The effects of age, sex, and habitat on body size and shape of the blackstripe topminnow, *Fundulus notatus* (Cyprinodontiformes: Fundulidae) (Rafinesque 1820)

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Lake and stream habitats pose a variety of challenges to fishes due to differences in variables such as water velocity, habitat structure, prey community, and predator community. These differences can cause divergent selection on body size and/or shape. Here, we measured sex, age, length, and eight different morphological traits of the blackstripe topminnow, *Fundulus notatus*, from 19 lake and stream populations across four river drainages in central Illinois. Our goal was to determine whether size and shape differed consistently between lake and stream habitats across drainages. We also considered the effects of age and sex as they may affect size and morphology. We found large differences in body size of age 1 topminnows where stream fish were generally larger than lake fish. Body shape mainly varied as a function of sex. Adult male topminnows had larger morphological traits (with the exception of body width) than females, in particular longer dorsal and anal base lengths. Subtle effects of habitat were present. Stream fish had a longer dorsal fin base than lake fish. These phenotypic patterns may be the result of genetic and/or environmental variation. As these lakes are human-made, the observed differences, if genetic, would have had to occur relatively rapidly (within about 100 years). © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 784–789.

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INTRODUCTION

Lake and stream habitats differ in many ecologically important characteristics including both biotic (predator and prey assemblages) and abiotic (spatial complexity and water velocity) factors (Jackson, Peres-Neto & Olden, 2001). Rivers/streams have a higher water velocity, are generally more heterogeneous habitats (Eadie *et al*., 1986), and contain lower predator abundances (Larimore & Bayley, 1996) than lakes. Studies comparing lakes and streams have found differences in fish body size and shape (Hendry, Taylor & McPhail, 2002; Collin & Fumagalli, 2011). The general prediction is that individuals will be more 'streamlined' in river/stream habitats (Langerhans, 2008), but there is evidence that this is not universally true (Brinsmead & Fox, 2002; McGuigan *et al*., 2003). Differences in size and shape may also arise from intrinsic factors, such as sex or age (Parker, 1992; Simonovic *et al*., 1999; Hendry *et al*., 2006; Spoljaric & Reimchen, 2011).

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Our goal was to explore body size and shape differences of the blackstripe topminnow, *Fundulus notatus*, between lake and stream populations across two ages and both sexes. We also examine the consistency of such patterns by utilizing populations from four different river drainages. Drainage was found to be important in determining the body shape of *F. notatus* in another study, although that study was only conducted in rivers (Schaefer, Duvernell & Kreiser, 2011). Gene flow is probably higher between populations within a drainage than between populations across drainages and, thus, examining patterns across multiple drainages allows for an assessment of the generality and consistency of the pattern. Consistent correlations between an organism's phenotype and their environment provide potential clues as to the adaptive significance of these traits (Reznick & Travis, 1996).

METHODS

A total of 572 blackstripe topminnows were collected with seines and dipnets from eight sites (four lakes and four streams) in 2008 and 17 (seven lakes and ten streams) in 2010, of which five sites were sampled in both years. The data from the two years were analysed together. The sites were chosen so that at least one lake and one stream were from each of four different river drainage systems in central Illinois (Supporting Table S1, Fig. S1).

Prior to measurement, fish were killed with an overdose of MS-222, preserved in formalin, and then stored in 70% ethanol. Following the methods of Grünbaum *et al*. (2007), fish were measured for body size (standard length) and eight standard linear morphometric measurements: body height, body width, caudal peduncle height, dorsal fin base, anal fin base, head height, head width, and eye diameter (Fig. S2). All measurements were made to the nearest 0.1 mm using Vernier calipers. Fish were aged to the nearest year using growth rings on their scales observed under light microscopy (DeVries & Frie, 1996). Sex was determined visually by examining lateral stripe patterning and fin ray length (Carranza & Winn, 1954) (Fig. S3).

STATISTICAL ANALYSIS

Individuals were classified into age classes corresponding to the number of full years they completed (i.e. age 0 are those individuals less than 1 year of age, age 1 are individuals over 1 but less than 2 years, etc.). We used a chi-square analysis to test for differences in the age distribution as a function of habitat as well as to test for differences in the distribution of the sexes between the two habitat types.

We analysed size and shape separately. For all analyses, we only considered individuals in age classes 0 and 1 because many populations only contained these two ages (see Table S1). To examine patterns in size, we compared body size between age-classes and sexes using a general linearized model. To examine patterns in shape, we regressed each of the eight morphometric traits on body size and then analysed the residuals in a principal components analysis using the correlation matrix. We focused on the first four principal components because together they accounted for almost 90% of the overall variation (Table S2).

For both the size and the shape analyses, the main terms were tested using either population or sex*population as the level of replication. We modelled the effects of habitat, drainage, sex, habitat*drainage, and population nested within the interaction of habitat*drainage. We consider drainage and population(habitat*drainage) to be random. We analysed the data separately for each age class and only included those populations that had at least two individuals of both sexes for a particular age. The error degrees of freedom were generated using a Satterthwaite approximation, which incorporates a weighted average of two components (the population (habitat*drainage) or sex*population (habitat* drainage) and the mean square error) (Sattertwaite, 1946). All analyses were performed in SAS V.2 (Cary, NC, USA). More information about the statistical analyses can be found in the Supplemental Methods. All raw data have been deposited in Dryad (Dryad data repository doi:10.5061/dryad.j7th2).

RESULTS

AGE AND BODY SIZE

Lakes consisted of 59% age 0 individuals, 39% age 1, and 2% age 2, while streams consisted of 57% age 0, 37% age 1, 5% age 2, and 1% age 3 individuals (Table S1). The age distribution did not differ between habitat types, regardless of whether we excluded age 3 ($\chi_2^2 = 3.8332$, $P = 0.1471$) or combined ages 2 and 3 into one age class ($\chi_2^2 = 5.4417$, $P = 0.0658$). The latter analysis trended toward a pattern where lakes were shifted towards a younger age distribution and streams towards an older distribution. The number of individuals of each sex did not differ between habitat types, as both lakes and streams consisted of 56% males and 44% females ($\chi_2^2 = 0.0391$, $P = 0.8432$).

Body size did not differ between the two sexes, but did differ among habitat types and drainages (Table S3). For age class 0, body size differed among habitat types, but the pattern depended on drainage, such that stream fish were larger in the Vermilion and
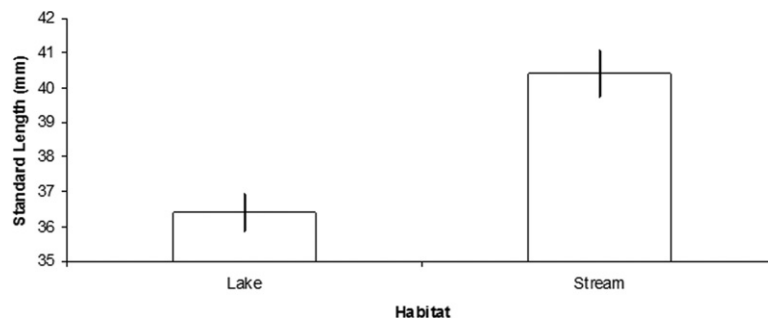
Figure 1. Standard length of fish from age class 1 as a function of habitat. Error bars are ±1 standard error.
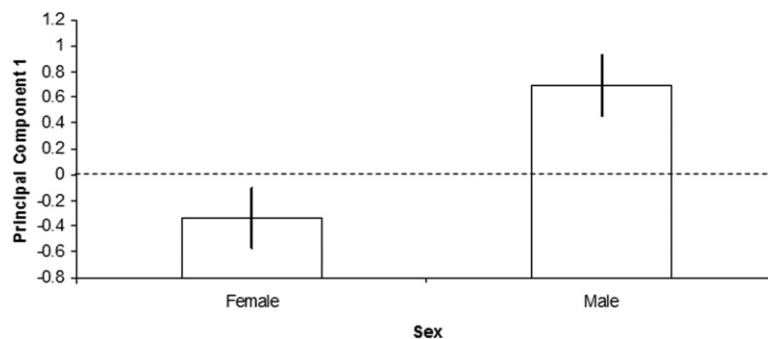

Figure 2. PC1 of fish from age class 1 as a function of sex. Error bars are ±1 standard error.

Kaskaskia drainages and lake fish were larger in the Embarras and Sangamon drainages ($F_{3,8} = 2.19$, $P = 0.0138$). In age class 1, stream fish were significantly larger than lake fish ($F_{1,8} = 7.86$, $P = 0.0228$) (Fig. 1).

BODY SHAPE

The first four principal components (PCs) together accounted for 88.5% of the variation (Table S2). The first principal component accounted for 61.5% of the variation. All traits with the exception of body width loaded positively onto PC1. In age class 1, males were more 'robust' (i.e. had more positive PC scores) than females ($F_{1,8} = 7.69$, $P = 0.0223$) (Fig. 2). The drainages which flow into the Ohio River (Vermillion and Embarras) had more robust age 1 fish than those which flow into the Mississippi River ($F_{3,8} = 8.56$, $P = 0.0066$) (Table S4).

PC2 was positively associated with body and head width (Table S2). We found no effect of habitat, drainage, sex, or any of their interactions for either age class (Table S5).

PC3 was positively associated with eye diameter and negatively associated with dorsal fin base length (Table S2). Lake individuals had larger PC3 scores (i.e. larger eye diameter and smaller dorsal fin base length) than stream individuals in both age class 0 ($F_{1,8} = 15.03$, $P = 0.0043$) and age class 1 ($F_{1,8} = 5.69$,

$P = 0.0435$). For age class 1, females had larger eyes and smaller dorsal fin base length than males ($F_{1,8} = 5.32$, $P = 0.0454$) (Table S6).

PC4 was positively associated with anal and dorsal fin base lengths and negatively associated with head width (Table S2). For age class 1, males had greater PC4 values (i.e. larger anal and dorsal fin base lengths and smaller head widths) than females ($F_{1,8} = 14.09$, $P = 0.0047$) (Table S7).

DISCUSSION

Our goals were to explore body size and shape differences of the blackstripe topminnow, *Fundulus notatus*, between lake and stream populations across two ages and both sexes and to examine the consistency of such patterns. Body size was driven by habitat, but differences in shape primarily resulted from sexual dimorphism. These differences mainly appeared in the later age class and were largely consistent across the four river drainages. We discuss these results in more detail below.

Adult (age class 1) stream fish were generally larger than adult lake fish. This may be explained by differences in predation and/or water velocity between lakes and streams. The lake sites sampled for this study are often stocked with predatory game fish

(Larimore & Bayley, 1996). High predation on larger fish/adults would select for smaller size at maturation (Charlesworth, 1980; Conover & Munch, 2002). Water velocity also affects body size through growth, although different species respond differently (Davison, 1997; Bhagat, Fox & Ferreira, 2006; Grünbaum, Cloutier & Le Francois, 2008; Fischer-Rousseau, Chu & Cloutier, 2010).

There was an effect of sex on three of the four principal component axes, suggesting that there is substantial sexual dimorphism in body shape of topminnows, particularly in the older age class. This is not surprising, as sexual dimorphism in body shape is common among many fish species (Parker, 1992). In general, male topminnows have larger traits, in particular greater anal and dorsal fin base lengths, than females. Differences in the fins had previously been qualitatively described and were attributed to the sex-specific roles of these fins in courtship and spawning (Carranza & Winn, 1954).

There was an effect of habitat on body shape, where individuals from lakes had a larger eye diameter and smaller dorsal fin base length than those from streams. Dorsal fins aid manoeuvring and stabilization in the water column (Standen & Lauder, 2007) and this is presumably more important for fish facing higher water velocities. However, there is interspecific variation in the use of the dorsal fin under different swimming speeds (Drucker & Lauder, 2005) and, thus, it is unknown whether the differences observed here are related to water velocity or some other environmental difference between lakes and streams. Interpreting the eye pattern is also difficult, as some fish have large eyes when they occur in clear water for picking zooplankton out of the water column (Huber *et al.*, 1997) while others have larger eyes when living in low light environments (Warrant & Locket, 2004). Either of these scenarios could apply to lake topminnows, as they spend most of their time at the water surface but overwinter in deeper water (Carranza & Winn, 1954).

It is unknown whether these phenotypic patterns are the result of genetic and/or environmental variation. Because all of our lake sites are human-made and less than 100 years old, the observed differences among populations within drainages, if genetic, would have had to develop fairly quickly. Impounding streams to form lakes is starting to be recognized as a potentially important driver of morphological change (Haas, Blum & Heins, 2010; Franssen, 2011). Our study was not designed to explicitly test this, as only four of our lake sites were created through impoundments, but rapid evolution has been documented in many taxa (Thompson, 1998; Simberloff *et al.*, 2000; Reznick & Ghalambor, 2005) including another cyprinodontiform (Collyer *et al.*, 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Supplemental Methods. The effects of age, sex, and habitat on body size and shape of the blackstripe topminnow, *Fundulus notatus*.

Figure S1. Map of the river system of Illinois with the four river drainages used in this study labelled. Enlarged sections show the counties in east-central Illinois and the sites used in this study (triangles are lakes, rectangles are streams). Two pairs of sites (Homer Lake/Homer Dam and Clear Lake/Long Lake) are grouped together because of close geographical proximity. Numbers refer to the 'map number' in Table S1. The star indicates the location of the University of Illinois.

Figure S2. Morphometric traits measured. Body width and head width are not shown.

Figure S3. Photographs of an approximately size-matched female (top) and male (bottom). Males have a more jagged/diamond-shaped lateral stripe and larger, more 'pointed' dorsal and anal fins than females. Background consists of 1×1-mm grid for scale.

Table S1. Habitat type and number of specimens collected at each site. Map number corresponds to the number on Fig. S1. For the lakes, the approximate year built is included in parentheses. For streams, the cumulative drainage area (CDA) is provided as a descriptor of stream size and local hydrology.

Table S2. Results from principal component analysis on the size-regressed traits using the correlation matrix. Eigenvectors of each morphometric trait for the first four principle component axes are shown. Eigenvalues and the proportion of variation accounted for are listed below.

Table S3. F-values from analyses of variance on standard length of (A) age 0 and (B) age 1 individuals. Significant ($P < 0.05$) factors are indicated with an asterisk (*). Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

Table S4. F-values from analyses of variance on PC1 of (A) age 0 and (B) age 1 individuals. Significant ($P < 0.05$) factors are indicated with an asterisk (*). Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

Table S5. F-values from analyses of variance on PC2 of (A) age 0 and (B) age 1 individuals. Significant ($P < 0.05$) factors are indicated with an asterisk (*). Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

Table S6. F-values from analyses of variance on PC3 of (A) age 0 and (B) age 1 individuals. Significant ($P < 0.05$) factors are indicated with an asterisk (*). Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

Table S7. F-values from analyses of variance on PC4 of (A) age 0 and (B) age 1 individuals. Significant ($P < 0.05$) factors are indicated with an asterisk (*). Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.