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Natural and anthropogenic sources of habitat variation influence exploration behaviour, stress response, and brain morphology in a coastal fish

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Abstract

1. Evolutionary ecology aims to better understand how ecologically important traits respond to environmental heterogeneity. Environments vary both naturally and as a result of human activities, and investigations that simultaneously consider how natural and human-induced environmental variation affect diverse trait types grow increasingly important as human activities drive species endangerment.

2. Here, we examined how habitat fragmentation and structural habitat complexity affect disparate trait types in Bahamas mosquitofish *Gambusia hubbsi* inhabiting tidal creeks. We tested a priori predictions for how these factors might influence exploratory behaviour, stress reactivity and brain anatomy.

3. We examined approximately 350 adult Bahamas mosquitofish from seven tidal-creek populations across Andros Island, The Bahamas that varied in both human-caused fragmentation (three fragmented and four unfragmented) and natural habitat complexity (e.g. fivefold variation in rock habitat).

4. Populations that had experienced severe human-induced fragmentation, and thus restriction of tidal exchange from the ocean, exhibited greater exploration of a novel environment, stronger physiological stress responses to a mildly stressful event and smaller telencephala (relative to body size). These changes matched adaptive predictions based mostly on (a) reduced chronic predation risk and (b) decreased demands for navigating tidally dynamic habitats. Populations from sites with greater structural habitat complexity showed a higher propensity for exploration and a relatively larger optic tectum and cerebellum. These patterns matched adaptive predictions related to increased demands for navigating complex environments.

5. Our findings demonstrate environmental variation, including recent anthropogenic impacts (<50 years), can significantly affect complex, ecologically important traits. Yet trait-specific patterns may not be easily predicted, as we found strong support for only six of 12 predictions. Our results further highlight the utility of simultaneously quantifying multiple environmental factors—for example had we failed to account for habitat complexity, we would not have detected the effects of fragmentation on exploratory behaviours. These responses, and

their ecological consequences, may be complex: rapid and adaptive phenotypic responses to anthropogenic impacts can facilitate persistence in human-altered environments, but may come at a cost of population vulnerability if ecological restoration was to occur without consideration of the altered traits.

1 | INTRODUCTION

1.1 | Background

Environmental variation, whether natural or anthropogenic, can drive rapid phenotypic variation, even among populations in close geographic proximity (Hendry et al., 2008; Palumbi, 2001; Wellborn & Langerhans, 2015). Structural habitat complexity, the three-dimensional diversity of the space an organism inhabits, represents a key environmental factor known to drive major evolutionary patterns of trait change. Variation in structural habitat—ranging from simple, open environments to spatially complex ones like dense vegetation and coral reefs—has driven phenotypic divergence in numerous organisms, including classic adaptive radiations in *Anolis* lizards and cichlid fishes (Langerhans & Reznick, 2010; Losos, 2009; Streelman & Danley, 2003). However, contemporary wild populations must cope with widespread impacts of human-induced environmental change in addition to natural variation.

Habitat fragmentation represents a pervasive anthropogenic impact on species' ecology (Fahrig, 2003; Fischer & Lindenmayer, 2007; Foley et al., 2005). Most work on fragmentation has centred on the effects of smaller population sizes and reduced genetic diversity and gene flow (Blanchet et al., 2010; Ewers & Didham, 2006; Fahrig, 2003), but fragmented environments often exhibit modified ecological conditions that alter natural regimes of selection, such as changes in temperature, water flow, predation and resource competition (Araujo et al., 2014; Giery et al., 2015; Haas et al., 2010; Langerhans & Kern, 2020; Riesch et al., 2015; Waples et al., 2007). Although the phenotypic impacts of habitat complexity and fragmentation are well-recognized, studies rarely address them simultaneously. Yet natural environmental gradients could obscure human impacts on organismal phenotypes (Derryberry et al., 2016). Because trait variation can have important ecological consequences (Hendry, 2017; Ohgushi et al., 2013), a better understanding of phenotype–environment associations will facilitate the integration of evolutionary principles within conservation activities (Carroll et al., 2014; Dawson et al., 2011; Smith et al., 2014).

Less studied in this context are changes in traits associated with how animals perceive and manage their environment, such as complex behaviours (e.g. exploration, learning), acute stress responses (e.g. fight or flight responses) and brain anatomy (e.g. size of the brain and specific brain regions). Yet, because of their obvious links to individual fitness, these phenotypes can have substantial ecological and evolutionary consequences (Brown et al., 2005; Heinen-Kay et al., 2016; Kondoh, 2010; Kotrschal, Buechel, et al., 2015).

Here we investigate how natural variation in structural habitat complexity and human-induced fragmentation, resulting from reduced hydrologic connectivity, influences the expression of exploratory behaviour, stress responses and brain anatomy in a coastal fish. Bahamas mosquitofish *Gambusia hubbsi*, a small livebearing fish (family Poeciliidae), inhabit tidal creeks that vary markedly in abiotic and biotic factors (Giery et al., 2015; Heinen-Kay et al., 2014; Layman et al., 2004; Valentine-Rose et al., 2007). Tidal creeks range widely in structural habitat complexity—from simple habitats, dominated by mud bottoms, to highly complex, rock-dominated systems—which can impact the traits required to adaptively navigate these contrasting environments to find food, interact with conspecifics and avoid predators. Further, human-induced fragmentation of tidal creeks is widespread, with the restriction of hydrologic connectivity causing major changes in tidal dynamics and predation regime. In this study, we generate and test a set of a priori predictions of phenotypic change in response to these environmental agents.

1.2 | Study system and predictions

Bahamian tidal creeks are shallow, tidally influenced systems typically characterized by a relatively narrow creek mouth at the ocean that broadens landward. Bahamas mosquitofish are most abundant along the shorelines, which are generally mud-bottomed with varying amounts of rock and red mangrove *Rhizophora mangle*. Water flux arises from tidal exchange (freshwater input only provided via rainfall and aquifer percolation), so salinities in unfragmented systems are typically around 30–35 ppt and the biotic communities comprise marine taxa (Araujo et al., 2014; Layman et al., 2004; Valentine-Rose et al., 2007).

The fragmentation of Bahamian tidal creeks—the process by which connectivity with the ocean is restricted or cut off entirely—is principally caused by road construction, and results in strong and persistent ecological change. Tidal-creek fragmentation on Andros Island mostly occurred during the 1960s and 1970s dramatically reduced tidal dynamics and altered the predation and competition

regime for Bahamas mosquitofish (see Supporting Information). Fragmentation has resulted in a ~75%–100% reduction of tidal amplitude, much higher densities of Bahamas mosquitofish and much lower densities of their primary predators (Araujo et al., 2014; Layman et al., 2004; Riesch et al., 2015). However, fragmentation has not led to consistent changes in other potentially relevant environmental parameters (e.g. salinity, dissolved oxygen; see Supporting Information).

We generated simple a priori predictions of phenotypic differentiation for six traits across tidal-creek environments varying in fragmentation status and degree of structural habitat complexity (Table 1). All predictions derived from the hypotheses of divergent natural selection on the traits across varying environments were supported by prior work, and assumed trait independence, ample genetic variance and a relatively closed system for simplicity (see Supporting Information for prediction details). Briefly, we predicted greater exploratory behaviour in (a) fragmented tidal creeks, owing to foraging advantages of exploration under high intraspecific resource competition, sexual advantages of exploratory behaviours for males and selection against exploratory behaviours in environments with abundant predators; and (b) sites with greater structural complexity, where behavioural exploration can have positive impacts on both food acquisition and finding refuge from antagonists (Table 1; Supporting Information).

TABLE 1 Predictions of phenotypic differentiation among populations of Bahamas mosquitofish inhabiting tidal creeks varying in human-induced fragmentation (Frag: predicted directions given for fragmented sites) and natural variation in structural habitat complexity (Complex: predicted directions given for sites with higher complexity; see Supporting Information for further details); results from this study are summarized with squares (results matched predictions) and circles (results opposite to predictions) drawn with solid (significant support) or dashed (suggestive support) lines

Trait	Frag	Complex	Reasoning
1. Exploration of a novel environment	↑ (square)	↑ (square)	Fragmentation: reduced predation and increased intraspecific competition[1–3] Complex habitat: increased demands and advantages for exploring unfamiliar regions for resource acquisition and avoidance of predators and competitors[4–6]
2. Stress response	↑ (square)	↓ (dashed circle)	Fragmentation: reduction of chronic stress caused by predation risk and temporal environmental dynamics via tidal exchange[7–10] Complex habitat: regular exposure to heterogeneous habitat[7]
3. Telencephalon	↓ (square)	↑	Fragmentation: reduced demands for emotional and spatial memory and learning with reduced predation risk and reduced tidal dynamics[11–14] Complex habitat: increased demands for spatial memory and learning[15–20]
4. Optic tectum	↓ (dashed square)	↑ (square)	Fragmentation: reduced demands for complex responses to visual stimuli with reduced predation risk[13–14,21] Complex habitat: elevated demands for responding to visual stimuli[13–14,21]
5. Cerebellum	↓	↑ (square)	Fragmentation: reduced demands for fear memory and motor skills and associative learning with reduced predation risk and reduced biotic diversity[13–14,22] Complex habitat: increased demands for motor skills and associative learning[15–16,18–19,22]
6. Total brain size	↓ (dashed square)	↑	Fragmentation: reduced demands for cognitive processing in less dynamic environment with lower predation risk[14,23] Complex habitat: overall greater demands for cognitive processing[15–17,19,24–25]

Note: Araujo et al. (2014)[1]; Heinen-Kay et al. (2016)[2]; Lapiedra et al. (2018)[3]; Mettke-Hofmann et al. (2002)[4]; Mikheev et al. (2010)[5]; Ingley, Billman, et al. (2014)[6]; Wingfield (2003)[7]; Brown et al. (2005)[8]; Archard et al. (2012)[9]; Fischer et al. (2014)[10]; Striedter (2005)[11]; Burns and Rodd (2008)[12]; Broglio et al. (2011)[13]; Kotrschal et al. (2017)[14]; Pollen et al. (2007)[15]; Yopak et al. (2007)[16]; Lisney et al. (2008)[17]; Gonda et al. (2009)[18]; Shumway (2010)[19]; White and Brown (2015)[20]; Huber and Rylander (1992)[21]; Heap et al. (2013)[22]; Reddon et al. (2018)[23]; Huber et al. (1997)[24]; Safi et al. (2005)[25]

Because of the negative effects of repeated or prolonged stress (e.g. Clinchy et al., 2004; Romero, 2004), we predicted that fish being routinely exposed to environmental stressors—such as high levels of predation threat and temporal variability due to greater tidal amplitudes in unfragmented sites, or the environmental heterogeneity of structurally complex environments—would exhibit attenuated physiological responses to mild stressors (Table 1; Supporting Information). We predicted fish should have relatively larger brains, as well as larger brain regions associated with emotional learning, escape responses, and spatial memory, decision-making, and learning (telencephalon), responding to visual stimuli and egocentric orientation (optic tectum), and fear memory, motor control and learning (cerebellum; Broglio et al., 2011; Bshary et al., 2002; Burns & Rodd, 2008; Kotrschal et al., 1998; Striedter, 2005), in (a) sites with greater structural habitat complexity and (b) unfragmented tidal creeks. This is because selection should more strongly favor these cognitive abilities in (a) more spatially complex environments and (b) sites with greater predation risk, higher temporal environmental variation due to tidal dynamics, and higher biotic diversity (Table 1; Supporting Information).

2 | MATERIALS AND METHODS

2.1 | Subject collection

We examined a total of 356 adult Bahamas mosquitofish collected from seven tidal creeks across Andros Island (Figure 1; Table S1). We selected these tidal creeks based on fragmentation status (three severely fragmented sites and four unfragmented sites), variation in structural habitat complexity, geographic location (spanning the extent of the northern island of Andros while not confounding geography with fragmentation status) and prior work demonstrating consistent environmental conditions within creeks over more than 15 years of personal observations (e.g. Riesch et al., 2015; see Supporting Information). Other measured environmental factors, that is, salinity, turbidity, pH and dissolved oxygen, either do not consistently differ among these seven sites (pH, dissolved oxygen) or do not covary with fragmentation or structural habitat complexity (see Supporting Information). During 26 May–12 June 2017, fish were collected using dip nets, minnow traps and seine in a manner designed to minimize the potential bias of behavioural phenotypes (see Supporting Information), and phenotyped on site as described
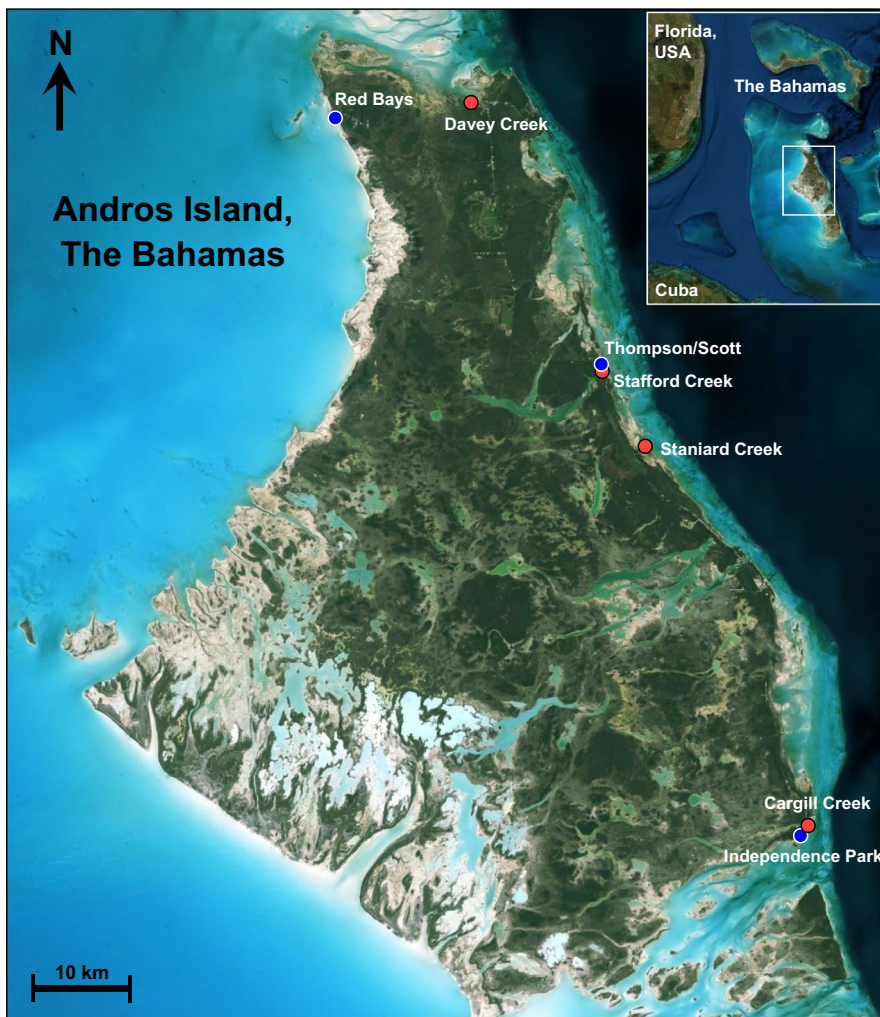

FIGURE 1 Map of study sites on Andros Island, The Bahamas. Red (light grey, black border) circles: unfragmented tidal creeks; blue (dark grey, white border) circles: fragmented tidal creeks. Maps created with National Geographic MapMaker Interactive and Zoom Earth

below. After phenotyping, specimens were euthanized with quinaldine and preserved in 95% ethanol.

2.2 | Structural habitat complexity

We estimated structural habitat complexity at each site using a randomized quadrat method. We randomly selected twenty 1-m² replicate quadrats per site within the region where Bahamas mosquitofish were observed and collected, with the condition that quadrats could not be within 1 m of each other. We recorded the presence/absence of four common habitat types within each quadrat—mud substrate, rock, mangrove and woody debris. Rock, complex limestone and mangrove habitats are highly structurally complex, whereas mud substrate is very simple and woody debris is intermediate (and less common). We calculated the average frequency of each habitat type in each site (proportion of quadrats where the respective habitat type was present), and used three variables to estimate habitat complexity in our analyses (see Supporting Information)—frequency of rock habitat, frequency of mangrove habitat and habitat diversity (Shannon's Diversity Index, which incorporates the relative frequencies of all four habitat types; Shannon, 1948). Our study sites spanned a wide range of structural complexity, from relatively simple to very complex (Table S1).

2.3 | Exploration behaviour

Following capture, fish were individually assayed for exploration using an established novel tank assay (e.g. Cachat et al., 2010; Wong et al., 2012). This protocol is explicitly designed to measure the propensity of individuals to explore novel environments, effectively capturing behaviours characterizing stress-coping styles and anxiety-like behaviours in small fish (Cachat et al., 2010; Egan et al., 2009; Heinen-Kay et al., 2016). Prior work in Bahamas mosquitofish has employed this procedure, using the same tanks and methods to document divergence in exploratory behaviour between predation regimes, repeatability and heritability of exploratory behaviours (Heinen-Kay et al., 2016). The experimental arena was a 2.5-L polycarbonate tank (22 × 9.5 × 12 cm, L × W × H) filled with water from the relevant site (rinsed and replaced between trials). Three sides of the tank were lined with opaque white plastic, and the entire arena was inside a portable photo studio light tent (80 × 80 cm) to reduce environmental disturbance and minimize glare and reflections. Each trial started when one fish was gently transferred to the experimental arena followed by 5 min of video recording using a Canon Vixia HF-R52 HD digital camcorder mounted on a tripod and positioned to record the side view of the tank. Experiments were conducted in close proximity to capture sites and we strived to minimize the time elapsed between fish capture and assay (the vast majority of fish were assayed within 10 min of capture, and holding time had no detectable effects on the exploratory behaviours measured; see Supporting Information). The start time of

all trials was recorded for inclusion as a possible covariate in analyses described below, and all trials occurred between 9:52 and 17:25, with substantial overlap among populations (Table S2). We also estimated hourly air temperature for inclusion as a possible covariate (see Supporting Information).

From the videos, we used automated video analysis software (EthoVision® XT 11.5, Noldus Information Technology) to track each fish and extract behavioural data. Based on inter-trait correlations among the 10 measured behaviours, we reduced the number of traits examined to four—total distance moved, top-half duration, mean meander and area covered (see Supporting Information). Greater distance travelled, more time spent close to the surface, a more meandering swimming pattern characterized by a greater turning angle per distance travelled and more area of the novel tank explored were considered more exploratory behaviours (Cachat et al., 2010; De Winter et al., 2016; Egan et al., 2009; Heinen-Kay et al., 2016; Kotrschal et al., 2014).

2.4 | Stress response

We characterized individual stress reactivity by quantifying the ventilation rate (opercular beat frequency) in response to a mild stressor (confinement). In fishes, confinement generally induces a ventilation response, which provides a sensitive and effective measure of the physiological response to stress (e.g. Bell et al., 2010; Brown et al., 2005). Immediately following the exploration assay, we transferred each individual to a polypropylene beaker (diameter: 9-cm) filled with 100 ml of water from the collection site (always rinsed and replaced between trials), and recorded 1 min of video from above using a tripod-mounted DSLR camera (Canon Rebel T3i or EOS 70D). Using VLC version 2.2.6, we magnified the opercular region of the fish in the videos to facilitate analysis, and recorded the time taken for 50 opercular beats to occur. With these data, we then calculated opercular beats per minute as our measure of ventilation rate.
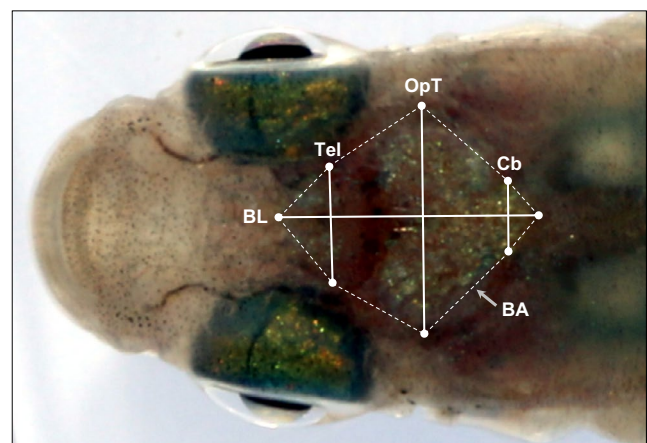

FIGURE 2 Dorsal-view brain measurements: BL, total brain length; Tel, telencephalon width; OpT, optic tectum width; Cb, cerebellum width, BA, brain area (area enclosed by the eight landmarks, depicted with dashed line)

2.5 | Brain morphology and body size

After completing the stress-response trial, we captured a dorsal-view photograph of each individual using the aforementioned tripod-mounted cameras. A small ruler was included in each image for scale. We used tpsDig2 (Rohlf, 2017) to digitize landmarks for measurements of the body, brain and brain region sizes. We measured standard length (SL) as our estimate of body size, defined as the length between the tip of the snout and the posterior end of the vertebrae. We employed a recently described method for non-invasive brain measurements in small fish with semi-transparent heads using digital photography (Näslund, 2014). We digitized eight landmarks on dorsal-view photographs of the brain to derive five measurements of brain morphology—telencephalon width, optic tectum width, cerebellum width, total brain length and brain area (Figure 2). We did not incorporate the olfactory bulb, hypothalamus or medulla oblongata, as these regions were not (fully) visible.

2.6 | Statistical analysis

We $\log_{10}$-transformed SL, mean meander, ventilation rate and brain measurements to meet normality assumptions; the remaining exploratory behaviours did not require transformation. Body size greatly overlapped among all populations for each sex (Table S1; Figure S2) and small differences in average body size among populations did not appear to influence any of our results (see Supporting Information). To obtain average trait values independent of body size for each sex within each population, we calculated population means for all traits for each sex, statistically controlling for any effects of body size using general linear models. To do so, we separately examined all 10 trait variables as dependent variables, and included $\log_{10}$-transformed SL as a covariate, and sex, population and the interaction between sex and population as fixed effects. For the estimates of exploration behaviours and ventilation rate, we additionally included the time of day and hourly air temperature as covariates. Population differences were evident in all cases (all $p < 0.01$), while effects of body size were evident in all cases except for exploration behaviours. Time of assay was positively associated with the distance moved during exploration trials ($p = 0.0004$), the area covered ($p = 0.0007$) and ventilation rate ($p = 0.0374$), but not the top-half duration ($p = 0.30$) or meander ($p = 0.12$). Hourly air temperature was negatively associated with the top-half duration ($p = 0.0004$), but had no apparent influence on any other trait (all $p > 0.16$). We found that the heterogeneity of slopes (i.e. interactions between body size and sex or population) had minimal influence on the estimation of relevant least-squares means (see Supporting Information for allometric details), and thus only present results assuming the homogeneity of slopes (i.e. similar allometries among populations and sexes) where trait means were estimated at a common body size for all populations.

Using these size-independent population means, we tested our hypotheses using model selection and multimodel inference. We employed this information theoretic approach because it is well-suited to the goals of the study (e.g. see Table 1); it simultaneously examines competing hypotheses to identify the best set of hypotheses that explain the data using information criteria, and quantitatively measures the relative support for the competing hypotheses (e.g. Burnham et al., 2011; Grueber et al., 2011). We constructed general linear models where our dependent variables were the 10 trait variables (four exploratory behaviours, ventilation rate and five brain measurements), and the possible independent variables were fragmentation regime (fragmented vs. unfragmented), rock habitat frequency, mangrove habitat frequency, habitat diversity, sex and interactions between sex and the other terms. We used Akaike's information criterion corrected for small samples sizes (AIC_c; Burnham et al., 2011) to select final models, and conducted model averaging using the subset of models with $\Delta AIC_c \leq 2.0$ (see Supporting Information, Table S5). Model averaging was not performed for four traits because no alternative models exhibited a $\Delta AIC_c < 2$ in these cases (total distance moved, duration in top half, telencephalon width, cerebellum width; see Results). For the six other traits, the number of models used in model averaging ranged between two and four (see Supporting Information). We assessed support for competing hypotheses in two ways: (a) we inspected the standard statistical support of model terms in the top model(s) for each trait, and (b) evaluated the statistical support for model-averaged coefficients deviating from zero. All p-values were adjusted to control for a study-wide false discovery rate of 5% (Benjamini & Hochberg, 1995). See Supporting Information for further details.

3 | RESULTS

3.1 | Model selection overview

Model selection and model averaging revealed a variety of effects of hydrologic connectivity and structural habitat complexity on exploration behaviour, ventilation rate and brain morphology (Tables 2 and 3; Table S5). With respect to our a priori predictions, evidence matched the predictions for six of the 12 predictions, with suggestive evidence for another two predictions (Table 1). Meanwhile, we found suggestive evidence opposite to one prediction (Table 1). Effect sizes presented as per cent differences are provided in Table S6.

3.2 | Exploration behaviour

We found greater distance moved and duration spent in the top half of the novel tank in populations from tidal creeks with greater occurrence of rock habitat (Tables 2 and 3; Figure 3a,b). We observed relatively weak evidence for a similar pattern with average meander, and slightly stronger, but suggestive, evidence for a similar pattern with the area covered during exploration trials (Tables 2 and 3; Figure 3c,d). We also found greater use of the top half of the tank

TABLE 2 Statistical results of the top model selected for each trait based on AIC_c. We additionally present the second-best model results for cases with $\Delta AIC_c < 1$, where the model does not represent a nested form of the top model (see Supporting Information for details of all models). All p-values adjusted for a false discovery rate of 5%

Category	Trait	Fragmentation	Rock habitat	Mangrove habitat	Habitat diversity	Sex	Sex × fragmentation
Exploration behaviour	Total distance moved	$F_{1,9} = 9.23$	$F_{1,9} = 138.42$			$F_{1,9} = 14.40$	$F_{1,9} = 19.91$
		$p = 0.0321$	$p < 0.0001$			$p = 0.0160$	$p = 0.0082$
	Duration in top half	$F_{1,10} = 10.47$	$F_{1,10} = 60.79$	$F_{1,10} = 47.46$			
		$p = 0.0261$	$p < 0.0001$	$p < 0.0001$			
	Average meander		$F_{1,12} = 3.32$				
			$p = 0.1037$				
	Area covered	$F_{1,10} = 7.04$	$F_{1,10} = 5.35$	$F_{1,10} = 7.90$			
		$p = 0.0451$	$p = 0.0594$	$p = 0.0379$			
Stress response	Ventilation rate	$F_{1,11} = 8.11$	$F_{1,11} = 4.97$				
		$p = 0.0343$	$p = 0.0594$				
Brain morphology	Telencephalon width	$F_{1,11} = 9.40$				$F_{1,11} = 10.04$	
		$p = 0.0292$				$p = 0.0261$	
	Optic Tectum width		$F_{1,12} = 5.98$				
			$p = 0.0528$				
		$F_{1,12} = 4.85$					
		$p = 0.0594$					
	Cerebellum width				$F_{1,11} = 12.10$	$F_{1,11} = 35.37$	
					$p = 0.0178$	$p < 0.0001$	
	Dorsal brain length					$F_{1,12} = 12.83$	
						$p = 0.0156$	
				$F_{1,10} = 5.29$	$F_{1,10} = 7.08$	$F_{1,10} = 19.89$	
				$p = 0.0594$	$p = 0.0451$	$p = 0.0077$	
	Dorsal brain area	$F_{1,11} = 4.73$				$F_{1,11} = 14.47$	
		$p = 0.0618$				$p = 0.0132$	

and less area covered in populations having greater occurrence of mangrove habitat. This latter finding regarding the area covered seemed to largely depend on a single study site, Thompson/Scott. Exclusion of that site removed any evidence of this particular effect, and thus we interpret this effect with caution. These findings generally matched our a priori prediction of increased exploration behaviour in environments with greater structural habitat complexity.

For males only, we found greater distance moved in the novel tank in fragmented tidal creeks compared to sites without restriction of hydrological connectivity (Tables 2 and 3 interaction term; Figure 3a). Both sexes tended to spend more time in the top half of the novel tank and cover less area in fragmented sites (Figure 3b,d). The effect of fragmentation on top-half duration was only apparent after statistically adjusting for an effect of mangrove habitat, while fragmentation's effect on area covered seemed to once again largely depend on the single site of Thompson/Scott. Fish from that

particular fragmented site—which had an especially high frequency of mangrove habitat—primarily used the top half of the tank, which consequently resulted in a relatively small area covered. We again interpret these results with caution. Overall, results are consistent, but partially sex specific, with our a priori prediction of increased exploration behaviour in fragmented tidal creeks.

3.3 | Stress response

Bahamas mosquitofish tended to exhibit a higher ventilation rate in response to a mildly stressful event in fragmented tidal creeks (Tables 2 and 3), matching our a priori prediction. Although, visual inspection of results revealed that one fragmented tidal creek (Red Bays) did not exhibit this shift (Figure 4a). We also observed a trend for increased ventilation rate in sites having greater occurrence of

TABLE 3 Standardized model coefficients, standard errors and p-values based on model averaging across subsets of models with $\Delta AIC_c < 2$ for each trait. *denotes cases where model averaging was not performed because only a single best model was identified (coefficients from the top model presented). All p-values adjusted for a false discovery rate of 5%

Category	Trait	Fragmentation	Rock habitat	Mangrove habitat	Habitat diversity	Sex	Sex × fragmentation
Exploration behaviour	Total Distance Moved*	0.27 ± 0.09	1.03 ± 0.09			0.27 ± 0.08	0.32 ± 0.06
		0.0321	<0.0001			0.0160	0.0082
	Duration in top half*	0.41 ± 0.14	0.99 ± 0.13	0.71 ± 0.10			
		0.0261	<0.0001	<0.0001			
	Average meander		0.47 ± 0.25			0.34 ± 0.26	
			0.1067			0.2417	
	Area covered	−0.60 ± 0.22	0.53 ± 0.24	−0.46 ± 0.16			
		0.0321	0.0594	0.0321			
Stress response	Ventilation rate	0.74 ± 0.34	0.63 ± 0.28		0.38 ± 0.23		
		0.0594	0.0594		0.1453		
Brain morphology	Telencephalon width*	−0.56 ± 0.18				−0.57 ± 0.18	
		0.0292				0.0261	
	Optic tectum width	−0.52 ± 0.24	0.58 ± 0.24				
		0.0594	0.0496				
	Cerebellum width*				0.45 ± 0.13	−0.78 ± 0.13	
					0.0178	<0.0001	
	Dorsal brain length			−0.41 ± 0.18	0.41 ± 0.21	−0.72 ± 0.19	
				0.0594	0.0835	0.0044	
	Dorsal brain area	−0.39 ± 0.18			0.34 ± 0.19	−0.69 ± 0.18	
		0.0618			0.1250	0.0077	

rock habitat (Tables 2 and 3; Figure 4a), and model averaging uncovered weak evidence for a positive association with habitat diversity (Table 3). These patterns were opposite to our a priori prediction.

3.4 | Brain morphology

Bahamas mosquitofish exhibited a relatively smaller telencephalon width in fragmented tidal creeks (Tables 2 and 3; Figure 4b), consistent with our prediction, but showed no association with habitat complexity, contrary to predictions. Females had a relatively wider telencephalon than males (Tables 2 and 3; Figure 4b). The optic tectum tended to be wider in tidal creeks with greater occurrence of rock habitat, as well as in fragmented tidal creeks, although these effects were not especially strong (Tables 2 and 3; Figure 4c). Females had a relatively larger cerebellum width than males, while both sexes exhibited a wider cerebellum in tidal creeks with greater structural habitat diversity (Tables 2 and 3; Figure 4d), as predicted. The cerebellum width showed no effects of fragmentation. Females had a relatively longer brain length and larger brain area than males (Tables 2 and 3; Figure 4e,f). We found relatively weak, suggestive evidence that relative brain length tended to increase with habitat diversity,

but decrease with mangrove habitat (Tables 2 and 3; Figure 4e). Meanwhile, we found relatively weak, suggestive evidence that relative brain area was larger in unfragmented tidal creeks, and especially weak evidence for larger brains in sites with greater habitat diversity (Tables 2 and 3; Figure 4f).

4 | DISCUSSION

We uncovered a range of phenotype–environment associations, indicating both natural and human-induced environmental heterogeneity shape complex vertebrate trait variation. Not only has natural variation in habitat complexity influenced a variety of ecologically important phenotypes, but anthropogenic habitat modification has led to changes in how a native animal interacts with its environment, altering its exploration behaviour, stress response and brain anatomy during the past 20–50 years. Data from natural populations relating multiple agents of selection to multiple ecologically and evolutionarily important traits are still not commonplace. Both habitat fragmentation and habitat complexity affected phenotypic variation, but not for all traits, and not always in the manner predicted. We found relatively clear support
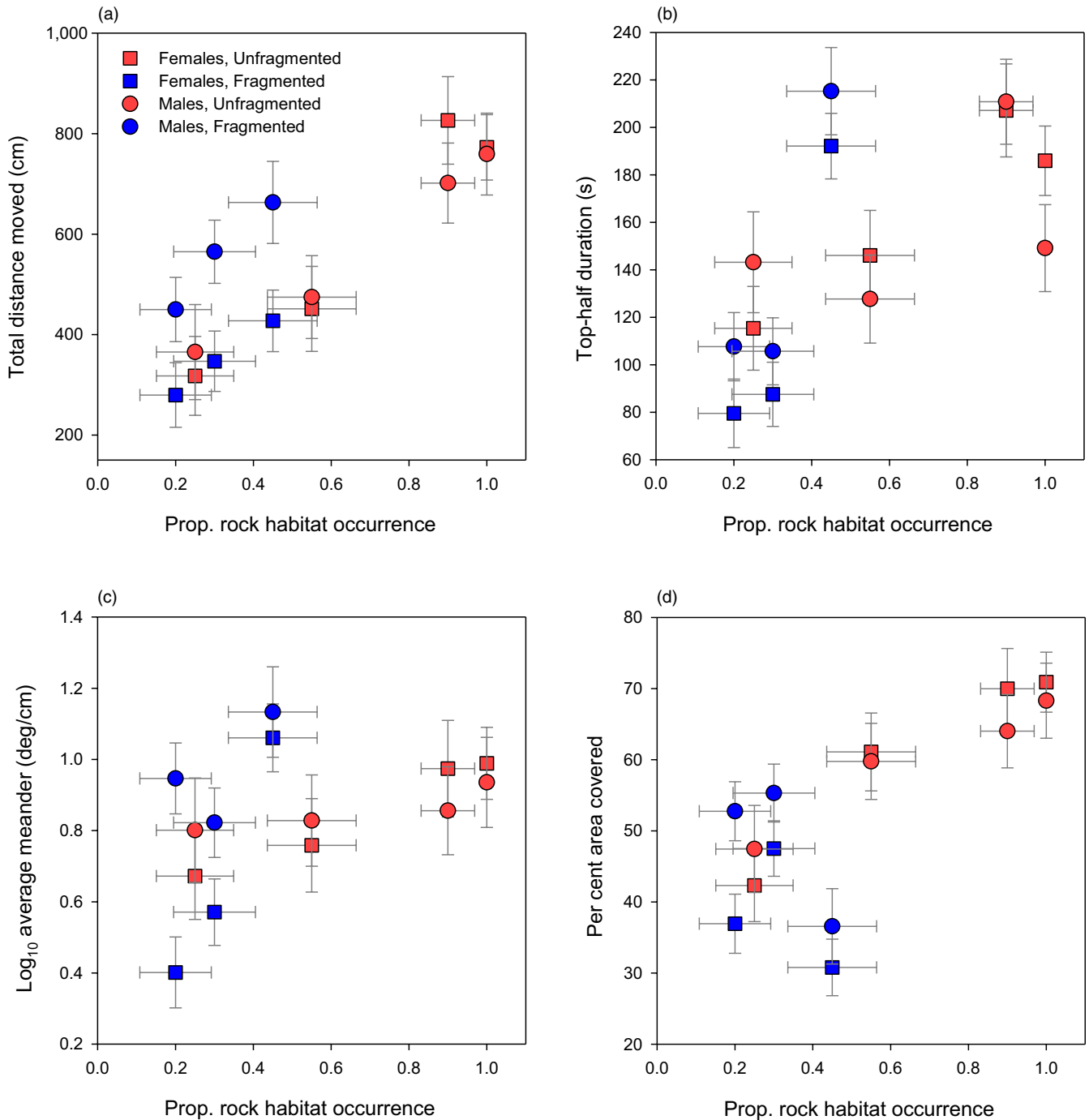
FIGURE 3 Among-population associations between (a) distance moved, (b) top-half duration, (c) average meander and (d) per cent area covered during exploration trials for females (squares) and males (circles) inhabiting unfragmented (red) and fragmented (blue) tidal creeks with varying levels of habitat complexity (rock habitat illustrated here). Means ± *SE*

for six of our 12 a priori predictions. Our approach and findings not only contribute to understanding how ecological agents can drive changes in complex traits, but also shed new light on our ability to accurately predict the nature of those changes, and amplify the call to better understand human-caused trait variation considering the recent recognition of the critical effects of rapid trait changes on ecological dynamics (e.g. Hendry, 2017; Ohgushi et al., 2013).

To date, we know little about how habitat complexity might affect population differentiation in exploratory behaviour within species, as most prior studies have examined within-population plasticity or differences among species, or confounded habitat variation with covariation in predation risk (Ingley, Billman, et al., 2014; Johnson et al., 2020; Mettke-Hofmann et al., 2002; Mikheev et al., 2010). Yet, one of the clearest and strongest findings of this study is that fish inhabiting tidal creeks with greater structural habitat complexity

exhibited a greater propensity to explore a novel environment, irrespective of fragmentation status. This matched our predictions based on selection favouring more exploratory behaviours in more spatially complex habitats to gather information and more effectively acquire resources and reduce encounters with potential competitors and predators. The only evidence we observed contrary to this prediction derived from a single study site with high mangrove frequency—fish from that population, however, covered relatively little area of the novel tank because they also primarily used the top half of the tank. Owing to the paucity of studies to date, we need more studies at the intraspecific level to begin determining how broadly these results might apply.

Our finding that fish in fragmented tidal creeks showed increased exploration of a novel environment suggests that human-induced changes in predation risk can lead to predictable changes in exploratory behaviours, and highlights the utility of simultaneously considering alternative environmental factors. This result is consistent with our hypothesis of stronger selection for exploratory behaviours in fragmented sites, owing to associated foraging advantages in more competitive environments and relaxed selection from predators. This further matches prior findings in Bahamas mosquitofish inhabiting blue holes that also differ in predation regime (Heinen-Kay et al., 2016). However, this trend contrasts with some previous findings with respect to differences in predation threat (Archard & Braithwaite, 2011; Archard et al., 2012; Ingley, Billman, et al., 2014; Ingley, Rehm, et al., 2014). But in these studies, increased predation risk typically correlated positively with increased habitat complexity. Had we ignored variation in structural habitat complexity in the present study, we would not have uncovered any effects of fragmentation on exploratory behaviours, illustrating the importance of concurrently addressing multiple key environmental factors. Moreover, while most prior studies investigating anthropogenic impacts on behavioural responses to unfamiliar situations have centred on direct human impacts, such as human presence and artificial structures or resources (e.g. Jarjour et al., 2020; Lowry et al., 2013; Miranda et al., 2013; Tryjanowski et al., 2016), we uncovered indirect effects of human activities via their ecological consequences. Overall, these results are consistent with the notion that animals may adaptively increase exploration behaviours in less risky environments where competition for resources is often more intense.

The stronger effects of fragmentation status on exploration behaviour in males could be explained by sex differences in exploratory motivation. Male exploration could be motivated by social and sexual interactions to a greater extent than in females; male Bahamas mosquitofish are heavily engaged in searching for, chasing and courting females, and in male–male interactions associated with social rank. In less risky environments, males may increase the frequency of these behaviours (Heinen et al., 2013; Heinen-Kay et al., 2016), leading to increased exploration in search of conspecifics. Prior work in Bahamas mosquitofish inhabiting blue holes found that both sexes showed much greater exploratory behaviours in low-predation environments, but did find weak evidence that males showed larger differences than females (Heinen-Kay et al., 2016), matching the trend found here. Female exploration, on the other hand, may be primarily motivated by food intake and minimizing risk (e.g. higher foraging rate, Heinen et al., 2013). If so, the advantages of exploration in the much more dynamic unfragmented sites (e.g. for food acquisition and seeking refuge) may partially balance out the advantages of exploration in the less risky but temporally consistent fragmented sites, leading to smaller differences between fragmentation regimes in females. Regardless, it appears that exploratory behaviours may respond in a sex-specific manner to environmental variation, but more research is needed to disentangle the underlying causes.

This study provides support for the elevation of acute stress responses in low-predation and less physically dynamic environments. Our finding of a stronger stress response in fragmented tidal creeks matched our prediction that the reduction of certain frequent stressors subsequent to fragmentation—predatory fish, tidal dynamics—would lead to an elevated stress response. Because Bahamas mosquitofish densities are higher in fragmented sites, our results suggest that predation and tidal dynamics serve as greater chronic stressors than high-density conditions. Although this mirrors the patterns documented in other tropical poeciliid fishes inhabiting divergent predation regimes (Archard et al., 2012; Brown et al., 2005; Fischer et al., 2014), it contrasts with those reported for three-spined sticklebacks (*Gasterosteus aculeatus*; Bell et al., 2010). Patterns are also mixed in taxa other than fish (e.g. Berger et al., 2007; Clinchy et al., 2004; Dahl et al., 2012; Robertson et al., 2011), suggesting that species-specific ecologies, covarying environmental factors, variation in costs of the stress response or limitations to evolutionary responses (e.g. gene flow, genetic [co]variation, timeframe) may underlie the heterogeneity of results to date. Even here, it is puzzling why fish in one particular fragmented site (Red Bays) did not exhibit the pattern observed in other fragmented sites. One possible explanation is that the effects of structural habitat complexity (see below) overwhelmed the effects of fragmentation in this site: Red Bays appears to represent the simplest, most open habitat in our study, showing a combination of the lowest values for habitat diversity and habitat richness, along with the highest occurrence of mud habitat.

Elevated chronic stress in more open habitats may explain why fish tended to show weaker stress responses in sites with lower frequency of rock habitat, a trend contrary to our original prediction. While this pattern was not especially clear (FDR-adjusted $p \approx 0.06$), it is consistent with some prior work in other prey taxa showing

FIGURE 4 Among-population associations between (a) ventilation rate in stress response trials, (b) relative telencephalon width, (c) relative optic tectum width, (d) relative cerebellum width, (e) brain length and (f) brain area for females (squares) and males (circles) inhabiting unfragmented (red) and fragmented (blue) tidal creeks with varying levels of habitat complexity (rock habitat and habitat diversity illustrated here). Means ± *SE*
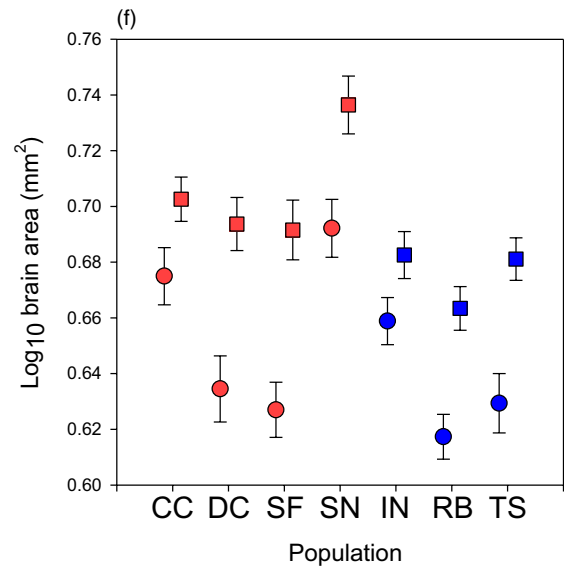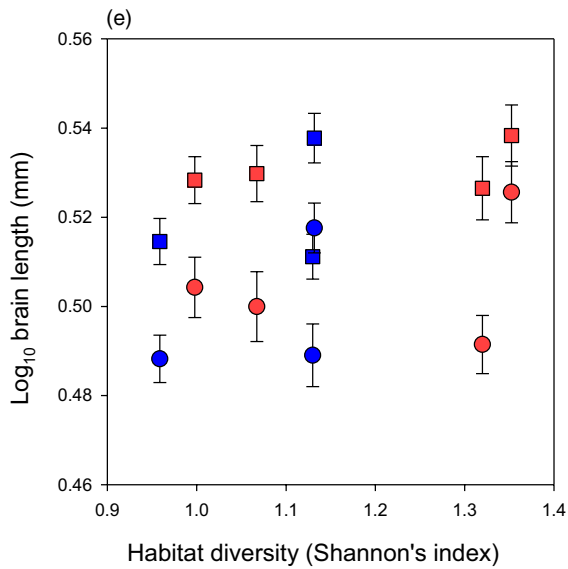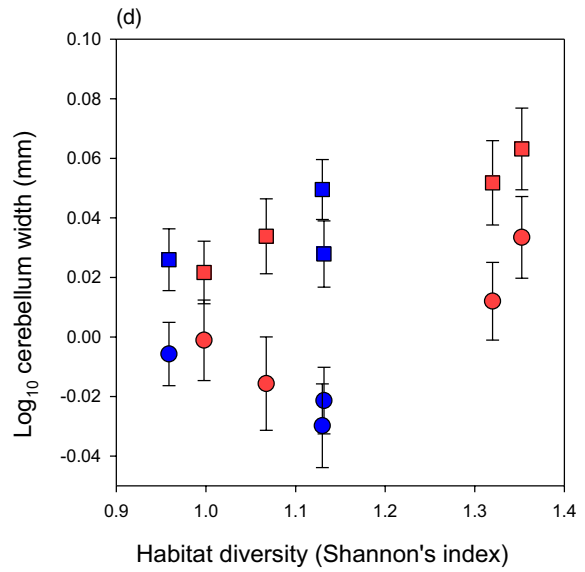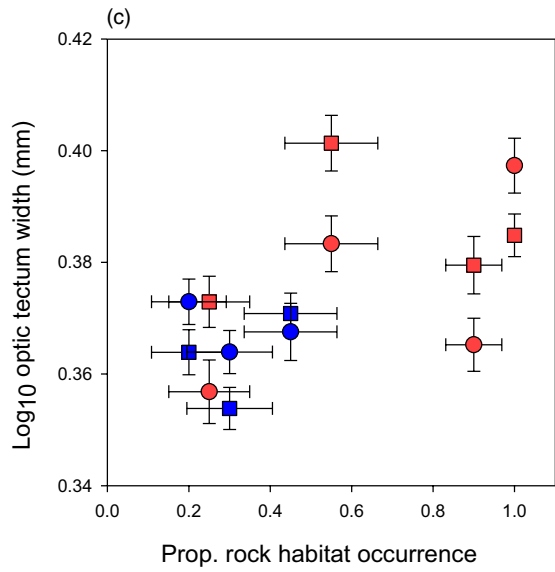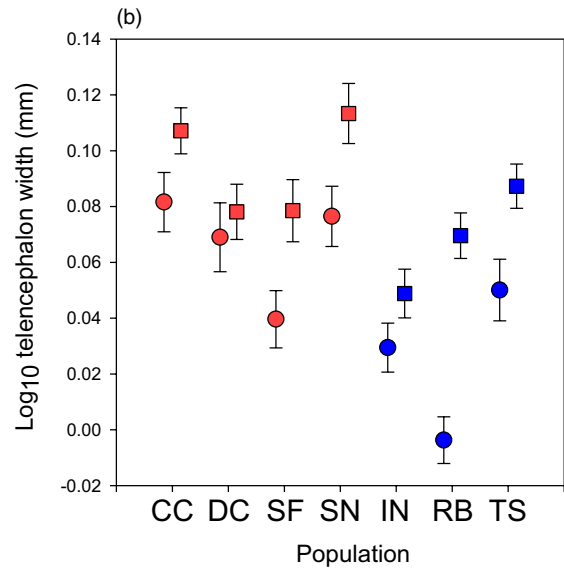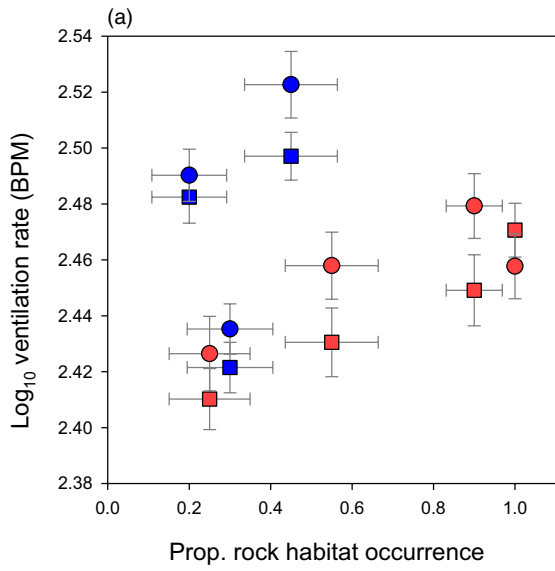
lower chronic stress levels in habitats with greater availability of cover/refuge (Bauer et al., 2013; Mateo, 2007; Navarro-Castilla & Barja, 2019). This leads to a testable hypothesis for future work, as higher structural habitat complexity may offer stress-alleviating effects in Bahamas mosquitofish. This sort of taxon-specific knowledge can prove useful in conservation and restoration efforts.

The relatively wider telencephalon observed in unfragmented tidal creeks may reflect an adaptive response to elevated demands for emotional learning and memory; escape responses; and spatial memory, decision-making and learning in these high-risk, temporally variable environments (e.g. Broglio et al., 2011; Burns & Rodd, 2008; Striedter, 2005). Considering the established role of the telencephalon in fear conditioning and escape behaviours, this pattern may involve divergent needs for fear-based learning in different predation regimes. Matching our a priori prediction, this result is consistent with recent research in Trinidadian guppies that found a larger telencephalon in natural populations with higher apparent threat from predatory prawns *Poecilia reticulata* (Kotrschal et al., 2017), but not with a recent translocation experiment (Mitchell et al., 2020). Gonda et al. (2009) and Gonda et al. (2011) showed that nine-spined sticklebacks *Pungitius pungitius* have evolved a larger telencephalon in marine populations compared to pond populations, which they suggest is related to higher predation risk and higher habitat complexity. To date, little research has examined this topic, and further work is needed to determine the generality of these patterns. Regardless, we show that anthropogenic environmental change can indeed lead to changes in brain anatomy.

The lack of association between telencephalon size and habitat complexity was somewhat surprising, given that larger telencephala have been observed in a range of fishes living in more structurally complex habitats (Bauchot et al., 1977; Gonda et al., 2009; Lisney et al., 2008; Pollen et al., 2007; White & Brown, 2015; Yopak et al., 2007; but see Park & Bell, 2010). Yet, nearly all of this work has examined variation among species, not population differences within species. Moreover, the telencephalon is complex, integrating many sources of information to modulate learning and memory, affecting disparate behaviours such as social, sexual, foraging, and anti-predator behaviours (e.g. Broglio et al., 2011; Striedter, 2005). Telencephalon size may thus be affected by numerous factors, partially explaining our results here. Park and Bell (2010) found that telencephalon shape may diverge between habitat types, with three-spined stickleback fish exhibiting a more laterally convex telencephalon in more complex habitats. Here, we only quantified the dorsal width of the telencephalon, and thus differentiation in other aspects of telencephalon morphology could have gone undetected.

Several different factors might explain why Bahamas mosquitofish only exhibited suggestive evidence for the predicted larger optic tectum in unfragmented tidal creeks. First, fragmented and unfragmented localities may only show small differences in overall demands for visual acuity, as many activities rely heavily on vision in this species, including predator detection, searching for food and mates, and social and sexual interactions with conspecifics. Second, because we only measured optic tectum width, we could

have missed unmeasured changes in size or shape. Finally, prior work showing a larger optic tectum in female Trinidadian guppies inhabiting stream sites with greater predator diversity and biomass (Kotrschal et al., 2017) could have been influenced by covariation between predator communities and other environmental factors, such as habitat complexity. If we had neglected to consider structural habitat variables in the present study, we would have uncovered a stronger and clearer effect of fragmentation status on optic tectum width. The trend for Bahamas mosquitofish showing a relatively wider optic tectum in sites with greater occurrence of rock habitat is intuitive and consistent with our a priori prediction—but with little prior research addressing this topic (Gonda et al., 2013), the subject deserves more attention.

Variation in spatial complexity may drive stronger differences in demands on the cerebellum than variation in predation risk or tidal dynamics—this could explain why Bahamas mosquitofish did not show any differences in the cerebellum width between fragmentation regimes, but did exhibit a relatively wider cerebellum in sites with greater habitat diversity. Although contrary to our prediction, the lack of differentiation across fragmentation regimes does match the lack of association between cerebellum size and predation risk found in Trinidadian guppies (Kotrschal et al., 2017; Mitchell et al., 2020). Variation in predation risk may not elicit divergent selection on cerebellum size, but our results further suggest that neither may tidal dynamics. On the other hand, wider cerebella in sites with greater habitat diversity matched our predictions based on divergence in the requirements of sensory-motor integration among habitats of varying complexity. Although a larger cerebellum in organisms inhabiting more complex habitats has been suggested before (e.g. Pollen et al., 2007; Yopak et al., 2007), our study extends previous findings by documenting this relationship using intraspecific variation, and while controlling for major differences in other factors. Some work suggests that the degree of foliation in the cerebellum may be more important than its size (Lisney et al., 2008)—because we only measured the cerebellum width here, we cannot address how other components of cerebellum morphology might be affected by these environmental agents. Regardless, we argue that the pattern observed may reflect adaptive variation, where a relatively larger cerebellum offers enhanced navigational abilities in spatially complex environments.

We did not find strong evidence for any association between overall brain size and environmental factors. Currently, there is no consensus regarding how predation risk impacts overall brain size, with some studies reporting larger brains (Kotrschal et al., 2017) and others reporting smaller brains in high-predation environments (Gonda et al., 2010; Mitchell et al., 2020; Walsh et al., 2016). A larger brain can confer fitness benefits in high-risk environments (Kondoh, 2010; Kotrschal, Buechel, et al., 2015; Kotrschal et al., 2013b), and we did observe a suggestive trend for larger dorsal brain area in unfragmented tidal creeks. But because brain size represents a composite of different regions, each with different functions, combined with the costs of large brains such as prolonged juvenile stages and lower reproductive output (Kotrschal, Corral-Lopez, et al., 2015; Kotrschal

et al., 2013a, 2013b), inter-population differences in the wild may be complex. Moreover, while many prior studies have observed larger brains in more structurally complex environments (Bauchot et al., 1977; Lisney et al., 2008; Pollen et al., 2007; Safi et al., 2005; Yopak et al., 2007), these patterns typically derive from cross-species comparisons, and not from inter-population studies. We only examined total dorsal length and area of the brain here, and thus we cannot rule out that different patterns may exist for alternative measures, such as total brain mass.

Both phenotypic plasticity and genetic differentiation may play significant roles in explaining the patterns observed here, but we cannot yet partition their relative importance. These traits can exhibit phenotypic plasticity, but genetically based differences in these traits are known (e.g. Di Poi et al., 2016; Herczeg & Välimäki, 2011; Kotrschal et al., 2012; Walsh et al., 2016). Because the trait differences between fragmented and unfragmented tidal creeks must have arisen during the prior 20–50 years, either plasticity or genetic evolution (or both) are plausible, as both can underlie phenotypic responses to anthropogenic impacts at this timescale (e.g. Brans et al., 2018; Hendry et al., 2008; Johnson & Munshi-South, 2017; Kern & Langerhans, 2018). Thus, future work is required to determine the plastic or genetic sources of trait variation observed here.

In conclusion, human-caused ecosystem fragmentation has induced changes in exploratory behaviours, physiological stress response and brain anatomy. Combined with prior work showing differentiation in traits as diverse as growth rate, male genital morphology and muscle mass (Araujo et al., 2014; Heinen-Kay et al., 2014; Riesch et al., 2015), it seems that the fragmentation of tidal creeks serves as a major anthropogenic factor influencing multiple aspects of the phenotype in Bahamas mosquitofish. Further, natural variation in structural habitat complexity resulted in changes in exploratory behaviour, optic tectum width and cerebellum width, with suggestive effects for physiological stress response. Such trait variation can have strong ecological consequences regardless of its plastic or genetic origin (e.g. Hendry, 2017; Ohgushi et al., 2013), but we do not yet know how these traits might influence ecological properties in tidal creeks. Moreover, despite the clear evidence of trait differentiation documented here, we only found clear support for six of our 12 a priori predictions. This success rate adds to the growing body of evidence pointing to a wide variation in our ability to predict trait change across environmental gradients in closely related populations (Langerhans, 2018; Oke et al., 2017), something that will influence the use of evolutionary principles in conservation efforts (Smith et al., 2014).

CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

R.B.L. and K.H. designed the study; M.R.J., J.M.C., A.R.C., K.H. and R.B.L. conducted fieldwork and collected the data; R.B.L. analysed the data. All the authors participated in writing the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.j0zpc86f6 (Langerhans et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supporting Information for:

"Natural and anthropogenic sources of habitat variation influence exploration behaviour, stress response and brain morphology in a coastal fish"

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Contents

SUPPLEMENTARY BACKGROUND
Predictions of phenotypic differentiation

For six types of traits in Bahamas mosquitofish, we formulated directional predictions of differentiation across environments based on hypotheses of divergent natural selection (see Table 1 in main text). All predictions derive from a combination of the following sources of information: 1) natural history knowledge regarding how the organisms are believed to interact with their environment, 2) how certain traits are believed to influence performance attributes and fitness components in the relevant environments, and 3) prior empirical research on the effects of relevant environmental factors on phenotypic expression or differentiation (for prediction generation, see e.g. Langerhans 2008; Langerhans 2010; Martin, McGee, & Langerhans 2015). Future work should test the hypotheses that these predictions rely on, but this study provides the important first step of testing for phenotype-environment associations consistent with particular hypotheses.

   Considerable research has investigated the evolution of exploratory behaviours because of their potentially strong effects on fitness by altering the outcomes of social, competitive, and predator-prey interactions (e.g. Cole & Quinn 2012; Mettke-Hofmann, Winkler, & Leisler 2002; Mikheev, Afonina, & Pavlov 2010; Smith & Blumstein 2008; van Overveld & Matthysen 2010).

In this study, we made two *a priori* predictions for differentiation of exploratory behaviours: increased exploration of novel environments in 1) fragmented, compared to unfragmented, tidal creeks and 2) environments with more structurally complex habitat. These predictions primarily rest on four hypotheses: *i*) exploratory behaviours should generally increase foraging performance in organisms that actively search and feed on patchy resources, like Bahamas mosquitofish, *ii*) exploration may offer key benefits to males by enhancing mating success, as male Bahamas mosquitofish regularly interact with rival males, and search for, chase, and court females throughout the day, *iii*) exploratory behaviours can reduce survivorship in the presence of visually-oriented predators by increasing encounter frequencies, and *iv*) exploration of heterogeneous environments can aid in identifying refuge from predators or competitors. If accurate, the first three hypotheses suggest that selection should favor greater exploratory behaviours in fragmented tidal creeks where conspecific densities are much higher (resulting in stronger intraspecific resource competition), foraging and feeding rate may be higher (at least in females), and predation risk is much lower (Araujo et al., 2014; Heinen-Kay et al., 2016; Heinen et al., 2013; Lapiedra et al., 2018; Pärssinen et al., 2021). The first, second, and fourth hypotheses together suggest that selection should more strongly favor increased exploration of unfamiliar areas in more structurally complex habitats to better locate food and mates, and avoid predators and competitors (Ingley, Rehm, & Johnson 2014; Johnson et al., 2020; MacKenzie & Cormier 2012; Mettke-Hofmann, Winkler, & Leisler 2002; Mikheev, Afonina, & Pavlov 2010).

To date, few studies have examined population differentiation in exploratory behaviours in these contexts—most studies have examined within-population plasticity, differences among species, or confounded habitat variation with co-variation in density or predation risk (e.g. Ingley, Rehm, & Johnson 2014; Johnson et al., 2020; Mettke-Hofmann, Winkler, & Leisler 2002; Mikheev, Afonina, & Pavlov 2010; Sol, Lapiedra, & Gonzalez-Lagos 2013; Thompson et al., 2018). So far, evidence for the role of predation and habitat complexity in shaping exploratory behaviours has sometimes been nuanced, and occasionally opposite to the predictions here (e.g. Archard & Braithwaite 2011; Archard et al., 2012; Ioannou, Ramnarine, & Torney 2017; Johnson et al., 2020), emphasizing the need for more research in the future.

A large volume of studies has examined the vertebrate stress response owing to its importance to fitness. An acute stress response generally involves enhanced glucocorticoid secretion, increased blood glucose levels, and enhanced cardiovascular activity and ventilation rate, i.e. the requirements of a fight-or-flight response (Clinchy, Sheriff, & Zanette 2013; Hammerschlag et al., 2017; Sapolsky, Romero, & Munck 2000). While stress responses clearly offer critical fitness advantages, activation of stress-induced pathways also induce costs in terms of lost foraging or reproductive opportunities, reduced growth rates, and overall increased energy consumption (Cooke et al., 2003; Gregory & Wood 1999; Lankford et al., 2005). Thus, individuals should avoid producing unnecessary acute stress responses, and instead exhibit a magnitude of stress reactivity proportional to the relative importance of a possible stressor. The negative consequences associated with repeated or prolonged stress has led to the prediction that organisms should show an attenuated physiological response to stress in environments with chronically high levels of stressors (Caldji et al., 2000; Clinchy et al., 2004; McEwen 1998; Sapolsky, Romero, & Munck 2000). That is, organisms should adapt or habituate to environments with high frequencies of potentially stressful events, such as predatory encounters, and show reduced acute stress responses to mild stressors.

We predicted here that fish being routinely exposed to environmental stressors—such as high levels of predation threat and temporal variability due to greater tidal amplitudes in

unfragmented sites, or the environmental heterogeneity of structurally complex environments—would exhibit attenuated physiological responses to mild stressors (Archard et al., 2012; Brown, Gardner, & Braithwaite 2005; Fischer et al., 2014; Wingfield 2003; but see Bell, Henderson, & Huntingford 2010). While these predictions have some prior empirical support, they are somewhat tenuous because at present we do not currently know the relative stress experienced by Bahamas mosquitofish via various factors in this system. For instance, while high predation risk almost certainly induces chronically high stress, and greater environmental fluctuation likely leads to higher stress, we do not yet understand the stress-inducing roles of higher population densities or open (exposed) habitat. It is possible that open habitat is more chronically stressful than complex habitat in Bahamas mosquitofish, as this could represent greater exposure to a variety of stressors with fewer nearby refugia (Bauer et al., 2013; Mateo 2007; Navarro-Castilla & Barja 2019). Again, future work is needed to address these gaps in knowledge.

Because brain anatomy—especially the size of brain regions—can influence a range of cognitive abilities, personality, and antipredator behaviours (e.g. Broglio et al., 2011; Gronenberg & Couvillon 2010; Jerison 1985; Kotrschal et al., 2015a,b, 2014, 2013, 2012; Logan et al., 2018; Pike, Ramsey, & Wilkinson 2018; Striedter 2005; van der Bijl et al., 2015), comparative studies of brain/brain-region size in relation to various measures of social, dietary, or habitat variables has a long history (e.g. Barton & Harvey 2000; Barton, Purvis, & Harvey 1995; Freas et al., 2012; Gonzalez-Voyer, Winberg, & Kolm 2009; Kolm et al., 2009; Kotrschal et al., 2017; Kotrschal, Van Staaden, & Huber 1998; Punzo & Ludwig 2002; Roth & Pravosudov 2009; Zeng et al., 2016). Based on this work, and the high energetic costs associated with developing and maintaining brain tissue (e.g. Safi, Seid, & Dechmann 2005), we can hypothesize how selection might act on brain size and brain-region size in Bahamas mosquitofish across fragmentation regimes and among sites of varying levels of structural habitat complexity. We hypothesized that selection will favor generally increased cognitive capacities for mitigating the more dangerous, dynamic, and biotically diverse environments of unfragmented tidal creeks, as well as the more spatially and visually heterogeneous environments found in localities with more structurally complex habitat. Thus, we predicted that Bahamas mosquitofish will exhibit a larger overall brain size, and more specifically, larger telencephalon, optic tectum, and cerebellum regions (relative to body size) in these more risky and variable environments. To date, relatively few studies have directly investigated the role of predation in driving patterns of prey brain anatomy, and while substantial literature addresses the role of habitat complexity on brain morphology, few studies have examined intraspecific variation among populations (see below).

Our predictions derive from the role of overall brain size on a variety of cognitive capacities and the apparent functions of particular brain regions. The telencephalon appears important for emotional learning and memory, reproductive behaviours, escape responses, and spatial cognition, memory, decision making, and learning (e.g. Broglio et al., 2011; Burns & Rodd 2008; Striedter 2005). Some evidence suggests that higher predation pressure from certain predators can lead to a larger telencephalon (Kotrschal et al., 2017), and developmental and comparative studies have sometimes found a larger telencephalon in organisms living in more spatially complex environments (Gonda, Herczeg, & Merila 2009; Huber et al., 1997; Kotrschal et al., 2012; Lisney et al., 2008; Näslund et al., 2012; Pollen et al., 2007; Shumway 2008, 2010; von Krogh et al., 2010; White & Brown 2015; Yopak et al., 2007). The optic tectum is important for processing and responding to visual stimuli and orienting the body relative to other objects, and some previous empirical work suggests that predation risk and habitat features can lead to changes in its size (Broglio et al., 2011; Fong et al., 2019; Huber & Rylander 1992; Huber et al.,

1997; Kotrschal et al., 2017). The cerebellum appears important in fear memory and motor coordination and learning (Broglio et al., 2011; Gomez et al., 2010, 2016; Heap et al., 2013), with some prior evidence suggesting that its size can be influenced by predation risk (Kotrschal et al., 2017) and habitat complexity (Gonda et al., 2009; Pollen et al., 2007; Yopak et al., 2007; Shumway 2008, 2010). Finally, overall brain size can influence a broad range of cognitive abilities, and incorporates all of the aforementioned regions; previous studies have sometimes found larger brains under higher predation risk (Kotrschal et al., 2017; Reddon et al., 2018; but see Burns & Rodd 2008; Gonda et al., 2010; Walsh et al., 2016) and in environments with greater structural habitat complexity (Bauchot et al., 1977; Budeau & Verts 1986; Huber et al., 1997; Lisney et al., 2008; Pollen et al., 2007; Safi & Dechmann 2005; Shumway 2008; Shumway 2010; Yopak et al., 2007; but see Bennett & Harvey 1985). The most obvious gap in our ability to formulate *a priori* predictions of variation in brain size involves the relative importance of cognitive abilities for Bahamas mosquitofish inhabiting the relatively high-density, low-predation environments of fragmented tidal creeks. We hypothesize stronger selection on a large range of cognitive abilities in unfragmented tidal creeks, but reproductive behaviours, visual acuity and orientation for prey detection and capture, and motor coordination for social and feeding behaviours could all prove quite important in the low-risk, high-predation situations of fragmented tidal creeks as well. This topic deserves future research.

SUPPLEMENTARY METHODS
Fragmentation of Bahamian tidal creeks and study site information
The bulk of road construction that caused tidal-creek fragmentation on Andros Island occurred during the 1960s and 1970s (Layman et al. 2004). Fragmentation dramatically reduced tidal exchange (tidal amplitude ~0–10 cm vs. ~40–70 cm in unfragmented creeks), leading to increased sedimentation rates, reduced animal biomass, reduced species diversity, and changes in the community composition of fishes, macroinvertebrates, plants, and macroalgae (Araujo et al., 2014; Valentine-Rose et al., 2007a; Valentine-Rose & Layman 2011; Valentine-Rose et al., 2007b; Valentine-Rose, Rypel, & Layman 2011). For the focal seven tidal creeks examined in this study, hydrologic connectivity is acutely restricted in the fragmented sites, with mean May tidal amplitudes of 7.2 cm compared to 48.9 cm in the unfragmented sites. Subsequent to fragmentation, small, generalist species including Bahamas mosquitofish (*Gambusia hubbsi*) and sheepshead minnow (*Cyprinodon variegatus*) increased in abundance, while piscivorous fishes, including the primary predators of Bahamas mosquitofish such as great barracuda (*Sphyraena barracuda*) and needlefish (*Strongylura* spp.), experienced marked declines in density or extirpation (Araujo et al., 2014; Layman et al., 2004; Riesch et al., 2015; Valentine-Rose et al., 2007a). Previous work suggests that these drastic changes in competition and predation represent the major drivers of rapid phenotypic change in Bahamas mosquitofish in tidal creeks (Araujo et al., 2014; Giery, Layman, & Langerhans 2015; Heinen-Kay et al., 2014; Riesch et al., 2015).

The water chemistry and biotic communities of many Bahamian tidal creeks have been studied during the past two decades, revealing consistent effects of fragmentation on some variables, but not others, and uncovering temporal repeatability of some, but not all, variables. Prior work across >40 tidal creeks on six different Bahamian islands has shown that the density of mosquitofish is consistently higher in fragmented tidal creeks, while the density of piscivorous fishes is consistently higher in unfragmented tidal creeks (e.g. Araujo et al., 2014; Heinen-Kay et al., 2014; Riesch et al. 2015). On the other hand, no consistent differences between fragmentation regimes in salinity, turbidity, pH, or dissolved oxygen have been observed across

all islands—and no consistent differences between fragmentation regimes occur for any of these variables on Andros Island (Heinen-Kay et al., 2014; Riesch et al. 2015). Moreover, for a subset of 12-20 tidal creeks, the strongest temporal repeatabilities within sites have been documented for salinity, piscivore density, and mosquitofish density (Heinen-Kay et al. 2014). For the seven tidal creeks on Andros Island examined in this study, we took multiple measurements of potentially relevant environmental variables (salinity, turbidity, pH, and dissolved oxygen) between 2002 and 2019. We tested for repeatability of each variable using the intraclass correlation coefficient (Lessells & Boag 1987), and included time of day as a covariate for the examination of dissolved oxygen content (dissolved oxygen content increases throughout the day, $P = 0.0036$). We found that for these seven study sites, salinity shows strong repeatability ($n = 44$, $r = 0.88$, $P < 0.0001$), turbidity shows moderate repeatability ($n = 36$, $r = 0.31$, $P = 0.0159$), and neither pH ($n = 40$, $r = 0.00$, $P = 0.47$) nor dissolved oxygen ($n = 17$, $r = 0.17$, $P = 0.34$) exhibit repeatability. Thus, while study sites consistently differ from one another in salinity, and moderately so for turbidity, temporal variation within sites is greater than differences among sites in pH and dissolved oxygen content. All sites exhibit relatively similar pH values (8.24 ± 0.05) and dissolved oxygen content (5.87 mg/L ± 0.45). To ensure that none of our key factors of interest in this study—fragmentation and structural habitat complexity—was confounded with variation in salinity or turbidity, we tested for associations between these two variables and fragmentation regime (*t*-test), proportion rock habitat, proportion mangrove, and habitat diversity (Pearson correlation). We found no significant associations (all $P > 0.05$), suggesting no major covariation among these environmental variables.

Field collections

We provide summary information regarding sample sizes, body size, and habitat-complexity estimates in Table S1. Sample sizes were relatively balanced. For instance, the average ratio of the largest sample size to other sample sizes across all sexes and trait categories was only 1.5. Unequal sample sizes can lead to unequal variances among groups, but we did not find this to be the case in this study. We found no evidence for correlations between sample size and standard deviation for any trait, whether sexes were analyzed together or separately (all $P > 0.13$). Thus, unequal sample sizes in this study should have minimal influence on results.

Each study site was visited on 1 or 2 days during May-June 2017 for this study, and we measured water temperature and salinity using a YSI Pro2030 (Yellow Springs, OH) immediately prior to the start of the first exploration trial at each site on each day (Table S2). All collections and phenotyping occurred on site during the day: >3.5 hours after sunrise and >2.5 hours before sunset (Table S2). We estimated hourly air temperature using data from the nearest weather station in Nassau, The Bahamas, for inclusion as a possible covariate in analyses of behavioural and stress-response assays. Weather was clear during all trials, with air temperature only ranging from $30.0 – 33.9°C$ in all cases, and mean air temperature was correlated with our measurements of water temperature ($n = 10$, $r = 0.65$, $P = 0.0412$).

To minimize potential bias in the behavioural phenotypes of subjects collected, we collected fish from each site in a manner designed to capture representative fish at each site irrespective of behaviour. That is, prior to collection, we recognized that an individual's behavioural type could influence its probability of detection and capture, and thus explicitly sought to minimize this potential effect with our capture methods. To accomplish this, at each site we collected fish using active (dip nets, seines) and passive (minnow traps) sampling in all habitats utilized by *Gambusia* (e.g. relatively open regions, rock habitat, mangrove). We

captured most fish at each site using large, hand-held dip nets after visually locating the fish, sneaking within 1-2 m, and rapidly thrusting the net to capture the fish (i.e. fish were not chased). This method should minimize potential association between behavioural phenotypes and capture probability. However, in case this method biased against subjects that tended to hide in refuge more frequently (e.g. temporarily seeking shelter after spotting the human collectors), we additionally used un-baited minnow traps and seines placed near possible refugia. The traps could capture fish as they ventured out of refugia, or while swimming near the complex habitat. Using seines, we placed them along the substrate just outside of possible refugia, and pulled it upward to capture fish swimming out of the complex habitat and over the seine net. These latter methods captured a relatively small number of fish at each site, likely owing to the fact that most Bahamas mosquitofish spend most of their time during the day actively swimming within all habitats in these sites, not hiding in refuge. To further minimize variation in collection methods among sites, the same two people collected all fish at all sites (KH and RBL), including one collector (RBL) with 15 years of prior experience collecting this species in tidal creeks on Andros Island.

We conducted exploration and stress-response assays on-site, within 30m of the capture location of each fish. Fish generally entered exploration assays rapidly after collection: 94% of fish entered the novel tank within 10 min of collection. Immediately prior to examination, fish were temporarily held in an Igloo Marine Ultra cooler (45.6L; $66 \times 37 \times 37$ cm) filled with water from the respective site. While we did not record the exact amount of time subjects were held prior to experimentation (we had focused on trying to keep the holding time to <10 min), we did record the times for subjects that were held 10 min or longer. Twenty-one fish from four sites (two fragmented, two unfragmented) were held $\geq$ 10 min. To test whether holding time might have influenced exploration behaviours, we included a categorical variable for holding time (0 = less than 10 min, 1 = 10 min or longer) in our statistical models employed to estimate site-sex average values described in the text. We found no association between holding time and any behaviour (all P > 0.56), and thus excluded it from the models.

Measurement of structural habitat complexity
To estimate habitat complexity, we initially calculated the average frequency of all four habitat types in each site (proportion of quadrats where the respective habitat type was present), and additionally calculated habitat richness (average number of habitat types present in each quadrat) and habitat diversity (Shannon's Diversity Index, which incorporates relative frequencies of all four habitat types; Shannon 1948). Upon inspection of correlations among these six variables, three habitat variables exhibited high correlations with others (average $|r|$ = 0.59, maximum $|r|$ = 0.97), and we thus excluded those variables to reduce multicollinearity in statistical models (i.e. excluded mud, wood, habitat richness). In our final analyses, we used three variables to estimate habitat complexity for each site: frequency of rock habitat, frequency of mangrove habitat, and habitat diversity. These variables provide intuitive measurements of structural complexity experienced by Bahamas mosquitofish, and exhibited relatively low correlations with one another ($|r|$ = 0.10 – 0.45).

Measurement of exploration behaviour
The use of "open-field" tests—comprising a novel, open environment—has a long history in the study of animal exploration and anxiety-like behaviours (Cachat et al., 2010; Egan et al., 2009; Hall 1934; Heinen-Kay et al., 2016; Mikheev & Andreev 1993; Mok & Munro 1998; Reale et

al., 2007; Warren & Callaghan 1975). The utility of this method for assessment of exploration in Bahamas mosquitofish has been previously demonstrated (Heinen-Kay et al., 2016), although future work is still needed to better understand how exploratory behaviours measured in the assay translate to behaviours and fitness in the wild. Here we used this assay to measure exploration of novel environments in Bahamas mosquitofish inhabiting tidal creeks. Video files of exploration assays performed in the field were analyzed in EthoVision software (EthoVision XT 11.5, Noldus Information Technology) to automatically track each fish and measure a range of behavioural variables designed to capture exploratory behaviours. We watched each video and manually set the start time for subject tracking of each fish to begin immediately after the fish entered the experimental arena. Because exploratory behaviour was partially measured by distance moved and average velocity (see below), we wished to avoid the tracking of any "panic" or "frantic" swimming, as this could be incorrectly interpreted as exploratory behaviour. Fortunately, Bahamas mosquitofish very rarely exhibit such behaviour, and instead typically freeze when showing anxiety-like behaviours in novel situations (e.g. Heinen-Kay et al., 2016). In the present study, only a few fish exhibited rapid, darting behaviour during the first 1-2 sec after entering the arena, and we initiated subject tracking immediately after the behaviour. We further viewed the subject tracking of each trial within EthoVision to ensure accurate tracking. This involved watching the video of each trial with the auto-tracked point and path overlaid onto the video, ensuring the subject tracked was always the focal fish and not some other object, and that the subject was not lost during the video.

Using EthoVision, we quantified 10 behavioural traits of Bahamas mosquitofish in each assay, selected based on their potential to capture varying aspects of exploratory behaviour: total distance moved (cm), mean velocity (cm/s), percent body mobility (%), movement duration (s), frozen duration (s), transitions among nine equal-sized quadrants spanning the arena (#), unique area covered (% of arena), duration in the top-half (s), mean absolute meander (deg/cm), and mean absolute angular velocity (deg/s). The latter two measurements were expressed as "absolute" metrics so that we captured average turning information without regard to whether the fish was turning left or right; these two variables were $\log_{10}$-transformed to improve normality. No other behavioural variables required transformation. All variables except unique area covered were calculated directly from the EthoVision software. To calculate the area of the novel tank explored by the fish during each assay, we measured the area of the heatmaps generated by EthoVision using the color threshold feature of ImageJ (https://imagej.nih.gov/ij/) (De Winter et al., 2016; Lievens 2012; Fig. S1). We specifically estimated the area covered using heatmaps (employing "per heatmap" color coding with 25px smoothing) and not center-point tracing because we assumed the fish had explored the immediate vicinity surrounding its body.

Because of high correlations among some behavioural variables, we reduced these traits to four variables with comparatively low pairwise correlations with each other. Specifically, we examined the correlation matrix of the 10 variables (Table S3), and determined that four "modules" appeared to be present. First, six traits describing overall movement tendency were all very highly correlated with one another (mean $|r| = 0.98$), but less so with other traits (mean $|r| = 0.43$). We retained the total distance moved as the behavioural variable to represent this module in analyses because of its intuitive link to exploration. Second, the area covered exhibited comparatively lower correlation with the six movement traits (mean $|r| = 0.73$) and low correlation with all other traits (mean $|r| = 0.26$). Despite its association with movement traits, we retained the area covered variable because of its clear distinction from the movement traits both in its average reduction of correlation coefficient by ~0.25, as well as its conceptual distinction

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of capturing a potentially different aspect of exploration than movement traits (e.g. fish could repeatedly move through the same area or only move when moving into unique areas). Third, top-half duration exhibited low correlations with all other variables (mean $|r| = 0.24$). Fourth, the two variables that involved turning angles ($\log_{10}$ meander and $\log_{10}$ angular velocity) had high correlation with each other ($r = 0.95$), but relatively low correlation with other traits (mean $|r| = 0.32$). We retained $\log_{10}$ meander to represent this module as it most intuitively captures exploratory behaviour, as higher turning angles per distance traveled describes fish that swim in more meandering, tortuous paths, as expected for an individual exploring a new environment.

Statistical analysis
Because body size distributions greatly overlapped among populations (Table S1), we could appropriately adjust trait values relative to a common body size for analysis (average trait value for the same sized fish across all populations), assuming homogeneity of slopes. Even so, for the size-associated traits, if average body size was confounded with ecological drivers, then the effects of these drivers on trait values could be related to changes in body size and not necessarily changes in relative trait size per se (i.e. differences in body size without concomitant differences in the trait result in apparent differences in relative trait size). To confirm average body sizes were not associated with our ecological factors of interest, we conducted general linear mixed models separately for each sex, using $\log_{10}$-transformed standard length as the dependent variable, population as a random effect, and either fragmentation regime, proportion rock habitat, proportion mangrove, or habitat diversity as an independent variable (8 total models). We found that body size was unrelated to most ecological factors ($P > 0.05$, uncorrected for multiple tests), with one exception: males showed a smaller average body size in populations with more mangrove habitat ($F_{1,5.52} = 8.45$, $P = 0.0299$). This latter finding was strongly influenced by one particular population (Thompson/Scott)—the significant relationship was eliminated with the exclusion of that site. Regardless, this means that effects of mangrove habitat on size-associated traits could be related to variation in body size. We only found one effect of mangrove habitat on a size-associated trait (dorsal brain length, see main text), but this effect was not sex-dependent, nor did we find a relatively longer brain in sites with more mangrove as would be expected if body size variation explained the pattern. That is, while populations inhabiting sites with more mangrove habitat tended to show a relatively smaller body, they did not tend to consequently show a relatively longer brain because of this—they tended to show the opposite. Thus, we found no evidence that variation in body size among populations explains any of our results.

When estimating population mean trait values for each sex, we tested for heterogeneity of slopes in our general linear models described in the text (interaction between $\log_{10}$-transformed standard length and either population or sex; no 3-way interaction was significant). We found significant evidence for allometric variation among populations for four traits (interaction between population and standard length; ventilation rate: $P = 0.0430$, optic tectum width: $P = 0.0430$, brain length: $P = 0.0223$, brain area: $P = 0.0311$), and among sexes for three traits (interaction between sex and standard length; ventilation rate: $P = 0.0168$, optic tectum width: $P = 0.0004$, brain area: $P = 0.0139$) (all P-values uncorrected for multiple tests). In all cases, heterogeneity of slopes had minimal effect on estimates of population means for each sex, as the correlations between estimates that either included or excluded the interaction terms were very high (all $r > 0.97$). Thus, we only present results in the main text that excluded interaction terms, assuming homogeneity of slopes. However, we provide all relevant log-log slopes to allow for

inspection of allometries in Table S4. We further provide bivariate plots for all size-associated traits in Figure S2 to allow for visual inspection of trait and size (co)variation among populations. We note, however, that this study was not designed for detailed assessment of allometric variation, so we do not provide reduced major axis regression.

We examined each of the 10 focal traits separately in our model-selection and multimodel inference analyses, as the study was not designed for multivariate examination—e.g. with 10 traits, two sexes, seven populations, and four putative environmental drivers, we have insufficient statistical power for multivariate analyses. Justifying a univariate approach is the fact that inter-individual trait correlations were generally low (average $|r| = 0.19$), and even among-population trait correlations were generally low to moderate (average $|r| = 0.37$). Among individuals, correlations among exploratory behaviours are addressed above, while ventilation rate was largely independent of all other traits (average $|r| = 0.06$), and the relative sizes of the three brain regions were not tightly correlated with one another (average $r = 0.24$; using log-log residuals from regressions on standard length; telencephalon-optic tectum $r = 0.28$, telencephalon-cerebellum $r = 0.18$, optic tectum-cerebellum $r = 0.27$). Relative brain length and brain area were highly correlated with one another ($r = 0.84$), but we examined both traits because they should capture slightly different aspects of overall dorsal brain size (indeed, we uncovered different results for these two traits). Multicollinearity was relatively low, with all VIFs < 1.76 in selected models. None of our conclusions would change if we forced the inclusion of the sex term in our models to account for sex regardless of whether trait differences were apparent. Although we had directional (i.e. one-tailed) predictions in all cases, we report two-tailed, FDR-adjusted P-values as a conservative approach.

For model averaging, we chose to use a subset of models having $\Delta AIC_c \leq 2.0$. We chose this cutoff by inspecting the AIC_c results to determine a reasonable threshold that would capture relatively important models but minimize the inclusion of potentially spurious models with low support. This cutoff resulted in the inclusion of all models with Akaike weights > 0.1. To determine whether our results were sensitive to this choice of cut-off, we also conducted model averaging using models with cumulative Akaike weight < 0.95. Results were very similar using this alternative set of models, with the only difference being suggestive effects of Rock × Sex interactions for top-half duration and mean meander, where males might exhibit a steeper slope than females. Model selection results are presented in Table S5. R code for the analyses is given in Appendix S1.

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TABLE S1 Sample sizes of Bahamas mosquitofish examined for each trait category, average body size of specimens, and summary habitat-complexity information for our study sites. Standard error is provided in parentheses.

Population	Fragmentation	Exploration		Stress Response		Brain Morphology		Standard Length (mm)		Prop.	Prop.	Habitat
		Females	Males	Females	Males	Females	Males	Females	Males	Rock	Mangrove	Diversity
Independence Park	Fragmented	32	30	32	30	31	29	23.74 (0.60)	20.20 (0.54)	0.20	0.40	1.13
Red Bays		34	31	32	32	34	32	22.02 (0.54)	20.44 (0.52)	0.30	0.40	0.96
Thompson/Scott		33	19	36	19	36	19	22.04 (0.53)	18.49 (0.61)	0.45	0.85	1.13
Cargill Creek	Unfragmented	34	19	33	20	34	20	22.97 (0.57)	19.47 (0.63)	1.00	0.30	1.00
Davey Creek		21	14	23	15	23	15	19.78 (0.59)	18.89 (0.70)	0.25	0.85	1.07
Stafford Creek		18	22	18	22	18	22	21.29 (0.72)	18.64 (0.57)	0.90	0.80	1.32
Staniard Creek		20	20	20	20	20	20	23.59 (0.76)	18.48 (0.59)	0.55	0.60	1.35
	Totals:	192	155	194	158	196	157					

TABLE S2 Summary information for field collections performed in this study in seven tidal creeks on Andros Island, The Bahamas.

Population	Fragmentation	Date(s) Examined	Time of Trials	Air Temp. Range (°C)	Avg. Water Temp. (°C)	Avg. Salinity
Independence Park	Fragmented	1 June 2017	10:36 – 15:25	30.0 – 32.8	29.7	47.8
Red Bays		2 June 2017	10:51 – 15:45	30.0 – 32.8	29.3	40.6
Thompson/Scott		26, 27 May 2017	11:13 – 16:21	30.0 – 33.3	31.7	20.2
Cargill Creek	Unfragmented	29 May 2017	11:33 – 17:25	31.1 – 33.9	31.6	38.2
Davey Creek		28 May, 3 June 2017	11:52 – 15:14	31.1 – 32.2	30.3	35.2
Stafford Creek		30 May 2017	11:37 – 17:07	32.8 – 33.9	30.7	25.0
Staniard Creek		6, 12 June 2017	9:52 – 13:50	30.0 – 32.2	29.7	31.4

TABLE S3 Correlation matrix for the ten behavioural variables measured in the exploration trials.

	Total Dist. Moved	Mean Velocity	Mean % Mobility	Movement Dur.	Frozen Dur.	Quadrat Trans.	Area Covered	Top-half Dur.	Log_{10} Meander
Mean Velocity	1.00								
Mean % Body Mobility	0.96	0.96							
Movement Duration	0.99	0.99	0.95						
Frozen Duration	-0.99	-0.99	-0.95	-1.00					
Quadrat Transitions	0.98	0.98	0.96	0.98	-0.97				
Area covered	0.72	0.72	0.69	0.75	-0.74	0.75			
Top-half Duration	0.28	0.28	0.20	0.30	-0.30	0.23	0.17		
Log_{10} Meander	0.31	0.30	0.25	0.30	-0.30	0.27	0.26	0.21	
Log_{10} Angular Velocity	0.42	0.42	0.37	0.41	-0.42	0.39	0.36	0.21	0.95

TABLE S4 Allometry information for stress response and brain morphology. Ordinary least-squares regression log-log slopes of six traits regressed on standard length depicted (analysis of ventilation rate included time and air temperature as covariates). Superscripts denote significant evidence ($P < 0.05$) for allometric differences between populations (P) and sexes (S). Bold indicates $P < 0.05$.

Population	Ventilation Rate[P,S] F	M	Telencephalon Width F	M	Optic Tectum Width[P,S] F	M	Cerebellum Width F	M	Brain Length[P] F	M	Brain Area[P,S] F	M
Independence Park	**-0.63**	-0.01	**1.08**	**0.93**	0.44	0.61	0.56	0.40	**0.40**	0.32	1.07	1.08
Red Bays Pond	**-0.26**	-0.29	**0.85**	**0.77**	0.33	0.40	0.57	0.30	**0.55**	0.57	1.04	1.06
Thompson/Scott	**-0.81**	**-0.98**	0.98	1.17	0.46	0.80	0.94	0.70	0.66	1.03	1.28	1.86
Cargill Creek	**-0.44**	0.30	**0.88**	**1.00**	0.36	0.65	0.68	0.97	**0.44**	0.65	0.95	1.46
Davey Creek	**-0.36**	-0.52	**0.93**	**1.08**	0.48	0.62	0.71	0.65	**0.51**	0.69	1.11	1.36
Stafford Creek North	**-0.54**	-0.26	**0.99**	**0.94**	0.51	0.57	0.80	0.88	**0.62**	0.72	1.28	1.79
Staniard Creek	**-0.46**	-0.17	**0.98**	**0.82**	0.45	0.68	0.49	0.66	**0.44**	0.50	1.06	1.18
Pooled	-0.42		0.94		0.47		0.66		0.54		1.17	

TABLE S5 Model selection results examining competing hypotheses to explain variation in 10 phenotypes among seven tidal creeks in Bahamas mosquitofish. Possible model terms included fragmentation regime (F), frequency of rock habitat (R), frequency of mangrove habitat (M), habitat diversity (HD), Sex (S), and interactions with Sex. We present the terms included in each model (denoted with "X"), number of fitted parameters (k), AIC_c value, ΔAIC_c value, Akaike weight (w), cumulative Akaike weight (Cum w), and the evidence ratio (ER; comparing each model to the next-best model) for all models with cumulative Akaike weight < 0.95 for each trait. Bold models are those with $\Delta AIC_c < 2$ included in model averaging.

Trait	F	R	M	HD	S	FxS	RxS	MxS	HDxS	k	AIC_c	Δ	w	Cum w	ER
Distance moved	**X**	**X**			**X**	**X**				**6**	**19.00**	**0.00**	**0.82**	**0.82**	**18.29**
	X	X	X		X	X				7	24.90	5.82	0.05	0.87	1.32
	X	X			X	X	X			7	25.40	6.38	0.03	0.90	2.00
		X			X		X			5	26.90	7.82	0.02	0.92	1.06
	X	X			X		X			6	26.90	7.88	0.02	0.94	1.00
Top-half duration	**X**	**X**	**X**							**5**	**23.20**	**0.00**	**0.77**	**0.77**	**11.64**
	X	X	X		X		X			7	28.10	4.90	0.07	0.83	1.03
		X	X							4	28.20	4.98	0.06	0.90	1.45
	X	X	X		X					6	28.90	5.71	0.04	0.94	1.38
Mean meander		**X**								**3**	**43.70**	**0.00**	**0.18**	**0.18**	**1.06**
										2	**43.80**	**0.11**	**0.17**	**0.35**	**2.22**
					X					**3**	**45.40**	**1.70**	**0.08**	**0.42**	**1.06**
		X			**X**					**4**	**45.50**	**1.82**	**0.07**	**0.50**	**1.14**
		X	X							4	45.80	2.07	0.06	0.56	1.03
		X			X		X			5	45.80	2.14	0.06	0.62	1.09
			X							3	46.00	2.34	0.06	0.68	1.27
	X	X								4	46.50	2.82	0.04	0.72	1.26
				X						3	47.00	3.27	0.04	0.76	1.03
	X									3	47.00	3.33	0.03	0.79	1.26
	X	X	X							5	47.40	3.77	0.03	0.82	1.13
		X		X						4	47.70	4.04	0.02	0.84	1.26
			X		X					4	48.20	4.52	0.02	0.86	1.00
		X	X		X					5	48.20	4.53	0.02	0.88	1.46
		X	X		X		X			6	48.90	5.23	0.01	0.89	1.08
	X	X			X					5	49.10	5.43	0.01	0.90	1.09
			X	X	X					4	49.30	5.59	0.01	0.91	1.00
	X				X					4	49.30	5.65	0.01	0.93	1.38
		X	X	X						5	50.00	6.33	0.01	0.93	1.14
			X	X						4	50.00	6.37	0.01	0.94	1.00
	X			X						4	50.00	6.37	0.01	0.95	1.00
Area covered	**X**	**X**	**X**							**5**	**33.40**	**0.00**	**0.29**	**0.29**	**1.60**
	X		**X**							**4**	**34.30**	**0.94**	**0.18**	**0.47**	**1.30**
		X								**3**	**34.90**	**1.47**	**0.14**	**0.60**	**1.60**

Trait						n	AICc	ΔAICc	wᵢ	Σwᵢ	ER
		X	X			4	35.80	2.41	0.09	0.69	1.41
	X	X				4	36.50	3.09	0.06	0.75	1.74
	X		X	X		5	37.60	4.18	0.04	0.79	1.40
	X	X	X	X		6	38.30	4.85	0.03	0.81	1.00
		X			X	4	38.30	4.86	0.03	0.84	1.09
	X	X	X		X	6	38.50	5.08	0.02	0.86	1.00
	X		X		X	5	38.50	5.09	0.02	0.88	1.15
		X	X	X		5	38.70	5.33	0.02	0.90	1.05
		X		X		4	38.80	5.44	0.02	0.92	1.06
	X					3	38.90	5.49	0.02	0.94	1.80
		X	X		X	5	40.00	6.65	0.01	0.95	1.43
Ventilation rate	**X**	**X**				**4**	**43.10**	**0.00**	**0.22**	**0.22**	**1.41**
						2	**43.80**	**0.68**	**0.15**	**0.37**	**1.29**
	X					**3**	**44.30**	**1.18**	**0.12**	**0.49**	**1.24**
	X	**X**		**X**		**5**	**44.70**	**1.61**	**0.10**	**0.58**	**1.71**
	X	X	X			5	45.80	2.68	0.06	0.64	1.12
	X			X		4	46.00	2.90	0.05	0.69	1.11
					X	3	46.20	3.12	0.05	0.73	1.18
	X	X		X		5	46.60	3.48	0.04	0.77	1.09
			X			3	46.70	3.64	0.04	0.81	1.03
		X				3	46.80	3.69	0.03	0.84	1.13
			X			3	47.00	3.94	0.03	0.87	1.11
	X				X	4	47.30	4.16	0.03	0.90	1.42
	X		X			4	48.00	4.87	0.02	0.92	1.90
	X	X		X	X	6	49.20	6.06	0.01	0.93	1.25
	X			X	X	5	49.80	6.69	0.01	0.94	1.14
				X	X	4	49.90	6.79	0.01	0.94	1.00
		X			X	4	50.00	6.85	0.01	0.95	1.17
Telencephalon width	**X**			**X**		**4**	**36.90**	**0.00**	**0.53**	**0.53**	**9.02**
	X			X	X	5	41.30	4.39	0.06	0.59	1.05
		X		X		4	41.40	4.49	0.06	0.65	1.06
				X		3	41.50	4.60	0.05	0.70	1.02
	X	X		X		5	41.60	4.67	0.05	0.75	1.21
	X					3	41.90	5.03	0.04	0.80	1.00
	X		X	X		5	41.90	5.05	0.04	0.84	1.02
	X			X	X	5	41.90	5.05	0.04	0.88	2.47
						2	43.80	6.89	0.02	0.90	1.42
		X				3	44.40	7.56	0.01	0.91	1.00
				X	X	4	44.50	7.56	0.01	0.92	1.50
			X	X		4	45.30	8.43	0.01	0.93	1.33
	X	X				4	45.80	8.88	0.01	0.94	1.00
	X	X		X		5	45.80	8.95	0.01	0.94	1.00
	X		X			4	46.00	9.08	0.01	0.95	1.00

	1	2	3	4	5	6	7	8	k					
Optic tectum width		**X**							**3**	**41.40**	**0.00**	**0.24**	**0.24**	**1.57**
	X								**3**	**42.30**	**0.90**	**0.16**	**0.40**	**2.07**
									2	43.80	2.35	0.08	0.47	1.03
	X		X						4	43.80	2.39	0.07	0.55	1.24
	X	X							4	44.30	2.82	0.06	0.61	1.07
		X		X					4	44.40	2.99	0.06	0.66	1.22
		X	X						4	44.80	3.39	0.05	0.71	1.22
			X						3	45.20	3.79	0.04	0.74	1.03
		X			X				4	45.20	3.81	0.04	0.78	1.33
			X	X					4	45.80	4.39	0.03	0.81	1.04
	X			X					4	45.90	4.47	0.03	0.83	1.13
	X		X	X					5	46.10	4.69	0.02	0.85	1.00
	X				X				4	46.20	4.73	0.02	0.88	1.10
			X						3	46.30	4.89	0.02	0.90	1.31
		X	X	X					5	46.90	5.43	0.02	0.91	1.00
					X				3	46.90	5.50	0.02	0.93	1.45
	X	X	X						5	47.60	6.15	0.01	0.94	1.57
	X		X		X				5	48.60	7.18	0.01	0.95	1.17
Cerebellum width				**X**	**X**				**4**	**27.70**	**0.00**	**0.59**	**0.59**	**5.93**
	X			X	X				5	31.30	3.56	0.10	0.69	1.43
		X		X	X				5	32.00	4.26	0.07	0.76	1.11
			X	X	X				5	32.20	4.47	0.06	0.83	1.31
				X	X		X		5	32.80	5.05	0.05	0.87	1.92
				X					3	34.10	6.34	0.03	0.90	1.14
	X			X					4	34.30	6.60	0.02	0.92	1.47
			X	X	X		X		6	35.10	7.38	0.02	0.94	1.25
	X			X	X	X			6	35.50	7.75	0.01	0.95	1.71
Brain length				**X**					**3**	**36.90**	**0.00**	**0.25**	**0.25**	**1.23**
		X	**X**	**X**					**5**	**37.30**	**0.41**	**0.20**	**0.45**	**1.56**
			X	**X**					**4**	**38.20**	**1.29**	**0.13**	**0.59**	**1.60**
	X			X					4	39.10	2.22	0.08	0.67	1.37
		X		X					4	39.80	2.84	0.06	0.73	1.22
	X	X	X	X	X				7	40.20	3.27	0.05	0.78	1.44
		X		X					4	40.90	4.01	0.03	0.81	1.31
		X	X	X	X				6	41.40	4.51	0.03	0.84	1.24
	X		X	X					5	41.90	5.00	0.02	0.86	1.31
	X	X		X					5	42.40	5.50	0.02	0.87	1.07
	X			X	X				5	42.60	5.66	0.02	0.89	1.00
	X		X	X	X				6	42.60	5.67	0.02	0.90	1.15
		X		X	X				5	42.90	5.94	0.01	0.92	1.18
			X	X				X	5	43.20	6.30	0.01	0.93	1.22
		X	X	X			X		6	43.60	6.66	0.01	0.94	1.13
		X	X	X				X	6	43.70	6.83	0.01	0.94	1.00

	C1	C2	C3	C4	C5	C6	C7	C8						
Brain area	**X**				**X**				**4**	**37.00**	**0.00**	**0.27**	**0.27**	**1.62**
					X				**3**	**38.00**	**0.96**	**0.16**	**0.43**	**1.36**
				X	**X**				**4**	**38.60**	**1.57**	**0.12**	**0.55**	**1.44**
			X	X	X				5	39.30	2.29	0.08	0.63	1.38
	X		X		X				5	39.90	2.95	0.06	0.70	1.30
	X			X	X				5	40.50	3.48	0.05	0.74	1.04
		X			X				4	40.60	3.57	0.05	0.79	1.25
	X		X	X	X				6	41.00	3.98	0.04	0.82	1.24
			X		X				4	41.40	4.45	0.03	0.85	1.26
	X			X	X	X			5	41.90	4.90	0.02	0.88	1.10
	X	X			X				5	42.00	5.04	0.02	0.90	1.50
		X		X	X				5	42.80	5.84	0.01	0.91	1.27
				X	X			X	5	43.40	6.43	0.01	0.92	1.22
									2	43.80	6.78	0.01	0.93	1.13
			X	X	X		X		6	44.10	7.12	0.01	0.94	1.33
	X								3	44.70	7.71	0.01	0.94	1.20
	X		X		X		X		6	44.90	7.86	0.01	0.95	1.25

TABLE S6 Effect sizes presented as percent differences between groups based on estimated means from relevant statistical models (see main text). While we estimated structural habitat complexity using continuous variables in these analyses, here we compared mean trait values in high complexity vs. low complexity to aid interpretation. Rock Habitat: 0.3 vs. 0.8 proportion of rock occurrence; Mangrove Habitat: 0.4 vs. 0.8 proportion of rock occurrence; Habitat Diversity: 1.0 vs. 1.3 Shannon's Diversity Index. Percent differences were calculated as the larger value divided by the smaller value. The superscript "M" indicates the pattern was only observed in males.

Category	Trait	In Fragmented Tidal Creeks	In High Rock Hab.	In High Mangrove	In High Hab. Div.
Exploration Behaviour	Total Distance Moved	43.5% greater^M	78.8% greater		
	Duration in Top-Half	29.0% greater	265.8% greater	140.8% greater	
	Average Meander		41.0% greater		
	Area Covered	27.7% less	14.6% greater	25.9% less	
Stress Response	Ventilation Rate	12.6% greater	8.1% greater		
Brain Morphology	Telencephalon Width	8.0% smaller			
	Optic Tectum Width	3.4% smaller	3.1% larger		
	Cerebellum Width				6.5% larger
	Dorsal Brain Length			3.0% shorter	4.1% larger
	Dorsal Brain Area	6.2% smaller			

FIGURE S1 Examples of the calculation of percent area covered by fish during exploration assays using (left panel) heatmaps generated by EthoVision and corresponding (right panel) image processing performed in ImageJ.
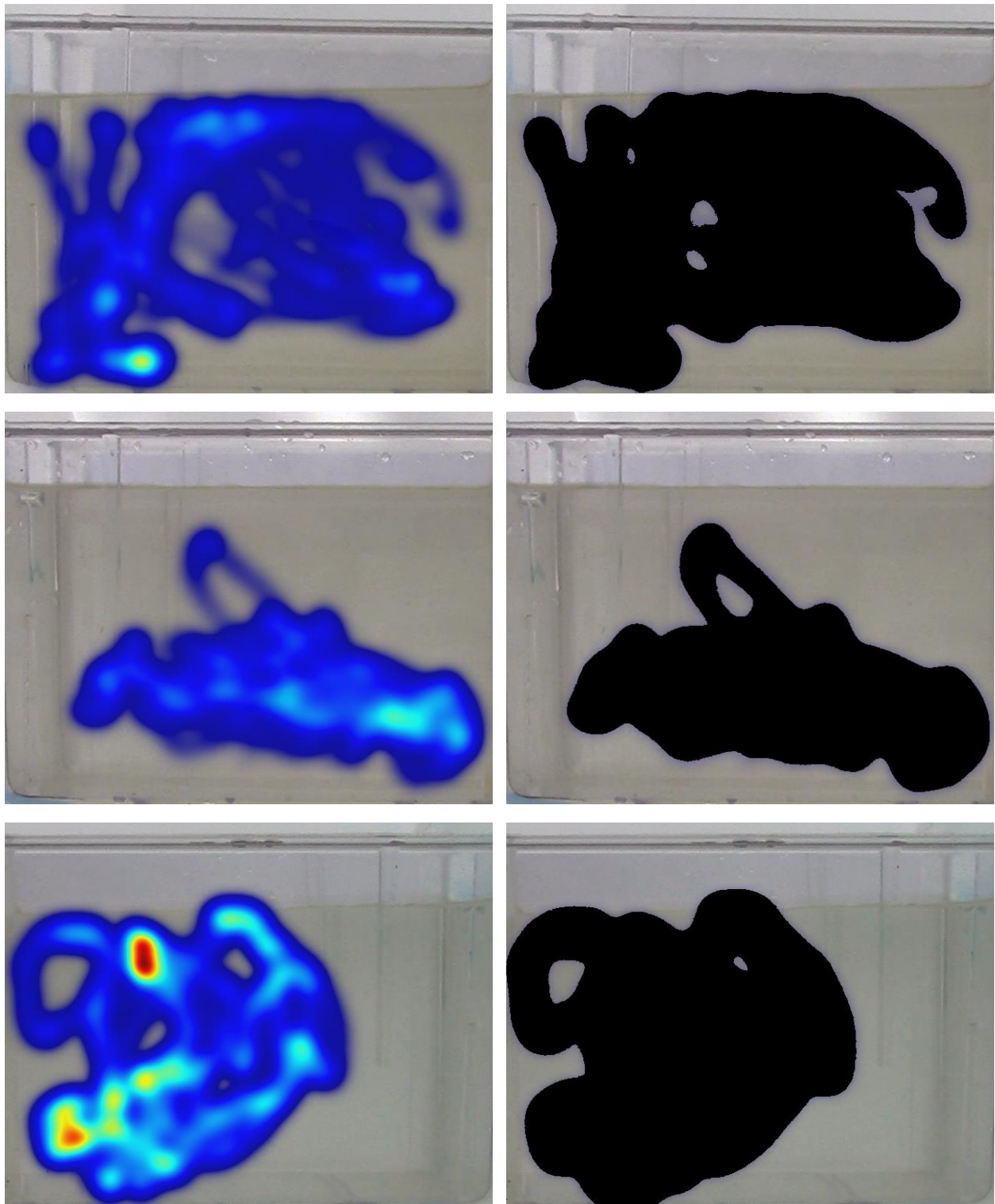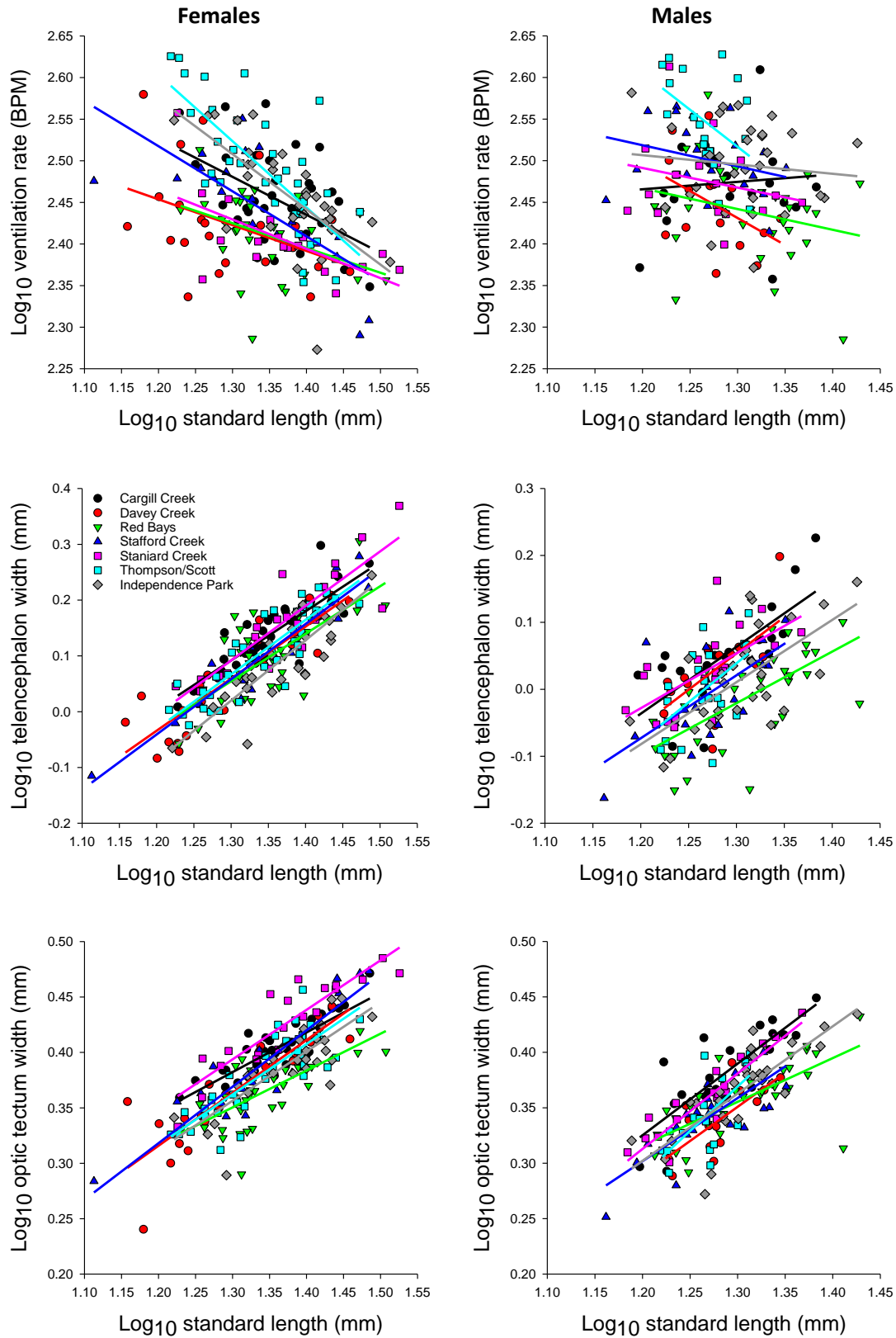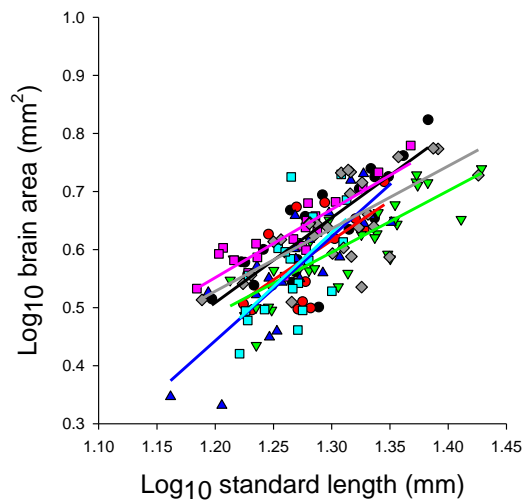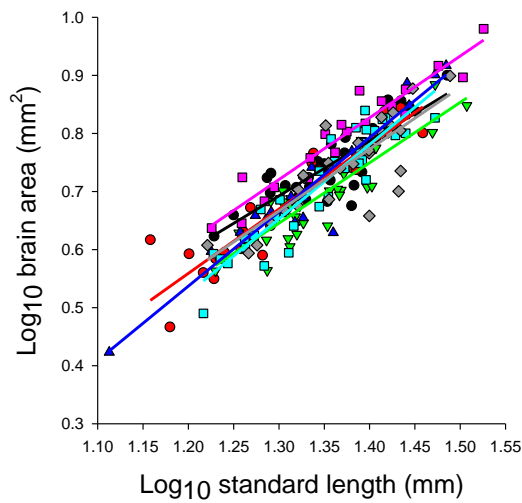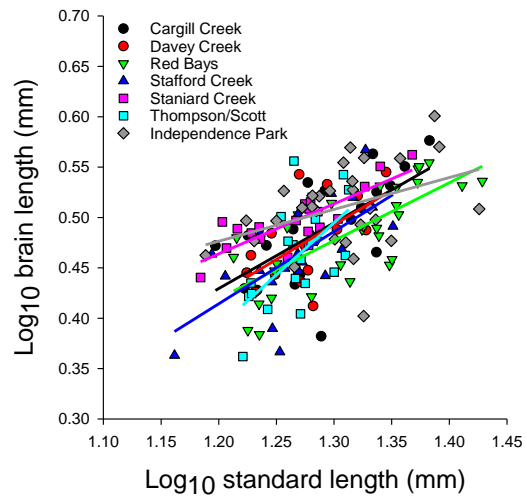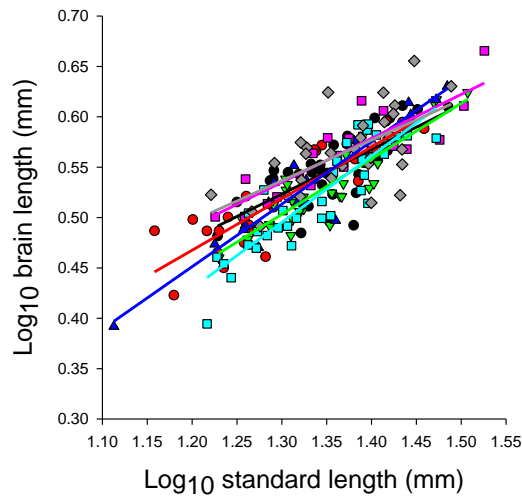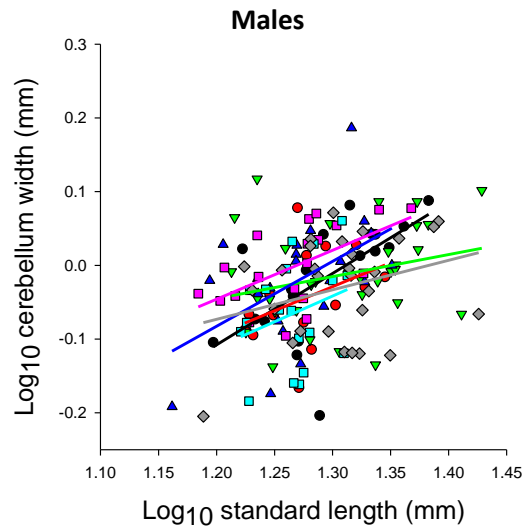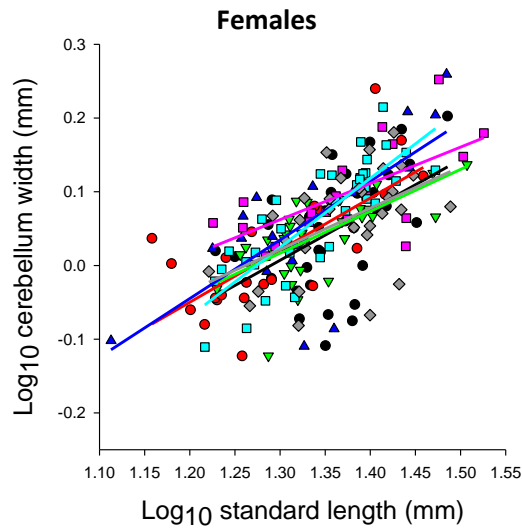
FIGURE S2 Linear regressions depicting the relationships between body size the six size-associated traits for each population in females (left) and males (right).

Females / **Males**

Legend:
- Cargill Creek
- Davey Creek
- Red Bays
- Stafford Creek
- Staniard Creek
- Thompson/Scott
- Independence Park

APPENDIX S1 R code for model selection and model averaging analyses.

```
library(MuMIn)
#Full Models
Dist<-lm(Dist ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Top<-lm(Top ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Area<-lm(Area ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Meander<-lm(Meander ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag
+ Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Vent<-lm(Vent ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
BL<-lm(BL ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
BA<-lm(BA ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Telen<-lm(Telen ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Optic<-lm(Optic ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
Cereb<-lm(Cereb ~ Mangrove + Rock + HabDiv + Frag + Sex + Sex*Frag +
Sex*Mangrove + Sex*Rock + Sex*HabDiv, data=data, na.action=na.pass)
#Gather models sorted by AICc
selected.models.Dist<-dredge(Dist, rank="AICc")
selected.models.Top<-dredge(Top, rank="AICc")
selected.models.Area<-dredge(Area, rank="AICc")
selected.models.Meander<-dredge(Meander, rank="AICc")
selected.models.Vent<-dredge(Vent, rank="AICc")
selected.models.BL<-dredge(BL, rank="AICc")
selected.models.BA<-dredge(BA, rank="AICc")
selected.models.Telen<-dredge(Telen, rank="AICc")
selected.models.Optic<-dredge(Optic, rank="AICc")
selected.models.Cereb<-dredge(Cereb, rank="AICc")
#Save AICc info
capture.output(selected.models.Dist,file="Dist models.txt")
capture.output(selected.models.Top,file="Top models.txt")
capture.output(selected.models.Area,file="Area models.txt")
capture.output(selected.models.Meander,file="Meander models.txt")
capture.output(selected.models.Vent,file="Vent models.txt")
capture.output(selected.models.BL,file="BL models.txt")
capture.output(selected.models.BA,file="BA models.txt")
capture.output(selected.models.Telen,file="Telen models.txt")
capture.output(selected.models.Optic,file="Optic models.txt")
capture.output(selected.models.Cereb,file="Cereb models.txt")
#Model Averaging
average.models.Dist<-model.avg(get.models(selected.models.Dist, subset
= delta <= 2), res.var=TRUE)
average.models.Top<-model.avg(get.models(selected.models.Top, subset =
delta <= 2), res.var=TRUE)
```

```
average.models.Area<-model.avg(get.models(selected.models.Area, subset
= delta <= 2), res.var=TRUE)
average.models.Meander<-model.avg(get.models(selected.models.Meander,
subset = delta <= 2), res.var=TRUE)
average.models.Vent<-model.avg(get.models(selected.models.Vent, subset
= delta <= 2), res.var=TRUE)
average.models.BL<-model.avg(get.models(selected.models.BL, subset =
delta <= 2), res.var=TRUE)
average.models.BA<-model.avg(get.models(selected.models.BA, subset =
delta <= 2), res.var=TRUE)
average.models.Telen<-model.avg(get.models(selected.models.Telen,
subset = delta <= 2), res.var=TRUE)
average.models.Optic<-model.avg(get.models(selected.models.Optic,
subset = delta <= 2), res.var=TRUE)
average.models.Cereb<-model.avg(get.models(selected.models.Cereb,
subset = delta <= 2), res.var=TRUE)
#Save model averaging summaries
capture.output(summary(average.models.Dist),file="Dist model
average.txt")
capture.output(summary(average.models.Top),file="Top model
average.txt")
capture.output(summary(average.models.Area),file="Area model
average.txt")
capture.output(summary(average.models.Meander),file="Meander model
average.txt")
capture.output(summary(average.models.Vent),file="Vent model
average.txt")
capture.output(summary(average.models.BL),file="BL model average.txt")
capture.output(summary(average.models.BA),file="BA model average.txt")
capture.output(summary(average.models.Telen),file="Telen model
average.txt")
capture.output(summary(average.models.Optic),file="Optic model
average.txt")
capture.output(summary(average.models.Cereb),file="Cereb model
average.txt")
```