



Substrate type induces plastic responses in the craniofacial morphology of a winnowing cichlid

Michelle C. Gilbert  · Sofia N. Piggott ·
R. Craig Albertson

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Abstract Understanding how local populations respond to specific changes in the environment can help us better predict how populations respond to such change. With this topic in mind, we followed up on a previous study by exploring the capabilities of a Geophagini cichlid, known for its unique feeding strategy, to mount a plastic response. We exposed *Satanoperca daemon*, a winnowing cichlid, to three different substrate types, two of which encouraged winnowing behaviors and a third that prevented winnowing entirely. Using geometric morphometrics, we quantified aspects of craniofacial anatomy to test for morphological differences between the treatments and to test for the integration of different traits across the head. We found significant differences across our experimental populations in both shape and disparity. We report evidence in support of wide-spread integration across craniofacial traits. A notable exception

to this pattern was the epibranchial lobe, a structure unique to the Geophagini, which exhibited more modular variation. Since anthropogenic alterations such as the damming of rivers can impact substrate type, these data offer insights into how Geophagini cichlids may respond to environmental change. In addition, this work further illuminates the functional morphology of winnowing foraging behaviors.

Keywords Plasticity · Morphology · Geometric · Morphometrics · Novelty

Introduction

The Cichlidae contain an enormous diversity of fishes that have exploited different niches, environments, and behaviors. Cichlids are distributed across Africa, South America, India, and Madagascar and have been the focus of study for decades, becoming a modern model for understanding complex evolutionary and ecological processes and for digging into evo-devo concepts (Muschick et al., 2012; Arbour & López-Fernández, 2013; Feilich, 2016; McGee et al., 2016; Powder & Albertson, 2016; Navon et al., 2020). Much of the work regarding adaptive radiations has focused on African cichlids (Turner, 2007), undoubtedly due to the nature of the rift lake system. However, it has been shown that South American cichlids have also gone through rapid radiation events, most

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M. C. Gilbert (✉)
Organismic and Evolutionary Biology Graduate Program,
University of Massachusetts, Amherst, MA 01003, USA
e-mail: michellecgilbert@outlook.com

S. N. Piggott · R. C. Albertson
Biology Department, Morrill Science Center, University
of Massachusetts, Amherst, MA 01003, USA

notably within the Geophagini clade (López-Fernández et al., 2013; Burress et al., 2018, 2022).

Plasticity in cichlids has been extensively noted and includes numerous aspects of craniofacial morphology (Chapman et al., 2000; Muschick et al., 2011). Because of this, plasticity has become incorporated as a key concept of how the diversity of cichlids has arisen, particularly through the feeding apparatus representing a flexible stem (West-Eberhard, 1989, 2003). This extends to the South American cichlids where plasticity has been demonstrated in the Geophagini cichlid genus *Geophagus* (Wimberger, 1991, 1992, 1993). Here, through a series of experiments, Wimberger was able to induce a plastic response in two species (*Geophagus steindachneri* and *G. brasiliensis*) by changing the prey available to experimental populations, thereby altering the predatory approach of the different populations. The Geophagini are so named (i.e., “earth eating”) due to their unique behavior of winnowing, a process of taking in mouthfuls of sediment and sifting out detritus and invertebrates. Distinctive to this group is a highly modified epibranchial lobe on the first gill arch that is speculated to have evolved to facilitate winnowing (López-Fernández et al., 2012; Weller et al., 2022), although its exact kinematic role remains elusive.

The epibranchial lobe possesses some degree of variation tied to diet and ecomorphology (López-Fernández et al., 2012). However, it is unknown how specific aspects of the morphology of the epibranchial lobe associate with different substrates and/or prey items. Further, the degree to which the epibranchial lobe is plastic remains largely unknown, as winnowing was not explored in early experiments by Wimberger (1991, 1992, 1993). Previously published work detailed significant morphological change in the winnowing cichlid species *Satanoperca jurupari* (Heckel 1840) and *Geophagus neambi* (Lucinda, Lucena and Assis 2010) following the damming of a major river system in Brazil, suggesting that radical environmental alterations had a substantial effect on the (external) cranial morphology of this species (Gilbert et al., 2020).

Because the alteration of a riverine system to a lacustrine system can substantially alter sediment type (Trautman, 1939; Allan & Flecker, 1993; Lau et al., 2006; Helfman, 2007), we sought to understand if different substrates (i.e., rock, sand, or substrate-free) could induce a plastic morphological change

in a winnowing cichlid. Specifically, we exposed the winnowing species, *Satanoperca daemon* (Heckel 1840), to alternate substrate types and quantified morphological variation across a suite of foraging-related traits. We predicted that the population exposed to larger sediments would develop deeper heads, larger muscles, and altered oral cavities, including the epibranchial lobes, to sift through the heavy, coarse substrate. Next, we tested for correlations between various craniofacial traits to identify sets of traits that exhibited an integrated plastic response. We predicted that functionally related traits, such as the oral jaws and *adductor* muscles as well as the oral cavity and epibranchial lobes, would covary. Together, these results will aid in discerning how winnowing species may adapt their feeding architecture in response to rapid environmental change, such as damming. In addition, levels of phenotypic integration across various aspects of foraging anatomy, may provide further insights into the kinematics of winnowing behavior including roles for the novel epibranchial lobe.

Methods

Experimental design

We obtained wild caught *Satanoperca daemon* juveniles from local importers who specialized in acquiring fishes from the Central and South Americas. Upon arrival, we parsed fish into one of three different 40 gallon aquaria. To evaluate the effect of substrate composition on winnowing morphology, we modified the substrate in each tank. Tank one ($n=15$) contained no substrate and fish were fed twice daily with bloodworms provided on the surface of the water. The lack of substrate in this tank prevented winnowing from occurring at all, even when food was absent. Tank two ($n=17$) possessed sand substrate, fish were fed twice daily with bloodworms that had been covered with sand in a 2" deep Pyrex dish. Tank three ($n=11$) possessed a substrate comprised of aquarium gravel and fish were fed twice daily with bloodworms that had been covered with identical gravel in a 2" deep Pyrex dish. Since *Satanoperca* had been previously observed to winnow casually, even outside of feeding hours, tanks two and three had substrate in their tanks continuously to reinforce winnowing behaviors even while food was

absent. However, tank one never possessed substrate of any kind to prevent the mechanical process of winnowing entirely. This experiment was kept active for 27 weeks and were fixed following MS-222 (Tricaine methane sulfonate) overdose coupled with ice shock. The body size (standard length) ranged from 5 cm to about 9 cm.

Specimen preparation and data collection

Fixed fish were stained using an alizarin solution in 75% EtOH to visualize osteological elements but preserve muscle integrity and morphology for data acquisition. We then carefully dissected all specimens to reveal the *adductor mandibularis Pars malaris* (A1) and *Par rictalis* (A2), and subsequent layers of muscles and bone were systematically removed, and collected, to ensure imagery of the dentary, angulo-articular, lachrymal, first epibranchial, and buccal cavity (including parasphenoid and hyoglossal—pharyngeal jaws; Fig. 1; Davoto & Vari, 2013).

We collected two-dimensional geometric morphometric data for the structures using *Stereomorph* v1.6.7 (Olsen & Westneat, 2015) in *R* (R Core Team, 2018). These two-dimensional morphometrics allowed us to encapsulate relevant shape data in a planar fashion. This collection method enabled us to analyze the craniofacial region in descending levels and keep the planes of analysis consistent to create an overarching view of this region of interest. In total, we collected morphometric data across 7 unique image sets. For *adductor mandibularis* and the orbit, five fixed and 24 semi-landmarks, 12 of which formed a continuous curve. Lachrymal, 10 fixed and six semi-landmarks. Anguloarticular, four fixed and nine semi-landmarks. Quadrate, four fixed landmarks and eight semi-landmarks. Dentary, six fixed and 12 semi-landmarks. For the first gill arch, including the epibranchial, four fixed and 10 semi-landmarks. For the oral cavity, six fixed and 10 semi-landmarks. See Fig. 2.

Geometric morphometric analyses

Unless otherwise stated, all analyses here were conducted using *geomorph* v3.3.6 (Adams et al., 2014, 2018). All geometric morphometric data were initially put through a generalized Procrustes analysis (GPA; Goodall, 1991) utilizing bending energy in

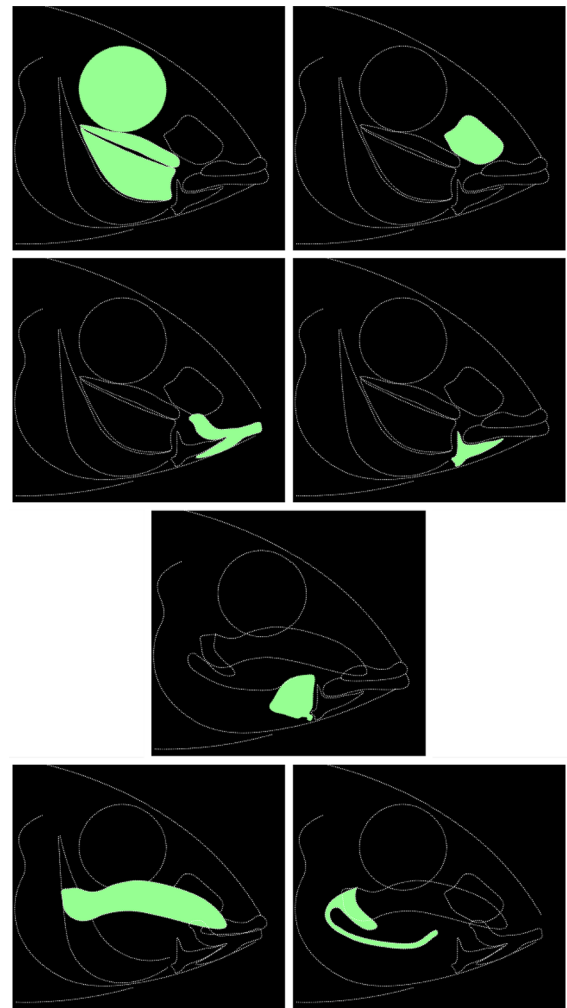
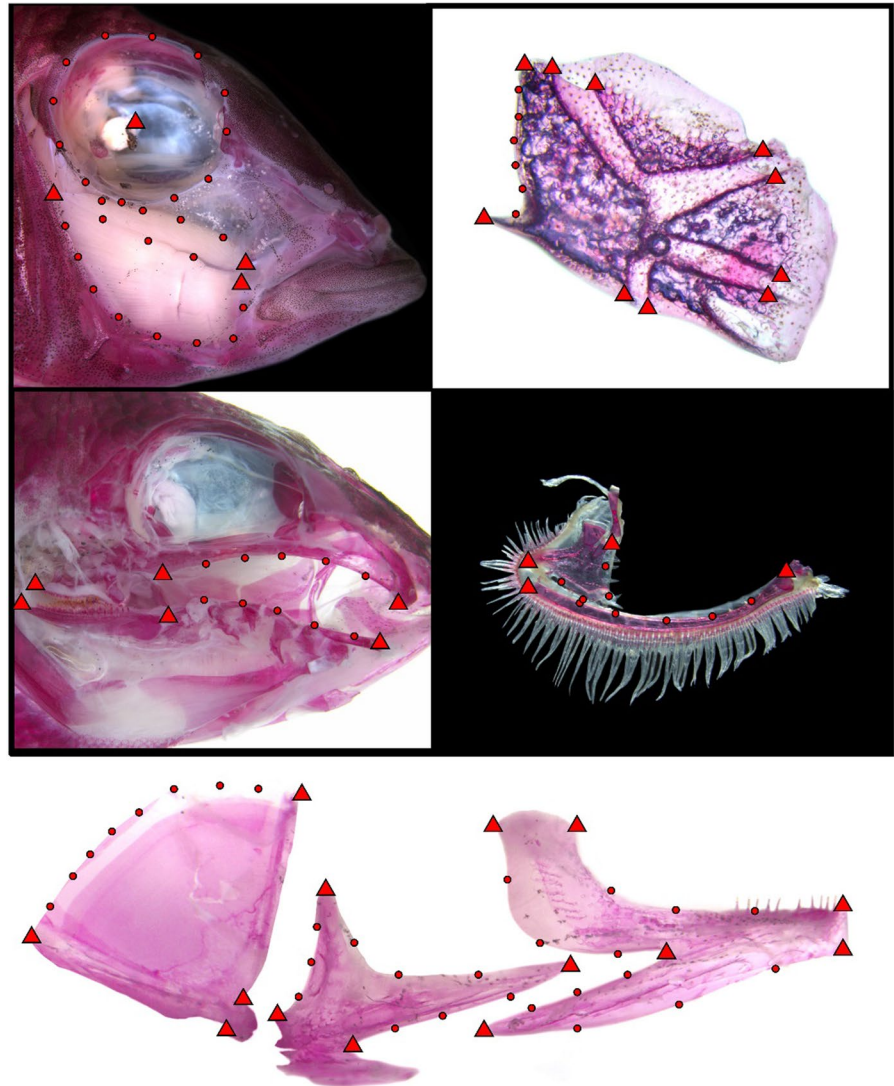


Fig. 1 Graphical illustration of the various aspects of anatomy that were assessed in this study. **A** Orbit and *Adductor mandibularis*, **B** Lacrymal, **C** Dentary, **D** Anguloarticular, **E** Quadrate, **F** Oral cavity, **G** First gill arch and epibranchial lobe

geomorph (gpagen). Using a Procrustes ANOVA, we tested for differences in mean shape between treatment groups within each GM dataset [e.g., (shape~centroid size+treatment)] using an RRPP procedure (Collyer & Adams 2018) and 9999 + 1 iterations. We then regressed PC1 values against centroid size to visualize differences in allometry across the seven unique image sets. Branching from the initial Procrustes ANOVA, we conducted post-hoc pairwise comparisons using the function `advanced.procD.lm` in *geomorph*. Tests of morphological disparity were conducted using the function `morphol.disparity` in *geomorph*. Lastly, using the

Fig. 2 Landmark configurations for each aspect of anatomy that we digitized, excluding whole body morphology. Triangles represent fixed, circles represent semi-landmarks



geomorph function two.b.pls (Rohlf & Corti, 2000; Adams & Collyer, 2016, 2019), we tested for correlations between each of the different anatomical elements included in this study [e.g., (two.b.pls (shape 1, shape 2, iter=9999)]. All post-hoc tests were adjusted by calculating a Bonferroni *p*-value using the function *p.adjust* in *R* (R Core Team, 2018).

Results

Foraging environment induces mean shape differences for numerous traits

Our feeding treatments had a significant effect on 5/7 anatomical components investigated (Table 1), the two exceptions being the anguloarticular ($p=0.138$) and quadrate ($p=0.986$). When compared to the *z*-score of centroid size, the effect of substrate had the greatest influence on the oral cavity ($z=3.245$ compared to CS, $z=1.957$), whereas it had the lowest effect on the quadrate ($z=-2.089$ compared to CS, $z=2.946$). Centroid size was

Table 1 Results of Procrustes ANOVA across all eight anatomical elements testing for the effect of treatment or centroid size

	RSq	z	p
AM, O			
CS	0.2471	4.9481	0.0001
Tr	0.0928	3.5694	0.0003
CS * Tr	0.0797	2.4236	0.0074
LAC			
CS	0.2942	5.1016	0.0001
Tr	0.0388	1.6197	0.0528
CS * Tr	0.0365	0.5528	0.2914
AA			
CS	0.1152	3.1433	0.0003
Tr	0.0627	1.0924	0.1380
CS * Tr	0.0519	0.6827	0.2500
D			
CS	0.1488	3.8882	0.0001
Tr	0.0720	2.2501	0.0064
CS * Tr	0.0487	0.7844	0.2182
Q			
CS	0.1378	2.9465	0.0016
TR	0.0112	−2.0894	0.9856
CS * Tr	0.0232	−0.9159	0.8184
GA			
CS	0.0628	1.6573	0.0379
Tr	0.1027	1.8120	0.0255
CS * Tr	0.0560	0.7128	0.2435
OC			
CS	0.0555	1.9570	0.0187
Tr	0.1473	3.2450	0.0004
CS * Tr	0.0348	−0.1463	0.5525

AM, O *Adductor mandibularis* and orbit, LAC lachrymal, AA anguloarticular, D dentary, Q quadrate, GA first gill arch and epibranchial lobe, OC oral cavity

For significance testing, $\alpha=0.05$

found to be significant in all treatments, but the interaction of centroid size and treatment was significant only in the orbit plus *adductor mandibulae* (Table 1). A plot of allometry (Fig. 3) shows a combination of differences, suggesting that the allometries of various aspects of craniofacial anatomy differ in shape depending on the treatment. For example, allometries of the three different treatments appear parallel to one another in both the lachrymal and dentary (relatively so for the quadrate), but the rock treated population diverges from

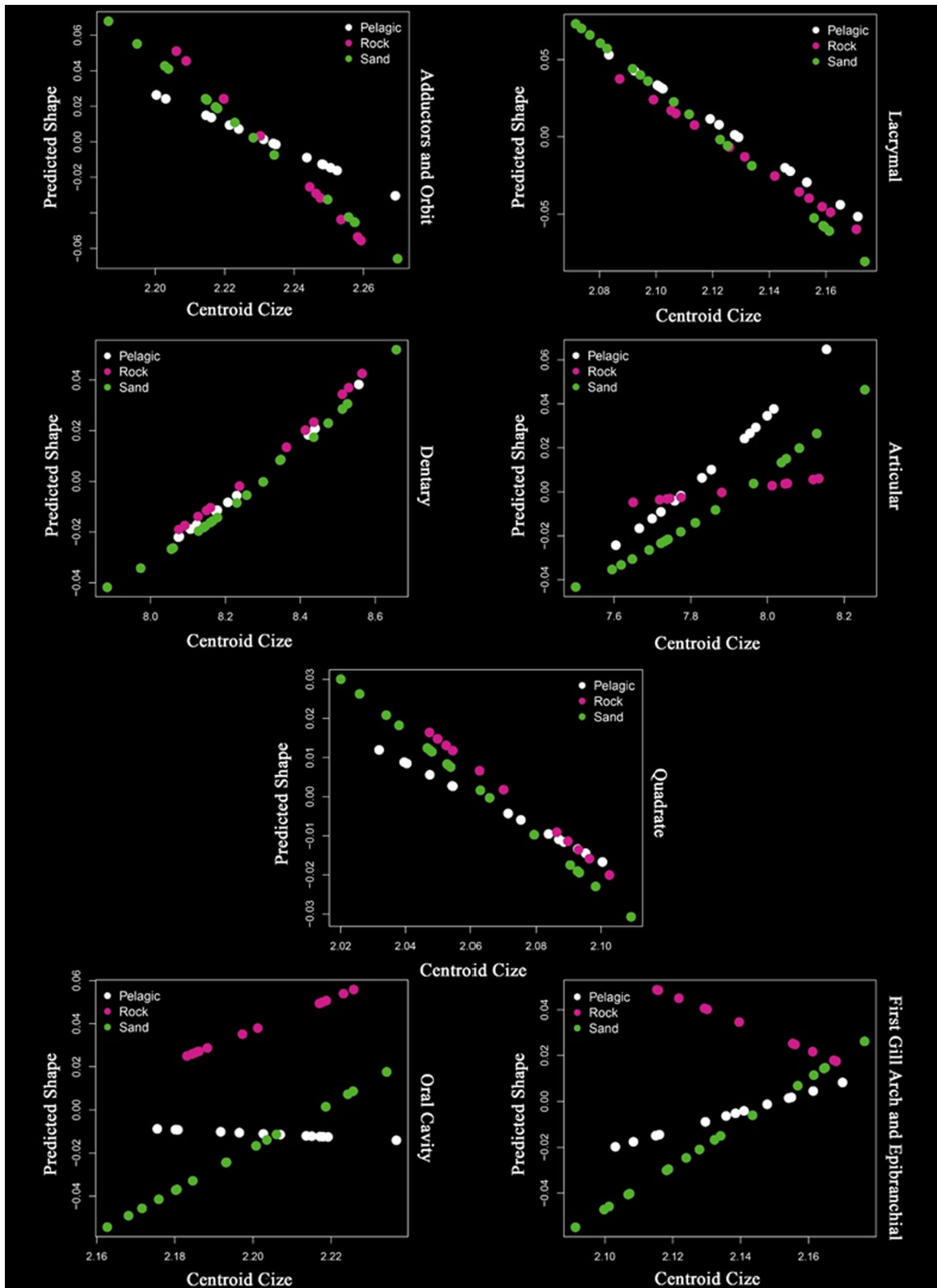
this in the articular. Conversely, the pelagic treated fish diverge from the parallel allometries in the orbit plus *adductor mandibulae* and none of treatments appear to have parallel allometries for the oral cavity and first gill arch plus epibranchials.

Post-hoc pairwise comparisons (Table 2) showed pelagic treated fish differed significantly from both sand and rock treated populations ($p=0.013$, 0.001 respectively; rock only following adjustment, $p=0.0077$) in orbit plus *adductor mandibulae* shapes. Pelagic treated fish also differed in both dentary and oral cavity shapes when compared to rock treated fish ($p=0.018$, 0.002 ; oral cavity only following adjustment, $p=0.0112$). Fish from the rock and sand treatment differed in oral cavity and gill arch shapes ($p=0.0012$, 0.0426 ; oral cavity only following adjustment, $p=0.0084$).

Deformation grids of substrate groups showed that our sand winnowing population often expressed an intermediate phenotype compared to pelagic feeding and rock winnowing fish (Fig. 4). Overall, the rock winnowing population was characterized by larger *adductor* muscles relative to the orbit, a gracile coronoid process, truncated but stout epibranchial lobes, and a steeply curved parasphenoid and ventrally shifted hyoid series creating a relatively large oral cavity. The pelagic feeding population is characterized by reduced *adductor* muscles relative to the orbit, a somewhat robust coronoid process, elongated epibranchial lobes, and a substantially smaller oral cavity.

Foraging environment induces few differences in morphological disparity between populations

Across all seven anatomical components, only two pairwise comparisons (both involving the lachrymal) showed significantly different levels of disparity (Table 3). The sand winnowing population had significantly more variance ($\sigma^2=0.00467$) in lachrymal shape than both the pelagic feeding population ($\sigma^2=0.00261$, $p=0.0161$) and rock winnowing population ($\sigma^2=0.00285$, $p=0.0418$). However, following a Bonferroni adjustment, no comparisons were found to be statistically significant with $\alpha=0.05$.



◀**Fig. 3** Regressions of PC1 values (Predicted shapes) against centroid size for each of the seven image sets. Dark green represents the sand population, dark pink represents rock population, and white represents the pelagic population

Orbit plus adductor shape is correlated with numerous other traits whereas the epibranchial lobe is largely modular

We implemented numerous partial least squares (PLS) tests to assess the degrees of integration in plasticity between each aspect of anatomy (Table 4, Fig. 5). Because quadrate shape was statistically the same across all three test populations, this component was not included in the PLS assay. Of the 15 PLS tests, 7 revealed a significant correlation. The orbit/adductor mandibulae had the greatest number of significant shape correlations (4/5 tests being significant before and after Bonferroni adjustment). Of these, the strongest correlation, and the strongest correlation of all trait comparisons, was with the lachrymal ($R^2=0.6727$, $p<0.0001$). The trait that had the fewest significant shape correlations was the first gill arch including the epibranchial lobe, which only correlated with oral cavity shape ($R_2=0.2972$, $p=0.022$; no significant correlation post Bonferroni adjustment).

Discussion

Substrate size induces plasticity in winnowing cichlid anatomy

The results of morphological analyses on multiple elements of craniofacial anatomy suggest that different substrate composition alone is sufficient to induce plastic responses in various aspects of feeding morphology in the winnowing cichlid *Satanoperca daemon*. Winnowing, a behavior that utilizes suction feeding to bring large amounts of sediment into the oral cavity, parsing of sediments and food items, and subsequent ejection of sediment (Weller et al., 2017) is an underexplored feeding strategy. While common among the Geophagini cichlids, it is rare across the Cichlidae family. Comparisons of mean morphological shape across three different foraging environments show that winnowing in rocky sediments induces the most drastic changes. Of six significant pairwise

differences in mean shape, five involved the rock treatment. Only a single pairwise comparison, orbit plus muscles, was significant for sand vs pelagic treatments. Thus, if we consider plasticity to be a proxy for kinematic demands, rock-winnowing appears to be the most challenging treatment, whereas sand-winnowing and pelagic foraging seem to impose similar demands on the feeding apparatus.

Lab-based results are similar to patterns of morphologic change following a major anthropogenic disturbance

The construction of the Tucuruí Hydroelectric Dam in 1984 introduced numerous changes in the system (Araújo-Lima et al., 1995; Eletrobrás/Dnaee, 1997; Fearnside, 2001), altering it from a riverine to lacustrine that included shifts in sediment composition (Trautman, 1939; Allan & Flecker, 1993; Lau et al., 2006; Helfman, 2007). Our previous work in this system documented statistically significant differences in the gross morphology, pre- and post-dam construction, within two Geophagini cichlids, *Geophagus neambi* and *Satanoperca jurupari* (Gilbert et al., 2020). We speculated that changes in sediment, from rocky to silty, had influenced the morphology of the Geophagini, possibly through phenotypic plasticity. It is also possible that a deeper reservoir habitat would provide greater opportunity for pelagic modes of feeding (e.g., ram/suction). Here, we show that substrate type alone (including substrate-free habitat) is indeed sufficient to induce morphological change in a closely related winnowing cichlid. It is therefore possible that the changes in morphology that we reported in 2020 were, at least in part, due to alterations of the substrate. However, we did not previously report on specific elements of craniofacial anatomy and next steps should work to directly compare anatomical elements of specimens from the two studies. The trend of effectively shifting mean shape values in phenotypic space but keeping interpopulation variation stable was also consistent with our previous paper (Gilbert et al., 2020), where we reported differences in whole body shape means, but not disparity, among cichlid species.

Table 2 Pairwise comparisons of shape across treatment groups

Ini	Pelagic	Rock	Sand	Pelagic	Rock	Sand
	AM, O			Lac		
Pelagic		4.0063	2.7111		0.5908	0.7973
Rock	0.0011		−0.1117	0.2516		0.2988
Sand	0.0133	0.4916		0.2047	0.3425	
	AA			D		
Pelagic		0.3396	1.4616		2.3468	0.7017
Rock	0.3363		0.1548	0.018		1.6532
Sand	0.0876	0.398		0.2368	0.0604	
	Q			GA		
Pelagic		−1.2756	−1.6939		1.5764	1.2330
Rock	0.9466		−0.9756	0.0755		1.9564
Sand	0.9944	0.8584		0.1219	0.0426	
	OC					
Pelagic		3.76857	1.4429			
Rock	0.0016		3.88693			
Sand	0.0892	0.0012				
Adj	<i>Pelagic</i>	<i>Rock</i>	<i>Sand</i>	<i>Pelagic</i>	<i>Rock</i>	<i>Sand</i>
	AM, O			Lac		
Pelagic		4.0063	2.7111		0.5908	0.7973
Rock	0.0077		−0.1117	1.0000		0.2988
Sand	0.0931	1.0000		1.0000	1.0000	
	AA			D		
Pelagic		0.3396	1.4616		2.3468	0.7017
Rock	1.0000		0.1548	0.1260		1.6532
Sand	0.6132	1.0000		1.0000	0.4228	
	Q			GA		
Pelagic		−1.2756	−1.6939		1.5764	1.2330
Rock	1.0000		−0.9756	0.5285		1.9564
Sand	1.0000	1.0000		0.8533	0.2982	
	OC					
Pelagic		3.76857	1.4429			
Rock	0.0112		3.88693			
Sand	0.6244	0.0084				

Z scores above, *p* values below diagonal. Abbreviations same as Table 1. *Ini* in the top left of mirrored tables indicates *P* values prior to Bonferroni adjustment, *Adj* indicates *p* values following said adjustment. For significance testing, $\alpha = 0.05$

Functional implications of plastic changes

Morphological plasticity in fishes, especially cichlids, has been heavily documented (Chapman et al., 2000; Alexander & Adams, 2004; Lema & Nevitt, 2006; Muschick et al., 2011) and others have reported plasticity in related taxa, such as *Geophagus brasiliensis* and *steindachneri* (Wimberger, 1991, 1992). To our knowledge, this is the first report of sediment-induced morphological plasticity in a winnowing cichlid. Understanding the specific mechanical forces that have precipitated these changes will be an important

topic for future study. However, the patterns of variation (and covariation) observed here lead to a number of predictions concerning the functional morphology of winnowing.

The differences observed in morphology are likely related to the effort required to suction and process substrates of radically different size. *Satanoperca* that winnowed rock, compared to the non-winnowers (pelagic), had substantially larger *adductor* muscles, larger buccal cavities, and parasphenoids with a higher arch. These traits could

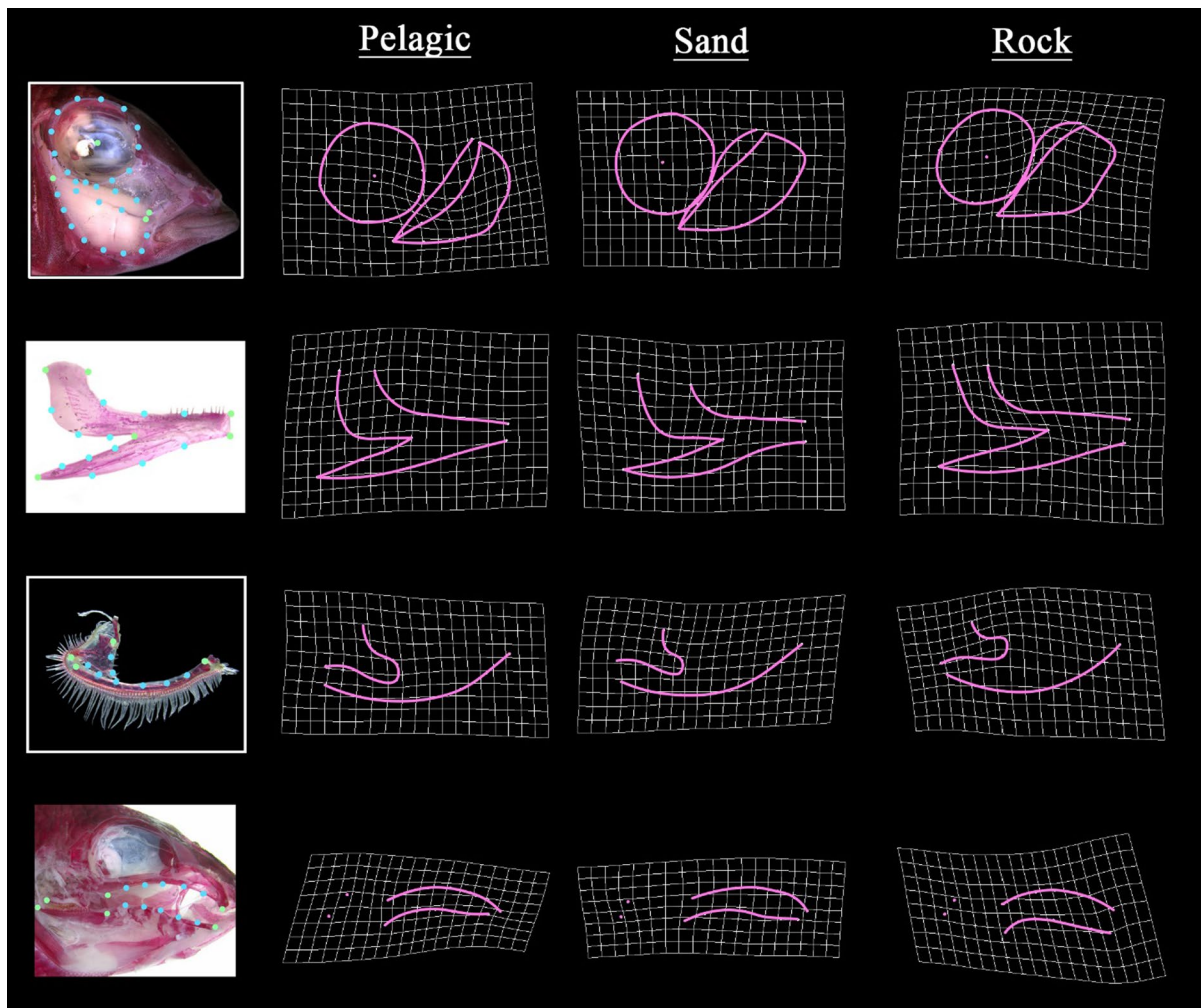


Fig. 4 Deformation grid matrix of the three different feeding treatments. Graphical examples of traits in the left column. Curves are outlined on deformation grids in pink, fixed landmarks not associated with curves are represented by pink

circles. Lachrymal, anguloarticular, and quadrate were not included since no significant pairwise comparison existed in our dataset

be associated with the cycling motions of the jaw when winnowing is occurring and room to generate increased water flow and turbulence to parse food items out of substrate (Van Wassenbergh et al., 2015; Brooks et al., 2018; Weller et al., 2022). Further, fish from both the sand and pelagic treatments had relatively wider coronoid processes of the dentary, compared to fish that winnowed rock. Since this process is where the ligament that connects the upper and lower jaws inserts, we speculate that a wider insertion point is due to forces

being propagated to the coronoid during jaw protrusion/suction feeding, consistent with suction being an important first step in winnowing fine (but not coarse) substrate (Weller et al., 2017). Winnowers had shorter epibranchial lobes, with more room between the lobe itself and the rest of the gill arch, compared to non-winnowing fish. This is intuitive, given that this is the area where food items are being parsed from substrate. Anecdotal evidence in support of direct contact between substrate and the epibranchial lobe includes the observation that rock winnowing cichlids possessed gill rakers that were

Table 3 Pairwise comparisons of shape disparity across treatment groups

Ini	Pelagic	Rock	Sand	Pelagic	Rock	Sand
	<i>AM, O</i>			<i>Lac</i>		
Pelagic		0.00004	0.00072		0.00024	0.00206
Rock	0.9281		0.00067	0.8105		0.00181
Sand	0.1082	0.1661		0.0161	0.0418	
	<i>AA</i>			<i>D</i>		
Pelagic		0.00193	0.00111		0.00011	0.00036
Rock	0.0559		0.00083	0.8539		0.00047
Sand	0.2442	0.4148		0.5291	0.435	
	<i>Q</i>			<i>GA</i>		
Pelagic		0.00023	0.00012		0.00043	0.00058
Rock	0.4250		0.00036	0.7722		0.00101
Sand	0.6487	0.1911		0.6789	0.4692	
	<i>OC</i>					
Pelagic		0.00064	0.00226			
Rock	0.706		0.00161			
Sand	0.0816	0.2673				
Adj	Pelagic	Rock	Sand	Pelagic	Rock	Sand
	<i>AM, O</i>			<i>Lac</i>		
Pelagic		0.00004	0.00072		0.00024	0.00206
Rock	1.0000		0.00067	1.0000		0.00181
Sand	0.7574	1.0000		0.1127	0.2926	
	<i>AA</i>			<i>D</i>		
Pelagic		0.00193	0.00111		0.00011	0.00036
Rock	0.3913		0.00083	1.0000		0.00047
Sand	1.0000	1.0000		1.0000	1.0000	
	<i>Q</i>			<i>GA</i>		
Pelagic		0.00023	0.00012		0.00043	0.00058
Rock	1.0000		0.00036	1.0000		0.00101
Sand	1.0000	1.0000		1.0000	1.0000	
	<i>OC</i>					
Pelagic		0.00064	0.00226			
Rock	1.0000		0.00161			
Sand	0.5712	1.0000				

Differences in absolute values above, *p* values below diagonal. Abbreviations same as Table 1

|Var| Absolute variance. *Ini* in the top left of mirrored tables indicates *P* values prior to Bonferroni adjustment, *Adj* indicates *p* values following said adjustment, For significance testing, $\alpha = 0.05$

generally more mineralized than those from other treatments (gill raker number did not vary between treatments—data not shown). Finally, we found no correlation in morphology between the first gill arch/epibranchial lobe with the other traits, consistent with a modular plastic response relative to other aspects of the head (Cheverud, 1996; Cheverud et al., 1997; Klingenberg, 2008; Navon et al., 2020).

Modular vs. integrated plasticity and paths of least evolutionary resistance

This work demonstrates how plasticity can facilitate studies of morphological integration, concepts that are not normally considered together (but see Mate-sanz et al., 2021; Earley et al., 2012). For instance, since the study of co-variation (e.g., integration)

Table 4 Pairwise comparisons of partial least squares, testing for correlation between the different anatomical units

Ini	AddMus	Lac	AA	D	EL	OC
AddMus		0.6727	0.4485	0.5756	0.1868	0.4165
Lac	0.000		0.2979	0.3495	0.1790	0.3663
AA	0.001	0.072		0.3185	0.1359	0.2410
D	0.000	0.048	0.075		0.1411	0.2412
EL	0.398	0.377	0.595	0.835		0.2972
OC	0.007	0.010	0.167	0.361	0.022	
Adj	AddMus	Lac	AA	D	EL	OC
AddMus		0.6727	0.4485	0.5756	0.1868	0.4165
Lac	0.001		0.2979	0.3495	0.1790	0.3663
AA	0.007	0.429		0.3185	0.1359	0.2410
D	0.001	0.287	0.450		0.1411	0.2412
EL	1.000	1.000	1.000	1.000		0.2972
OC	0.041	0.061	1.000	1.000	0.134	

R^2 above, p values below diagonal

Var1 Absolute variance. *Ini* in the top left of mirrored tables indicates p values prior to Bonferroni adjustment, *Adj* indicates p values following said adjustment. For significance testing, $\alpha = 0.05$

relies on there being sufficient levels of variation (Gonzalez et al., 2011a,b; Hallgrímsson et al., 2002), and plasticity can produce novel sources of variation (West-Eberhard, 1989, 2003), it can be an effective tool in this realm. Moreover, since craniofacial/skeletal plasticity generally occurs during later life-history stages, its application can help to decouple the forces that may affect morphological integration, developmental versus functional, in particular. Of course, it is important to acknowledge that given the hierarchical nature of organismal development and growth, factors that influence morphological integration during early life-history will “carry-over” into later stages. Hallgrímsson has used a palimpsest metaphor to describe this (Hallgrímsson, 2009), whereby information is repeatedly written and erased from the same tablet but traces remain each time. Thus, anatomical units that participate in the same function are likely to become morphologically integrated, owing to a common load regime, but the strength of integration may also be influenced by a shared developmental history.

Regardless of the source, the subdivision of different biological systems into different modules is hypothesized to facilitate the evolvability of various traits (Wagner et al., 2007; Larouche et al., 2018; Zelditch & Goswami, 2021). Modular traits are often

evolutionarily decoupled (less integrated), allowing the two or more traits to evolve independently to serve multiple, even disparate, functions (Cheverud, 1996). The independence of traits can be explained by the weakening of evolutionary constraints between them (Jacob, 1977; Wagner & Misof, 1993; Klingenberg, 2008; McGhee, 2007; Hallgrímsson et al., 2009; Sheftel et al., 2013). Indeed, it has been hypothesized that the evolution of novel traits is, in part, the result of an increasingly modular system developing (Prum, 2005a; Kuratani, 2009; Niwa et al., 2010; Gilbert et al., 2021). Thus, it is plausible that increasing modularity can lead to increased rates of evolution in lineages, thereby promoting speciation in diverse, complex environments (West-Eberhard, 1989, 2003; Foote, 1997).

Challenging fishes with various foraging environments provides an excellent opportunity to quantify magnitudes of both shape variation and covariation, thereby enabling assessments of phenotypic integration (Conith and Albertson, 2021; Navon et al., 2020). We document various levels of integration across craniofacial traits. On one end of the spectrum, orbit-adductor mandibulae shape is integrated with most other traits, suggesting that these traits are more likely to co-evolve along an evolutionary path of least resistance (Schluter, 1996; Foote, 1997; Conith & Albertson, 2021). Alternatively, the first gill arch including

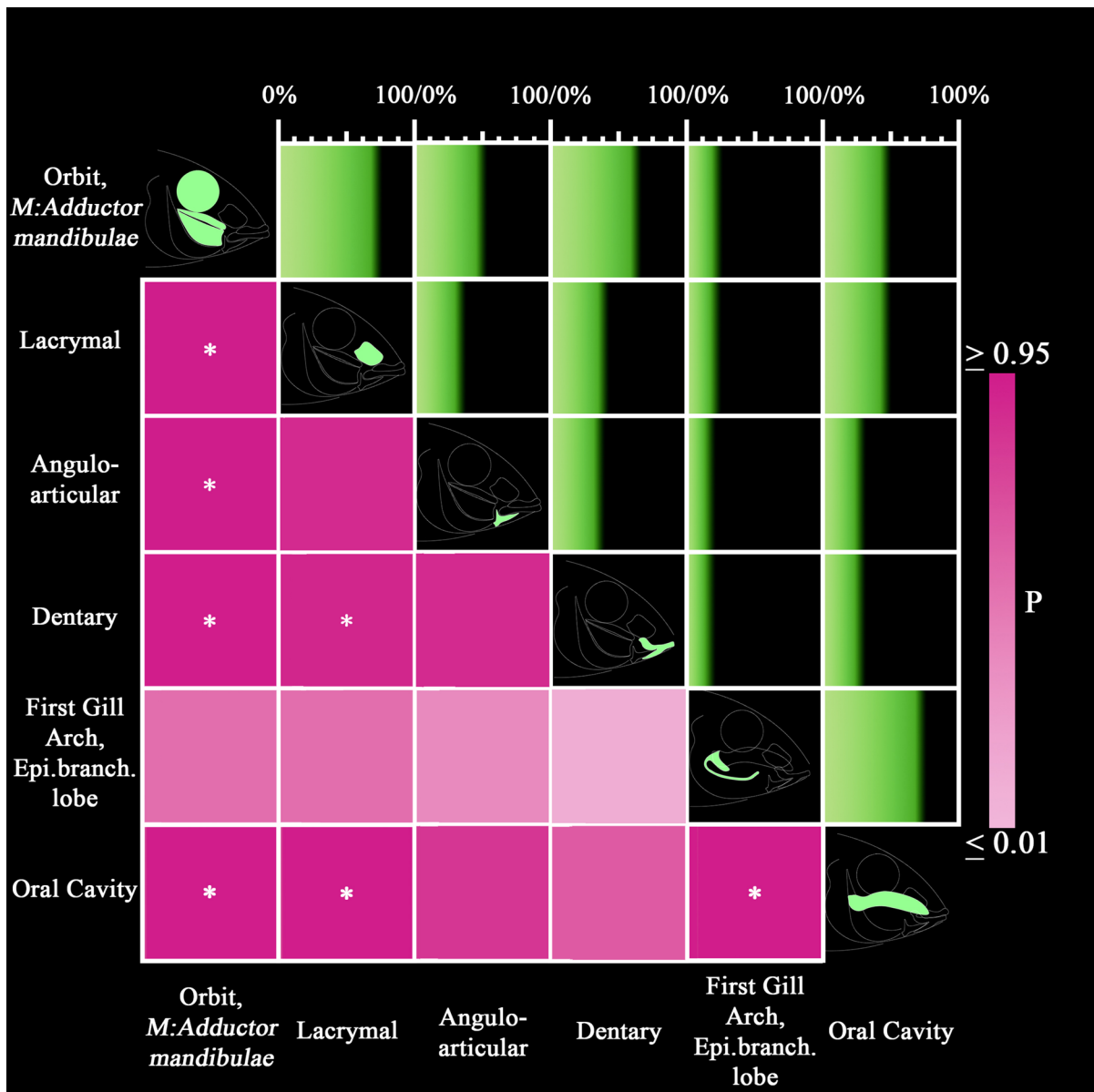


Fig. 5 Graphical scatterplot matrix illustrating the results of various partial least squares tests to assess degrees of correlation between two anatomical units (or complexes). R^2 values are depicted as percentages above the diagonal in green, while

the p values (prior to Bonferroni correction) are below in pink. Darker shades of pink represent increasing confidence (values closer to or beyond 0.05), lighter represents decreasing confidence. Asterisks denote statistical significance ($p \leq 0.05$)

the epibranchial lobe is less integrated with the other aspects of anatomy measured, only covarying with the oral cavity. We hypothesize that the morphological decoupling of the first gill arch from other craniofacial elements and its plasticity have allowed, in part, for the Geophagini to take advantage of a novel niche environment. Given phenotypic plasticity can

lead to the evolution of phenotypic novelties (Moczek, 2008), it is possible that this mechanism facilitated the evolution of the epibranchial lobe (a novelty specific to Geophagini cichlids), and that its decoupled, seemingly modular nature allowed it to be modified without influencing other aspects of the head. Further, we speculate that the retention of plasticity

in the epibranchial lobe paved the way for multifunctionality to evolve in certain lineages, including the co-option of this functional complex for mouthbrooding, without altering other aspects of prey processing, a hypothesis recently posed by Weller et al., (2022).

Conclusion

All in all, we show that a simple, but ecologically relevant, shift in foraging habitat can lead to subtle but widespread shifts in anatomy. These lab-based experiments have the potential to inform patterns observed in nature, both in terms of how species may adapt (or not) to rapid, human-induced, environmental change, as well as the evolutionary origin and diversification of complex phenotypes. Further, we demonstrate that the quantification of phenotypic (co)variation following the introduction of foraging challenges is a useful paradigm for understanding the kinematics of feeding. Finally, we suggest that while winnowing represents a strange and somewhat enigmatic foraging strategy, it holds excellent potential as a study system to draw connections between ecology, development, anatomy, function and evolution.

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Data availability Data publicly available on GitHub at <https://github.com/DrMermaid-MichelleGilbert/Satanoperca-daemon-2022>.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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References

- Adams DC, Collyer ML, Otarola-Castillo E 2014 Geomorph Software for geometric morphometric analysis
- Adams, D. C. & M. L. Collyer, 2016. On the comparison of the strength of morphological integration across morphometric datasets. *Evolution*. 70: 2623–2631.
- Adams, D. C. & M. L. Collyer, 2019. Comparing the strength of modular signal, and evaluating alternative modular hypotheses, using covariance ratio effect sizes with morphometric data. *Evolution*. 73: 2352–2367.
- Adams, D., M. L. Collyer & A. Kaliontzopoulou, 2018. Geomorph: software for geometric morphometric analysis. R Package Version 3: 6.
- Allan, J. D. & A. S. Flecker, 1993. Biodiversity conservation in running waters. *Bioscience* 43: 32–43.
- Alexander, G. D. & C. E. Adams, 2004. Exposure to a common environment erodes inherited between-population trophic morphology differences in Arctic charr. *J. Fish Biol.* 64: 253–257.
- Araújo-Lima C, Agostinho A, Fabré N 1995 Trophic aspects of fish communities in Brazilian rivers and reservoirs. 105–136 in *Limnology in Brazil*. Brazilian Academy of Sciences, Rio de Janeiro
- Arbour, J. H. & H. López-Fernández, 2013. Ecological variation in South American geophagine cichlids arose during an early burst of adaptive morphological and functional evolution. *Proc. R. Soc. B Biol. Sci.* 280: 20130849–20130849.
- Brooks, H., G. E. Haines, M. Carly Lin & S. Laurie Sanderson, 2018. Physical modeling of vortical cross-step flow in the American paddlefish, *Polyodon spathula*. *PLoS One* 13: 1–25.
- Burress, E. D., F. Alda, A. Duarte, M. Loureiro, J. W. Armbruster & P. Chakrabarty, 2018. Phylogenomics of pike cichlids (cichlidae: crenicichla): the rapid ecological speciation of an incipient species flock. *J. Evol. Biol.* 31: 14–30.
- Burress, E. D., L. Piálek, J. Casciotta, A. Almirón & O. Řičan, 2022. Rapid parallel morphological and mechanical diversification of South American pike cichlids (crenicichla). *Syst. Biol.* <https://doi.org/10.1093/sysbio/syac018>.
- Chapman, L. G., F. Galis & J. Shinn, 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecol. Lett.* 3: 387–393.
- Cheverud, J. M., 1996. Developmental integration and the evolution of pleiotropy. *Am. Zool.* 36: 44–50.
- Cheverud, J. M., E. J. Routman & D. J. Irschick, 1997. Pleiotropic effects of individual gene loci on mandibular morphology. *Evolution*. 51: 2006–2016.
- Collyer, M. L. & D. C. Adams, 2018. RRPP: an r package for fitting linear models to high—dimensional data using

- residual randomization. *Methods Ecology Evolution*. 2018: 1772–1779.
- Conith, A. J. & R. C. Albertson, 2021. The cichlid oral and pharyngeal jaws are evolutionarily and genetically coupled. *Nat. Commun.* 12: 1–11.
- Davoto, A. & R. Vari, 2013. The jaw adductor muscle complex in teleostean fishes: evolution, homologies and revised nomenclature (osteichthyes: actinopterygii). *PLoS One*. 8(4): 0060846.
- Earley, R. L., A. F. Hanninen, A. Fuller, M. J. Garcia & E. A. Lee, 2012. Phenotypic plasticity and integration in the mangrove rivulus (*Kryptolebis marmoratus*): a prospectus. *Integrat Comp Biol*. 52(6): 814–827.
- Eletróbrás/Dnaee 1997 Manual de Inventário Hidrelétrico de Bacias Hidrográficas. Brasília
- Fearnside, P. M., 2001. Environmental impacts of Brazil's Tucuruí Dam: unlearned lessons for hydroelectric development in Amazonia. *Environ. Manage.* 27: 377–396.
- Feilich, K. L., 2016. Correlated evolution of body and fin morphology in the cichlid fishes. *Evolution (NY)*. 70: 2247–2267.
- Foote, M., 1997. The evolution of morphological diversity. *Annu. Rev. Ecol. Syst.* 28: 129–152.
- Gilbert, M. C., A. Akama, C. Cox & R. C. Albertson, 2020. Rapid morphological change in multiple cichlid ecotypes following the damming of a major clearwater river in Brazil. *Evolution Appl* 13: 2754–2771.
- Gilbert, M. C., A. J. Conith, C. S. Lerose, J. K. Moyer, S. H. Huskey & R. C. Albertson, 2021. Extreme morphology, functional trade-offs, and evolutionary dynamics in a clade of open-ocean fishes (perciformes: bramiidae). *Integr. Org. Biol.* 3: 003.
- Gonzalez, P. N., B. Hallgrímsson & E. E. Oyhenart, 2011a. Developmental plasticity in covariance structure of the skull: effects of prenatal stress. *J Anatomy* 218: 243–257.
- Gonzalez, P. N., E. E. Oyhenart & B. Hallgrímsson, 2011b. Effects of environmental perturbations during postnatal development on the phenotypic integration of the skull. *J Experiment Zool.* 316: 547–561.
- Goodall, C., 1991. Procrustes methods in the statistical analysis of shape. *J. r. Stat. Soc.* 53: 285–339.
- Hallgrímsson, B. H., K. Willmore & B. K. Hall, 2002. Canalization, developmental stability, and morphological integration in primate limbs. *Yearbook Phy Anthropol.* 45: 131–158.
- Hallgrímsson, B., H. Jamniczky, N. M. Young, C. Rolian, T. E. Parsons, J. C. Boughner & R. S. Marcucio, 2009. Deciphering the palimpsest: Studying the relationship between morphological integration and phenotypic covariation. *Evol. Biol.* 36: 355–376.
- Heckel, J. J. 1840. Johann Natterer's neue Flussfische Brasilien's nach den Beobachtungen und Mittheilungen des Entdeckers beschrieben (Erste Abtheilung, Die Labroiden). *Annalen des Wiener Museums der Naturgeschichte*. 2: 325–471, Pls.29–30.
- Helfman, G., 2007. *Fish Conservation: A guide to understanding and restoring global aquatic biodiversity and fishery resources*, Island Press, Washington, D.C.:
- Jacob, F., 1977. *Evolution and tinkering*. Science. 196: 1161–1166.
- Klingenberg, C. P., 2008. Morphological integration and developmental modularity. *Annu. Rev. Ecol. Evol. Syst.* 39: 115–132.
- Kuratani, S., 2009. Modularity, comparative embryology and evo-devo: developmental dissection of evolving body plans. *Dev. Biol.* 332: 61–69.
- Larouche, O., M. L. Zelditch & R. Cloutier, 2018. Modularity promotes morphological divergence in Lau JK, Lauer TE, Weinman ML 2006 Impacts of channelization of stream habitats and associated fish assemblages in east central Indiana. *Am. Midl. Nat.* 156:319–330. Ray-Finned Fishes. *Sci. Rep.* 8: 1–6.
- Lema, S. C. & G. a Nevitt., 2006. Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. *J. Exp. Biol.* 209: 3499–3509.
- López-Fernández, H., K. O. Winemiller, C. Montaña & R. L. Honeycutt, 2012. Diet-morphology correlations in the radiation of south american geophagine cichlids (perciformes: cichlidae: cichlinae). *PLoS One*. 7: 33997.
- López-Fernández, H., J. H. Arbour, K. O. Winemiller & R. L. Honeycutt, 2013. Testing for ancient adaptive radiations in neotropical cichlid fishes. *Evolution (NY)*. 67: 1321–1337.
- Lucinda, P., C. Lucena, N. Assis, 2010. Two new species of cichlid fish Genus *Geophagus* Heckel from the Rio Tocantins Drainage (Perciformes: Cichlidae). *Zootaxa*. 2429: 29–42
- Matesanz, S., M. Blanco-Sánchez, M. Ramos-Muñoz, M. de la Cruz, R. Benavides & A. Escudero, 2021. Phenotypic integration does not constrain phenotypic plasticity: differential plasticity of traits is associated to their integration across environments. *New Phytologist*. 231: 2359–2370.
- Mcgee, M. D., B. C. Faircloth, S. R. Borstein, J. Zheng, C. D. Hulsey, P. C. Wainwright, M. E. Alfaro, W. Pc & A. M. E. Replicated, 2016. Replicated divergence in cichlid radiations mirrors a major vertebrate innovation. *Proceed Royal Soc B Biologic Sci* 1–6: 20151413.
- McGhee, G. R., 2007. *The geometry of evolution: adaptive landscapes and theoretical morphospaces*, Cambridge University Press, Cambridge:
- Moczek, A. P., 2008. On the origins of novelty in development and evolution. *BioEssays* 30: 432–447.
- Muschick, M., M. Barluenga, W. Salzburger & A. Meyer, 2011. Adaptive phenotypic plasticity in the midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol. Biol.* 11: 116.
- Muschick, M., A. Indermaur & W. Salzburger, 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* 22: 2362–2368.
- Navon, D., I. Male, E. R. Tetrault, B. Aaronson, R. O. Karlstrom & R. Craig Albertson, 2020. Hedgehog signaling is necessary and sufficient to mediate craniofacial plasticity in teleosts. *Proc. Natl. Acad. Sci. USA*. 117: 19321–19327.
- Niwa, N., A. Akimoto-Kato, T. Niimi, K. Tojo, R. Machida & S. Hayashi, 2010. Evolutionary origin of the insect wing via integration of two developmental modules. *Evol. Dev.* 12: 168–176.

- Olsen, A. & M. Westneat, 2015. StereoMorph: an R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods Ecol. Evol.* 6: 351–356.
- Powder, K. E. & R. C. Albertson, 2016. Cichlid fishes as a model to understand normal and clinical craniofacial variation. *Dev. Biol.* 415: 338–346.
- Prum, R. O., 2005a. Evolution of the morphological innovations of feathers. *J. Exp. Zool. Part B Mol. Dev. Evol.* 304: 570–579.
- R Core Team, 2018. R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria:
- Rohlf, F. J. & M. Corti, 2000. Use of two-block partial least-squares to study covariation in shape. *Syst. Biol.* 49: 740–753.
- Schluter, D., 1996. Adaptive radiation along genetic lines of least resistance. *Evolution (NY)*. 50: 1766.
- Sheftel, H., O. Shoval, A. Mayo & U. Alon, 2013. The geometry of the Pareto front in biological phenotype space. *Ecol Evol.* 3: 1471–1483.
- Trautman, M., 1939. The Effects of man-made modifications on the fish fauna in lost and gordon Creeks, Ohio, between 1887–1938. *Ohio J. Sci.* 39: 275–288.
- Turner, G. F., 2007. Adaptive radiation of cichlid fish. *Curr. Biol.* 17: 827–831.
- Van Wassenbergh, S., N. Z. Potes & D. Adriaens, 2015. Hydrodynamic drag constrains head enlargement for mouthbrooding in cichlids. *J. r. Soc. Interface.* 12: 20150461.
- Wagner, G. P. & B. Y. Misof, 1993. How can a character be developmentally constrained despite variation in developmental pathways? *J. Evol. Biol.* 6: 449–455.
- Wagner, G. P., M. Pavlicev & J. M. Cheverud, 2007. The road to modularity. *Nat. Rev. Genet.* 8: 921–931.
- Weller, H. I., C. D. McMahan & M. W. Westneat, 2017. Dirt-sifting devilfish: winnowing in the geophagine cichlid *Satanoperca daemon* and evolutionary implications. *Zoomorphology.* 136: 45–59.
- Weller, H., H. López-Fernández, C. D. Mc Mahan & E. L. Brainerd, 2022. Relaxed feeding constraints facilitate the evolution of mouthbrooding in neotropical cichlids. *Am. Nat.* 199: E197.
- West-Eberhard, M. J., 2003. Developmental plasticity and evolution, Oxford University Press, New York, NY:
- West-Eberhard, M. J., 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20: 249–278.
- Wimberger, P. H., 1993. Effects of vitamin C deficiency on body shape and skull osteology in *geophagus brasiliensis*: implications for interpretations of morphological plasticity author (s): Peter H. Wimberger published by : American society of Ichthyologists and herpetologis. *Copeia* 1993: 343–351.
- Wimberger, P. H., 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *geophagus* (pisces: cichlidae). *Biol. J. Linn. Soc.* 45: 197–218.
- Wimberger, P. H., 1991. Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasiliensis* and *Geophagus steindachneri*. *Evolution (NY)*. 45: 1545–1563.
- Zelditch, M. L. & A. Goswami, 2021. What does modularity mean? *Evol. Dev.* 23: 377–403.

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