Evolutionary Ecology Research (revision 2021-8-24)

Frequent habitat migration, phenotypic plasticity, and residual ecophenotypy revealed by isotope-based natal habitat inference in bluegill sunfish, *Lepomis macrochirus*

Joshua R. Mays[1, 2], Thomas J. DeWitt[3*], Prarthana Dharampal[1,4], C. Fred T. Andrus[5], Robert H. Findlay[1]

[1]Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA
[2]Department of Science, Gaston College, Dallas, NC 28034, USA
[3]Department of Ecology & Conservation Biology, Texas A & M University, College Station, TX 77843, USA
[4]Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, USA
[5]Department of Geological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA

[*]*Corresponding author. Current e-mail: tdewitt@tamu.edu*

ABSTRACT

Background: Forced or chosen habitat changes drive the evolution of strategies by which organisms tolerate or mitigate functional tradeoffs among habitats. Phenotypic plasticity is a powerful adaptation to mitigate the effects of habitat change but it may be limited by the constraint of prior phenotypes developed in former environments. Addressing this question in animals has been difficult as it is hard to quantify time spent in different habitats over several years of life in natural environments. Recent advances in otolith isotope analysis however, has made possible such environmental reconstruction.

Study system: Bluegill sunfish (*Lepomis macrochirus*) from three habitats (main river channel; oxbow lake; beaver pond) in the Sipsey River watershed in Alabama, USA.

Goals: We address the following questions. 1) Do bluegills move between habitats in the study system? 2) Does higher fecundity of fish inhabiting floodplain lakes result in higher recruitment within or migration to the system as a whole? 3) Do fish in the alternative habitats evince differences in diet? 4) Has previously observed morphological differentiation persisted? 5) If habitat associated ecophenotypy is observed, is it due to phenotypic plasticity? 6) If morphological differentiation is evident and due to plasticity, is there a residual of natal ecophenotypy in adults that migrated to new habitats?

Methods: We collected fish from the three focal environments and assessed body shape as reflected by the positions of twelve landmarks of fish external morphology. We measured morphometry and inferred natal habitat by isotope discriminant patterns in recently-laid bone (otolith edges) compared with first-laid bone (otolith core). We assessed the trophic positions of fish in different habitats using amino-acid specific differentials in ^{15}N isotopes. We estimated diet composition using ^{13}C, ^{18}O, and ^{15}N isotopes. Multivariate shape analysis was used to define body shapes associated with habitat of capture and isotope-inferred residual morphology due to natal habitat.

Results: 1) Sixty-five percent of fish were captured in a habitat differing from their natal habitat. 2) Habitat-specific fecundity differentials did not drive measurable differences in recruitment among habitats. 3) Isotope inferred diets differed among habitats but did not result in differences in summative trophic levels. 4) Fish morphology varied with habitat of capture—individuals from dammed ponds had deeper mid-bodies, elongated snouts, and larger heads. Fish from the oxbow lake had larger eyes, disciform bodies, posteriorly shifted pelvic fins, short caudal peduncles and short dorsal fin bases. 5) Fish that changed habitats had differing morphology than those not changing, indicating phenotypic plasticity. 6) Natal habitats left residual morphological signatures very similar to current-environment effects. We discerned that residual morphology would impose functional costs and thus implies a limit to the evolution of plasticity.

Keywords:
Environmental change, habitat choice, habitat migration, costs and limits of phenotypic plasticity, geometric morphometrics, stable isotope analysis

INTRODUCTION

Habitat heterogeneity drives the evolution of local adaptation, migration, environmental tolerance, and phenotypic plasticity in diverse organisms (Dobzhansky 1951, Bradshaw 1965, Levins 1968). Such heterogeneity may stem from environmental change out of an organism's influence or from movement (dispersal and migration).

Habitat changes made by choice can be a strategic means to exploit alternative resource levels (Rosenzweig 1973, Wilson & Yoshimura 1994). Differing challenges imposed by alternative habitats with conflicting demands on organismal phenotypes can be at least partly offset by environment-dependent trait expression (phenotypic plasticity or physiological adaptation) in response to the alternative biotic and/or abiotic conditions (Bradshaw 1965, DeWitt & Scheiner 2004). Migration itself, if induced by environmental cues or behavioral choice of preferred habitat, may be considered phenotypic plasticity (Sih 2004, Ghalambor et al. 2010). Advantageous phenotypes in environments that induce them, to the extent they have a heritable basis, lead to the evolution of adaptive plasticity or physiology (Waddington 1953, Via & Lande 1985, DeWitt 2016). Adaptive plasticity has been particularly well documented to have evolved in relation to resources, predators, or physical environmental conditions (Bradshaw 1965, West-Eberhard 1989, Pfennig & McGee 2010) and among vertebrates, particularly so in fishes (e.g., Meyer 1987, Brönmark & Miner, 1992, Wimberger 1992, Day et al. 1994, Ruehl & DeWitt 2005, Crispo & Chapman 2010, DeWitt 2016, Díaz-Gil 2020).

Traits that mitigate a given environmental challenge often entail reduced performance in other domains—an ecological concept known as functional "trade-offs" (Levins 1968, Futuyma & Moreno 1988, DeWitt & Scheiner 2004, Pfennig & McGee 2010). In fishes, trade-offs have been well documented for traits such as morphology (Day et al. 1994, Robinson & Wilson 1996, Svanbäck & Eklöv 2003, Langerhans 2009), intrinsic growth rate (Arendt & Wilson 2000), and other traits of consequence with respect to habitat differences (Werner et al. 1983, Fraser & Bernatchez 2007, Arendt 2015). There is a large body of literature specifically documenting movement of fish within and across diverse watershed habitats (Ross & Baker 1983, Ru & Liu 2013, Sparks 2016). Many studies have also documented replicated differentiation among fishes from alternative habitats (Schluter & McPhail 1993, Robinson & Wilson 1996, Johnson & Belk 2001, Langerhans & DeWitt 2004, Tobler et al. 2008, Ruehl et al. 2011). Less well-studied is the interplay of movement patterns per se and phenotypic differentiation.

Stable isotope and trace elemental analysis have been widely used to determine movement patterns in a variety of organisms (Elsdon et al. 2008, Walther et al. 2008, Gibson-Reinemer et al. 2009). Otoliths, the stalwart of fish sclerochronology, not only capture information on fish growth rates, but also reflect the environment of the individual at the time of accretion for each new layer of bone (Zitek et al. 2010, Zeigler & Whitledge 2011). The recognition that both annual and daily growth rings are recorded, and the development of highly sensitive instrumentation has led to the ability to define specific isotopic signatures for even very fine increments of a fish's lifetime (Campana & Thorrold 2001). These advances create an opportunity to unite the study of movement patterns and trait differentiation for fish in multiple-habitat systems.

In the floodplain systems of the Sipsey (AL, USA) and Pearl (MS, USA) Rivers, Rypel et al. (2012) noted morphological differences in bluegill sunfish (*Lepomis macrochirus*), hereafter 'bluegills', from floodplain lakes and the main river channel. Lake fish had benthic-oriented jaws, large caudal fins and eyes, and relatively deep bodies—especially at mid-body, whereas main channel fish had relatively fusiform bodies, small eyes and pelagic-oriented jaws (Rypel et al. 2012). Further, bluegills captured in the floodplain lakes of the Sipsey River were larger, grew faster, and had a higher gonadosomatic index (GSI), which traits were inferred to translate into increased fitness and survivorship for individuals occupying floodplain lakes (Rypel et al. 2012). The hydroperiod of Sipsey River floodplain lakes included intermittent events in which lakes completely dried and that these drying events may occur, on average, every ~5 years, when mean annual discharge of the river falls below ~18 m^3s^{-1} (Rypel et al. 2012). While floodplain lake habitats could potentially provide short-term increases in fitness, on a long-term exclusive basis these habitats are unviable. Rypel et al. (2012) speculated that bluegills spending some, *but not all,* of their life in the floodplain lakes would be expected to have higher fitness than permanent main channel residents.

While it is likely that fish migration occurs in this study system, this has not to the best of our knowledge been documented or studied in the context of ecophenotypy. Research reported in the present paper was intended to address the following questions: 1) Do bluegills move between habitats in the study system? 2) Does higher fecundity of fish inhabiting floodplain lakes result in higher recruitment within or migration to the system as a whole? 3) Do fish in the alternative habitats evince differences in diet? 4) Has the previously observed morphological differentiation persisted? 5) If habitat associated ecophenotypy is observed, is it discernably due to phenotypic plasticity? 6) If morphological differentiation is evident and due to plasticity, is there a residual of natal ecophenotypy evident in adults that migrated to new habitats? If systematic residual ecophenotypy were present, it would support the case for accuracy of natal habitat inferences from isotopes, provide evidence for plasticity as a mechanism of phenotypic differentiation, and also imply limits to the adaptive value of the plasticity.

MATERIAL AND METHODS

Study Site and Collection

Fish were collected from the Sipsey River main channel and two associated floodplain lakes within the Alabama Forever Wild Sipsey Tract, near Elrod, Alabama between November and December 2012. The Sipsey River is a sixth-order free-flowing river that originates near Glen Allen, Alabama and flows to the southwest, emptying into the upper Tombigbee River. It spans approximately 175 km of tupelo-cypress wetlands, marshes, and bottomland hardwood floodplains throughout the coastal plain of western Alabama (Ward et al. 2005, Rypel et al. 2008, 2012). Mean annual discharge and gage height during 2012 were 17.95 m^3s^{-1} and 2.44 m, respectively (USGS gaging station 02446500). Of the two floodplain lakes, one was a series of active beaver ponds (BP) and the second was a natural oxbow lake (OX). The third site was the main channel of the Sipsey River (MC). We collected bluegills by boat electrofishing (n = 23) in MC and barge electrofishing in OX (n = 11) and BP (n = 10). We immediately anesthetized and euthanized fish in the field by submersion in a tricaine methane sulfonate (MS-222) solution, after which we bagged, placed on ice, and transported them to the laboratory.

Muscle Isotope (Trophic Position) Analysis

Boneless, skinless fish fillets were frozen (-80° C), lyophilized (at -86° C and -0.010 mBar using the Labconco, Freezone 2.5 plus analytical kit), and ground to powder under liquid N_2. Resulting samples (0.4–0.7 mg) were packed into 4 × 6 mm tin capsules and combusted in a Costech EA 4010 Elemental Analyzer, coupled to a Thermo Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS). We ran all analyses in duplicate and measured mean isotope ratios noting that the coefficient of variation for all analyses averaged 3.90 %. We reported isotope ratios in standard notation relative to the Vienna PeeDee Belemnite and atmospheric N_2 for carbon and nitrogen respectively, using the equation:

$$\delta X(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \cdot 10^3$$

where X = ^{13}C or ^{15}N, and R is the ratio of the heavy to light isotope (McKinney et al. 1950).

Otolith Isotope (Habitat Use) Analysis

Sagittal otoliths were removed from each individual, rinsed in deionized water, and stored in dry polypropylene microcentrifuge tubes. We used polyoxymethylene forceps for all handling to avoid trace metallic elemental contamination. Otoliths were mounted individually in Buehler® EpoThin™ epoxy alongside a small identifier tag. Mounted otoliths were thin sectioned on the transverse plane using a low-speed saw with diamond wafering blade and then fixed onto standard glass microscope slides using cyanoacrylate glue. We used waterproof silicon carbide paper (2400 grit) to polish thin sections to the depth of the nucleus for subsequent sclerochronological analyses. Although otolith-based aging of bluegill sunfish is highly repeatable, even among observers (Hales & Belk 1992), we independently assessed otolith ages four times: two using transverse sectioned images and two using whole-view images. A second analyst confirmed each age determination.

A New Wave micromill with sub-micron position control was used to remove otolith material (40–105 µg) from the outer edge and core of each thin section. We collected the resulting powder onto a clean razor blade and transferred it to weighing paper. We weighed samples to the nearest 0.1 µg and placed them into labeled 4.5 ml borosilicate Exetainers® capped with a pierceable septum. All materials were cleaned with compressed air and a dry wipe between samples to minimize cross contamination. Isotopic analysis for otolith $\delta^{18}O$ and $\delta^{13}C$ was conducted using a Thermo GasBench II coupled with a Thermo Delta V Plus IRMS using previously described methods (Paul & Skrzypek 2007). We expressed all isotope ratios using the standard δ notation relative to the Vienna Pee Dee Belemnite (VPDB) scale via the NBS-19 standard performing calculations as previously described except that R = ^{y+2}X / ^{y}X for oxygen. Precision (1σ) was ± 0.1 ‰ for both $\delta^{18}O$ and $\delta^{13}C$ based on 14 NBS-19 standards measured in each run. No aragonite oxygen isotope correction was applied. Of the 44 individuals collected, one was lost in processing and one was discarded from ^{18}O analysis due to insufficient spectrometer beam size.

Morphology and Gonadosomatic Index

All individuals were laid beside a 30 cm ruler and photographed in lateral perspective using an

8.0 MP digital camera (Apple iPhone 4s) at 2880 pixels per mean body length. We prevented parallax to the best of our ability by standardizing camera position relative to samples. This was achieved via a bracing system in which the camera remained stationary and samples were centered directly below the lens. We used geometric shape analysis to assess body shapes (Kendall 1977). Twelve landmarks were defined using the software program tpsDig2 (v. 2.3; © F.J. Rohlf 2017) as indicated in Figure 1. Two helper landmarks were added to better define shape of the dorsal surface. We did this by creating chords between full-rank landmarks on the dorsum, between the head and the origin, and the origin and insertion of the dorsal fin. The chords were rotated 90° on center and used as visual guides to digitize the helper landmarks where the rotated chords crossed the dorsum (Fig. 1). These helper landmarks were dimensionally deficient; each had only one dimension of shape information because their definition was based on the geometry of other landmarks. Thus, they were treated as semilandmarks during superimposition, which is the centering, scaling, and rotational fitting of landmark conformations to a standard (Kendall 1977). Semilandmarks were slid during superimposition into a position of minimal leverage per Bookstein (1997) using the program tpsRelw (v. 1.7; © F.J. Rohlf, 2019). Landmark conformations were scaled to unit centroid size of lateral profile landmarks and slid semilandmarks. We saved original centroid size of the profile landmarks for use as a covariate in analyses. Centroid size was used as a covariate in statistical analyses to statistically control for allometry to reduce error and to compensate any differential in size by habitat or age class. Superimposed coordinates were subjected to principal components analysis to remove null dimensions and reduce the number of shape variables to the two major components, which in this case summarized 54.1 % of inter-individual variation in shape.

Eye diameter was measured digitally with tpsDig2 (v. 2.3; © F.J. Rohlf, 2017). For visualization of body shape, the eye center landmark was replaced in a version of the superimposed coordinate dataset with two helper landmarks set radius-length away from the original center point, in the horizontal dimension so that both had the same vertical coordinate as the original center point. This approach maintains three dimensions of information (per eye location and diameter) but allows for visualization of eye diameter with the landmark suite.

Sex was identified by dissection and gonadosomatic index (GSI) was calculated as the ratio of gonad mass to body mass × 100. We measured body mass to the nearest gram, gonad mass to the nearest mg, and total length to the nearest mm.

Statistical Analysis

Muscle tissue isotope data ($\delta^{13}C$, $\delta^{15}N$) were analyzed using multivariate analysis of covariance (MANCOVA) for effects due to capture site and sex using the natural logarithm of the cube root of mass as a covariate. Interactions were initially included in the statistical model but removed for lack of significance.

Isotopic composition ($\delta^{13}C$, $\delta^{18}O$) of the most recent otolith growth increment for fish from the three habitats was characterized using quadratic discriminant analysis (QDA). QDA makes fewer assumptions than linear discriminant analysis and allows groups to have characteristically different variances and covariances per patterns in the actual data. The resulting discriminant functions were used to infer natal habitats for each individual based on their otolith core (natal bone) isotope signatures, with the assumption that the core isotope ratios reflect the habitat of birth. Classification to recent (capture) habitat was set proportional to occurrence and classification to natal habitats was not restricted with prior probabilities (i.e., all fish could be assigned to a single natal habitat if warranted by otolith core isotope data). We performed QDA on all fish for estimating migration frequencies. We performed a separate QDA for the subset used in the morphometric analysis to infer natal habitats. Natal habitat assignments were used to tested for residual morphological effects associated with prior environments. Goodness of fit χ^2 was used to assess capture site classification success and to test for differential retention of or export of fishes from natal habitats.

Morphological data comprising the two major geometric principal components of body shape variation and eye diameter was assessed with a multivariate analysis of covariance (MANCOVA). We tested for differences in morphology across habitats of collection, inferred natal habitats, and their interaction. Centroid size was treated as a covariate. The three-way interaction was tested but dropped from the model for lack of significant effect. Three dependent variables were used for analysis to provide that the main effect degrees of freedom would be tested with an excess over 5-fold the number of residual degrees of freedom less *p* (the number of dependent variables).

GSI was analyzed separately by sex due to the asymmetric investment in gametes by sex. We used analysis of covariance (ANCOVA) to test for effects of capture-site, size, and their interaction. If not significant, interactions were removed from the final analytical model.

Significant habitat effects in any of the parametric analyses were followed with planned contrasts of the floodplain lakes versus the river channel, and BP versus OX. A nonsignificant χ^2 result bearing on one of the research questions was tested *post hoc* for power to detect an effect size equivalent to a correlation of $r = 0.5$ following Cohen (2013). We calculated parametric analyses with JMP Pro (v. 15; SAS Inc.) and partial effect sizes for multivariate tests, η_p^2, and χ^2 tests with Excel (v. 2004; Microsoft, Inc.). Data and calculations in Excel were archived at The OAK Trust (Mays & DeWitt 2020); https://hdl.handle.net/1969.1/195012

evolutionary-ecology.com/data/3235Appendix.pdf

RESULTS

Individuals Captured

Fishes sampled from the three sites were biased in age ($\chi^2_6 = 26.9$, P = 0.0002) but not in representation by sex ($\chi^2_2 = 3.40$, P = 0.18). The fish ranged from 1 year (MC, $n = 3$; BP, $n = 8$; OX, $n = 2$) to 4 years (BP, $n = 1$) with a majority of individuals being intermediate in age (2 years: MC, $n = 5$; BP, $n = 1$; OX, $n = 6$; 3 years: MC, $n = 15$; OX, $n = 3$). Full tabulation is provided in Table A1. Although a parametric analysis of fish size by age and habitat was not advisable given imbalance in representation, qualitative results of regression were informative. Fish size ($\ln \sqrt[3]{g}$) strongly increased with age (log worth 9.3), but habitat differences were minor (log worth 3.6) and interaction of age and habitat was not noteworthy (log worth 1.4). The empirical pattern is given in Figure A1.

Muscle Isotope (Trophic Position) Analysis

There were significant differences in bulk $\delta^{13}C$ and $\delta^{15}N$ of fish muscle across habitats and for fish of different size, but no sex or interaction effects were evident (Table 1A). Planned contrasts showed that the proportion of heavy isotopes was higher in MC compared to the floodplain lakes (14.2% higher for $\delta^{13}C$ and 8.8 5 higher for $\delta^{15}N$), with no difference noted between OX and BP (Table 1A; Fig. A2A). Larger fish also had a higher proportion of both isotopes, but more so for $\delta^{15}N$ than $\delta^{13}C$ (canonical correlations 0.21 and 0.33 respectively).

Otolith Isotope (Habitat) Analysis

Otolith powders from the growing edge of otoliths were strongly differentiated in $\delta^{18}O$ and $\delta^{13}C$ by habitat (Table 1B; Fig. A2B) but did not differ significantly by fish size or sex. QDA correctly classified 95 % of fish to correct capture site (Table A2A). The strong differentiation in otolith edge isotopes among fish captured in alternative habitats was also noted for the subset of fish used in the morphometric analysis ($F_{4,56} = 27.9$, $P < 10^{-12}$). Correlations of isotope values with canonical discriminant scores, illustrated in Figure A2B as 'biplot rays', indicated that each isotope provided different contributions to the discrimination (if they were redundant, their rays would be coincident). Otoliths of individuals collected from BP showed the greatest proportion of ^{18}O, while otoliths of individuals from OX were intermediate and those from MC were relatively low. Otolith powders from the growing edge of otoliths of fish captured in OX were relatively high in $\delta^{13}C$ compared to those from the flood plain lake habitats (OX and BP), which did not differ from each other.

Classifications of individuals to putative natal habitats, based on discriminant functions derived in the analysis of otolith edge isotope data indicated significant movement of fish among habitats versus a null hypothesis of perfect site fidelity ($\chi^2_1 = 18.2$, $P = 0.0002$). Classification numbers by capture and inferred natal sites are given in Table A2B. Sixty-five percent of fish were inferred to have been born in a site unlike that in which they were captured, however there did not appear to be any significant retention or differential migration among specific habitats ($\chi^2_1 = 2.29$, $P = 0.32$).

Morphological Traits

Geometric morphometrics indicated that individuals showed characteristic morphologies based on their site of capture ($F_{6,42} = 7.02$, $P < 10^{-4}$, $\eta_p^2 = 0.50$; Fig. 2A). Both planned contrasts were significant (MC v. OX/BP, $F_{3,21} = 8.55$, $P < 10^{-2}$; OX v. BP, $F_{3,21} = 6.04$, $P = 0.004$). Bluegills collected from the floodplain lakes had greater body depth at mid length, relatively elongate snouts, and large heads. Fish from OX had a more disciform lateral profile, large eyes, and posteriorly placed pelvic fins, as well as shorter caudal peduncles and dorsal fin bases. Fish from MC had the flattest ventrum profile and most vaulting anterior dorsum. Fish from BP had slightly elongated peduncles

and pronounced small eyes.

In addition to morphological differences by collection habitat, morphology differed among the inferred natal sites (Table 1C). The main effect of inferred natal site was significant by one tailed hypothesis test, which would be reasonable given that patterns fit prior results and ecomorphological expectations ($F_{6,42}$ = 1.94, P = 0.096, η_p^2 = 0.22). Post-hoc tests were not conducted due to the marginal P-value for the inferred natal site main effect. There was a significant interaction between the capture and inferred natal site main effects ($F_{12,55.9}$ = 2.35, P = 0.016, η_p^2 = 0.34). Based on canonical centroid values (multivariate least square means) from the interaction term decomposition, natal environmental associations with adult fish shape were characteristically similar to the main canonical decomposition (Fig. 2B; columns 1-3 and 5). However, capture and natal site interactions demonstrated heterogeneous variances, most notably due to the lack of discernable natal environment effect among fish captured in MC (Fig. 2B; column 4).

GSI in male bluegills exhibited no patterns given our plan of analysis. The interaction of capture site and size was first removed for lack of significance and then the reduced model did not exhibit a significant omnibus test. For females, GSI values were radically higher (mean $\ln^3\sqrt{g}$ = 0.729 v. males at 0.087). Female GSI did not exhibit a capture site by size interaction but both main effects were significant. Capture site was most strongly associated with female GSI ($F_{2,14}$ = 15.2, P = 0.0003). Female bluegills in the floodplain lakes had greater GSI than those in the MC ($F_{1,14}$ = 28.6, P < 0.0001), but the difference between BP and OX was not significant ($F_{1,14}$ = 2.73, P = 0.12). Female body mass was related to GSI less strongly than capture site, but the relationship was significant ($F_{2,14}$ = 8.53, P = 0.01). Analysis results for female GSI are given in Table A3.

DISCUSSION

Movement among habitats was revealed as a norm by isotope analysis, affirming the first of our research questions. Sixty-five percent of sampled individuals apparently spent the first year of life in a habitat other than that in which they were captured. This contradicts several previous studies reporting high site fidelity and small home range sizes for sunfishes (*L. megalotis*, Berra & Gunning 1972; *L. gibbosus*, Coleman & Wilson 1996; McCairns & Fox 2004; *L. cyanellus*, Gatz 2007). Yet if our habitat assignments were wrong, there would be no vestigial morphology systematically attributable to isotope-inferred natal habitat assignments and it would be especially improbable that randomly accrued effects would happen to parallel among-site differentiation. Greater site fidelity reported in previous studies may reflect environmental stability associated with basin habitats (lakes, ponds) with minor fluvial influence, or relatively brief focal time scales (< 1 yr). Some basin studies nevertheless have found varied movement patterns by sunfish. Paukert et al. (2004) reported bluegill home range sizes up to 172 ha in a 332-ha lake. It is also common for sunfish species to change habitat ontogenetically as the ability to exploit different niches changes with growth (Osenberg et al. 1988). Over short durations, considerable movement was seen in longear sunfish even in a small river system. Berra & Gunning (1972) performed a mark-recapture study in which 70 % of recovered marked longear sunfish were found in the site of release, suggesting strong site fidelity. However, the number of fishes *not recovered*, 81 %, suggests migration as a norm. As sunfishes are common in diverse habitats, it appears that the dynamics of movement may simply differ widely by habitat system and time frames considered.

The variable fluvial dynamics of moderate and large rivers like the Sipsey River generate dynamic mosaics of off-channel habitats including temporary and permanent lakes, ponds, sloughs and variously conformed retention habitats within the floodplain. Monthly variation in rainfall and evapotranspiration typically cause the Sipsey River to inundate the surrounding floodplain in winter (December-March) and episodically following large rain events. Periods of inundation are critical natural events that connect fish communities in time and space (Junk et al. 1989, Ward et al. 2005) and the specific hydrological regime of these ecosystems directly affects year-class success or failure (Welcomme 1979). Increased recruitment is common during years that backwater habitats are accessible to juveniles (Nannini et al. 2012), as these habitats offer increased temperature and zooplankton availability which in turn increase growth rates and fecundity (Yamamoto & Siah 2013, Santucci & Wahl 2003, Wahl et al. 2008). As well, backwater habitats can serve as refugia from high flow dynamics and predation relative to the main channel (Rypel et al. 2012). Typically, centrarchids are regarded as backwater specialists (Galat & Zweimüller 2001, Csoboth & Garvey 2008) as they often select these habitats for reproductive processes and at larval and juvenile stages (Nannini et al. 2012). This would suggest differential recruitment within habitats or export of post-yearling fishes to other floodplain lakes or the river channel. In the present study, backwater bluegill showed higher fecundity, but this advantage did not present as differential retention or export within or among habitats, and hence did not

affirm our second research question. The absence of signal for differential recruitment/migration may be due to low sample size, although power to detect a strong effect was 75 % (Cohen 2013). Thus, migration was the rule in this system, but it was not detectably biased with respect to the three focal habitats.

In addition to differential fecundity, dietary differences were evident across habitats. The high muscle $\delta^{13}C$ of main channel bluegills, +4.5 ‰ relative to other habitats, indicated heavy reliance on carbon originating from the riparian and forested areas of the floodplain. This is unsurprising, as terrestrially derived organic carbon contributes up to 50% of the total carbon pool in riverine fish (Nowlin et al. 2007, Pingram et al. 2012). In contrast, the $\delta^{13}C$ of floodplain lake bluegills was consistent with a diet strongly subsidized by submerged macrophytes (France 1995). Submerged macrophytes can contribute > 75 % of producer carbon assimilated by omnivorous fish in shallow lakes (Jepsen & Winemiller 2007, Mendonça et al. 2012, Yu et al. 2016). Despite this inference, difference between the bulk $\delta^{15}N$ of main channel and floodplain lake fish, ~ 1 ‰, was ecologically trivial, given that consumers enrich by ~ 3.4 ‰ per trophic level (Minagawa & Wada 1984). However, fish diet and isotope profiles can vary in myriad patterns even where fish occupy comparable trophic positions (Dharampal & Findlay 2017; McCutchan et al. 2003). For instance, fish consuming a greater amount of plant material may also be eating higher order predators (e.g. odonates), potentially resulting in net trophic levels similar to fish eating low order herbivores (e.g. plankton). Lacking specific information on sources of heavy isotopes in this system, we will not attempt to make conclusions about specific diet or trophic position. Rather, we can be confident that the strong among-habitat variation in isotopes relative to within habitat variation indicates that diets differed, apparently strongly so, among the habitats studied (Fig. A1). This affirms our third research question.

Differences in diet imply different demands on feeding morphology. Structural and other habitat differences may also impose alternative demands on fish morphology. Many studies show that fishes meet such alternative demands with inducible morphology (e.g., Meyer 1987, Brönmark & Miner 1992, Day et al. 1994, Svanbäck & Eklöv 2003, Parsons & Robinson 2007, Ruehl & DeWitt 2005, 2007). Alternatively, phenotypic matching to environments may reflect genetic differentiation (Langerhans et al. 2004, Rundle et al. 2010). Morphological differences in the bluegills from habitats in the present study parallel well-known ecomorphological patterns seen in other studies. For example, the disciform shape typical of bluegills from OX is known to be adaptive in low energy, structurally complex environments (Webb 1984), which characters are an apt description of oxbow conditions (Winemiller et al. 2000). Rypel et al. (2012) previously observed many of the morphological distinctions in bluegills among these habitats (see also Papadopoulos 2010). The present study affirmed persistence of those differences, although we noted that eye size was only enlarged in the oxbow (not both types of floodplain lakes). Thus with minor exception we affirmed our fourth research question. Yet we also revealed additional aspects of differentiation. For example, we observed relatively flat ventrums and anteriorly shifted maximum depth in the lotic (MC) habitat relative to that in the more lentic OX and BP. These aspects of body shape paralleled those for sunfish in the Brazos River and associated oxbows in Texas, USA (Papadopoulos 2010) and the cichlid *Biotodoma wavrini* in river channel and variously connected lagoon habitats in the Río Cinaruco of Venezuela (Langerhans et al. 2003). As well, elongated snouts as observed in OX and BP are expected among fish that feed by picking small pelagic prey items such as plankton from the water column (Hegrenes 2001, Ruehl & DeWitt 2005), which prey are typical to lentic habitats (like OX and BP). To the extent that habitat-associated morphology increases adaptive matching to conditions in the respective habitat, a logical inference is that changing from one habitat to another may leave fish ill adapted to the new habitat. Thus, opportunities associated with moving among habitats (e.g., fast growth or high fecundity in backwaters) may entail costs due to environmentally mismatched phenotypes (DeWitt et al. 1998).

Plasticity can increase phenotype-habitat matching where multiple habitats could be experienced during life, regardless of whether habitat change is driven by choice or by circumstance (DeWitt & Scheiner 2004). This may explain why plasticity is so pervasive in fishes. For sunfish, plasticity is known to play a strong role in resource exploitation by habitat. Robinson & Wilson (1996) directly observed induction of adaptive trophic morphology of pumpkinseed sunfish raised in benthic and limnetic environments and noted genetic components of trophic morphology as well. Yonekura et al. (2007) documented well-matched (adaptive) ecophenotypy in introduced bluegill sunfish across connected habitats of a single lake, in which genetic variation was extremely low (effective population size < 15; Kawamura et al. 2006). Bhagat et al. (2011) found a similar result for trophic ecophenotypy of introduced pumpkinseed sunfish (*L. gibbosus*) in Iberia. Lack of genetic variation in these latter two cases leaves plasticity as the likely explanation for ecophenotypy. Although we did not assay genetic variation in this study, the morphological signature of natal habitats demonstrated that morphology changed within individual lifetimes and therefore supports plasticity as a mechanism underlying ecophenotypy and affirms our fifth research question.

The ability to discern natal environments not only allows tests for migration and plasticity, but also enables finer inference on potential limits to adaptive plasticity. We speculated as a final research question that if there were predictable ecophenotypy due to plasticity, then there may be 'residual ecophenotypy' still detectable in fish developed in alternative natal environments. This was affirmed by the natal habitat effect observed. The natal habitat main effect was significant by directional hypothesis test, yet its interaction with that for the habitat of capture was also significant. Interaction terms can be significant for two reasons: 1) one factor has a fundamentally different effect depending on the value of another factor or 2) an effect is similar in nature across values of another effect but has heterogeneous variance (Fry 1992). In the present case, the issue seemed to be the latter—similar effect nature with inconsistent magnitude (Fig. 2B). For fish captured in a given habitat, when natal effects were evident their nature was similar to that defined for each habitat by the capture site effect (Fig. 2A,B). Thus, it appears that even young fish were expressing phenotypes expected to be adaptive for their respective environments. This logically implies some degree of phenotypic mismatch for migrants entering a new habitat.

As vital an adaptation as plasticity may be for fish in dynamic mosaic environments, the residual ecophenotypy we have described logically has two concomitant limits: 1) when an individual moves to a new habitat with traits developed elsewhere, it may be functionally mismatched to the new habitat for the duration, and to the extent, that the vestiges of plastic development past remain. This represents a 'developmental range' limit (DeWitt et al. 1998, Weinig & Delph 2001, Hoverman & Relyea 2015). As plastic development advances toward phenotype-environment matching, residual ecophenotypy may take time to 'undo', which time represents a 'developmental lag-time' limit (West-Eberhard 1989, Moran 1992, Padilla & Adolf 1996). Despite such implied limits, bluegills in the Sipsey River habitat mosaic appear to have evolved, or at least maintained, morphological and possibly 'dispersal' plasticity (Arendt 2015). Our results, taken with those of Rypel et al. (2012), suggest that phenotypic plasticity in bluegill sunfish extends well into ontogeny, allowing organisms with indeterminant growth to adapt morphologically to environmental change even as adults. These joint capacities are likely important for any organisms in changing environments and speak to the need for further work to understand how they enhance or limit performance of various organisms.

Rypel et al. (2012) speculated that bluegill should migrate on annual or super-annual intervals to maximize growth and longevity and although our results roughly support this idea, it is unclear the degree to which migration is determined by habitat choice or fluvial circumstance. Conservation efforts in critical habitats such as floodplain rivers may be enriched by a deeper understanding of how organisms move across space and time, and developmentally adjust to enable persistence in complex habitat systems (Tamario et al. 2019). At larger scales, ecophenotypy fosters evolutionary diversification and increases geographic range potential (Pfennig & McGee 2010) which may largely determine those species that make it through the Anthropocene (Crutzen 2006). In the Sipsey River system, fish moved commonly, by choice or circumstance, and developmentally adjusted in each case over short life intervals, arguably making them relatively well pre-adapted for the increasing pace of environmental change that we are currently experiencing.

DECLARATIONS

JM is responsible for project design, fieldwork, morphometric measurement, and otolith ^{13}C and ^{18}O isotope analysis. JM and TD developed the conceptual framework for this paper. TD oversaw morphometric characterization and conducted multivariate analysis. JM, TD, and RF wrote the manuscript. FA provided funding and equipment necessary for isotope analysis. PD conducted muscle ^{13}C and ^{15}N isotope analysis and fieldwork. The Central Analytical Facility at the University of Alabama provided access to facilities for sample preparation. Drs. Jon Benstead and Phil Harris provided use of field equipment and Cameron Craig, Kimberly Mays, Mark Dedmon, RF and PD assisted with fieldwork. This work was conducted under Alabama scientific collection permit AL-2013000023968680 and University of Alabama Institutional Animal Care and Use protocol IACUC-APN 12-372-1. Funding was provided by a GAANN fellowship and a research grant provided by the Department of Biological Sciences at the University of Alabama to JM.

DATA ACCESSIBILITY STATEMENT

Data are archived and publicly available in Excel spreadsheet format at the OAK Trust:
Mays, J. R., & DeWitt, T. J. 2020. Dataset and morphometric analysis Bluegill ecophenotypy. Available electronically from https://hdl.handle.net/1969.1/195012.

REFERENCES

Arendt, J. D. (2015). Effects of dispersal plasticity on population divergence and speciation. Heredity **115**, 306- 311.

Arendt, J. D., & Wilson, D. S. (2000). Population differences in the onset of cranial ossification in pumpkinseed *Lepomis gibbosus*, a potential cost of rapid growth. Canadian Journal of Fisheries and Aquatic Sciences **57**, 351- 356.

Berra, T. M., & Gunning, G. E. (1972). Seasonal movement and home range of the longear sunfish, *Lepomis megalotis* (Rafinesque) in Louisiana. American Midland Naturalist **88**, 368- 375.

Bhagat, Y., Fox, M. G., & Ferreira, M. T. (2011). Trophic polymorphism in introduced pumpkinseed *Lepomis gibbosus* inhabiting Iberian reservoirs. Environmental Biology of Fishes **91**, 203- 217.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. Medical Image Analysis **1997**, 225- 243.

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity. Advances in Genetics **13**, 115- 155.

Brönmark, C., & Miner, J. G. (1992). Predator-induced phenotypical change in body morphology in crucian carp. Science **258**(5086), 1348- 1350.

Campana, S. E. and Thorrold, S. R. (2001). Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences **58**, 30- 38.

Crispo, E., & Chapman, L. J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. Journal of Evolutionary Biology **23**, 2091- 2103. https://doi.org/10.1111/j.1420-9101.2010.02069.x

Cohen, J. (2013). Statistical Power Analysis for the Behavioral Sciences, 2 Ed. Academic press.

Coleman, K., & Wilson, D. S. (1996). Behavioral and ecological determinants of home range size in juvenile pumpkinseed sunfish *Lepomis gibbosus*. Ethology **102**: 900- 914.

Crutzen, P. J. (2006). Earth system science in the Anthropocene. In: Ehlers E, Krafft T (eds.), The “Anthropocene”, pp. 13- 18. Springer. https://doi.org/10.1007/3-540-26590-2_3

Csoboth, L. A., & Garvey, J. E. (2008). Lateral exchange of larval fish between a restored backwater and a large river in the east-central USA. Transactions of the American Fisheries Society **137**: 33- 44.

Day, T., Pritchard, J., & Schluter, D. (1994). A comparison of two sticklebacks. Evolution, **48**(5), 1723- 1734.

DeWitt, T. J. (2016). Expanding the phenotypic plasticity paradigm to broader views of trait space and ecological function. Current Zoology **62**: 463- 473.

DeWitt, T. J., & Scheiner, S. M. (2004). Phenotypic plasticity: functional and conceptual approaches. Oxford University Press.

DeWitt, T. J., Sih, A., Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution **13**: 77- 81.

Dharampal, P. S., & Findlay, R. H. (2017). Mercury levels in largemouth bass (*Micropterus salmoides*) from regulated and unregulated rivers. Chemosphere **170**: 134- 140.

Díaz-Gil, C., Alós, J., Arechavala-Lopez, P., Palmer, M., Riera-Batle, I., Grau, A., & Catalán, I. A. (2020). Reversible morphological changes in a juvenile marine fish after exposure to predatory alarm cues. Royal Society Open Science, **7**(5), 191945.

Dobzhansky, T. (1951). Genetics and the Origin of Species, 3 Ed. Columbia University Press, New York.

Elsdon, T. S. Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R. and Walther, B.D. (2008). Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology : An Annual Review **46**, 297- 330.

France, R. L. (1995). Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. Vegetatio **124**, 67- 72.

Fraser D. J. and Bernatchez, L. (2005). Adaptive migratory divergence among sympatric brook charr populations. Evolution **59**, 611- 624.

Fry, J. D. (1992). The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution **46**, 540- 550.

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. Annual Review of Ecology and Systematics **19**, 207- 234.

Galat, D. L., & Zweimüller, I. (2001). Conserving large-river fishes: is the highway analogy an appropriate paradigm? Journal of the North American Benthological Society **20**, 266- 279.

Gatz Jr., A. J. (2007). Homing ability of displaced stream-dwelling green sunfish. Journal of Freshwater Ecology **22**, 403- 409.

Ghalambor, C. K., Angeloni, L. M., & Carroll, S. P. 2010. Behavior as phenotypic plasticity. In *Evolutionary behavioral ecology*. Oxford University Press.

Gibson-Reinemer, D. K., Johnson, B. M., Martinez, P. J., Winkelman, D. L., Koenig, A. E., & Woodhead, J. D. (2009). Elemental signatures in otoliths of hatchery rainbow trout *Oncorhynchus mykiss*: distinctiveness and utility for detecting origins and movement. Canadian Journal of Fisheries and Aquatic Sciences **66**, 513- 524.

Hales, L. S., Jr & Belk, M. C. (1992). Validation of otolith annuli of bluegills in a southeastern thermal reservoir. Transactions of the American Fisheries Society **121**, 823- 830.

Hegrenes, S. (2001). Diet-induced phenotypic plasticity of feeding morphology in the orangespotted sunfish, *Lepomis humilis*. Ecology of Freshwater Fish **10**, 35- 42.

Hoverman, J. T., & Relyea, R. A. (2015). Prey responses to fine-scale variation in predation risk from combined predators. Oikos **125**, 254- 261.

Jepsen, D. B., & Winemiller, K. O. (2007). Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers. Ecology of Freshwater Fish **16**, 267- 281.

Johnson, J. B., & Belk, M. C. (2001). Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. Oecologia **126**, 142- 149.

Junk, W., Bayley, P. B., & Sparks, R. E. (1989). The flood pulse concept in river-floodplain-systems. Canadian Journal of Fisheries and Aquatic Sciences **106**, 110- 127.

Kawamura, K., Yonekura, R., Katano, O., Taniguchi, Y., & Saitoh, K. (2006). Origin and dispersal of bluegill sunfish, *Lepomis macrochirus*, in Japan and Korea. Molecular Ecology **15**, 613- 662

Kendall, D. G. (1977). The diffusion of shape. Advances in Applied Probability **9**, 428- 430.

Langerhans, R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. Journal of Evolutionary Biology **22**(5), 1057- 1075.

Langerhans, R. B., & DeWitt, T. J. (2004). Shared and unique features of evolutionary diversification. The American Naturalist, 164(3), 335-349.

Langerhans, R. B., Layman, C. A., Langerhans, A. K., & DeWitt, T. J. (2003). Habitat-associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society **80**, 689- 698.

Langerhans, R. B., Layman, C. A., Shokrollahi, A. M., & DeWitt, T. J. (2004). Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution **58**, 2305- 2318.

Levins, R. (1968). Evolution in changing environments: some theoretical explorations (No. 2). Princeton University Press.

Mays, J. R., & DeWitt, T. J. (2020). Dataset and morphometric analysis - Bluegill ecophenotypy. Available electronically from https : / /hdl .handle .net /1969 .1 /188375.

McCairns, R. S., & Fox, M. G. (2004). Habitat and home range fidelity in a trophically dimorphic pumpkinseed sunfish *Lepomis gibbosus* population. Oecologia **140**, 271- 279.

McCutchan, J. H. Jr Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**, 378- 390.

McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., & Urey, H. C. (1950). Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. Review of Scientific Instruments **21**(8), 724- 730.

Mendonça, R. S., Kosten, S., Lacerot, G., Mazzeo, N., Roland, F., Ometto, J.P., Paz, E.A., Bove, C.P., Bueno, N.C., Gomes, J.H.C., Scheffer, M. (2012). Bimodality in stable isotope composition facilitates the tracing of carbon transfer from macrophytes to higher trophic levels. Hydrobiologia **710**, 205- 218.

Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution **41**, 1357- 1369.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta **48**, 1135- 1140.

Nannini, M. A., Goodrich, J., Dettmers, J. M., Soluk, D. A., & Wahl, D. H. (2012). Larval and early juvenile fish dynamics in main channel and backwater lake habitats of the Illinois River ecosystem. Ecology of Freshwater Fish **21**, 499- 509.

Moran, N. A. (1992). The evolutionary maintenance of alternative phenotypes. American Naturalist **139**, 971- 989.

Nowlin, W. M., González, M. J., Vanni, M. J., Stevens, M. H. H., Fields, M. W., & Valente, J. J. (2007). Allochthonous subsidy of periodical cicadas affects the dynamics and stability of pond communities. Ecology **88**, 2174- 2186.

Padilla, D. K., & Adolph, S. C. (1996). Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evolutionary Ecology **10**, 105- 117.

Papadopoulos, A. (2010). An ecophysiological framework for the morphological evolution of bluegill sunfish. PhD Dissertation, Texas A&M University.

Parsons, K. J., & Robinson, B. W. (2007). Foraging performance of diet-induced morphotypes in pumpkinseed sunfish *Lepomis gibbosus* favours resource polymorphism. Journal of Evolutionary Biology **20**, 673- 684.

Paul, D., & Skrzypek, G. (2007). Assessment of carbonate-phosphoric acid analytical technique performed using GasBench II in continuous flow isotope ratio mass spectrometry. International Journal of Mass Spectrometry **262**, 180- 186.

Paukert, C. P., Willis, D. W., & Bouchard, M. A. (2004). Movement, home range, and site fidelity of bluegills in a Great Plains lake. North American Journal of Fisheries Management **24**, 154- 161.

Pfennig, D. W., & McGee, M. (2010). Resource polyphenism increases species richness: a test of the hypothesis. Phil. Transactions of the Royal Society of London, Series B **365**, 577- 591.

Pingram, M. K., Collier, K. J., Hamilton, D. P., David, B. O., & Hicks, B. J. (2012). Carbon sources supporting large river food webs: a review of ecological theories and evidence from stable isotopes. Freshwater Reviews **5**, 85- 103.

Robinson, B. W., & Wilson, D. S. (1996). Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish *Lepomis gibbosus*. Evolutionary Ecology **10**, 631- 652.

Rosenzweig, M. L. (1973). Habitat selection experiments with a pair of coexisting heteromyid rodent species. Ecology **54**, 111- 117.

Ross, S. T., & Baker, J. A. 1983. The response of fishes to periodic spring floods in a southeastern stream. American Midland Naturalist **109**, 1- 14.

Ru, H. J., & Liu, X. Q. (2013). River-lake migration of fishes in the Dongting Lake area of the Yangtze floodplain. Journal of Applied Ichthyology **29**, 594- 601.

Ruehl, C. B., & DeWitt, T. J. (2005). Trophic plasticity and fine-grained resource variation in populations of western mosquitofish, *Gambusia affinis*. Evolutionary Ecology Research **7**, 801- 819.

Ruehl, C. B., & DeWitt T. J. (2007). Trophic plasticity and foraging performance in red drum, *Sciaenops ocellatus* (Linnaeus). Journal of Experimental Marine Biology and Ecology **349**, 284- 294.

Ruehl, C. B., Shervette, V., & DeWitt, T. J. (2011). Replicated shape variation between simple and complex habitats in two estuarine fishes. Biological Journal of the Linnean Society **103**, 147- 158.

Rundle, H. D., Nagel, L., Boughman, J. W., & Schluter, D. (2000). Natural selection and parallel speciation in sympatric sticklebacks. Science **287**(5451), 306- 308.

Rypel, A. L., Arrington, D. A., & Findlay, R. H. (2008). Mercury in southeastern US riverine fish populations linked to water body type. Environmental Science & Technology **42**, 5118- 5124.

Rypel, A. L., Pounds, K. M., & Findlay, R. H. (2012). Spatial and temporal trade-offs by bluegills in floodplain river ecosystems. Ecosystems **15**, 555- 563.

Santucci Jr, V. J. & Wahl, D. H. (2003). The effects of growth, predation, and first-winter mortality on recruitment of bluegill cohorts. Transactions of the American Fisheries Society **132**, 346- 360.

Sih, A. (2004). A behavioral ecological view of phenotypic plasticity. In: DeWitt, T. J., & Scheiner, S. M. (Eds.) Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press.

Sparks, R. E. (2016). Need for ecosystem management of large rivers and their floodplains. BioScience **45**, 168- 182.

Svanbäck, R., & Eklöv, P. (2003). Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization? Oikos **102**, 273- 284.

Tamario, C., Sunde, J., Petersson, E., Tibblin, P., & Forsman, A. (2019). Ecological and evolutionary consequences of environmental change and management actions for migrating fish: a review. Frontiers in Ecology and Evolution **7**, 271. https://doi.org/10.3389/fevo.2019.00271

Tobler M., DeWitt, T. J., Schlupp, I., García de León, F. J., Herrmann, R., Feulner, P. G., Tiedemann, R., & Plath, M. (2008). Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution **62**, 2643- 2658.

Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution **39**, 505- 522.

Waddington, C. H. (1957). The Strategy of the Genes. Macmillan.

Wahl, D. H., Goodrich, J., Nannini, M. A., Dettmers, J. M., & Soluk, D. A. (2008). Exploring riverine zooplankton in three habitats of the Illinois River ecosystem: where do they come from? Limnology and Oceanography **53**, 2583- 2593.

Walther, B. D., Thorrold, S. R., & Olney, J. E. (2008). Geochemical signatures in otoliths record natal origins of American shad. Transactions of the American Fisheries Society **137**, 57- 69.

Ward, M. G., Harris, P. M., & Ward, A. K. (2005). Gulf coast rivers of the Southeastern United States. In: Rivers of North America. Elsevier.

Webb, P. W. (1984). Body form, locomotion and foraging in aquatic vertebrates. American Zoologist **24**, 107- 120.

Weinig, C. & Delph, L. F. 2001. Phenotypic plasticity early in life constrains developmental responses later. Evolution **55**, 930- 936.

Welcomme, R. L. (1979). Fisheries Ecology of Floodplain Rivers. Longman.

Werner, E. E., Gilliam, J. F., Hall, D. J., & Mittelbach, G. G. (1983). An experimental test of the effects of predation risk on habitat use in fish. Ecology **64**, 1540- 1548.

Osenberg, C. W., Werner, E. E., Mittelbach, G. G., & Hall, D. J. (1988). Growth patterns in bluegill (Lepomis macrochirus) and pumpkinseed (L. gibbosus) sunfish: environmental variation and the importance of ontogenetic niche shifts. Canadian Journal of Fisheries and Aquatic Sciences **45**(1), 17- 26.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics **20**, 249- 278.

Wilson, D. S., & Yoshimura, J. (1994). On the coexistence of specialists and generalists. American Naturalist **144**, 692- 707.

Wimberger, P. H. (1992). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). Biological Journal of the Linnean Society **45**, 197- 218.

Winemiller, K. O., Tarim, S., Shormann, D., & Cotner, J. B. (2000). Fish assemblage structure in relation to environmental variation among Brazos River oxbow lakes. Transactions of the American Fisheries Society **129**, 451- 468.

Yonekura, R., Kawamura, K., & Uchii, K. (2007). A peculiar relationship between genetic diversity and adaptability in invasive exotic species: bluegill sunfish as a model species. Ecological Research **22**, 911- 919.

Yamamoto, Y., & Siah, F. K. (2013) Potential importance of lagoons as breeding grounds for bluegill *Lepomis macrochirus* in Lake Biwa, Japan. Limnology **14**, 31- 37.

Yu, J., Liu, Z., He, H., Zhen, W., Guan, B., Chen, F., Li, K., Zhong, P., Teixeira-de Mello, F., & Jeppesen, E. (2016). Submerged macrophytes facilitate dominance of omnivorous fish in a subtropical shallow lake: implications for lake restoration. Hydrobiologia **775**, 97- 107.

Zeigler, J. M., & Whitledge, G. W. (2011). Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes. Hydrobiologia **661**, 289- 302.

Zitek, A., M., Sturm, M., Waidbacher, H., & Prohaska, T. (2010). Discrimination of wild and hatchery trout by natural chronological patterns of elements and isotopes in otoliths using LA-ICP-MS. Fisheries Management and Ecology **17**, 435- 445.

FIGURES

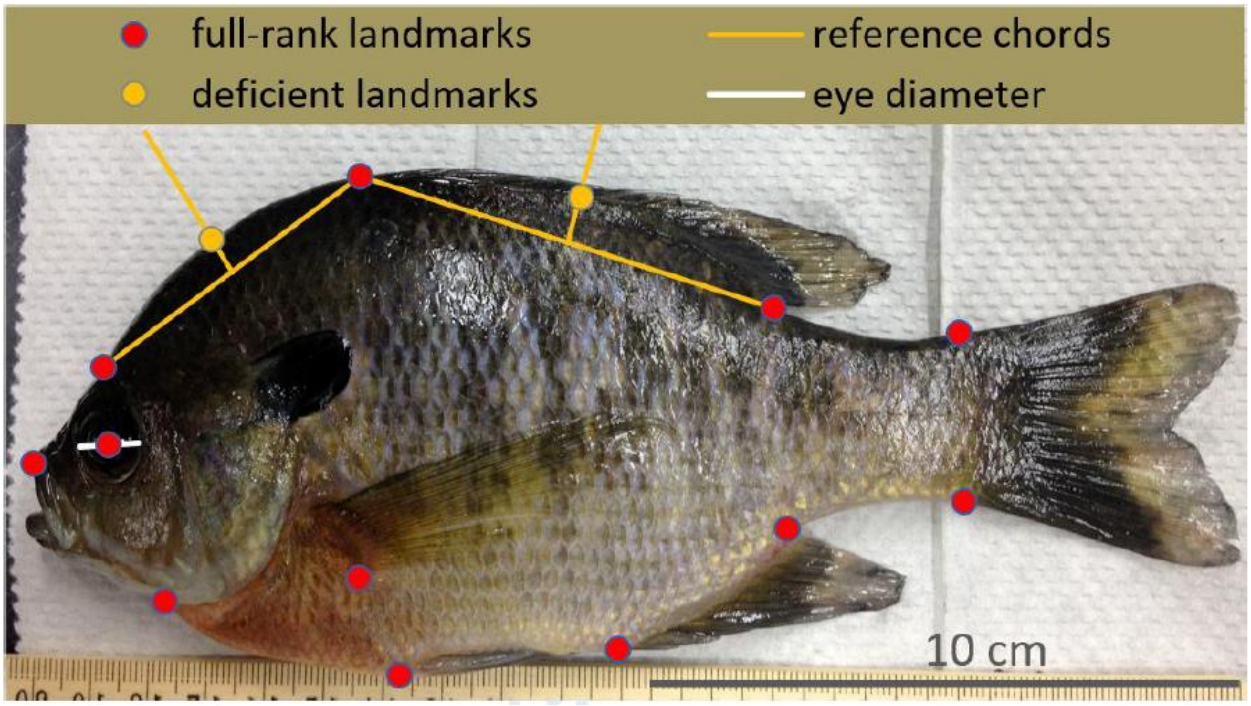

Figure 1. Landmarks and semilandmarks used for morphometric analyses. Reference chords were used as visual guides to place semilandmarks that help characterize dorsal shape.

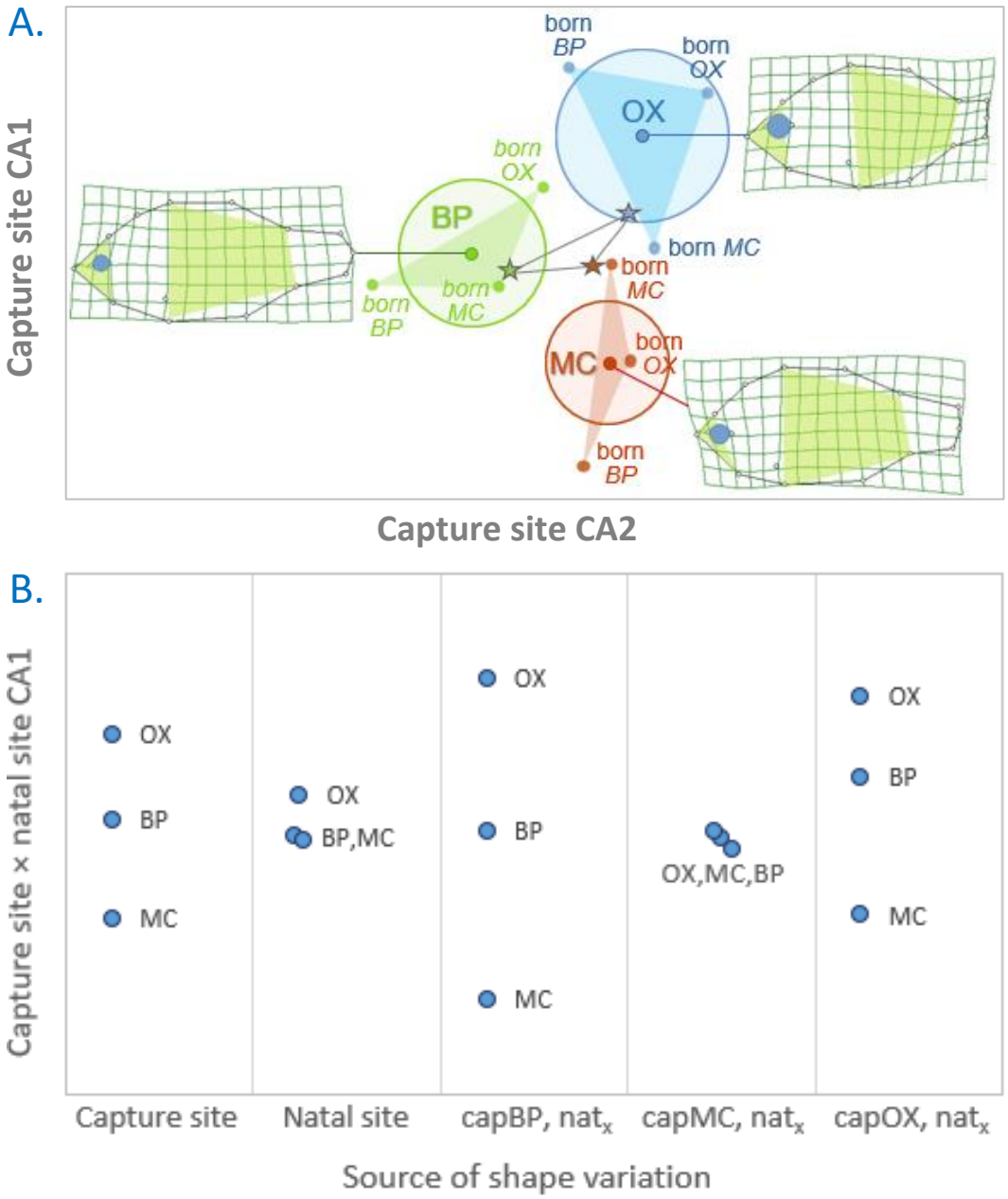

Figure 2. Bluegill body shape differentiation by nat$_x$al and capture sites. A. Capture site canonical ordination. Circles show 95% centroids for multivariate mean shape by capture site. Triangles connect multivariate mean shapes broken out for each natal site in general (grey) and per capture site (red, blue, green). Body shape illustrations depict true geometry (non-canonical least squares means) of bluegill body shapes from each capture site. B. Capture × natal site canonical means. Magnitude of variation in shape among capture and natal sites differed more than the nature of shape differentiation.

Table 1. Effect tests for chemical and body shape variation (MANCOVA results)

A. Muscle isotopes

effect	n	df_{effect}	df_{error}	p	s	df_{num}	df_{denom}	Λ	F	P	η_p^2
catch site	31	2	26	2	2	4	50	0.12	24.2	3E-11	0.66
sex		1			1	2	25	0.93	0.92	0.411	0.07
size		1			1	2	25	0.57	9.59	8E-04	0.43
planned contrast											
catch lake v. river		1			1	2	25		92.3	3E-12	
catch OX v. BP		1			1	2	25		0.05	0.951	

B. Otolith edge isotopes

effect	n	df_{effect}	df_{error}	p	s	df_{num}	df_{denom}	Λ	F	P	η_p^2
catch site	42	2	37	2	2	4	72	0.12	34.1	6E-16	0.65
sex		1			1	2	36	0.98	0.45	0.642	0.02
size		1			1	2	36	0.97	0.6	0.556	0.03
planned contrast											
catch lake v. river		1			1	2	36		88.4	1E-14	
catch OX v. BP		1			1	2	36		13.9	3E-05	

C. Morphometry

effect	n	df_{effect}	df_{error}	p	s	df_{num}	df_{denom}	Λ	F	P	η_p^2
catch site	33	2	23	3	2	6	42	0.25	7.02	3E-05	0.5
born site		2			2	6	42	0.61	1.94	0.096	0.22
catch x born		4			2.65	12	56.0	0.34	2.35	0.016	0.34
size		1			1	3	21	0.08	80.9	1E-11	0.92
planned contrast											
catch lake v. river		1			1	3	21	0.45	8.55	7E-04	0.55
catch OX v. BP		1			1	3	21	0.54	6.04	0.004	0.46