	1	0.095	0.761
error	20		

Table 3. Analyses of absolute growth for competition experiment 3. F, F-values, df = numerator degrees of freedom. Significant terms are bolded. Marginally non-significant terms italicized.

Effect	df	Absolute growth F	 p-value
Covariate:			
eye size	1	*3.485*	*0.066*
Fixed effects:			
population	1	**4.006**	**<0.05**
treatment	1	0.94	0.336
sex	1	2.199	0.143
population x treatment	1	0.149	0.7
population x eye size	1	0.638	0.427
treatment x eye size	1	0.329	0.568
population x treatment x eye size	1	0.96	0.331
Random effect:			
stream	1	0.011	0.916

FIGURES

Figure 1. Relationship between relative eye size and absolute growth under high (HD) and low (LD) density treatments from competition experiment 1. HD treatment is shown as closed circles and LD treatment is shown as open circles. We found a significant ($p < 0.05$) relationship between relative eye size and growth in high density treatments, but not in low density treatments.

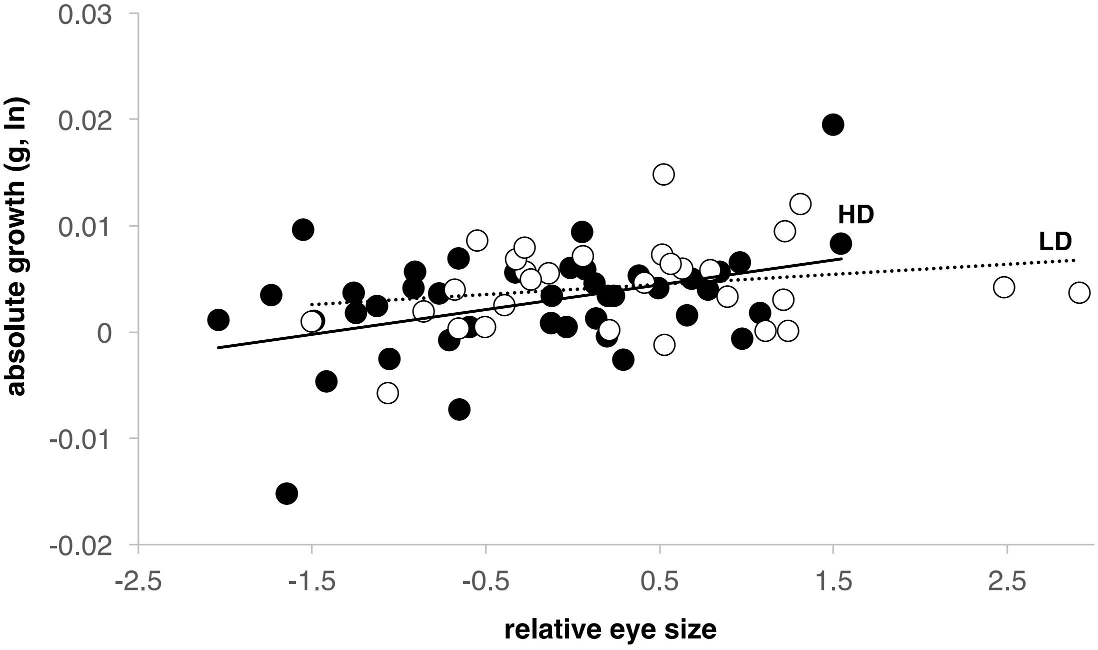


Figure 2. Variation in absolute growth in competition experiment 2 in small and large eyed fish. We observed no difference in growth between large and small eyed *Rivulus* under divergent competition treatments. Mean high density (HD) is represented with closed circles and mean low density (LD) is represented with open circles. Error bars = ± 1 S.E.

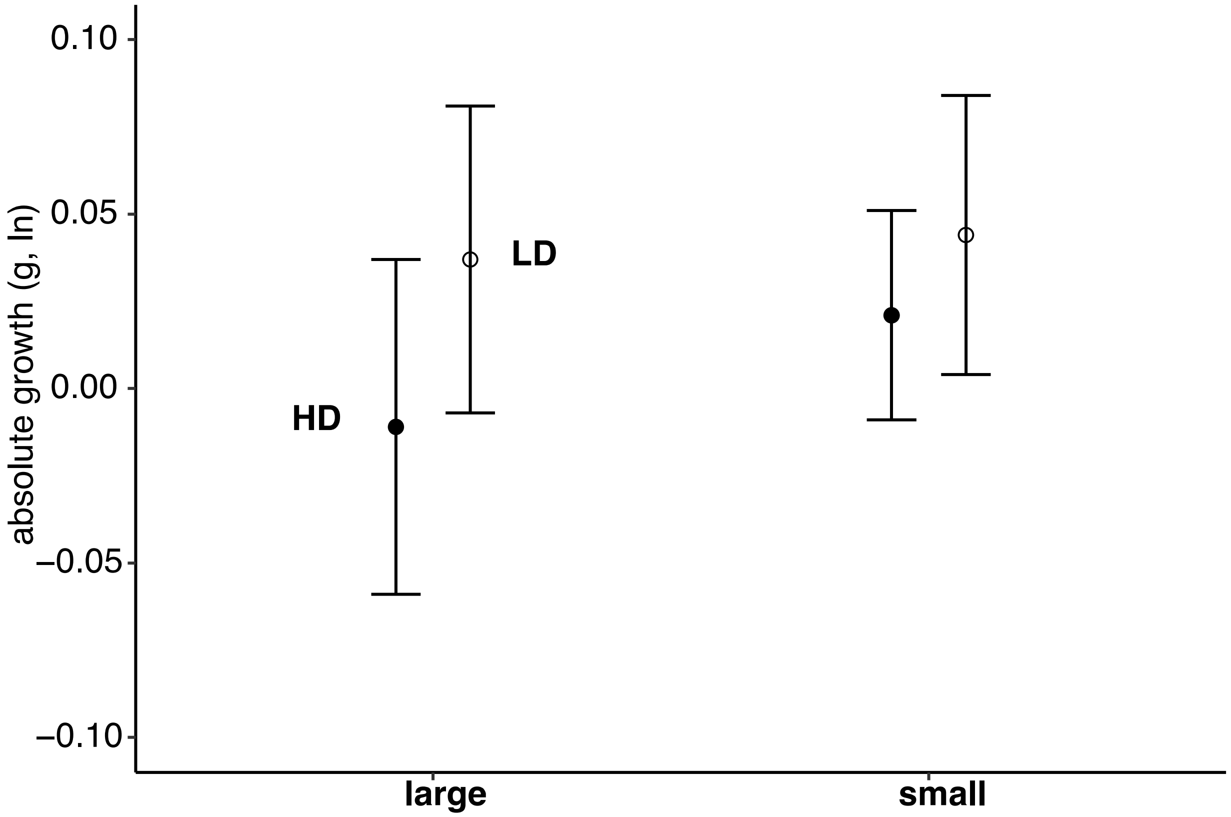
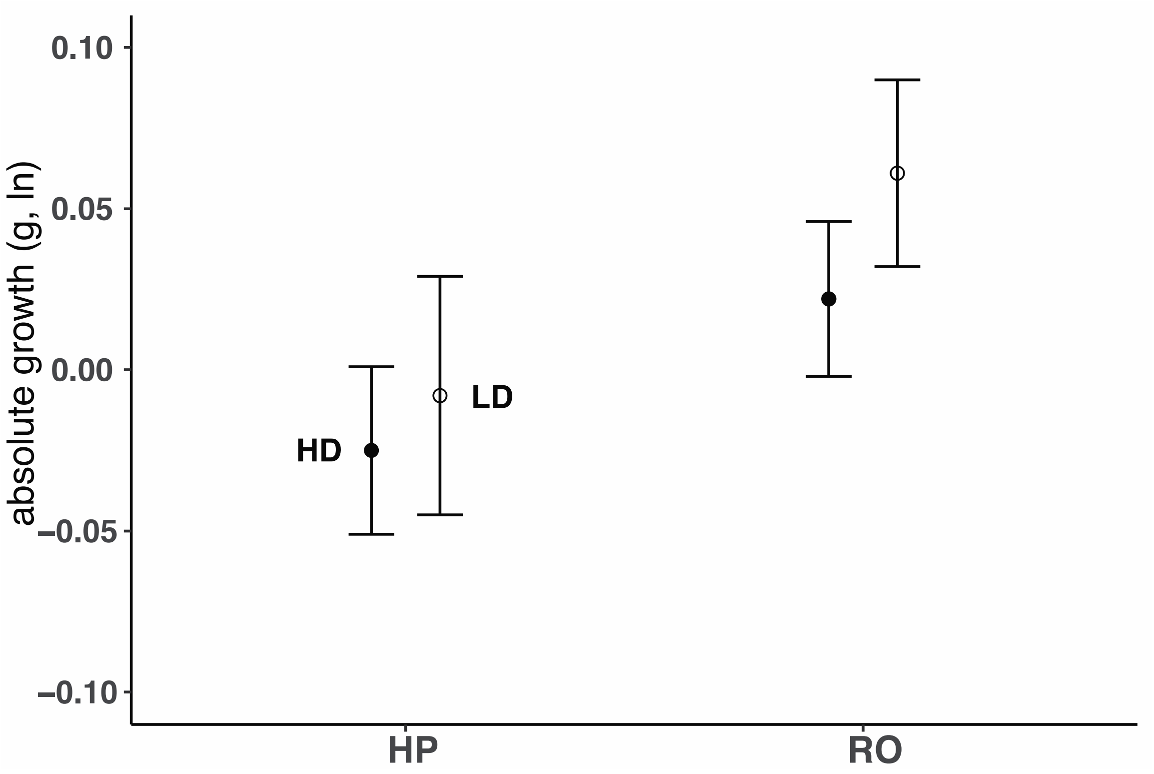


Figure 3. Variation in absolute growth rate for competition experiment 3. We observed a significant ($p < 0.05$) difference in growth between *Rivulus* from high predation (HP) and *Rivulus*-only (RO) sites, irrespective of whether fish were exposed to high density (HD) or low density (LD) treatments. Average absolute growth for HD treatments is represented with a closed circle, while average absolute growth for LD treatments is represented with an open circle. Error bars = ± 1 S.E.



CHAPTER 5:

THE EVOLUTION OF EYE SIZE IN RESPONSE TO INCREASED FISH PREDATION IN *DAPHNIA*§

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ABSTRACT

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.

INTRODUCTION

The size and structure of eyes vary extensively across taxa (Bauer et al. 1998; Fernald 2004 a,b; Land and Fernald 1992; Land and Nilsson 2012). Increased eye size is correlated with enhanced aspects of vision (Caves et al. 2017; Land and Nilsson 2012; Martin 1993; Møller and Erritzøe 2010; Motani et al. 1999; Ritland 1983), as well as shifts in foraging, mating behavior, and anti-predator responses (Brischoux et al. 2010; Garamszegi et al. 2002; Hall and Ross 2007; Liu et al. 2012; McCoy et al. 2015; Møller and Erritzøe 2010). 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; Hall 2008; Moser et al. 2004; Ross and Kirk 2007; Somanathan et al. 2009; Thomas et al. 2006; 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 Beston et al. 2017a; Brandon et al. 2015).

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 (Beston et al. 2017a; Brandon et al. 2015; Glazier and Deptola 2011). For instance, predators often target pigmented characteristics, such as eyes. Such mortality should lead to the evolution of a smaller eye in prey (Beston et al. 2017a; Lönnstedt et al. 2013; Zaret and Kerfoot 1975). Conversely, increased eye size is correlated with enhanced anti-predator 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 3 lakes with anadromous alewife (Bridge, Dodge, Gorton), 3 lakes with landlocked alewife (Amos, Long, Quonnipaug), and 3 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 ~1.0 mg C L^{-1} day^{-1}) (Photoperiod 14L:10D; 13ºC). *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 6 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 (13ºC) and photoperiod (L:D 14:10) as the previous generations. In the original experiment, all clones were replicated 3x per treatment (125 clones across all lakes x 3 replicates per treatment x 2 treatments x 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 amongst 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 non-significant ($p > 0.05$), 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 were 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: 2m, 4m, 6m, 8m; Dodge: 3.5m, 7m, 10.5m, 14m; Hayward: 2.5m, 5m, 7.5m, 9.75-10m; Gardner: 3m, 6m, 9m, 12m). We completed 10 samples (12 L x 10 samples = 120 L total volume) at each depth across all lakes (except for at Hayward 2.5m and Wyassup 8m 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 non-significant ($p > 0.05$).

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 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 non- significant ($p > 0.05$) (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 x 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 non-predator treatments in generation 1 but the absolute and relative eye size of *Daphnia* from the predator treatment was ~4% and ~2% smaller than the non-predator 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 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 non-significant 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 non-significant ($0.05 < p < 0.10$) '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 vs. depth in lakes

Daphnia densities differed by lake type ($F_{1,3.099} = 30.640$, $p < 0.05$). *Daphnia* were approximately 7 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 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 towards 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 (Wong-Riley 2010; Laughlin 2001; 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 are 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 (Beston et al. 2017a; Brandon et al. 2015; Glazier and Deptola 2011). Similar to the results of the current study, populations of amphipods (*Gammarus minus*) that co-occur 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 versus 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 (Beston et al. 2017a; Glazier and Deptola 2011). 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.

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TABLES

Table 1. Analyses of absolute and relative eye size for laboratory experiments. 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 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 x predator	2	1.6 (1127.8)	0.2	0.66 (1125)	0.52
Lake type x generation	2	0.71 (1128.7)	0.49	1.44 (1126.5)	0.24
Predator x generation	1	**12.37 (1125.2)**	**<0.001**	**4.17 (1130.9)**	**0.041**
Lake type x predator x 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**

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

FIGURES

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 grey circles represent the eye sizes for all replicate lakes. Letters denote significant differences based upon post-hoc tests. Error = ±1 s.e.

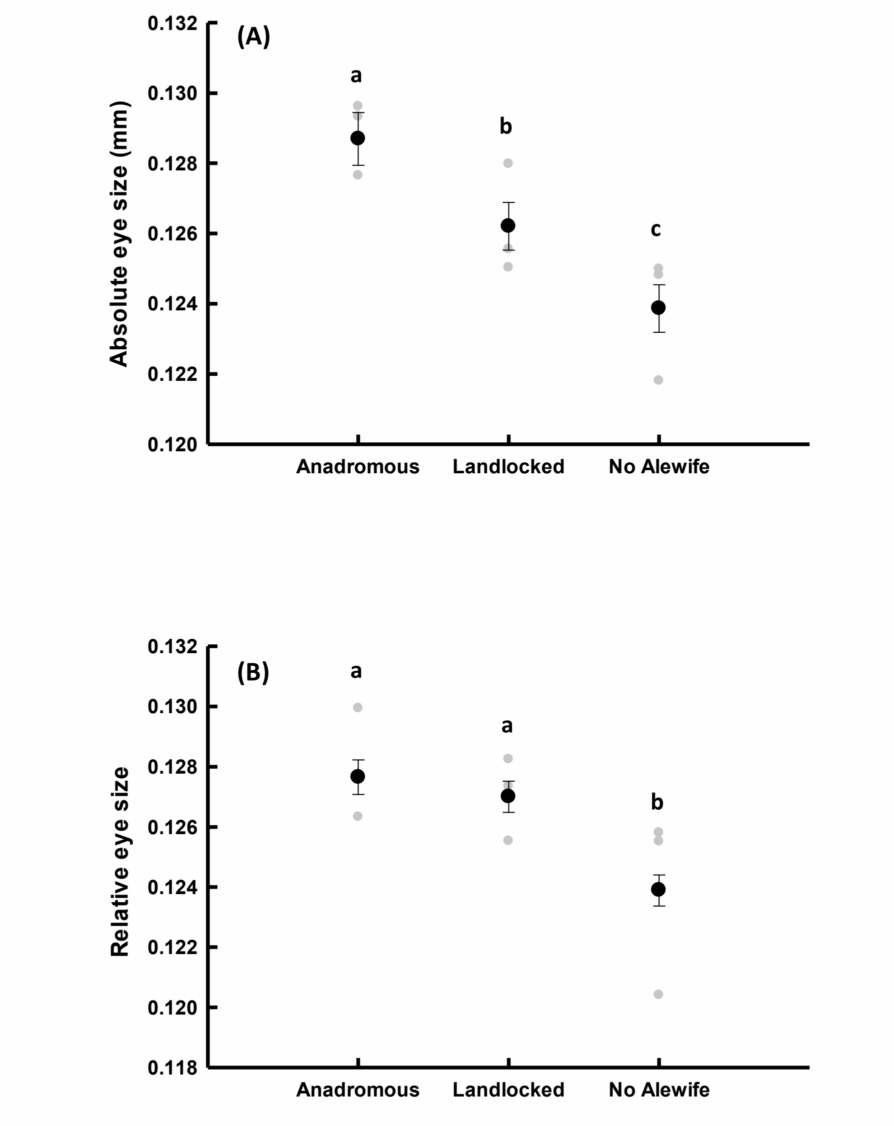


Figure 2. Predator-induced transgenerational plasticity in (A) absolute and (B) relative eye size. 'Predator' and 'No predator' denote the treatments where *Daphnia* were raised in the presence and absence of alewife predator cues in generation one, respectively. We found a significant ($p < 0.05$) 'predator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

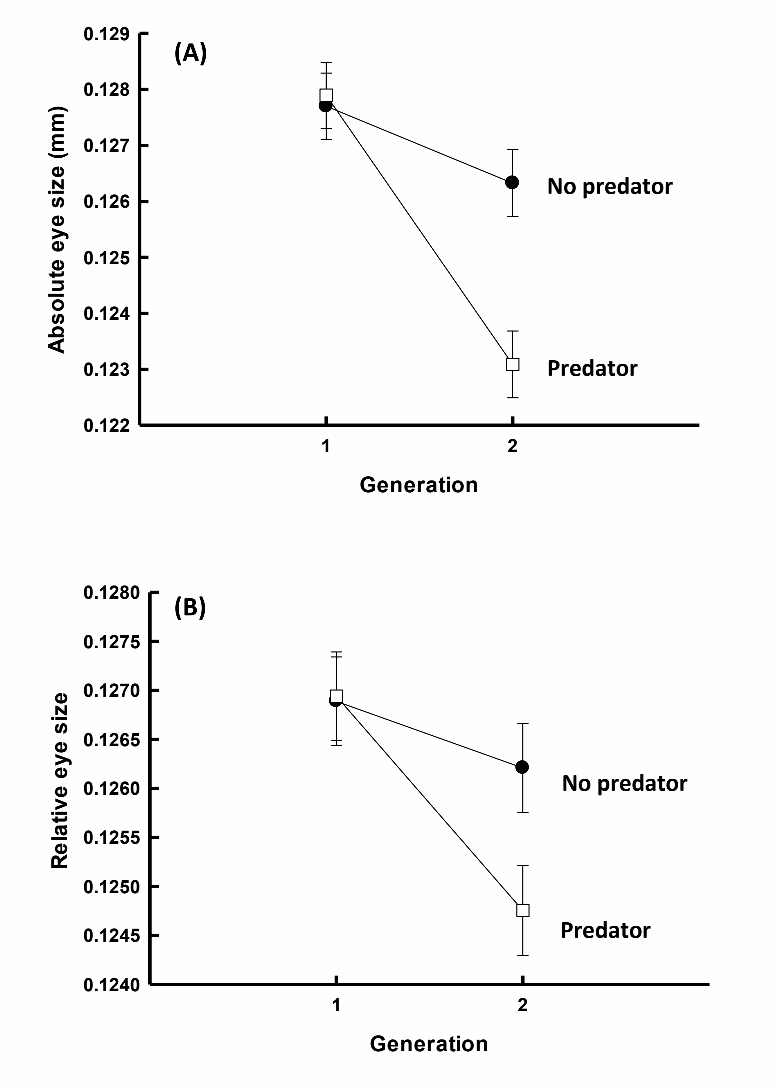
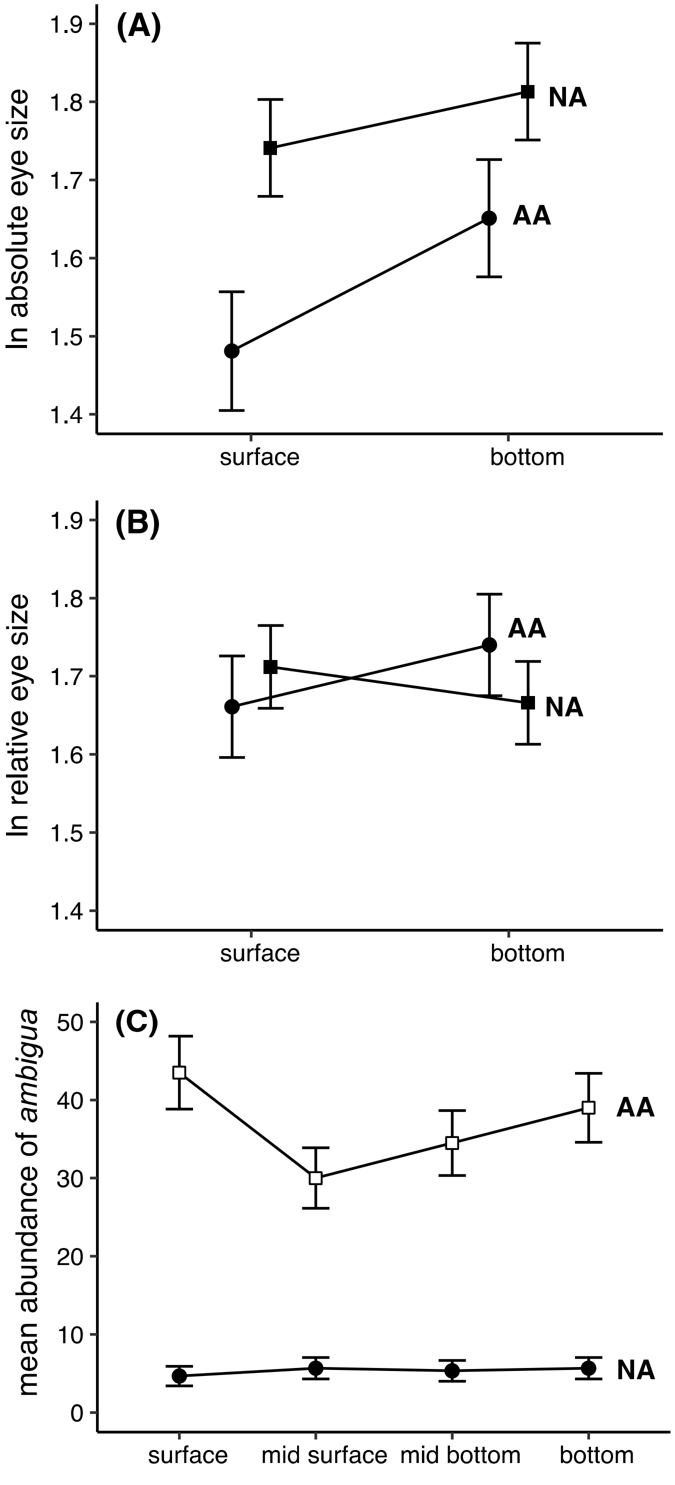


Figure 3. Variation in (A-B) eye size and (C) average densities across depths of wild-caught *Daphnia* populations. We found a moderately non-significant ($0.05 < p < 0.10$) and significant ($p < 0.5$) 'lake type x depth' interaction for absolute and relative eye size, respectively. In panels A and B, 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 s.e.


(A)
ln absolute eye size
1.9
1.8
1.7
1.6
1.5
1.4
NA
AA
surface
bottom
(B)
ln relative eye size
1.9
1.8
1.7
1.6
1.5
1.4
AA
NA
surface
bottom
(C)
mean abundance of *ambigua*
50
40
30
20
10
0
AA
NA
surface
mid surface
mid bottom
bottom

SUPPORTING INFORMATION

Table S1. Secchi depths across lakes and lake types. Secchi depth measurements were taken at the same time as *Daphnia* were sampled from each lake.

	Secchi depth (m)
Anadromous:	
Bride	1.5
Dodge	2.5
No alewife:	
Wyassup	2.25
Hayward	2.5
Gardner	2.5