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Urbanization drives contemporary evolution in stream fish

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Abstract

Human activities reduce biodiversity but may also drive diversification by modifying selection. Urbanization alters stream hydrology by increasing peak water velocities, which should in turn alter selection on the body morphology of aquatic species. Here, we show how urbanization can generate evolutionary divergence in the body morphology of two species of stream fish, western blacknose dace (*Rhinichthys obtusus*) and creek chub (*Semotilus atromaculatus*). We predicted that fish should evolve more streamlined body shapes within urbanized streams. We found that in urban streams, dace consistently exhibited more streamlined bodies while chub consistently showed deeper bodies. Comparing modern creek chub populations with historical museum collections spanning 50 years, we found that creek chub (1) rapidly became deeper bodied in streams that experienced increasing urbanization over time, (2) had already achieved deepened bodies 50 years ago in streams that were then already urban (and showed no additional deepening over time), and (3) remained relatively shallow bodied in streams that stayed rural over time. By raising creek chub from five populations under common conditions in the laboratory, we found that morphological differences largely reflected genetically based differences, not velocity–induced phenotypic plasticity. We suggest that urbanization can drive rapid, adaptive evolutionary responses to disturbance, and that these responses may vary unpredictably in different species.

KEYWORDS
creek chub, global environmental change, human impacts, morphology, phenotypic plasticity, *Rhinichthys obtusus*, *Semotilus atromaculatus*, urban streams, western blacknose dace

1 | INTRODUCTION

Cities are growing fastest in areas of the world with the highest biodiversity (Lepers et al., 2005) and are among the most detrimental types of land use to ecosystem services (Braat & ten Brink, 2008; Grimm, Faeth, et al., 2008; Grimm, Foster, et al., 2008; Paul & Meyer, 2001). Humans have been building cities for thousands of years, giving this type of environmental disruption a long history, and the spread of urban areas is currently accelerating at a record pace (United Nations, 2014). Urban development can cause rapid, adaptive evolution in the resilient species that persist in these altered environments (Alberti et al., 2017; Donihue & Lambert, 2015; Luther & Derryberry, 2012; Munshi-South, 2012). However, the evolutionary consequences of urbanization have so far received very little attention, especially compared to *ecological* consequences (Chadwick et al., 2006; Grimm, Faeth, et al., 2008; Grimm, Foster, et al., 2008; Marzluff, 2001). A better understanding of rapid evolution due to human impacts could help us mitigate the negative consequences of human disturbances (Carroll, Hendry, Reznick, & Fox, 2007; Hendry et al., 2011).

Urbanization disrupts both terrestrial and aquatic habitats, with especially drastic consequences for freshwater streams. Among other things, urbanization generates higher peak water velocities (especially during heavy precipitation events) caused by runoff from impervious surfaces, reduced riparian buffers, and stream channelization. Urban streams have more frequent and more intense high-flow events (Jacobson, 2011; Poff, Bledsoe, & Cuhaciyan, 2006; Walsh et al., 2005), largely due to an increase in impervious surface cover (Schueler, Fraley-McNeal, & Cappiella, 2009). Although the most salient impact of urbanization is loss of species richness (Marchetti, Light, & Feliciano, 2001; Paul & Meyer, 2001; but see Marchetti, Lockwood, & Light, 2006), urbanization may also influence the direction of selection on the species that remain behind and may even drive predictable evolutionary responses via altered hydrologic regimes. However, despite the worldwide nature of urban growth, we know almost nothing about how aquatic species evolve in impacted streams.

Water velocity is an important selective agent for fish (e.g., Hvidsten, 1985; Jensen & Johnsen, 1999), and fish populations have often diverged in morphology under different water velocity conditions (Brinsmead & Fox, 2002; Langerhans, 2008). For instance, fish have exhibited changes in body shape where anthropogenic activity has reduced water velocity, such as in water impoundments (Haas, Blum, & Heins, 2010; Lytle & Poff, 2004). These morphological shifts appear to reflect changes in selection, which generally favors steady-swimming abilities and high endurance capacities in fast-moving water, but favors maneuverability and burst-speed abilities in slow-moving water (Langerhans, 2008; Langerhans & Reznick, 2010; Nelson, Gotwalt, & Snodgrass, 2003). Based on empirical and theoretical work, we may be able to predict how fish respond to human-induced increases in water velocity. The simplest prediction is that in areas with rapidly flowing water, fish should become more streamlined—i.e. fusiform shape: deep/wide anterior body, tapering to shallow/narrow caudal peduncle—because this reduces the energetic cost of station-holding and can increase foraging efficiency (Langerhans, 2008). Conversely, in areas of low velocity (slow rivers, lakes, or lagoons) fish should be less streamlined since this improves maneuverability and burst-swimming ability and can increase fecundity (Langerhans, Layman, Langerhans, & Dewitt, 2003; McGuigan, Franklin, Moritz, & Blows, 2003). In essence, a general pattern should arise wherein fish in faster, flashier streams are more streamlined than their conspecific populations in slower, more stable habitats. A range of studies have indeed demonstrated that fish in slower-moving water have deeper bodies, while those in higher velocity habitats have more streamlined profiles, corresponding to reduced drag (Aguirre, 2009; Langerhans, 2008; Schaefer, Duvernell, & Kreiser, 2011). However, no study to date has investigated whether fish morphology responds to the increases in stream flashiness and maximum water velocity caused by urbanization.

Here, we test whether urbanization has led to rapid and predictable changes in the body morphology of two widespread North American minnows, the western blacknose dace (*Rhinichthys obtusus*; hereafter referred to as blacknose dace for brevity) and the creek chub (*Semotilus atromaculatus*). We focus on a particularly important region for freshwater fishes: the southeastern United States, which harbors the most diverse temperate freshwater fish fauna in the world (Hocutt & Wiley, 1986). We make three major comparisons: (1) between existing populations in urban and rural streams, (2) between existing populations and specimens from historical collections (some preurbanization), and (3) among juveniles from urban and rural populations raised in a common laboratory environment under different water velocities (Table 1, Figure 1). In part one, we use geometric morphometrics to assess the morphology of blacknose dace and creek chub in streams that are currently rural or urban. In part two, we compare creek chub collected before and after urbanization to provide a temporal test of the connection between land use and fish morphology. Finally, we raise creek chub from rural and urban streams in the laboratory to assess whether morphological divergence has a genetic basis.

2 | MATERIALS AND METHODS

2.1 | Dace and chub: Morphological divergence between urban and rural streams

To test whether the well-known increases in maximum flow rates caused by urbanization (Chadwick et al., 2006; Jacobson, 2011; Poff et al., 2006; Schueler et al., 2009) may cause changes in fish body morphology, we first evaluated present-day populations of common North American minnows inhabiting rural and urban streams. We examined two species of distantly related cyprinids, blacknose dace and creek chub, which are readily caught and are widespread in both urban and rural streams. These fish provide representative models for examining how resilient species might respond to human activities. Because of their short generation time (1–2 years to sexual maturity) these small minnow species could have evolved measurable responses to urbanization over the past several decades. Other work in fish has found divergence due to selection from anthropogenic

TABLE 1 Study overview

Test for:	Study component	Species	Site summary	*N*
Current patterns	Contemporary comparison	Blacknose dace	Five rural streams, three urban streams	165
		Creek chub	Two basins: 10 rural streams, 15 urban streams	528
Recent temporal trends	Historical (~50 years) comparison	Creek chub	Seven rural streams, seven recently urbanized streams, four historically urban streams	578
Genetics vs. plasticity	Lab-rearing experiment	Creek chub	One rural stream, two recently urbanized streams, two historically urban streams	58

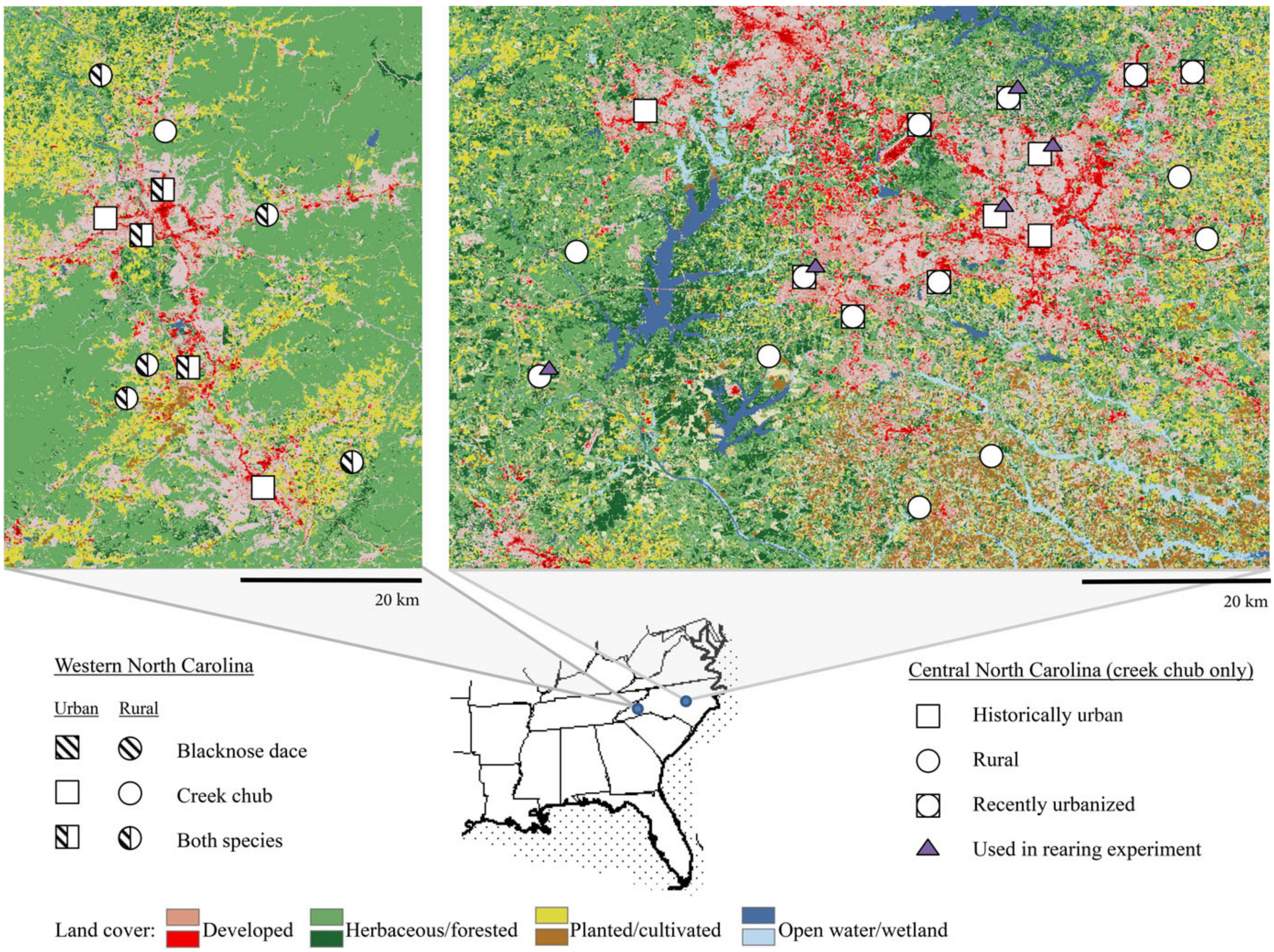

FIGURE 1 Study sites in western and central North Carolina, USA, for the two cyprinid fish species examined in this study

activity within as few as 13 generations (Hendry, Wenburg, Bentzen, Volk, & Quinn, 2000).

We examined a total of 693 fish from 25 North Carolina streams characterized as rural or urban (Figures 1, S1, Table 1). We used maps and satellite images to select streams of similar size that differed in their degree of watershed development. We quantified urbanization by measuring the percentage of developed land cover for a 2.6 km (1 mi) radius around each site using the 2011 National Land Cover Database (Homer et al., 2015) and QGIS. Urban and rural sites differed in amount of surrounding developed land cover (*t*-test, $p < .0001$). Urban sites had an average of 75.9% developed land cover, and rural sites had an average of 20.2%. The 2011 National Land Cover Database separates developed land cover into several categories, and for our analysis we included all of them.

Urban streams in our study area have flashier flow regimes than nearby rural streams (and do not have lower flows during base flow conditions; Brown et al., 2009; McMahon, Bales, Coles, Giddings, & Zappia, 2003). To confirm that our selected sites conform to this previously documented trend, we measured flashiness in a subset of 11 streams using HOBO data loggers to record the rate of water depth change during rain events. As expected, urban streams rose faster and more often than nearby rural streams measured during the same rain events (Figure S2, Table S1). We found a positive correlation between flashiness (defined as number of time intervals in which the stream rise rate exceeded nine times the average overall rise rate, following Brown et al. (2009)) and developed land cover ($\rho = .65$, $p = .0299$).

We captured fish from May 2012 to November 2014 using dip nets and a backpack electroshocker. We photographed each fish live at field sites using a small clear container (16.5 × 8 × 9 cm) within an aquarium and a high-resolution digital camera (Canon Rebel XTi, T3i, and XS) with a macro lens. For blacknose dace, we sampled eight sites (five rural, three urban) within the French Broad River basin (165 fish). For creek chub, we sampled 10 sites within the French Broad River basin in western North Carolina (213 fish) and 15 sites within the Neuse and Cape Fear river basins in central North Carolina (315 fish; see Table S2 for details). These study sites in western and central North Carolina are separated by the Eastern Continental Divide (rivers in the western region flow to the Gulf of Mexico while those in the east flow to the Atlantic Ocean). This substantial separation allows us to assess independent cases of phenotypic divergence between urban and rural sites, in both regions.

We used landmark-based geometric morphometrics to compare the body shape of fish from urban and rural sites. Geometric morphometrics is a tool for visualizing and quantitatively measuring shape variation, and advances in this field (Adams & Collyer, 2007; Collyer & Adams, 2007) have been applied to studying rapid morphological change caused by humans (Webster, Atton, Hart, & Ward, 2011). We digitized 12 anatomically homologous landmarks on two-dimensional lateral images of fish using tpsDig (Rohlf, 2013a) and performed generalized procrustes analysis to scale, rotate, and superimpose landmarks (removing isometric size effects and all other non-shape variation). Landmarks were positioned on the anterior tip of the snout, the center of the eye, the anterior and posterior margins of the eye, the insertion of the operculum on the ventral side, the back of the head, the anterior and posterior insertions of the dorsal fin, the anterior insertions of the anal and pelvic fins, and the anterior attachments of the dorsal and ventral insertions of the caudal fin (Figure 2a). We also digitized several points along the midline of the body in order to unbend images using tpsUtil (Rohlf, 2013b). The unbending step removes specimens' postural variation that would otherwise be interpreted as shape variation, and thus more accurately restricts analysis to true shape variation. The unbending points along the midline of the body are not anatomically homologous and were therefore not included in the analysis of shape. We saved relative warps (principal components of shape variation) using tpsRelw (Rohlf, 2010).

Using centroid size (the square root of the sum of squared distances from landmarks to their centroid) as an estimate of body size, we confirmed that body sizes greatly overlapped between urban and rural streams for each species (Table S3). This check is necessary because comparing size-independent shape requires allometric adjustment. We conducted a general linear mixed model to test for differences in centroid size between urban and rural streams (treating population as a random effect), and found no significant difference in either case (blacknose dace: $F_{1,6.91} = 0.06$, $p = .82$; creek chub: $F_{1,21.95} = 2.75$, $p = .11$). This makes our dataset highly amenable for testing for shape differences between habitat types.

We conducted multivariate statistical analysis using the relative warps (described above) to compare shape between rural and urban streams within each species. For blacknose dace we retained the first 15 relative warps, which explained 98.4% of the variance; for chub we used the first 15 relative warps, which explained 98.6% of the variance. For each species separately, we used a mixed-model multivariate analysis of covariance (MANCOVA) to investigate how body shape differs between rural and urban streams. In each case we included centroid size as a covariate to control for multivariate allometry. We $\log_{10}$-transformed centroid size to improve normality of residuals. We used site nested within urbanization status (urban or rural) as a random effect (see Hassell, Meyers, Billman, Rasmussen, and Belk (2012), Heinen-Kay and Langerhans (2013) and Riesch, Martin, and Langerhans (2013) for other examples of this statistical approach). The *p*-value for the effect of urbanization was determined using the MIXED procedure in SAS in order to treat populations as random effects (appropriate since population serves

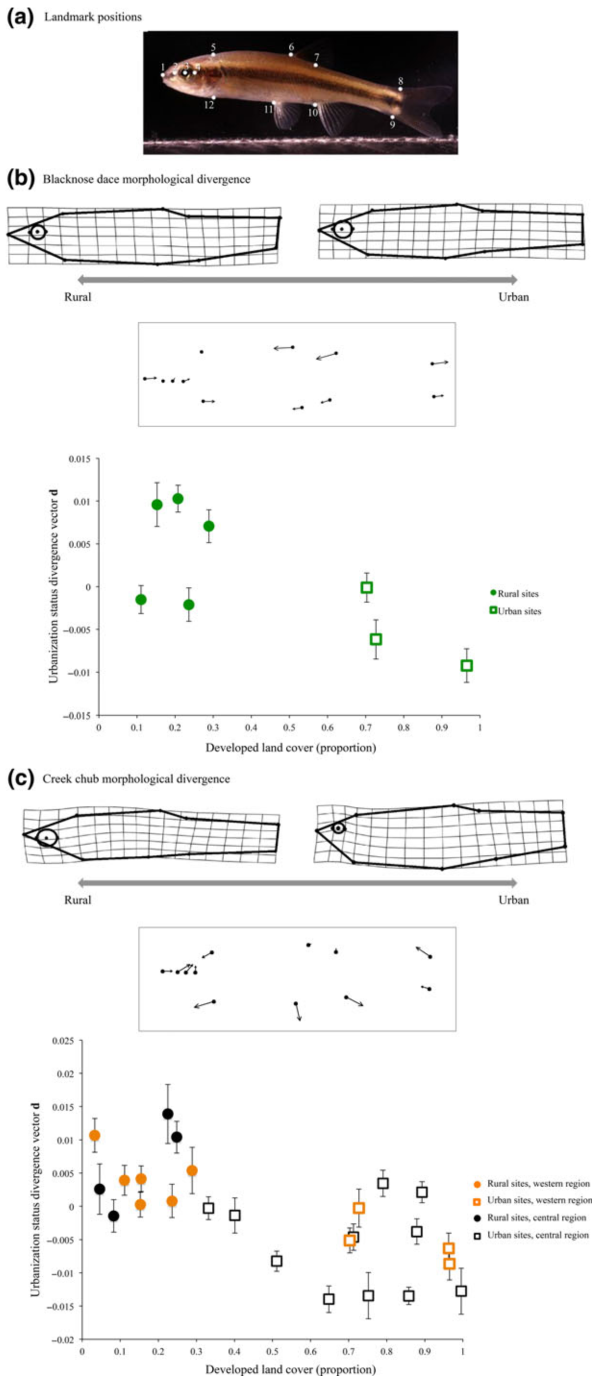

FIGURE 2 Morphological divergence between urban and rural populations of blacknose dace and creek chub. (a) Landmarks used for geometric morphometrics (juvenile creek chub shown). (b) Statistically significant morphological differences between urban and rural blacknose dace. Transformation grids are at the observed range (i.e., are not magnified) and illustrate urban and rural group means along the urbanization-status divergence vector, which describes morphological variation due to urbanization status (rural or urban). Landmark vectors (small arrows) indicate the direction and magnitude of change of the landmark locations, with arrows pointing in the rural-to-urban direction of change. Population means along the urbanization-status divergence vector are plotted against the proportion of developed land cover in a 2.6 km radius around each population's sampling location. Error bars are ±1 *SE*. (c) As in Figure 2b, for creek chub

as the unit of replication for this test of urbanization status). We measured both adults and juveniles, and included all individuals that were large enough to be confidently identified to species (1.3–15.0 cm standard length [SL]), thus spanning a large range in body size. Sexes were pooled due to lack of obvious external sexual dimorphism in nonbreeding fish. For creek chub, we additionally included a term for geographic region (testing for differences between western and central North Carolina) and for the interaction between region and urbanization status (testing for unique effects of urbanization across regions). Blacknose dace were present in only one region, so for the blacknose dace model we did not include a region term. We used η_p^2, an estimate of multivariate effect size, to compare the relative importance of each model term (Langerhans & DeWitt, 2004).

We produced visualizations that illustrate morphological differences by using the canonical axes or "divergence vectors" derived from the effect of interest (e.g., urbanization status) in each MANCOVA. Canonical axes, which were calculated in this case by performing principal components analysis on the relevant sums of squares cross products matrix, are linear combinations of the response variables and illustrate the greatest differences between groups while controlling for other model terms (details in Langerhans (2009) and Langerhans and Makowicz (2009)). Here, they describe in a multivariate fashion the morphological variation associated with urbanization status, with urban fish on one end of the axis and rural fish on the other. Morphological variation along the divergence vectors was visualized using thin-plate spline deformations, which depict the changes in landmark locations as points on warped grids (Bookstein, 1991).

Heterogeneity of slopes (interaction between centroid size and main terms of interest) was tested in all cases in this study, and was nonsignificant in the lab-rearing experiment but significant within other analyses. Although statistically significant ($p < .05$), these terms were of relatively minor importance (η_p^2 ranged from 25% to 68% as large as main effect), and did not alter the significance, nature, or magnitude of observed differences between urbanization regimes. All divergence vectors were highly correlated when including or excluding interaction terms with centroid size (r ranged from .95 to .00, all $p < .0001$), and thus we only present analyses excluding the terms throughout.

Since shape may vary with body condition, we checked for condition differences between urban and rural habitats. We measured SL and weight for 478 individuals (87 blacknose dace, 133 creek chub from western North Carolina, and 258 creek chub from central North Carolina). Weight relative to length (a proxy for condition) did not differ significantly between urban and rural habitats (blacknose dace: $F_{1,1.14} = 3.09$, $p = .31$; creek chub: $F_{1,11.78} = 0.06$, $p = .82$), and so it is unlikely that urban and rural fish diverge in shape because of differences in body condition.

To provide a quantitative metric comparable across studies for the degree of parallelism, or consistency of morphological differentiation between urban and rural streams, observed for each species/region, we followed Langerhans (2017) and Oke, Rolshausen, LeBlond, and Hendry (2017) in calculating the proportion of among-population trait variance explained by urbanization status (R^2).

2.2 | Creek chub: Morphological change through time

In addition to examining (above) how urban and rural fish differ in morphology among contemporary populations, we further investigated how creek chub have changed over time; i.e., before and after urbanization. For this component of the study we compared modern samples with museum samples collected from the same places several decades ago. Over this time period, some streams have stayed continuously rural, some have remained urban, and some have changed from rural to urban. The last category (recently urbanized sites) allows us to ask whether creek chub have changed in shape over time due to urbanization and what those changes involve. The other two categories (rural and historically urban) allow us to control for biologically relevant overall shape changes over time that might be shared across stream categories, and to account for preservation effects. Since historical samples were photographed as 28- to 53-year-old preserved specimens (collected during 1961–1986), whereas modern samples were photographed as live or recently preserved specimens, preservation might affect shape and create a spurious effect of "time." However, including sites that had been either continuously urban or continuously rural over both sampling times provides controls to avoid confounding time effects with urbanization effects.

We categorized sites by comparing current land cover estimates and satellite images with aerial photographs from the early 1980s. This allowed us to determine whether sites had changed substantially in urbanization level within the last 30 years. We categorized all sites as either rural (very little change), historically urban (already highly urban by 1983), or recently urbanized (showing a marked shift in land use from rural to urban between 1983 and 2012). We collected fish from 15 modern sites: five rural streams, four historically urban streams, and six recently urbanized streams. We compared these to collections in the North Carolina Museum of Natural Sciences representing 12 historical sample sites. Nine of these historical sites were from the same locations as our modern samples, and the remaining three were from nearby, similar streams. Of these 12 historical collections, five were rural, three were historically urban, and four were recently urbanized (but still rural at time of initial sampling).

After all fish were photographed, one researcher completed all the landmark digitization for geometric morphometrics (same methods as above). We used a mixed-model MANCOVA with relative warps (i.e., shape variables) as response variables and used $\log_{10}$-transformed centroid size, urbanization history (i.e., rural, recently urbanized, or historically urban), sample time (modern or historic), the interaction of urbanization history and sample time, and stream site nested within urbanization history (designated random effect) as independent variables. We used the first 15 relative warps, which explained 98.7% of the variation. For this analysis, the urbanization-history term tests for differences between the three habitat categories irrespective of time, the sample time term tests for overall changes in shape over time irrespective of urbanization history, and the interaction term between urbanization history and sample time tests whether change in fish shape over time depends on habitat alteration. This latter test is of primary interest because it directly tests our hypothesis that urbanization drives morphological change.

When a term of interest (such as the interaction between urbanization history and sample time) was significant, we calculated the divergence vector(s) for the term and generated thin-plate spline visualizations of the major differences along canonical axes (described above). This allowed us to visually examine how shape has changed over time due to increasing urbanization.

2.3 | Creek chub: Laboratory-rearing experiment

We caught very young juvenile creek chub (~1.5 cm SL) from five streams in central North Carolina during June and July of 2014. For this species, this period of time represents a late postlarval stage shortly after nest emergence (Buynak & Mohr, 1979; Washburn, 1948). Two stream sites were historically urban, two were recently urbanized, and one was rural. Fish were transported to facilities at North Carolina State University and each population was randomly divided into two water-velocity treatment groups designed to roughly mimic flow conditions of urban and rural streams. High-velocity treatment groups received 60 min of intensified water velocity once a week for 16 weeks, while low-velocity treatment groups did not. All fish (n = 58; Table S4), both high-velocity and low-velocity treatment groups, were kept in 10 L tanks in the same recirculating system (Aquatic Habitats; Apopka, FL), which allowed populations and treatment groups to experience identical water conditions (0.5 ppt salinity, 25°C) other than the weekly velocity treatment. Fish were allowed to acclimate to lab conditions for 2 weeks prior to initiation of the velocity treatment. The velocity treatment involved greatly increased water flow into the tanks, flushing the tanks at a constant rate of 4.2 L/min vs. 0.7 L/min at other times and throughout the low-velocity treatment. This created elevated, nonuniform water velocity within the tanks (~0.9–5.5 cm/s) and forced fish to swim against a moderate current to maintain position (~1–3.5 body lengths per second). Fish were fed brine shrimp and dry flakes (TetraMin pro) ad libitum daily throughout the experiment and kept on a 12:12 hr light-dark cycle.

Raising recently hatched fish for 18 weeks under common laboratory conditions should allow us to determine the heritable basis of morphological differences among populations because the first summer of a temperate stream fish's life is an especially formative period, and 16 weeks of treatment has uncovered phenotypic plasticity in other fish (Imre, McLaughlin, & Noakes, 2002). However, due to the long time to maturity in these species, the experiment was not designed to rule out maternal effects, which leaves them as a potential factor influencing fish shape.

After 16 weeks of treatment, fish were photographed for morphometrics, anesthetized with MS-222, weighed, and preserved in ethanol. We digitized the same 12 landmarks as above (Figure 2a) and performed geometric morphometrics to visualize differences and compare shape between populations and treatments.

To test for effects of population (genetically based morphological differences), velocity treatment (phenotypic plasticity in morphology), and their interaction (plasticity that differs among populations) on body morphology, we used a MANCOVA with $\log_{10}$-transformed centroid size, population, treatment, and the interaction between population and treatment as independent variables, with relative warps as dependent variables. We used the first 15 relative warps, which explained 98.7% of the variation.

With no significant evidence for velocity-induced morphological plasticity from the above MANCOVA, we pooled fish across treatments within populations to perform a direct test of urbanization on body shape of lab-raised fish. We conducted a MANCOVA using relative warps as dependent variables, $\log_{10}$-transformed centroid size as a covariate, urbanization history (historically urban, recently urbanized, rural) as a main effect, and population nested within urbanization history as a fixed effect because we wished to explicitly examine variation in body shape within these five populations for this analysis. Our primary interest for this analysis was to provide a direct test of the effects of urbanization history on body shape and to visualize this variation (using thin-plate spline transformations along divergence vectors as described above).

3 | RESULTS

3.1 | Dace and chub: Morphological divergence between urban and rural streams

Blacknose dace and creek chub both showed statistically significant morphological differences between urban and rural streams (Table 2). After controlling for multivariate allometry, we found that the two species showed different patterns of urban-rural differentiation. Urban blacknose dace had a longer and shallower midbody/caudal region, with more anteriorly-shifted dorsal, pelvic, and anal fins than rural blacknose dace. Urban blacknose dace also had an eye positioned closer to the snout compared to rural blacknose dace (Figure 2b). In creek chub, urban populations had a deeper midbody/caudal region owing to ventrally shifted pelvic and anal fin insertions, a longer midbody region resulting from a posteriorly shifted anal fin and a shorter head, and a smaller eye (Figure 2c). Within each species and region, morphological differences between urbanization regimes exhibited clear evidence of parallel phenotypic differentiation (R^2 values: dace = .41, creek chub = .46 within both regions) that was remarkably similar to recent studies investigating evidence of parallel evolution in fishes (overall average values of .41 and .46 in Langerhans (2017) and Oke et al. (2017) respectively).

In our MANCOVAs investigating how body shape differs between rural and urban streams, all other model terms (body size, region, and region x urbanization status) were highly significant as well (Table 2). There was a substantial effect of allometry in both blacknose dace and creek chub, as expected due to the large range

TABLE 2 Results of MANCOVAs testing body shape differences between urban and rural streams in contemporary populations of blacknose dace and creek chub

Model term	F	df	p	η_p^2 %
Blacknose dace				
Log centroid size	78.21	15, 142	<.0001	89.20
Urbanization status	4.71	14, 922	<.0001	35.34
Creek chub				
Log centroid size	238.17	15, 488	<.0001	87.98
Urbanization status	9.37	14, 2,965	<.0001	23.78
Region	11.21	14, 2,965	<.0001	24.67
Region × urbanization status	5.85	14, 2,965	<.0001	16.43

of body sizes in wild-caught fish. We also found regional morphological differences between western and central populations of creek chub, which is not surprising considering their long separation by the Eastern Continental Divide. Based on our measure of multivariate effect size and inspection of canonical axes, creek chub in both western and central regions exhibited largely consistent differences between rural and urban streams. (Region was not considered in the blacknose dace model because they are found in only one region.)

3.2 | Creek chub: Morphological change through time

In our comparison of modern creek chub populations with historical museum samples, all the model terms (size, urbanization history, sampling time [i.e., historical or modern], and the urbanization history by sampling time interaction) had a highly significant influence on body shape (Table 3). Allometry was again strongly evident. Based on our measures of effect size, the strongest gradient in the data other than allometry was that shape changed over time, irrespective of habitat change. After controlling for the effect of time (which includes any specimen preservation effects), we found consistent differences between habitat categories. Fish from historically urban sites strongly differed in body shape from other populations, regardless of time of sampling. Historically urban populations displayed a body morphology that was virtually identical to the urban fish described above (see part I) in the modern, urban-vs.-rural comparison of creek chub (deeper caudal region, ventrally shifted pelvic and anal fin insertions, longer midbody, and smaller eye; Figure S3).

Of key interest was the significant interaction between urbanization history and sampling time, meaning that the change in body shape over time depended on whether a stream changed from rural to urban or stayed the same. Because the interaction term tests our primary hypothesis, and because the divergence vectors derived from this term summarize the overall findings of this analysis, we inspect this term in more detail. The interaction term revealed that the effect of time on body shape was greatest for the recently urbanized sites (Figure 3). Morphological differences between historical and modern collections were greatest for recently urbanized sites along the first divergence vector, and the recently urbanized sites were the only habitat category that showed significant differences over time along the second divergence vector (Figure 3).

TABLE 3 Results of MANCOVA testing temporal shape variation in creek chub

Model term	F	df	P	η_p^2 %
Log centroid size	138.92	15, 542	<.0001	79.36
Urbanization history	13.01	28, 4,568	<.0001	20.36
Sampling time	37.56	14, 3,249	<.0001	55.72
Urbanization history × sampling time	7.15	28, 4,568	<.0001	9.79

The first divergence vector derived from the interaction term revealed that all populations tended to become more shallow-bodied with larger heads over time, and that sites experiencing urbanization during this time period showed the largest magnitude of change. The second divergence vector separated collections by urbanization history. All rural collections (modern and historical collections from continuously rural sites, and historical collections from recently urbanized sites) exhibited a similar morphology, characterized by a shallow midbody/caudal region, a short midbody region, and a large eye. Meanwhile, all collections (both modern and historical) from historically urban localities exhibited a morphology characteristic of that uncovered for urban creek chub in the above analysis of contemporary rural vs. urban streams; i.e., a deeper midbody/caudal region owing to ventrally shifted pelvic and anal fin insertions, a longer midbody region resulting from a posteriorly shifted anal fin and a shorter head, and a smaller eye. Finally, modern collections from recently urbanized sites exhibited a morphology intermediate between rural and historically urban sites. This suggests that urbanization has led to shape changes in creek chub, as only the recently urbanized sites experienced a change along the second divergence vector, with historical collections resembling fish in continuously rural streams, and with modern collections shifted toward resembling fish from historically urban sites. In summary, creek chub (1) became deeper bodied in streams experiencing recent urbanization; (2) were already deep-bodied, and showed no subsequent deepening over time, in streams which were urbanized more than 30 years ago; and (3) remained relatively shallow bodied in streams that remained rural (Figure 3).

3.3 | Creek chub: Laboratory-rearing experiment

In our laboratory rearing experiment the effect of population was highly significant, indicating strong differences between populations even after they were raised under common laboratory conditions (Table S5). Significance tests and our estimate of multivariate effect size showed that out of all the model terms, the population term clearly had the strongest influence on body shape. While no other term was significant, the interaction term between population and treatment was marginally nonsignificant and suggested that one site (Beaver Creek) exhibited some plasticity in shape in response to the velocity treatment.

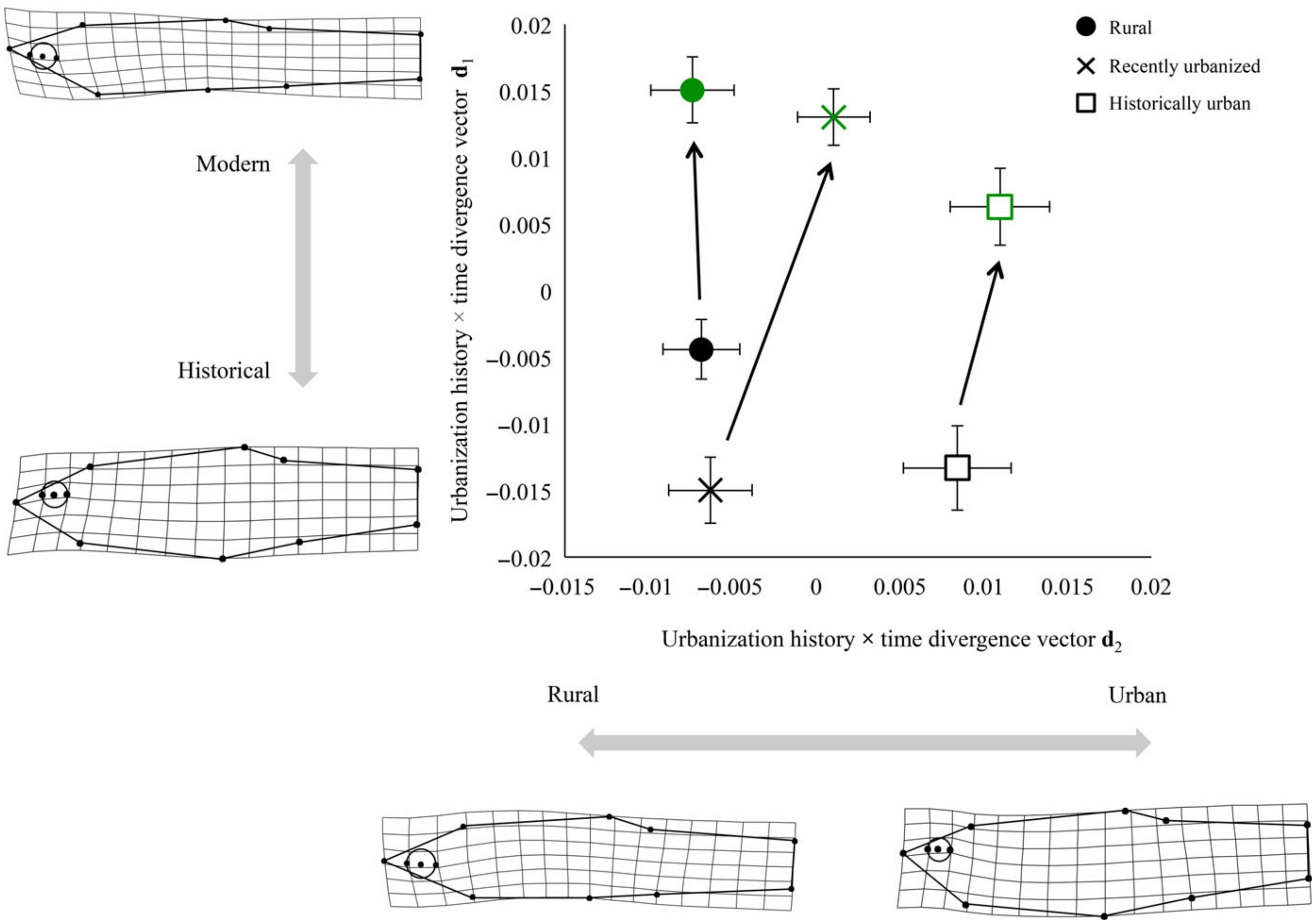

FIGURE 3 Visualization of the "urbanization history × sampling time" interaction term, depicting how creek chub body shape differs between habitat categories and over time. The axes are the first two divergence vectors from the interaction term of the MANCOVA ($\mathbf{d}_1$ and $\mathbf{d}_2$). $\mathbf{d}_1$ primarily illustrates the effect of time, with sites experiencing urbanization during this time period showing the strongest morphological changes. $\mathbf{d}_2$ clearly differentiates fish collections among habitats, with no morphological changes over time within rural or historically urban sites, but with a significant shift in recently urbanized sites, where fish went from being historically indistinguishable from rural sites to being intermediate between rural and urban fish in modern collections. Green symbols indicate modern collections; black indicates historical collections. Arrows between symbols show direction of change over time. Error bars are ±1 *SE*

In a test of the effect of urbanization on the morphology of lab-raised creek chub, we found that body shape significantly differed between habitats (Table 4; Figure 4). In this case, we found no evidence of allometry, likely owing to relatively small variation in body size in our rearing experiment. Visualizing the first divergence vector derived from the urbanization-history term revealed that the two historically urban populations exhibited deeper bodies—specifically, deeper and longer midbodies—than the rural population, with the two recently urbanized populations intermediate. This follows the patterns seen in the above analyses of wild-caught fish from additional populations. To directly compare this similarity, we projected wild-caught fish onto the first urbanization-history divergence vector derived from lab-raised fish, and tested for a correlation between the first urbanization-history divergence vector scores derived from wild-caught and lab-raised fish. We found these scores were highly correlated ($r = .72$, $p < .0001$), indicating that the manner in which urban and rural populations differ in shape after being reared in common conditions mirrors the way they differ in the field, where our observations included many more populations.

4 | DISCUSSION

In both species examined, we consistently found shape differences between urban and rural populations. The degree of consistency/parallelism observed within each species and region indicated a magnitude of parallelism generally regarded as quite strong, explaining

TABLE 4 Results of MANCOVA testing for differences between habitat types in lab-reared creek chub

Model term	*F*	*df*	*p*
Log centroid size	0.45	15, 38	.3501
Urbanization history	1.71	30, 76	.0315
Population [urbanization history]	2.45	30, 76	.0009

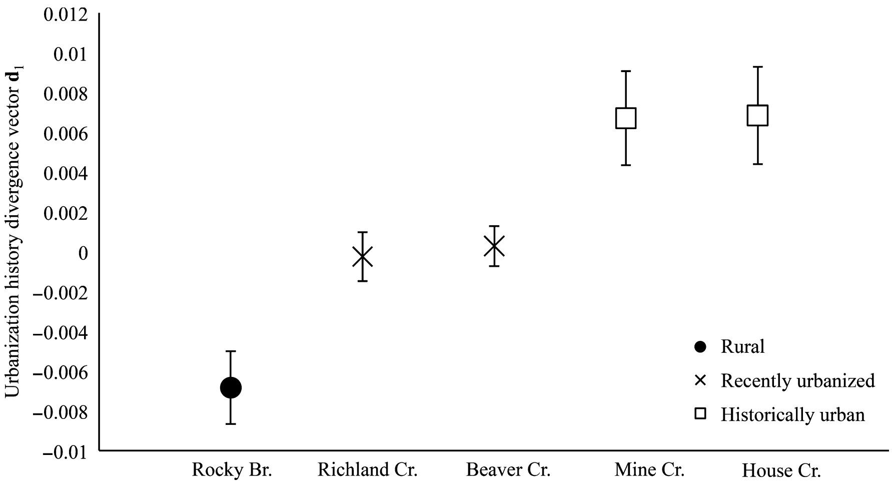

FIGURE 4 Variation in morphology of lab-reared creek chub from three habitat types along the first urbanization-history divergence vector from the MANCOVA of lab-raised fish. Error bars are ±1 *SE*

slightly less than half of among-population trait variance (Langerhans, 2017; Oke et al., 2017). In blacknose dace the nature of shape change matched predictions for morphological adaptation to urban stream flow regimes, while in creek chub the changes were strong and consistent but not in the direction expected. That both species—and both the western and central groups of creek chub—showed a morphological response to urbanization despite being widely separated phylogenetically, suggests that this might represent a widespread response to urbanization in fish that has hitherto gone unnoticed. From our laboratory rearing experiment, it appears that shape differences across populations are strongly genetically determined, which implies that human modification of the landscape influences the evolution of fish shape in urban streams. With our finding (in the historical comparison) that creek chub have diverged in body shape over time following recent urbanization, we can place the pace of phenotypic change observed here in the context of recent work showing that urbanization has typically resulted in phenotypic changes in organisms in the vicinity of 0.7–1.0 square-root haldane numerators (trait changes in standard deviation units; Alberti et al., 2017). We found that urbanization-driven morphological changes in creek chub (along the second divergence vector in Figure 3) directly correspond to these prior findings: 0.81 square-root haldane numerators for stream populations experiencing recent urbanization, compared to 0.05 for continuously rural populations and 0.23 for continuously urban populations.

Prior research has shown that variation in water velocity can lead to morphological changes in fish (Langerhans, 2008), and recent work has found that human-caused reductions in velocity, such as that imposed by dams, can rapidly result in altered fish morphology (Cureton & Broughton, 2014; Franssen, 2011; Franssen, Stewart, & Schaefer, 2013; Haas et al., 2010). But there is little or no data on the evolutionary impact of *increased* water velocity on fish shape, and to our knowledge this is the first study to examine the effects of periodic increases in velocity due to anthropogenic disturbance. The morphological shift we observed in blacknose dace matched our a priori predictions of increased streamlining in urban streams, and may reflect adaptive shifts to altered velocity regimes, consistent with theoretical work and empirical work examining sites that differ strongly in average velocity (Langerhans, 2008). Increased streamlining can offer the functional advantage of increased locomotor efficiency in flashier streams characterized by elevated maximum velocities. Prior research suggests that body shape divergence in fast- and slow-moving water probably arises because natural selection favors different morphologies in different environments due to performance tradeoffs: some habitats favor increased burst-speed capacity and maneuverability, while others favor steady-swimming ability and greater endurance (Nelson et al., 2003). The general biomechanical impossibility of simultaneously maximizing both capacities results, in most fish, in divergence (Langerhans & Reznick, 2010).

In creek chub, the deeper and longer midbody region observed in urban fish represents a morphological shift that is more difficult to explain based on adaptive hypotheses related to locomotor performance under varying velocity conditions. However, other studies have also described similar morphological adaptation (deeper bodies in faster moving waters) in cyprinid minnows, atherinopsid silversides, centrarchid sunfish, threespine stickleback, and poeciliid livebearing fish (Franssen et al., 2013; Stuart et al., 2017; Zúñiga-Vega, Reznick, & Johnson, 2007). This supports the contention that changes in velocity regime have led to the observed shape changes in creek chub inhabiting urban streams. This morphology might enhance steady-swimming performance by delaying boundary layer separation and allowing for greater musculature in the midbody and caudal region to enhance thrust production. Creek chub from urbanized streams do in fact exhibit higher locomotor efficiency during steady swimming, consistent with the notion of adaptive divergence in response to selection favoring such abilities (E. M. A. Kern & R. B. Langerhans, unpublished data), and recent work shows swimming performance associations with impervious surface cover in another stream fish (Nelson, Atzori, & Gastrich, 2015; Nelson, Gotwalt, Simonetti, & Snodgrass, 2008). Furthermore, in the face of increased peak velocities, selection may favor locomotor abilities that are more complex than simply "increased steady-swimming performance," and so urban creek-chub morphology may reflect a compromise between competing demands for both endurance and power or agility, since deepened midbody regions theoretically enhance maneuverability.

That creek chub and blacknose dace should respond differently to urbanization is puzzling, but not unprecedented. Two closely related minnow species, *Cyprinella lutrensis* and *C. venusta*, showed opposite responses to reduced water velocity, with one species becoming deeper bodied and the other becoming more streamlined (Franssen, 2011; Franssen et al., 2013). Similarly, two salmonids responded differently to increased water velocity: salmon became deeper bodied while brown trout became more streamlined (Pakkasmaa & Piironen, 2000). Species-specific responses to urbanization may often involve differences between species (e.g., behavior, habitat use, diet) that cause differences in selection regimes, but could also involve factors such as genetic (co)variances of traits.

In our comparison of historical and modern samples we found significant trends before and after urbanization that support a causal link between urbanization and morphological changes. Not only did historically urban fish differ from rural fish in ways nearly identical to the modern analysis, but fish from sites that had recently become urbanized shifted from a rural morphology to a morphology approximately halfway to the historically urban morphology.

Urbanization appears to have been affecting fish shape for many decades prior to our historical urban collections, since creek chub had already achieved an urban shape by the 1960s–1980s. Since historically urban sites did not progress further along the relevant divergence vector between historical and modern sampling times, fish may shift to a new adaptive peak in urban sites and remain there. For the recently urbanized streams, we may have caught this shift in the act, in which populations are either still evolving toward the new adaptive peak or are tracking environmental changes as they occur (in other words, adaptive peaks are transitioning from rural to urban morphologies as environments become urbanized). Inspecting the degree of urban development across sites, we find that the latter explanation is more likely: first, recently urbanized sites show intermediate levels of developed land cover (averages for sites in this analysis: rural = 9.5%, recently urbanized = 56.3%, urban = 87.1%,); and secondly, mean body shape (characterized by $\mathbf{d}_2$ from the interaction term of the MANCOVA) is positively correlated with developed land cover across all sites ($r = .67$, $p = .0025$). This means that if urbanization continues, then we might expect creek chub morphology to continue to change toward the morphology typical of fish from historically urban sites.

In our laboratory rearing experiment we noted strong evidence that shape divergence has a genetic basis, as fish from urban and rural populations raised in a common environment still showed morphological differences. Similarly, morphological changes due to impoundment have been shown to be genetically based in related fish species (Brinsmead & Fox, 2002; Haas et al., 2010; Langerhans, 2008; Langerhans et al., 2003; McGuigan et al., 2003). We found only weak evidence of plasticity in a recently urbanized site. It is possible that plasticity plays an important role early on during times of environmental change (e.g., soon after human disturbance), and that genetic assimilation follows later (Lande, 2009, 2015). Our results clearly support a genetic basis for shape, yet plastic and genetic responses are not mutually exclusive, and the number of populations we were able to test in a lab-rearing experiment is modest enough to warrant some caution in interpreting the nonsignificant results as the absence of plasticity. It is also conceivable that a more intense or more frequent velocity treatment than the one we imposed could have succeeded in inducing a plastic response.

This work demonstrates that urbanization appears to have a repeatable impact on fish body shape in multiple species and regions. We suggest water velocity as the most likely driver of shape divergence in urban streams because of its tight link with urbanization, its relationship with swimming efficiency and hydrodynamics, and because much past work has shown that for many fish, body shape varies predictably with water velocity. Still, urban and rural streams differ in more ways than one, and it is important to consider whether other urban stream features might also impact fish morphology. In our study region, known salient characteristics of urban streams involve changes in macroinvertebrate assemblages, pollution levels, and stream channel morphology, while work from other geographic regions indicates that higher temperatures and reduced habitat complexity may also be present (Brown et al., 2009; Walsh et al., 2005). Are any of these likely to produce morphological shifts like the ones we observed? For one thing, the shift in eye size and position that we found might be related to a change in fish diet (Tófoli, Alves, Higuti, Cunico, & Hahn, 2013). Additionally, it is conceivable that less complex channels and reduced organic debris in urban streams (Walsh et al., 2005) mean that there are fewer advantages to a deeper fish body shape suited for high maneuverability in those environments (Webb, 1982). Finally, there is some evidence that temperature influences fish shape and eye size (Georga & Koumoundouros, 2010; Georgakopoulou et al., 2007; Ramler, Mitteroecker, Shama, Wegner, & Ahnelt, 2014; Sfakianakis, Leris, Laggis, & Kentouri, 2011). However, data on the link between these additional factors and fish body morphology are limited compared to the well-documented connection between velocity and morphology, and thus require future investigation.

In conclusion, our results suggest that urbanization causes rapid evolutionary changes in body morphology in fish. The morphological changes were somewhat predictable based on principles of hydromechanics, although different species had unique features to their responses. Other factors in need of additional investigation might also contribute to shape change. Urbanization is a global phenomenon that may have a broad impact on the evolution of aquatic species, so care should be taken to examine its influence on contemporary evolution in order to make informed conservation decisions (Carroll et al., 2014; Smith, Kinnison, Strauss, Fuller, & Carroll, 2014). Understanding how fish respond evolutionarily to human disturbance could have diverse applications, such as predicting species persistence, measuring the rate of evolution in human-impacted systems, making recommendations for improving urban stormwater management to mitigate biodiversity loss, and developing evolutionary interventions for the management and preservation of biodiversity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Adams, D. C., & Collyer, M. L. (2007). Analysis of character divergence along environmental gradients and other covariates. *Evolution*, *61*, 510–515. https://doi.org/10.1111/j.1558-5646.2007.00063.x

Aguirre, W. E. (2009). Microgeographical diversification of threespine stickleback: Body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. *Biological Journal of the Linnean Society*, *98*, 139–151. https://doi.org/10.1111/j.1095-8312.2009.01267.x

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., … Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 8951–8956. https://doi.org/10.1073/pnas.1606034114

Bookstein, F. L. (1991). *Morphometric tools for landmark data: Geometry and biology*, Vol. 10. New York, NY: Cambridge University Press.

Braat, L., & ten Brink, P. (Eds.). (2008). *The cost of policy inaction: The case of not meeting the 2010 biodiversity target*. Wageningen, the Netherlands: Alterra.

Brinsmead, J., & Fox, M. G. (2002). Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. *Journal of Fish Biology*, *61*, 1619–1638. https://doi.org/10.1111/j.1095-8649.2002.tb02502.x

Brown, L. R., Cuffney, T. F., Coles, J. F., Fitzpatrick, F., McMahon, G., Steuer, J., … May, J. T. (2009). Urban streams across the USA: Lessons learned from studies in 9 metropolitan areas. *Journal of the North American Benthological Society*, *28*, 1051–1069. https://doi.org/10.1899/08-153.1

Buynak, G. L., & Mohr, H. W. (1979). Larval development of creek chub and fallfish from two Susquehanna River tributaries. *The Progressive Fish-Culturist*, *41*, 124–129. https://doi.org/10.1577/1548-8659(1979)41[124:LDOCCA]2.0.CO;2

Carroll, S. P., Hendry, A. P., Reznick, D. N., & Fox, C. W. (2007). Evolution on ecological time-scales. *Functional Ecology*, *21*, 387–393. https://doi.org/10.1111/j.1365-2435.2007.01289.x

Carroll, S. P., Jorgensen, P. S., Kinnison, M. T., Bergstrom, C. T., Denison, R. F., Gluckman, P., … Tabashnik, B. E. (2014). Applying evolutionary biology to address global challenges. *Science*, *346*(6207), 1245993–1245993. https://doi.org/10.1126/science.1245993

Chadwick, M. A., Dobberfuhl, D. R., Benke, A. C., Huryn, A. D., Suberkropp, K., & Thiele, J. E. (2006). Urbanization affects stream ecosystem function by altering hydrology, chemistry, and biotic richness. *Ecological Applications*, *16*, 1796–1807. https://doi.org/10.1890/1051-0761(2006)016[1796:UASEFB]2.0.CO;2

Collyer, M. L., & Adams, D. C. (2007). Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology*, *88*, 683–692. https://doi.org/10.1890/06-0727

Cureton, J. C., & Broughton, R. E. (2014). Rapid morphological divergence of a stream fish in response to changes in water flow. *Biology Letters*, *10*, 20140352–20140352. https://doi.org/10.1098/rsbl.2014.0352

Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, *44*, 194–203. https://doi.org/10.1007/s13280-014-0547-2

Franssen, N. R. (2011). Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evolutionary Applications*, *4*, 791–804. https://doi.org/10.1111/j.1752-4571.2011.00200.x

Franssen, N. R., Stewart, L. K., & Schaefer, J. F. (2013). Morphological divergence and flow-induced phenotypic plasticity in a native fish from anthropogenically altered stream habitats. *Ecology and Evolution*, *3*, 4648–4657. https://doi.org/10.1002/ece3.842

Georga, I., & Koumoundouros, G. (2010). Thermally induced plasticity of body shape in adult zebrafish *Danio rerio* (Hamilton, 1822). *Journal of Morphology*, *271*, 1319–1327. https://doi.org/10.1002/jmor.10874

Georgakopoulou, E., Sfakianakis, D. G., Kouttouki, S., Divanach, P., Kentouri, M., & Koumoundouros, G. (2007). The influence of temperature during early life on phenotypic expression at later ontogenetic stages in sea bass. *Journal of Fish Biology*, *70*, 278–291.

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., & Briggs, J. M. (2008). Global change and the ecology of cities. *Science*, *319*(5864), 756–760. https://doi.org/10.1126/science.1150195

Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., … Peters, D. P. (2008). The changing landscape: Ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, *6*, 264–272. https://doi.org/10.1890/070147

Haas, T. C., Blum, M. J., & Heins, D. C. (2010). Morphological responses of a stream fish to water impoundment. *Biology Letters*, *6*, 803–806. https://doi.org/10.1098/rsbl.2010.0401

Hassell, E. M. A., Meyers, P. J., Billman, E. J., Rasmussen, J. E., & Belk, M. C. (2012). Ontogeny and sex alter the effect of predation on body shape in a livebearing fish: Sexual dimorphism, parallelism, and costs of reproduction. *Ecology and Evolution*, *2*, 1738–1746. https://doi.org/10.1002/ece3.278

Heinen-Kay, J., & Langerhans, R. B. (2013). Predation-associated divergence of male genital morphology in a livebearing fish. *Journal of Evolutionary Biology*, *26*, 2135–2146. https://doi.org/10.1111/jeb.12229

Hendry, A. P., Kinnison, M. T., Heino, M., Day, T., Smith, T. B., Fitt, G., … Carroll, S. P. (2011). Evolutionary principles and their practical application. *Evolutionary Applications*, *4*, 159–183. https://doi.org/10.1111/j.1752-4571.2010.00165.x

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C., & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: Evidence from introduced salmon. *Science*, *290*(5491), 516–519. https://doi.org/10.1126/science.290.5491.516

Hocutt, C. H., & Wiley, E. O. (1986). *The Zoogeography of North American freshwater fishes*. New York, NY: Wiley.

Homer, C. G., Dewitz, J. A., Yang, L., Jin, S., Danielson, P., Xian, G., & Megown, K. (2015). Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*, *81*, 345–354.

Hvidsten, N. A. (1985). Mortality of pre-smolt Atlantic salmon, *Salmo salar* L., and brown trout, *Salmo trutta* L., caused by fluctuating water levels in the regulated River Nidelva, central Norway. *Journal of Fish Biology*, *27*, 711–718. https://doi.org/10.1111/j.1095-8649.1985.tb03215.x

Imre, I., McLaughlin, R. L., & Noakes, D. L. G. (2002). Phenotypic plasticity in brook charr: Changes in caudal fin induced by water flow. *Journal of Fish Biology*, *61*, 1171–1181. https://doi.org/10.1111/j.1095-8649.2002.tb02463.x

Jacobson, C. R. (2011). Identification and quantification of the hydrological impacts of imperviousness in urban catchments: A review. *Journal of Environmental Management*, *92*, 1438–1448. https://doi.org/10.1016/j.jenvman.2011.01.018

Jensen, A. J., & Johnsen, B. O. (1999). The functional relationship between peak spring floods and survival and growth of juvenile Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Functional Ecology*, *13*, 778–785. https://doi.org/10.1046/j.1365-2435.1999.00358.x

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, *22*, 1435–1446. https://doi.org/10.1111/j.1420-9101.2009.01754.x

Lande, R. (2015). Evolution of phenotypic plasticity in colonizing species. *Molecular Ecology*, *24*, 2038–2045. https://doi.org/10.1111/mec.13037

Langerhans, R. B. (2008). Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology*, *48*, 750–768. https://doi.org/10.1093/icb/icn092

Langerhans, R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology*, *22*, 1057–1075. https://doi.org/10.1111/j.1420-9101.2009.01716.x

Langerhans, R. B. (2017). Predictability and parallelism of multitrait adaptation. *Journal of Heredity*, *109*, 59–70. https://doi.org/10.1093/jhered/esx043

Langerhans, R. B., & DeWitt, T. J. (2004). Shared and unique features of evolutionary diversification. *The American Naturalist*, *164*, 335–349. https://doi.org/10.1086/422857

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. https://doi.org/10.1111/j.1095-8312.2003.00266.x

Langerhans, R. B., & Makowicz, A. M. (2009). Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. *Journal of Evolutionary Biology*, *22*, 2231–2242. https://doi.org/10.1111/j.1420-9101.2009.01839.x

Langerhans, R. B., & Reznick, D. N. (2010). Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In P. Domenici & B. G. Kapoor (Eds.), *Fish locomotion: An eco-ethological perspective* (pp. 200–248). Enfield, NH: Science Publishers. https://doi.org/10.1201/b10190

Lepers, E., Lambin, E. F., Janetos, A. C., Defries, R., Achard, F., Ramankutty, N., & Scholes, R. J. (2005). A synthesis of information on rapid land-cover change for the period 1981–2000. *BioScience*, *55*, 115–124. https://doi.org/10.1641/0006-3568(2005)055[0115:ASOIOR]2.0.CO;2

Luther, D. A., & Derryberry, E. P. (2012). Birdsongs keep pace with city life: Changes in song over time in an urban songbird affects communication. *Animal Behaviour*, *83*, 1059–1066. https://doi.org/10.1016/j.anbehav.2012.01.034

Lytle, D. A., & Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends in Ecology & Evolution*, *19*, 94–100. https://doi.org/10.1016/j.tree.2003.10.002

Marchetti, M., Light, T., & Feliciano, J. (2001). Homogenization of California's fish fauna through abiotic change. In J. L. Lockwood & M. L. McKinney (Eds.), *Biotic homogenization* (pp. 259–278). New York: Springer. https://doi.org/10.1007/978-1-4615-1261-5

Marchetti, M. P., Lockwood, J. L., & Light, T. (2006). Effects of urbanization on California's fish diversity: Differentiation, homogenization and the influence of spatial scale. *Biological Conservation*, *127*, 310–318. https://doi.org/10.1016/j.biocon.2005.04.025

Marzluff, J. M. (2001). Worldwide urbanization and its effects on birds. In J. M. Marzluff, R. Bowman, & R. Donnelly (Eds.), *Avian ecology and conservation in an urbanizing world* (pp. 19–47). Boston, MA: Springer. https://doi.org/10.1007/978-1-4615-1531-9

McGuigan, K., Franklin, C. E., Moritz, C., & Blows, M. W. (2003). Adaptation of rainbow fish to lake and stream habitats. *Evolution*, *57*, 104–118. https://doi.org/10.1111/j.0014-3820.2003.tb00219.x

McMahon, G., Bales, J. D., Coles, J. F., Giddings, E. M. P., & Zappia, H. (2003). Use of stage data to characterize hydrologic conditions in an urbanizing environment. *Journal of the American Water Resources Association*, *39*, 1529–1546. https://doi.org/10.1111/j.1752-1688.2003.tb04437.x

Munshi-South, J. (2012). Urban landscape genetics: Canopy cover predicts gene flow between white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Molecular Ecology*, *21*, 1360–1378. https://doi.org/10.1111/j.1365-294X.2012.05476.x

Nelson, J. A., Atzori, F., & Gastrich, K. R. (2015). Repeatability and phenotypic plasticity of fish swimming performance across a gradient of urbanization. *Environmental Biology of Fishes*, *98*, 1431–1447. https://doi.org/10.1007/s10641-014-0369-x

Nelson, J. A., Gotwalt, P. S., Simonetti, C. A., & Snodgrass, J. W. (2008). Environmental correlates, plasticity and repeatability of differences in performance among blacknose dace (*Rhinichthys atratulus*) populations across a gradient of urbanization. *Physiological and Biochemical Zoology*, *81*, 25–42. https://doi.org/10.1086/523304

Nelson, J. A., Gotwalt, P. S., & Snodgrass, J. W. (2003). Swimming performance of blacknose dace (*Rhinichthys atratulus*) mirrors home-stream current velocity. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*, 301–308. https://doi.org/10.1139/f03-023

Oke, K. B., Rolshausen, G., LeBlond, C., & Hendry, A. P. (2017). How parallel is parallel evolution? A comparative analysis in fishes. *The American Naturalist*, *190*(1), 1–16. https://doi.org/10.1086/691989

Pakkasmaa, S., & Piironen, J. (2000). Water velocity shapes juvenile salmonids. *Evolutionary Ecology*, *14*, 721–730. https://doi.org/10.1023/A:1011691810801

Paul, M. J., & Meyer, J. L. (2001). Streams in the urban landscape. *Annual Review of Ecology and Systematics*, *32*, 333–365. https://doi.org/10.1146/annurev.ecolsys.32.081501.114040

Poff, N. L., Bledsoe, B. P., & Cuhaciyan, C. O. (2006). Hydrologic variation with land use across the contiguous United States: Geomorphic and ecological consequences for stream ecosystems. *Geomorphology*, *79*, 264–285. https://doi.org/10.1016/j.geomorph.2006.06.032

Ramler, D., Mitteroecker, P., Shama, L. N. S., Wegner, K. M., & Ahnelt, H. (2014). Nonlinear effects of temperature on body form and developmental canalization in the threespine stickleback. *Journal of Evolutionary Biology*, *27*, 497–507. https://doi.org/10.1111/jeb.12311

Riesch, R., Martin, R., & Langerhans, R. B. (2013). Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *The American Naturalist*, *181*, 78–93. https://doi.org/10.1086/668597

Rohlf, F. J. (2010). *TpsRelw*. Stony Brook, NY: State University of New York.

Rohlf, F. J. (2013a). *TpsDig*. Stony Brook, NY: State University of New York.

Rohlf, F. J. (2013b). *TpsUtil*. Stony Brook, NY: State University of New York.

Schaefer, J., Duvernell, D., & Kreiser, B. (2011). Shape variability in topminnows (*Fundulus notatus* species complex) along the river continuum. *Biological Journal of the Linnean Society*, *103*, 612–621. https://doi.org/10.1111/j.1095-8312.2011.01660.x

Schueler, T. R., Fraley-McNeal, L., & Cappiella, K. (2009). Is impervious cover still important? Review of recent research. *Journal of Hydrologic*

Engineering, *14*, 309–315. https://doi.org/10.1061/(asce)1084-0699(2009)14:4(309).

Sfakianakis, D. G., Leris, I., Laggis, A., & Kentouri, M. (2011). The effect of rearing temperature on body shape and meristic characters in zebrafish (*Danio rerio*) juveniles. *Environmental Biology of Fishes*, *92*, 197. https://doi.org/10.1007/s10641-011-9833-z

Smith, T. B., Kinnison, M. T., Strauss, S. Y., Fuller, T. L., & Carroll, S. P. (2014). Prescriptive evolution to conserve and manage biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *45*, 1–22. https://doi.org/10.1146/annurev-ecolsys-120213-091747

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., ... Ahmed, N. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology & Evolution*, *1*, 0158. https://doi.org/10.1038/s41559-017-0158

Tófoli, R. M., Alves, G. H. Z., Higuti, J., Cunico, A. M., & Hahn, N. S. (2013). Diet and feeding selectivity of a benthivorous fish in streams: Responses to the effects of urbanization. *Journal of Fish Biology*, *83*, 39–51. https://doi.org/10.1111/jfb.12145

United Nations (2014). *World urbanization prospects: Highlights*. New York, NY: United Nations.

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & Morgan, R. P. (2005). The urban stream syndrome: Current knowledge and the search for a cure. *Journal of the North American Benthological Society*, *24*, 706–723. https://doi.org/10.1899/04-028.1

Washburn, G. N. (1948). Propagation of the creek chub in ponds with artificial raceways. *Transactions of the American Fisheries Society*, *75*, 336–350. https://doi.org/10.1577/1548-8659(1945)75[336:POTCCI]2.0.CO;2

Webb, P. W. (1982). Locomotor patterns in the evolution of actinopterygian fishes. *American Zoologist*, *22*, 329–342. https://doi.org/10.1093/icb/22.2.329

Webster, M. M., Atton, N., Hart, P. J. B., & Ward, A. J. W. (2011). Habitat-specific morphological variation among threespine sticklebacks (*Gasterosteus aculeatus*) within a drainage basin. *PLoS ONE*, *6*, e21060. https://doi.org/10.1371/journal.pone.0021060

Zúñiga-Vega, J. J., Reznick, D., & Johnson, J. (2007). Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. *Oikos*, *116*, 995–1005. https://doi.org/10.1111/j.0030-1299.2007.15763.x

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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