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Urbanization drives phenotypic evolution in mosquitofish

Zachary T. Wood[1], Caroline E. Booth[1], Rebecca R. Robinson[2], Rachel C. Van Gorden[1], Cowan J. Sikora[1], Mitchell R. Paisker[1], Eric P. Palkovacs[2] and Michael T. Kinnison[1]

[1]*School of Biology and Ecology, Ecology and Environmental Sciences Program, University of Maine, Orono, Maine, USA and* [2]*Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, California, USA*

ABSTRACT

Question: Urbanization can alter both the physical and biological dimensions of landscapes, potentially leading to novel environmental pressures, patterns of selection, and distinct phenotypes. Here we seek to understand how both physical and biological dimensions of urbanization interact to drive phenotypic evolution of mosquitofish morphology and behaviour against a backdrop of natural landscape drivers.

Study system: Western mosquitofish (*Gambusia affinis*) from 21 lakes of various sizes, eco-regions, introduced species regimes, and urban development levels in central California, USA.

Methods: We catch, measure, and observe the behaviour of wild mosquitofish. We examine the magnitude and direction of trait changes in relation to shoreline urban development and species introductions (predators and competitors). We also analyse trait variation that is associated with innate landscape attributes, including lake size and eco-region (desert or coast).

Results: Both physical and biological dimensions of urbanization – urban development and introductions of predators and competitors – lead to novel suites of mosquitofish traits associated with a niche shift towards sheltered fringe habitats. Effect sizes of urbanization on mosquitofish traits were comparable to those from innate landscape variables, suggesting that urbanization is a potent selective agent.

Keywords: *Gambusia affinis*, morphology, anthropogenic impacts, behaviour, environmental change, lake ecosystem alteration, introduced species.

INTRODUCTION

Background

Urbanization alters ecological communities in ways that are likely to cause contemporary evolution. But to what extent do urban environments select for truly novel phenotype

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Correspondence: Z.T. Wood, School of Biology and Ecology, Ecology and Environmental Sciences Program, University of Maine, Orono, ME 04469, USA. email: zachary.t.wood@maine.edu.
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combinations? And how important are human effects on trait variation of human-associated species compared to the effects of natural landscape features? In some ways, urban environments constitute comparable or more extreme conditions compared to those found elsewhere in nature (Paul and Meyer, 2001). But in other ways, they can be dramatically different. Urban environments are typically patchier (Wade *et al.*, 2003), more homogenized within patches (Jongman, 2002; McKinney, 2006), and less complex than their undeveloped counterparts (Zanette *et al.*, 2005). Simultaneously, urban environments often have less biodiversity but contain more non-native species (McKinney, 2008). As such, urbanization has the potential to act as a crucible for rapid and novel evolution, because isolated populations face fewer, stronger, and more consistent sets of selection pressures and therefore are more likely to exhibit contemporary evolution (Barton and Partridge, 2000; Urban and Skelly, 2006; Barraclough, 2015). Whether urbanization facilitates or stalls evolution is likely context-dependent, with strong selection occurring in taxa that are maladapted to humans, but with humans artificially increasing the fitness of some favoured taxa (Fugère and Hendry, 2018).

The eco-evolutionary impacts of urbanization are not limited to urban areas. Pollution – thermal (Råman Vinnå *et al.*, 2017), chemical (Arenas-Sánchez *et al.*, 2016), noise (Kunc *et al.*, 2016), and light (Gaston *et al.*, 2013) – can travel substantial distances to generate ecological impacts. Urban areas also serve as gateways for invasive species to enter nearby undeveloped environments (Aronson *et al.*, 2017; Cadotte *et al.*, 2017; Padayachee *et al.*, 2017). Human-adapted (i.e. domesticated) taxa can spread back into wild environments, leading to further ecological change (Jessup, 2004; Wood *et al.*, 2019).

The ecological and evolutionary changes associated with urbanization likely extend far beyond areas with very high human density and those that are heavily engineered. Such areas – with fewer, more diffuse effects of urbanization – are more likely to be overlooked, particularly when they occur in habitats or systems that are perceived as 'natural' or 'wild' places within urbanized settings, such as nature parks or ponds. But such places, with what we may term 'softer urbanization', might also influence evolution.

Lakes, in particular, are apt to be heavily impacted by urbanization due to their sensitivity to shoreline influences. While lakes do integrate watershed-wide (catchment-wide) processes (Schindler, 2009), disturbances at or near the shoreline have the greatest potential to influence lake ecology due to limited hydrological buffering space between the disturbance and the lake (Francis and Schindler, 2009; Larson *et al.*, 2011). Humans simultaneously disturb shoreline environments and reduce their hydrological buffering capacity (e.g. through impervious surface construction), creating profound ecological changes that could affect the evolution of local populations. For example, human-induced phosphorus loading into lakes can lead to cultural eutrophication, resulting in novel fish evolution that reinforces that eutrophication (Scheffer and van Nes, 2007; Tuckett *et al.*, 2013, 2017). So lakes can integrate, retain, and even amplify watershed impacts, and these impacts are likely to be evolutionarily relevant for aquatic organisms (Table 1). At the same time, lakes can be relatively confined and isolated habitats for many organisms like fish, leading to locally intense trophic and competitive interactions, especially when species are added or lost from these habitats (Estes *et al.*, 2011; Walsh *et al.*, 2016; Wood *et al.*, 2020). Not surprisingly, lake populations of many fishes often show considerable lake-to-lake phenotypic variation in traits associated with trophic interactions (Stockwell and Weeks, 1999).

Here we examine trait variation in western mosquitofish (*Gambusia affinis*) in central California, USA in response to two potentially interacting impacts of urbanization on lakes: shoreline urban development and introduced species.

Shoreline urban development

Urban development can cause significant hydrological change in watersheds, particularly through creation of impervious surfaces (Ceola *et al.*, 2014), which can increase runoff into streams and lakes. This increased runoff, which is typically higher in particulates and pollutants, generally results in a decline in water quality as well as a decline in fish and macroinvertebrate diversity (Paul and Meyer, 2001; Tixier *et al.*, 2011). The altered hydrology, chemistry, and clarity of urban waterbodies can select for differences in morphology, physiology, and behaviour in urban fish, especially those that enhance chemical tolerance and sensory abilities (Langerhans, 2008; Tuckett *et al.*, 2013; Kern and Langerhans, 2018). Note that by 'development' here we mean the human footprint, rather than shoreline complexity.

Introduced species

Urban lakes often receive non-native species because such species are frequently used by humans and become exposed to transport pathways (Dudgeon *et al.*, 2006; Strayer, 2010; Anderson *et al.*, 2015). Exotic species can be introduced unintentionally, including through ballast water, canals (Copp *et al.*, 2005), and escape from aquaculture facilities (Schröder and Garcia de Leaniz, 2011; Lin *et al.*, 2015). But many invaders are introduced intentionally. In the case of fishes, this is often for angling, including both the target game fish (Carpio *et al.*, 2019) and baitfish (Kilian *et al.*, 2012; Drake *et al.*, 2015). In addition, some species may be released from home aquaria, while others may be released at a large scale for biocontrol (Chandra *et al.*, 2008). Many non-native species are aggressive predators and competitors that are apt to select for detection, defence, avoidance, or life-history changes in other interacting species (Langerhans *et al.*, 2004; Rehage *et al.*, 2005; Moran and Alexander, 2014).

In this study, we link variation in morphology and behaviour across different landscape variables to two types of urbanization: physical shoreline development and biotic introduction of strong interacting predators and competitors. We compare the phenotypic effects of urbanization to those driven by two natural landscape variables: lake size and eco-region (coast or desert). This comparison is aided by the fact that all of the mosquitofish populations in our study were introduced, often with their predators and competitors, in the last century. We examine two sets of competing hypotheses:

1a: Change in mosquitofish traits in response to urbanization is analogous to existing patterns of natural trait variation because human activity leads to environmental and selective patterns that are analogous to natural systems.

1b: Mosquitofish trait change in response to urbanization generates novel suites of phenotypes, because human activity produces unique environmental conditions and regimes of selection (Wood *et al.*, 2019).

2a: Mosquitofish trait change in response to urbanization is greater than in response to other landscape factors due to stronger, more persistent, or simpler environmental pressures in urbanized places and so natural selection is stronger (Darimont *et al.*, 2009).

2b: Mosquitofish trait change in response to urbanization is less than in response to other factors, as the urban versus non-urban environmental dichotomy presents weaker environmental differences and selection or faces stronger constraints on adaptive variation (Fugère and Hendry, 2018).

As we were interested in the net phenotypic impact of urbanization, including genetic, plastic, epigenetic, and other gene-by-environmental effects, we used wild fish for this study.

Table 1. Urbanization generates ecological change in lakes and ponds, and this ecological change can lead to evolutionary change in aquatic organisms

Urbanization impact	Examples	Ecological change	Evolutionary effect of ecological change	References
Harvest	Commercial and recreational angling	Fewer fish, trophic cascade, regime change	Niche shifts	Persson *et al.* (2007), Audzijonyte *et al.* (2013), Wood *et al.* (2018)
Physical habitat change	Piers, canalization, dredging	Community change, niche overlap	Niche shifts, hybridization	Diehl (1988), Hasselman *et al.* (2014)
Invasive species introductions	Recreation, vector control, hitchhikers, escapees	Decreased biodiversity, trophic cascade, introgression	Anti-predator or anti-competitor evolution, niche shifts	Estes *et al.* (2011), Moran and Alexander (2014), Walsh *et al.* (2016)
Stocking	Recreation, conservation	Trophic cascade, regime change, introgression	Anti-predator or anti-competitor evolution, niche shifts, introgression	Hutchings (2014), Wood *et al.* (2019)
Pollution: acidification	Acid rain, CO_2-induced acidification	Fewer calcium-based organisms	Shell and carapace evolution, niche shifts	France (1987), Fischer *et al.* (2001), Shao *et al.* (2012), Hasler *et al.* (2018), Weiss *et al.* (2018)
Impoundment	Power, drinking water, recreation, flood control	Decreased oxygen, lower refreshing rate, increased turbidity, water level fluctuation	Life-history evolution, vision evolution, selection for oxygen tolerance, morphological change	Stearns (1983a, 1983b), Sturmbauer (1998), Sarch and Birkett (2000), Marchetti *et al.* (2001), Coops *et al.* (2003), Fischer and Öhl (2005), Haas *et al.* (2010), Chu *et al.* (2015)

Pollution: physical	Garbage, littering	Adjusted diets, starvation	Niche shifts	Crozier and Hutchings (2014)
Pollution: nitrogen and phosphorus	Agriculture runoff, soil erosion	Eutrophication	Niche shifts	Schindler (1974), Scheffer and van Nes (2007), Candolin (2009), Tuckett *et al.* (2013, 2017)
Pollution: heavy metals	Deposition	Bioaccumulation	Tolerance evolution	Maes *et al.* (2005), Huang *et al.* (2013)
Pollution: salt	Road salt runoff	Induction of meromixis	Niche shifts, salt tolerance evolution	Purcell *et al.* (2008), Dugan *et al.* (2017)
Pollution: thermal	Industrial runoff	Altered thermal regimes	Thermal tolerance evolution	Cherry *et al.* (1976), Meffe *et al.* (1995), Koschel *et al.* (2002), Sunday *et al.* (2012), Kirillin *et al.* (2013)
Pollution: particulate	Stormwater runoff	Increased turbidity, decreased oxygen	Vision evolution, selection for oxygen tolerance	De Robertis *et al.* (2003)
Climate alteration	Anthropogenic climate change	Hypolimnion warming, stronger stratification	Niche shifts, selection for hypoxia tolerance, thermal tolerance evolution	Beitinger and Fitzpatrick (1979), Stasio *et al.* (1996), Mohseni *et al.* (2003), Xenopoulos *et al.* (2005), Ohlberger *et al.* (2008)
Pollution: hormones	Drug by-product runoff	Altered fish sex ratios	Niche shifts, sex determination evolution	Arona and Kulshrestha (1984), Kime (1995), Yamaguchi *et al.* (2003)

METHODS

Data availability

All data collected, generated, and analysed in this work are available in supplementary files, which can be accessed at: evolutionary-ecology.com/data/3219Appendix.html.

Mosquitofish sources

We collected 889 mosquitofish from 21 lakes in central California (see Appendix Table S1) in summer 2015 (for morphology) and 2016 (for behaviour) using beach seines and hand netting (for full sample size details, including subsets used, see Table 2 and Appendix Table S-1). We classified these lakes based on lake surface area, eco-region (desert vs. coast), presence of introduced predators and competitors, and shoreline urban development. We calculated lake surface area (m^2) using the polygon tool in Google Earth. We classified lakes as belonging in the eastern desert (Inyo County) or western coastal (Santa Cruz County) regions of California. Lakes in the eastern desert eco-region are fed almost entirely by groundwater, and are generally oligotrophic and extremely clear, while lakes in the western coastal eco-region are fed by a mixture of runoff and groundwater, and are generally eutrophic and macrophyte-rich (Deacon and Minckley, 1974). These eco-regions thus may represent a strong landscape gradient in environmental conditions that shape both habitats and mosquitofish trait variation, and serve as a baseline to compare the magnitude of urbanization-linked trait variation. We determined the piscine predator and competitor regime in each lake using extensive summer seine netting and visual assessments. We classified lakes as having largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), both species, or neither. Largemouth bass prey on mosquitofish of all sizes, whereas bluegills consume smaller mosquitofish and act as exploitation competitors for invertebrate prey (Nowlin and Drenner, 2000; Langerhans *et al.*, 2004).

We used ArcGIS, ArcPro, and Geospatial Modeling Environment (GME) to calculate the proportions of urban development within a 500 foot (152.4 m) buffer of shoreline for the nine lakes in coastal California (Appendix Table S-1) for which data were available. All desert lakes were undeveloped, and so had no shoreline urbanization. We also used the National Land Cover Database 2011 (Homer *et al.*, 2011) to characterize land cover within the shoreline buffer. For the purposes of this study, we recognized four urbanization conditions: high development, medium development, low development, and open development. We used a standardized principal components analysis (PCA) to analyse these four conditions. The first principal component explained 76.1% of data variation, so we used it as our 'urbanization index' for subsequent analyses. For sample sizes and lake information, see Appendix Table S-1 and for PCA loadings, Appendix Table S-2.

The range of mosquitofish is nearly global because of their use in mosquito-borne disease control. So they exhibit contemporary evolution in response to many natural and anthropogenic factors (Hagen, 1964; Stearns, 1983a, 1983b; Meffe, 1991; Meffe *et al.*, 1995; Mulvey *et al.*, 1995; Langerhans *et al.*, 2004; Langerhans and Makowicz, 2009; Vera *et al.*, 2016; Wood *et al.*, 2019). Mosquitofish were introduced to California in the early twentieth century for mosquito control, and have since colonized numerous lakes in the region (Lenert, 1923). The dominant predators and competitors of mosquitofish in our study system – largemouth bass and bluegill – were also introduced in the twentieth century, and can have significant evolutionary effects on the morphology and behaviour of mosquitofish (Langerhans *et al.*, 2004; Wood *et al.*, 2019). In central California, bass and bluegill appear to be more common near urban areas (see below).

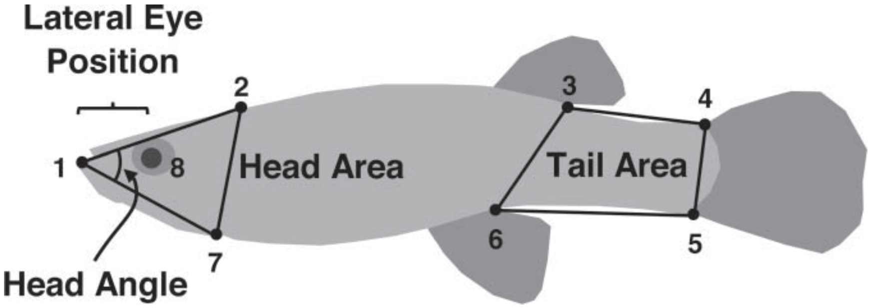

Fig. 1. Landmark positions and morphological traits measured in western mosquitofish.

Morphology

We obtained standardized lateral images of each mosquitofish immediately after euthanasia. We assigned eight landmarks to each fish (Figs. 1 and S-1), which we used to calculate four morphological traits:

1. Head area: the lateral area of the head.
2. Head angle: the angle formed by the top of the head and the bottom of the head.
3. Lateral eye position: the distance between the snout and eye.
4. Tail area: the lateral area of the caudal peduncle.

These traits have been linked to swimming performance, feeding ability, and feeding niche in mosquitofish (Langerhans *et al.*, 2004; Ruehl and DeWitt, 2005; Wood *et al.*, 2019). A subset of these measurements (lateral eye position, only, for 6 of 21 populations) is reported in Wood *et al.* (2019).

Exploratory behaviour

Exploratory behaviour can be defined as 'willingness to investigate novel environments' (Conrad *et al.*, 2011). We created an experimental arena consisting of a plastic tank (49 cm × 34 cm × 21.5 cm) with a cylindrical PVC refuge (20.5 cm tall, 11 cm diameter) secured inside the tank. We could remotely open a door in the PVC cylinder (refuge) by rotating the cylinder. We used cardboard around each arena to minimize exposure of the fish to outside movement and we observed fish through a small hole.

For each trial, we placed one female and one male fish inside the PVC refuge and allowed 5 minutes for acclimation. After acclimation, we opened the refuge door and recorded the time each fish remained in the refuge before leaving, with a maximum trial time of 10 minutes. For each population, we examined mosquitofish exploratory behaviour by measuring the time it took male–female pairs to leave a refuge. After each trial, we measured the total length (including caudal fin) of each fish. We calculated exploration latency for pairs of mosquitofish – a naturally schooling species (Arrington *et al.*, 2009) – as the average arcsine square-root transform of the time it took for each pair to leave the refuge. As with morphology, a subset of these data (6 of 16 populations) can be found in Wood *et al.* (2019), which also describes methods for this experiment in detail.

Analyses

Using generalized linear models (GLMs), we examined whether introduced fish species were more likely to occur in urban lakes. We correlated presence of introduced fish to shoreline urbanization using a binomial GLM:

$$I_B = \text{logit}^{-1}(\beta_0 + \beta_1 \times S) \tag{1}$$

where I_B = introduced fish presence / absence; U = shoreline urban development index; and β-terms are coefficients. We correlated the number of introduced fish species to shoreline urbanization using a Poisson GLM:

$$I_N = \exp(\beta_0 + \beta_1 \times S) \tag{2}$$

where I_N = number of introduced fish species; U = shoreline urban development index; and β-terms are coefficients.

We examined the relationship between mosquitofish traits and landscape and urbanization variables using general linear (mixed) models in R (Bates *et al.*, 2015; R Core Team, 2016). As not all lakes had associated shoreline urban development data, we used different models to analyse the effects of shoreline urban development and the three other environmental factors.

We fit the following models for mosquitofish morphology and behaviour from all lakes:

$$M = \beta_1 \times I + \beta_2 \times E + \beta_3 \times \ln(A) + \beta_4 \times \ln(L) + \beta_5 \times S + \beta_6 \times \ln(L) \times S + \sigma_{12} \times T \tag{3a}$$

$$B = \beta_1 \times I + \beta_2 \times E + \beta_3 \times \ln(A) + \beta_4 \times L_{♀} + \beta_5 \times L_{♂} \tag{3b}$$

where M = morphological trait value; B = pair mean exploration latency; I = introduced predators or competitors; E = eco-region (desert or coast); A = lake surface area; L = fish length; S = fish sex; T = morphology technician; and β- and σ-terms are fixed and random coefficients, respectively, which were determined during the model-fitting process (and take more than one value for factorial variables). All exploration latency trials were measured by the same technician. We used type II likelihood ratio tests to examine the significance of the I, E, and $\ln(A)$ terms.

We fit the following models for mosquitofish from lakes with additional shoreline urban development data:

$$M = \beta_0 \times U + \beta_1 \times I + \beta_2 \times E + \beta_3 \times \ln(A) + \beta_4 \times \ln(L) + \beta_5 \times S + \beta_6 \times \ln(L) \times S + \sigma_{12} \times T \tag{4a}$$

$$B = \beta_0 \times U + \beta_1 \times I + \beta_2 \times E + \beta_3 \times \ln(A) + \beta_4 \times L_{♀} + \beta_5 \times L_{♂} \tag{4b}$$

where U = shoreline urban development index; all other terms are the same as in (3a) and (3b). We used type II likelihood ratio tests to examine the significance of the U term.

To examine the relative strength of each landscape variable in determining mosquitofish phenotype, we calculated effect size as the partial R^2 for each variable of interest for each phenotype:

$$R^{2\text{p}} = 1 - SS_F / SS_R \tag{5}$$

where $R^{2\text{p}}$ = partial R^2; SS_F = full model sum of squares; SS_R = sum of squares for model with the variable of interest removed.

We used 1000 iterations of bootstrapping with sample size equal to the original datasets in order to generate the mean and standard error (i.e. standard deviation of the estimate) partial R^2 for each variable.

RESULTS

Measuring shoreline urbanization

We were able to assess shoreline urbanization for nine coastal lakes (out of 12). As there was essentially no variation in urbanization around our desert lakes, we did not attempt to assess shoreline urbanization for desert lakes. The first principal component of our shoreline urban development PCA explained 76.1% of the total variation in urban development, and had loadings of 0.51, 0.56, 0.54, and −0.37 for high, medium, low, and open (e.g. field) development, respectively. Both presence and number of introduced fish species were positively related to shoreline urbanization ($\chi^2 = 4.4$, $df = 1$, $P = 0.036$; $\chi^2 = 4.7$, $df = 1$, $P = 0.030$, respectively).

Morphology

Both effects of urbanization that we studied – shoreline urban development and introduced competitors and predators — resulted in similar morphological signals, i.e. smaller heads and caudal peduncles, shallower heads, and marginally more posterior eyes (Fig. 2, Table 2). Larger lakes and desert lakes were also associated with these morphological changes.

Exploratory behaviour

Both effects of urbanization – shoreline urban development and introduced species – resulted in less exploratory behaviour (higher exploration latency) by mosquitofish. However, unlike with morphology, larger lakes and desert lakes resulted in the opposite effect, i.e. fish with more exploratory behaviour (Fig. 3, Table 2).

Size of effects

Our two urbanization and two landscape variables had roughly similar effects (Fig. 4). Shoreline urban development had (marginally) the larger effect on head area and lateral eye position; eco-region had (marginally) the larger effect on caudal peduncle area and head angle. Interestingly,

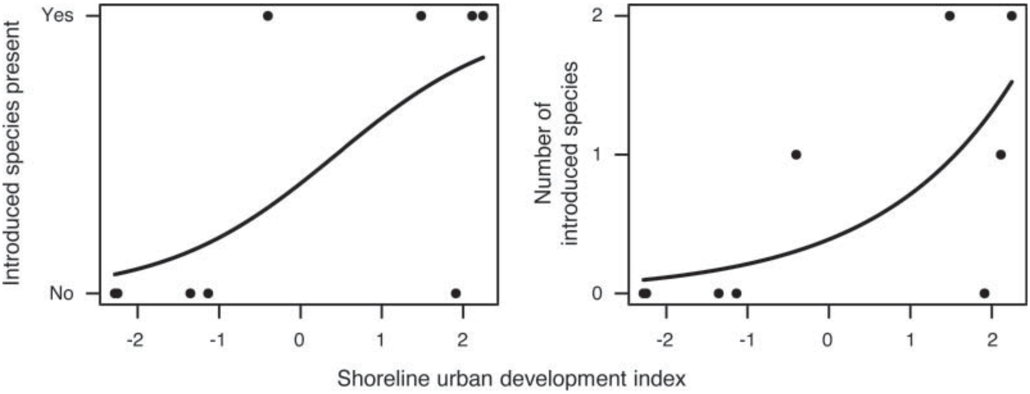

Fig. 2. Introduced fish species (bass and bluegill) are more common near urban environments. Lines show predictions from binomial (left) and Poisson (right) generalized linear models.

Table 2. General linear mixed model type II likelihood ratio tests for effects of landscape variables on mosquitofish traits

Trait	Desert/coast eco-region	Lake surface area	Introduced competitors and competitors	Shoreline urban development
Caudal peduncle area	$\chi^2 = 35.07$ $P < 0.001$	$\chi^2 = 21.92$ $P < 0.001$	$\chi^2 = 19.97$ $P < 0.001$	$\chi^2 = 9.21$ $P = 0.0024$
Head area	$\chi^2 = 11.85$ $P < 0.001$	$\chi^2 = 14.19$ $P < 0.001$	$\chi^2 = 16.74$ $P < 0.001$	$\chi^2 = 9.81$ $P = 0.0017$
Lateral eye position	$\chi^2 = 3.62$ $P = 0.06$	$\chi^2 = 6.60$ $P = 0.010$	$\chi^2 = 14.33$ $P = 0.0025$	$\chi^2 = 11.78$ $P < 0.001$
Head angle	$\chi^2 = 50.19$ $P < 0.001$	$\chi^2 = 0.36$ $P = 0.55$	$\chi^2 = 28.95$ $P < 0.001$	$\chi^2 = 8.39$ $P = 0.0038$
Exploration latency*	$\chi^2 = 28.83$ $P < 0.001$	$\chi^2 = 28.62$ $P < 0.001$	$\chi^2 = 14.15$ $P = 0.0027$	$\chi^2 = 8.05$ $P = 0.0045$
	$df = 1$ $N = 889$ (*307) $N_{pop} = 21$ (*16)	$df = 1$ $N = 889$ (*307) $N_{pop} = 21$ (*16)	$df = 3$ $N = 889$ (*307) $N_{pop} = 21$ (*16)	$df = 1$ $N = 345$ (*113) $N_{pop} = 9$ (*6)

Note: All morphological traits except head angle were ln-transformed for analysis; lake surface area was also ln-transformed. *Sample sizes (in pairs) for exploration latency experiments.

our urbanization and landscape variables explained somewhat more variation in exploratory behaviour than any morphological trait, with partial R^2 values ranging from 0.06 to 0.10 for exploration latency and from 0.00 to 0.04 for morphological traits.

DISCUSSION

We found that mosquitofish morphology and behaviour varied significantly with several landscape factors, including one conspicuous and one inconspicuous effect of urbanization (shoreline urban development and introduced predators and competitors, respectively). Furthermore, the effects of urbanization on mosquitofish traits were typically at least as great as those of other innate landscape variables.

Urbanization generates novel trait combinations

Our study demonstrates that urbanization can lead to novel combinations of mosquitofish traits. Interestingly, morphological responses to increased urbanization (both shoreline urban development and introduced species) tended to mimic morphological responses to larger lakes and desert lakes, while the opposite was true for behavioural responses. Thus, urbanization here leads to unique combinations of morphological and behavioural traits in mosquitofish, combinations not otherwise generated by natural landscape gradients. As such, no combination of natural landscape factors examined here could generate mosquitofish ‘pre-adapted’ to urbanization. Other work in this mosquitofish system has found that another human impact – adaptation to captive propagation facilities – also leads to novel suites of morphological and behavioural traits in mosquitofish (Wood *et al.*, 2019). These works reinforce the growing body of

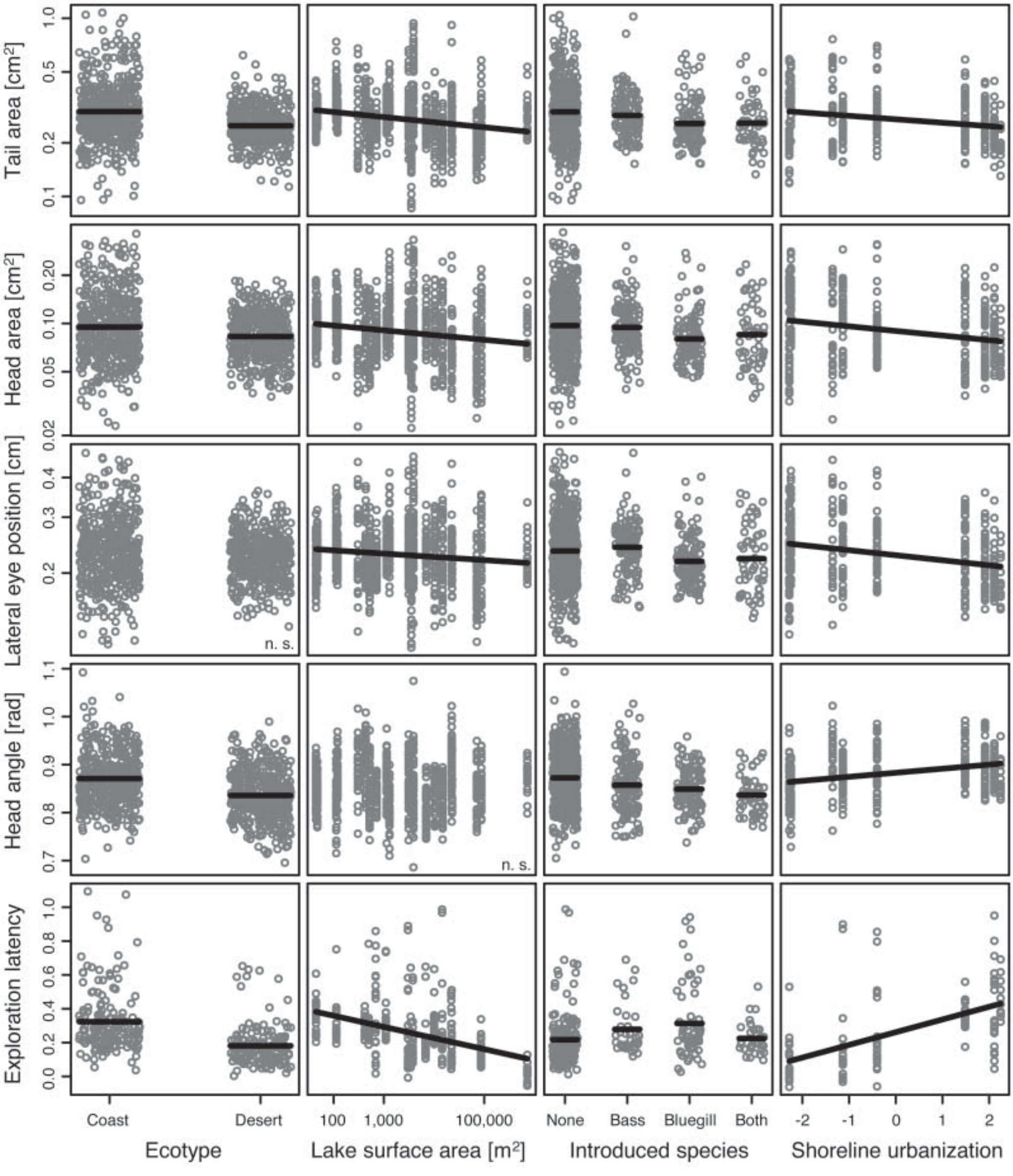

Fig. 3. Effects of landscape and urbanization variables on mosquitofish phenotypes. Black lines indicate statistically significant trends. Each column shows data with the effects of the other variables (columns) removed. For sample size and statistical significance, see Appendix Tables S-1 and S-2.

evidence that human activity pushes wild organisms into novel phenotypic trait space, which may generate new, even stronger ecological impacts (Palkovacs *et al.*, 2012; Tuckett *et al.*, 2017; Des Roches *et al.*, 2018).

Indeed, here urbanization appears to have shifted the ecological niche of mosquitofish. In this study, shoreline urban development led to smaller, more streamlined heads and caudal

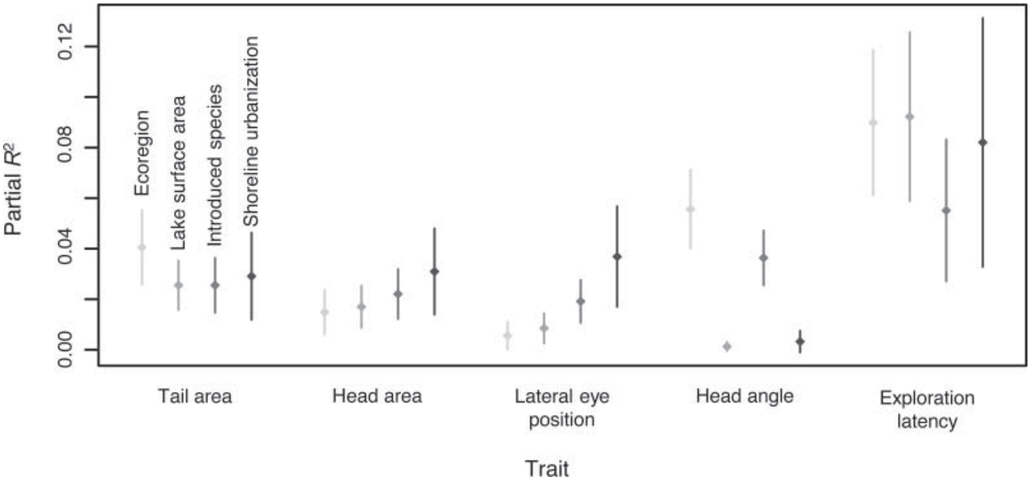

Fig. 4. Sizes of the effects of urbanization variables (introduced predators and competitors and shoreline urban development) are comparable to those of other landscape variables. Sizes here are bootstrapped partial R^2 of equations (3) and (4).

peduncles, blunter heads, more anterior eyes, and less exploratory behaviour (Fig. 2). Co-existence with predators and competitors – an inconspicuous effect of urbanization – led to a similar suite of traits, though not blunter heads. Taken together, these trait patterns suggest that both conspicuous and inconspicuous impacts of urbanization on lake ecosystems alter the functional niche of mosquitofish to favour conservative, fringe-feeding, rather than pelagic feeding. A shallower, streamlined body form in mosquitofish has been associated with efficient swimming (Langerhans, 2009) and fringe-feeding in macrophyte-rich environments (Ruehl and DeWitt, 2005), and is likely less metabolically expensive to maintain by virtue of its lower mass. Indeed, pelagic foraging in urban lakes is likely less profitable due to decreased visibility, yet riskier due to the presence of introduced predators (Table 1). This high-risk, low-reward scenario is also supported by our observations that mosquitofish from urban and high-predation lakes were less likely to explore out of cover (Fig. 3). Foraging in shallow fringes, away from predators, may also explain why our mosquitofish in high-predation environments showed traits that others have suggested may be maladaptive around predators, namely smaller caudal peduncles (reduced burst speed) and anterior eyes (predator detection) (Langerhans *et al.*, 2004; Langerhans, 2009). Avoiding predators altogether, as mosquitofish are known to do (Goodyear, 1973), by remaining in the weedy fringe may thus relax selection for anti-predator traits and allow for opposing selection for small caudal peduncles and anterior eyes, which likely aid in manoeuvrability and prey capture in structurally complex (weedy) fringe environments (Bianco *et al.*, 2011).

Because we surveyed wild individuals, phenotypic divergence observed in our mosquitofish was likely a combination of genetic and plastic effects, including potential epigenetic effects and gene-by-environment interactions. Based on other work demonstrating a heritable basis for the traits we studied (Langerhans and Makowicz, 2009), we suspect that genetic differentiation (including genetic evolution of plastic capacity) plays a significant role in the phenotypic trends we observed in this study. Future work should investigate whether plastic or genetic responses dominate adaptation to urbanization, as plastic responses may provide a more immediate (but less complete) response to rapid urban development (Price *et al.*, 2003).

Evolutionary effects of urbanization are as strong as other landscape factors

For the majority of our phenotypic variables, the effects of urbanization (introduced predators and competitors and shoreline urban development) were roughly as strong as other landscape variables (lake surface area and coast vs. desert). This trend suggests that humans are prominent drivers of phenotypic variation through conspicuous effects of their local changes to urbanizing environments and inconspicuous introductions of species to markedly different habitats. This pattern also suggests that anthropogenic rearranging of ecosystems fundamentally alters selection pressures on constituent populations sufficiently to overcome background stabilizing or directional selection tied to major landscape features.

Successful adaptation to urbanization is not guaranteed, and differences in adaptive capacity will likely dictate which taxa are able to persist in novel anthropogenic environments. For example, sufficient population size and standing genetic variation may be prerequisites for *in situ* genetic adaptation to some forms of urbanization. Populations that have already endured strong selection and genetic bottlenecks (i.e. at-risk taxa) may have poor prospects to adapt to a very different (i.e. urbanized) environment (Carlson *et al.*, 2014). Likewise, some taxa may have life histories that quickly offset these demographic and genetic limitations of low population size and low standing genetic variation. Western mosquitofish have generation times of only a few months and can form large populations very quickly in habitats that are marginal for many other species (Pyke, 2005), thus reducing their susceptibility to demographic stochasticity while increasing their ability to regenerate new genetic variation. Not surprisingly, they are therefore known for their ability to adapt to new and extreme environments (Cherry *et al.*, 1976; Stearns, 1983a, 1983b; Meffe *et al.*, 1995; Mulvey *et al.*, 1995; Vera *et al.*, 2016; Moffett *et al.*, 2018). Moreover, their adaptations strongly, reciprocally shape their environments in ways that can further influence their demography and fitness. For example, adaptation to bass predators in mosquitofish strongly reduces bass performance, further aiding mosquitofish persistence (Fryxell *et al.*, 2019; Wood *et al.*, 2020). As such, mosquitofish likely exist at the high end of a spectrum of both tolerance and eco-evolutionary potential for adaptation to urbanization, whereas many native species are not so capable.

We suggest that an eco-evolutionary approach to urban ecosystem management – one that focuses on adaptive capacity as a contribution to ecological resistance and resilience – will increase the success of management efforts. Furthermore, the definition of urban evolution should be expanded to encompass any evolutionary change caused by urban development, not merely evolution of organisms residing within urban areas. While such an expansion would mean that evolution in nearly any environment could potentially be attributed to urbanization, this new definition would more accurately represent the global eco-evolutionary footprint of urban development.

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