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Darter fishes exhibit variable intraspecific head shape allometry and modularity

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Abstract

Allometry, the relationship between anatomical proportions and body size, may either limit or facilitate the diversification of morphology. We examined the impact of allometry in darter fish morphology, which displays a variety of trophic morphologies. This study aimed to address (a) whether there was significant variation in darter head allometry, (b) if allometry contributed to head shape diversity in adults, and (c) if darters show head shape modularity associated with allometry. We used geometric morphometrics to quantify head shape across 10 different species and test for heterogeneity in allometric slopes. In addition, we quantified the degree of modularity between the preorbital and postorbital regions of the darter head, both before and after correction for body size. We found that different species have unique allometric slopes, particularly among the *Simoperca* subgenus, and that closely related darter species tend to show ontogenetic divergence, contributing to the diversity of head shapes observed in adults. We suggest that such a pattern may result from the similarity of juvenile diets due to gape limitation. We also found that several species show significant modularity in head shape but that modularity was evolutionarily labile and only sometimes impacted by head shape allometry. Overall, our work suggests that ontogenetic shape development may have been important to the evolution of head shape in darters, particularly in the evolution of foraging traits and microhabitat.

KEYWORDS

ecomorphology, Etheostomatinae, geometric morphometrics, ontogeny, Percidae

1 | INTRODUCTION

Understanding the mechanisms that cause phenotypic variation among taxa is important to macroevolutionary biology. Those intrinsic factors that structure how shape change covaries across anatomical structures may have significant implications for the rate and pattern of trait evolution (Arbour et al., 2021; Felice et al., 2018; Hulsey et al., 2018). Allometry describes the relationship between size and morphological traits and is tied to developmental, genetic, and physiological trade-offs (Esquerré et al., 2017; Klingenberg, 2016; Klingenberg & Marugán-Lobón, 2013). While some researchers have posited that allometry limits shape diversity, allometric relationships may in theory drive rapid diversification by allowing an "evolutionary path of least resistance" of coordinated changes in anatomical structures (Marroig & Cheverud, 2005). One may examine allometry across the size range/life span of a single organism (e.g., ontogenetic allometry) or across adult specimens of many species (evolutionary allometry).

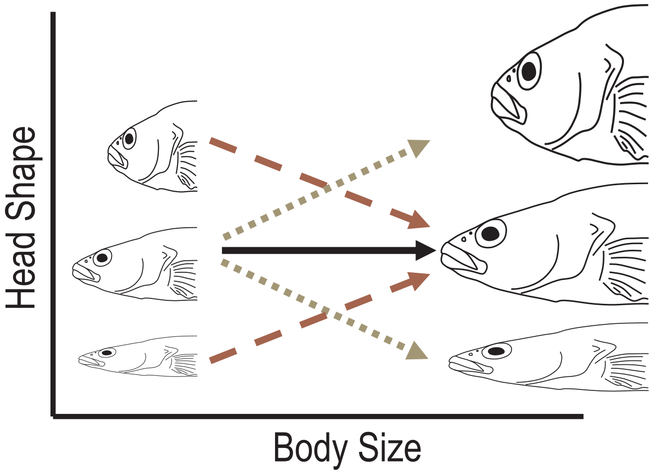

FIGURE 1 Possible patterns of intraspecific head shape allometry or isometry (solid line, no change with body size) in darters. Allometric relationships in darter may result in the development of convergent adult head shapes (dashed line) despite differing juvenile morphologies. Or allometry may result in divergent head shapes in adults (dotted line) from similar juvenile morphologies

Evolutionary allometry has been studied in broad comparative contexts across major clades of animals (Arbour et al., 2021; Bright et al., 2016; Cardini et al., 2004, 2015; Klingenberg & Marugán-Lobón, 2013; Piras et al., 2014). For example, a conserved pattern of cranial allometry (termed CREA) has been observed across numerous mammal clades and significantly impacts cranial shape variation across mammal clades of varying sizes (Arbour et al., 2021; Cardini et al., 2015). How ontogenetic allometry contributes to the morphological diversity of a clade from a comparative perspective is not as well understood (Esquerré et al., 2017). Shifts in ontogenetic allometry may result in changes in the scaling relationship of structures that either results in greater or lesser overlap in morphologies among adult specimens. *Ontogenetic convergence* results in two species that started at different shape configurations early in their lifetime and eventually converged onto a similar shape configuration (Figure 1, dashed line). On the other hand, *ontogenetic divergence* results in two species that started at a similar shape configuration early in their development and eventually diverged onto different shape configurations (Figure 1, dotted line). These both contrast with an isometric relationship, in which shape does not vary with body size (Figure 1, solid line). Even within a single clade, different anatomical regions or structures may show varying patterns of ontogenetic convergence or divergence. A recent study of shape diversification in 40 species of python snakes (Esquerré et al., 2017) showed that head shape allometry drove diversifying shapes with size (ontogenetic divergence), while juveniles tended to show greater body shape diversity compared to adults (ontogenetic convergence).

Allometry may also contribute to patterns of shape covariation between anatomical structures. Modularity and integration are terms representing extremes in the degree of shape covariation and independence between anatomical units. Modules are those anatomical structures or regions displaying strong trait covariation but that tend to vary independent of other modules (e.g., the fore and hind limbs of tetrapods). Integrated anatomical structures, on the other hand, show broad patterns of high shape covariation. Size-scaling relationships may significantly impact such patterns of regionalization of shape covariation. For example, the broad shape changes often associated with allometry may create a signature of integration and mask underlying shape modularity (Goswami & Polly, 2016). However, allometric trends may lead to modular shape variation if size-related changes are highly concentrated in particular structures (e.g., rostral elongation in non-echolocating bats) (Arbour et al., 2021). Tracking shape variation in relation to size variation, allometric trajectories are a crucial component to understanding the integration (or lack thereof) of shape configuration between modules.

Darters (Etheostomatinae; Percidae) are one of the most diverse clades of freshwater fish species endemic to North America (Carlson & Wainwright, 2010; Etnier & Starnes, 1993; Near et al., 2011; Page, 1983). Darters are chiefly a clade of small bodied (<20 cm), rheophilic fishes inhabiting streams and rivers (Carlson & Lauder, 2010, 2011; Carlson & Wainwright, 2010). While darters tend to have similar elongate body shape, owing to their swift water habitats, they show a surprising diversity in their head shapes (Carlson & Wainwright, 2010). In fact, the name of the largest genus (*Etheostoma*) is said to be derived from the term "many mouths." Darter species' head shapes vary in multiple ways: from elongate, sharply pointed heads; to conical, rounded snouts; and to blunt, downturned faces. It has been suggested that these differing head shapes allow darters to specialize in their tactics for foraging aquatic invertebrates across stream microhabitats (e.g., between crevices vs. overtop large flat rocks) (Carlson & Wainwright, 2010). However, our understanding of how head shape in this clade varies with body size is limited. Do darters show significant head shape allometry? And does allometry help to increase the diversity of head shapes, and potentially foraging, across this clade?

In this study, we used geometric morphometrics to quantify head shape variation across 10 species of darters (9 species of *Etheostoma* and 1 species of *Nothonotus*). We tested for significant intraspecific modularity across the species as a whole and contrasted whether closely related taxa tend to trend toward similar head shape configurations (convergence) or if different patterns are observed (divergence). We further tested whether partitioning of shape variation (modularity/integration) in the darter head was driven by variation in ontogenetic allometry.

2 | METHODS

2.1 | Sample collection

Using specimens from the zoological collection at Middle Tennessee State University, we sampled 191 individuals of 10 different darter species spanning the Etheostomatinae phylogeny. These included the greenside darter (*Etheostoma blennioides, n* = *20*), blenny darter (*Etheostoma blennius*, *n* = 12), snubnose darter (*Etheostoma simoterum*, *n* = 20), blackside snubnose darter (*Etheostoma duryi*, *n* = 16), rainbow darter (*Etheostoma caeruleum*, *n* = 20), fantail darter (*Etheostoma flabellare*, *n* = 23), fringed darter (*Etheostoma crossopterum*, *n* = 26), orangethroat darter (*Etheostoma spectabile*, *n* = 13), speckled darter (*Etheostoma stigmaeum*, *n* = 22), and redline darter (*Nothonotus rufilineatum*, *n* = 19). *Nothonotus* is typically supported as the sister clade to *Etheostoma* (Arbour & Stanchak, 2021; Near et al., 2011), and other major strongly supported subclades are indicated in Figure 5. As only the head was photographed for geometric morphometrics, we used standard length (SL) rather than centroid size to characterize body size. The SL (from the tip of the snout to the posterior most point on the caudal peduncle) was measured for each specimen using digital calipers to 0.01 mm. The sampled size ranges for each species were generally reflective of the observed size ranges for each species, and details are given in Table S1.

2.2 | Geometric morphometric analysis

Each specimen was photographed in lateral view from the anterior-most tip of the snout to approximately the insertion of the pectoral fin. All images were captured with a Leica DMC 4500 microscope in the software *LAS X*. A two-millimeter scale bar was digitally placed next to each specimen to quantify size. On each captured image, we plotted 17 landmarks and three curves (made of 25 sliding semi-landmarks) to characterize the head shape of each specimen (Figure 2, Table S2). The landmarks were digitized using tpsDig (Rohlf, 2015). A generalized Procrustes superimposition was conducted to remove the effect of rotation, scale, and position between landmarks, and retain only shape variation, using the function "gpagen" in the package *geomorph* v.4.0.1 (Adams & Otarola-Castillo, 2013) in R version 4.1.2. We allowed the sliding semi-landmarks to slide to minimize bending energy using the curves argument, as using Procrustes distance created artifacts in the positioning of semi-landmarks on the dorsal profile. We quantified overall head shape variation across all specimens using principal component analysis, as implemented in the *geomorph* function "gm.prcomp" (Adams & Otarola-Castillo, 2013). We selected a critical number of axes using a broken-stick model (Peres-Neto et al., 2005).

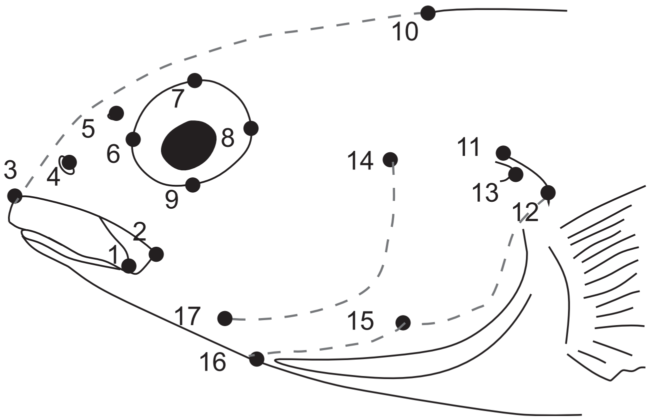

FIGURE 2 Geometric morphometric landmarks used in the analysis of head shape allometry in 10 darter species. Points indicate the position of landmarks, and dashed lines show the location of sliding semi-landmarks. See Table S2 for full description of landmark locations

2.3 | Analysis of ontogenetic allometry

We tested for an overall pattern of allometry in head shape in darters using the function "procD.lm" from the R package *geomorph*, which implements a Procrustes analysis of variance (ANOVA), or np-multivariate analysis of variance (MANOVA). The null hypothesis assumes isometry, while significant slopes are indicative of size-associated shape changes. We regressed Procrustes aligned head shape against log(SL) across all species and specimens to determine the overall impact of head allometry on shape variation. As some previous studies have found issues regarding power and error in the "distance-based" approaches used in Procrustes ANOVA (Clavel & Morlon, 2020), we also conducted a MANOVA on the PC scores produced above using the R function "manova" to confirm significance of the main effects and interaction term. We also conducted procD.lm analyses on each species individually to determine the variation in the strength of allometric relationships.

To test for differing allometric relationships across species, we implemented a homogeneity of slopes (HOSs)

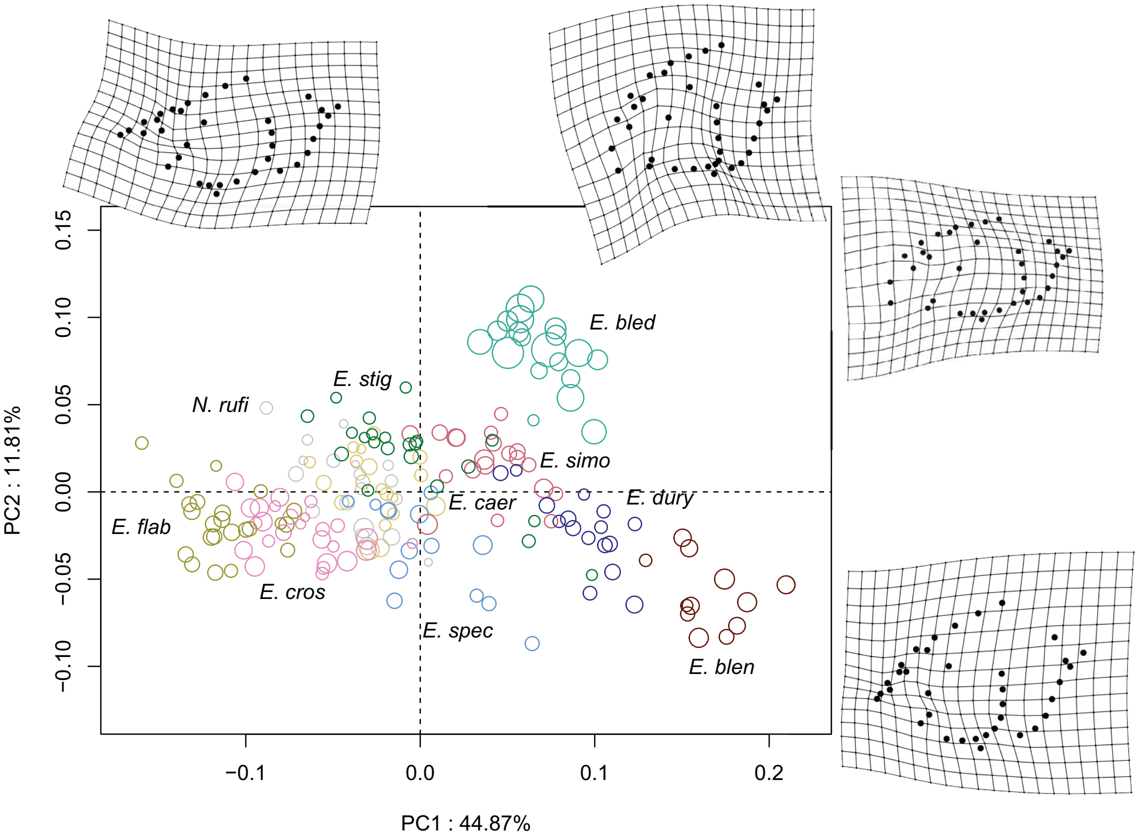

FIGURE 3 Principal component analysis of darter head shape across 17 landmarks and three curves in 10 species. First two axes illustrated, see Figure S1 for PC3 and PC4. Point size is scaled by standard length. Mesh grids illustrate the shape changes associated with the extreme values of each axis. Species observed included *Etheostoma blennioides* (*E. bled*), *Etheostoma blennius* (*E. blen*), *Etheostoma caeruluem* (*E. caer*), *Etheostoma crossopterum* (*E. cros*), *Etheostoma duryi* (*E. dury*), *Etheostoma flabellare* (*E. flab*), *Etheostoma simoterum* (*E. simo*), *Etheostoma spectabile* (*E. spec*), *Etheostoma stigmaeum* (*E. stig*), and *Nothonotus rufilineatum* (*N. rufi*)

test by comparing procD.lm models with and without an interaction between log(SL) and species using the R function “anova.” A significant result indicates that allometric slopes (of head shape on log(SL)) vary between species. To determine which slopes varied, we conducted pairwise procD.lm analyses using the same approach for the HOS test (Table S4). We also used the R package *RRPP* function “pairwise” to compare the strength of slope vectors (test.type = “DL”). Lastly, to ensure the relatively small sample sizes did not bias the results of procD.lm, we conducted 500 bootstrap replicates of these analyses at (a) the smallest species sample size (12), (b) the largest species sample size (26), and a larger sample size than observed (50) (and see Table S6).

We used the function “plotAllometry” to visualize changes in shape across species. This function conducts a PC analysis using the fitted values from the Procrustes ANOVA and uses the values from the first PC axis to represent the most important morphological trends associated with body size variation (but this PC analysis is not part of the original Procrustes ANOVA).

2.4 | Modularity analysis

To test for head shape modularity and the impact of allometry on intra-specific modularity, we used the function “modularity.test” in the R package *geomorph*. We divided the head into two putative modules (pre-orbital and post-orbital) based on previous work of head shape modularity in other Acanthopterygians and our observations of major patterns of head shape variation in darters (Cooper et al., 2010). Landmarks 1 through 5 and 18 through 20 were assigned to the preorbital module; landmarks 6 through 17 and 21 through 36 were assigned to the post-orbital module. The covariance ratio (CR) coefficient for each species was calculated using the function “modularity.test”. The CR coefficient is the ratio of the average covariation of landmark position between modules and the average covariation within modules. The CR value is low when modularity is high (the modules vary independently); the CR value is high when modules are integrated and covary. Significance is assessed through permutation, where landmarks are randomly assigned to modules and the distribution of CR values is taken (where $p =$ the number of randomized landmark configurations with a CR < the observed value).

To determine whether patterns of intra-specific modularity were driven by head shape, we extracted the residuals from the regression of head shape on log(SL). The residuals were taken from the full model with main effects for species and SL, and the interaction effect, to account for differences in allometric slopes. These were added to the consensus landmark configuration (mean coordinates per landmark) to produce a set of allometry-corrected landmark configurations. The CR coefficient and significance were calculated again for each species using the size-corrected landmarks and was contrasted with the original dataset's CR values.

3 | RESULTS

3.1 | Head shape variation in darters

Variation in darter head shape was highest in the snout/pre-orbital region. The first principal component of head shape variation (44.87%) across all 191 specimens characterized mouth position and jaw length, with positive values associated with shorter, inferior mouths, and negative values associated with terminal mouths with longer jaws (Figure 3). PC2 (11.81%) was more strongly impacted by snout length, with positive scores associated with longer snouts. Post-orbital shape variation was low, although high PC1 was associated with a more sharply angled preopercle. PC3 and PC4 were associated with more subtle variation in head shape (Figure S1). PC3 (9.10%) was associated with variation in the positioning of the eye and depth of the head, and PC4 (8.58%) was associated with the length and steepness of the dorsal profile of the head (Fig. S1).

Head shape was somewhat variable within species but showed low overlap on the first two critical axes, albeit with greater overlap on subsequent axes. Along the first two axes, *E. caeruleum* and *N. rufilineatum* showed the greatest overlap, with moderate PC1 and PC2 values and fairly generalized head shapes (moderately sized snouts with somewhat subterminal mouths). The *Goneaperca* species tended toward the most pointed head shapes with long, terminal jaws on PC1, while *Simoperca* species (*E. blennioides*, *E. blennius*, *E. duryi* and *E. simoterum*) trended toward more blunt head shapes

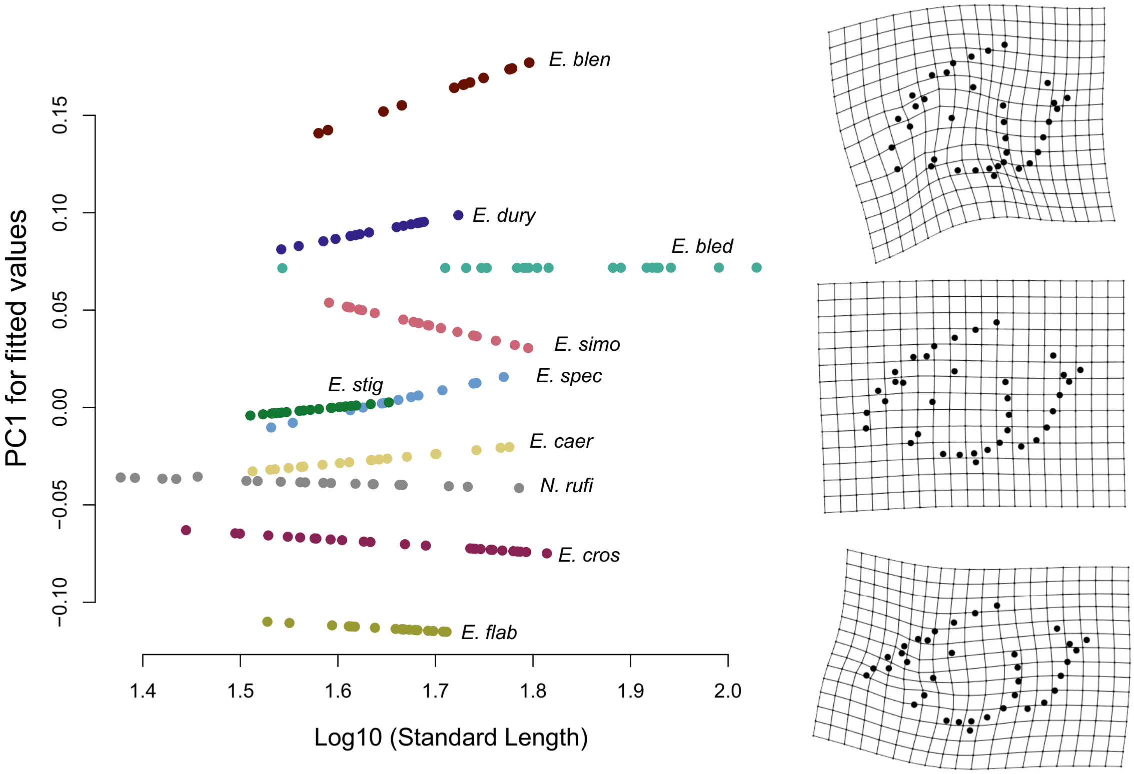

FIGURE 4 Allometric trends in head shape in 10 species of darters. Mesh grids illustrated the shapes associated with the extremes and moderate values on the first PC of fitted shape values. Species observed included *Etheostoma blennioides* (*E. bled*), *Etheostoma blennius* (*E. blen*), *E. caeruluem* (*E. caer*), *Etheostoma crossopterum* (*E. cros*), *Etheostoma duryi* (*E. duryi*), *Etheostoma flabellare* (*E. flab*), *Etheostoma simoterum* (*E. simo*), *Etheostoma spectabile* (*E. spec*), *Etheostoma stigmaeum* (*E. stig*), and *Nothonotus rufilineatum* (*N. rufi*)

with inferior mouths (high PC2 scores). The largest species, *E. blennioides*, was associated with generally high PC2 scores, but body size overall (as illustrated by point size) did not show a consistent association with either PC1 or PC2 across all species examined (Figure 3).

3.2 | Ontogenetic allometric analysis

Across all 10 of the species sampled, darters showed significant allometry in head shape using Procrustes ANOVA. We found that Log_{10}SL ($F = 33.951$, $p < .001$, $R^2 = 0.0635$) and the interaction between size and species ($F = 1.34$, $p = .03$, $R^2 = 0.0225$) significantly influenced the head shape of darters; however, differences in mean shapes between species had a considerable and larger impact on overall head shape ($F = 35.31$, $p < .001$, $R^2 = 0.594$). We found a similar result when conducting a MANOVA on the scores from the PCA of head shape. All main effects were found to be significant under this approach, (logSL–approximate $F = 42.08$, $p < .001$; species $F = 8.458$, $p < .001$) and the species were found to have different allometries ($F = 1.569$, $p < .001$). When examined individually, procD.lm showed significant allometry in seven of the 10 species (excluding *E. flabellare*, *E. spectabile* and *E. stigmaeum*), with goodness of fit values (R^2) from 11.4% to 22.3%, considerably higher than the combined analysis.

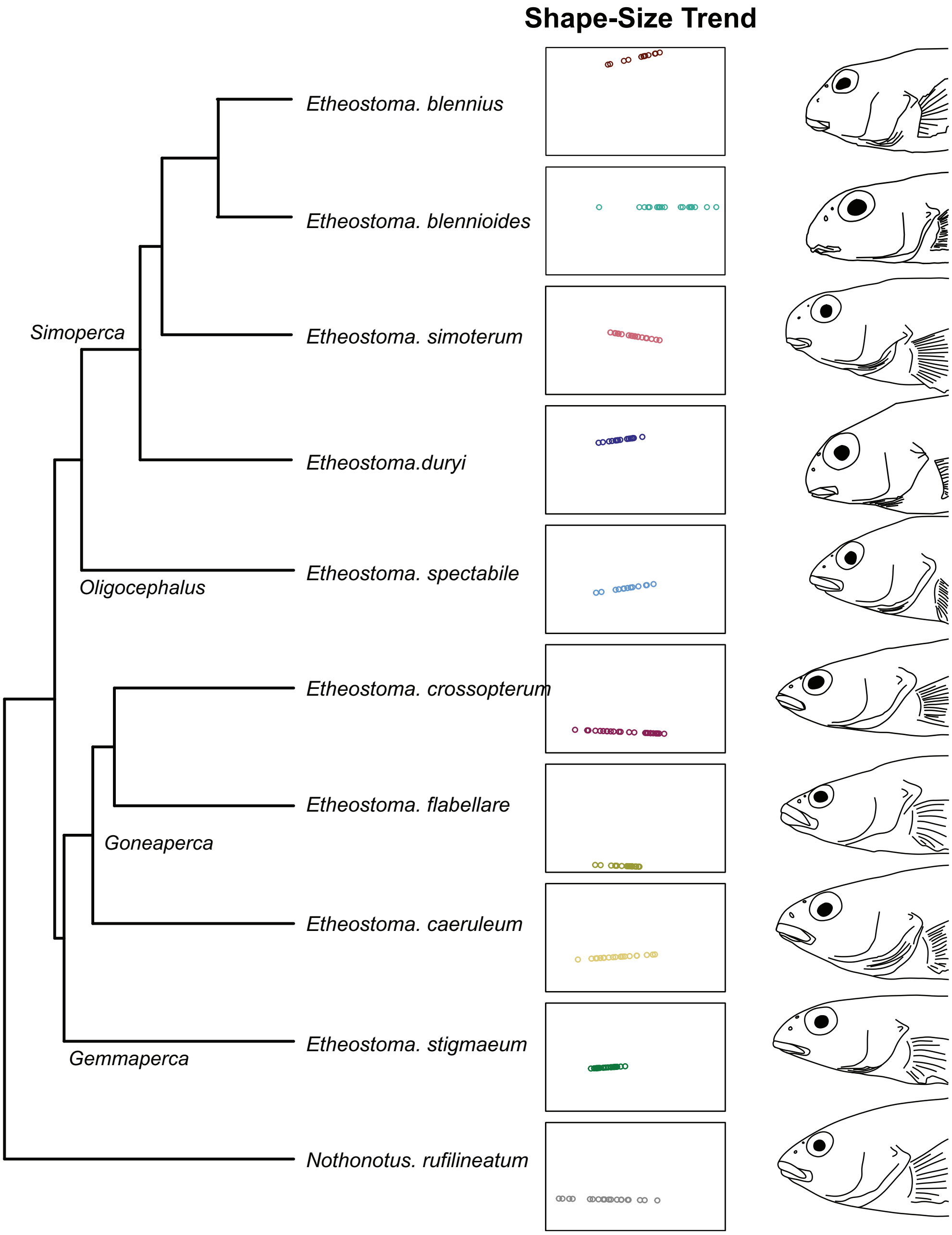

FIGURE 5 Phylogenetic comparison of allometric trends in head shape across darters. Trends correspond to those illustrated in Figure 4. Phylogeny is taken from Arbour and Stanchak (2021)

Based on the results generated by plotAllometry to visualize allometry trajectories (Figure 4), the head shape change associated with an increase in darter body size involves a transition from a terminal mouth to an inferior mouth and a more dorsally located eye as well as a broadening of the head and a transition to a shortened and blunter snout. Species like *E. simoterum*, *E. crossopterum*, trended toward a narrower, sharper snout with increasing body size (SL), while *E. blennius*, *E duryi*, and *E. caeruleum* trended toward a blunter snout (see Figure 3). Species level analyses showed that *N. rufilineatum* and *E. blennioides* exhibited significant allometry (*N. rufilineatum* among the highest R^2 values, Table S3), yet their allometric trajectories seemed fairly isometric in the plot of fitted values (Figure 4), suggesting that perhaps the allometric trends (smaller eyes and taller heads with size, results not shown) of this species are different from the major patterns seen across darters. Pairwise analyses showed that members of the *Simoperca* clade had the most significant differences in allometry from other species, as almost all significant contrasts included one species from this clade (Table S5 and S6).

When overall head shape and allometric trends (as visualized by "plotAllometry") are placed in a phylogenetic context, a pattern of ontogenetic divergence appears most common (Figure 5) but varies between the two major lineages examined. The two major clades appear to have undergone significant overall shifts in mean head shape, consistent with the high proportion of variation described by the "species" term in "procD.lm" analyses (see above). Among the two major clades defined in the Arbour and Stanchak (2021) phylogeny, here represented by the subgenera *Simoperca–Oligocephalus* vs. *Goneaperca–Gemmaperca* clades (Figure 5), there is a noticeable split in terms of overall head shape, with the S-O clade showing overall more blunt head shapes (Figure 5, top five species) and the G-G clade showing moderate to sharply pointed snouts (Figure 5, bottom five species).

Within the *Simoperca* clade, ontogenetic trends tended to result in species with more diverse adult head shapes and more similar juvenile shapes (Figures 4 and 5). Three species in this clade show very similar juvenile head shapes (*E. duryi*, *E. blennioides* and *E. simoterum*), but each trend toward different adult head shapes (*E. duryi*—more blunt, *E. blennioides*—fairly isometric, *E. simoterum*—more sharply pointed) (Figures 4 and 5). Even *E. blennius*, which has much more blunt faced juveniles than other *Simoperca* species, develops an even more blunt face as it grows. Overall, this results in highly diverse adult morphologies among the *Simoperca* subgenus.

Within the *Gemmaperca–Goneaperca* subclade, allometric divergence in adult head shapes is less significant. While qualitatively adults do appear to show a greater variability of head shapes (e.g., *E. spectabile* develops a blunter snout with growth, while *E. crossopterum* and *E. flabellare* develop slightly more sharp snouts), pairwise contrasts showed few significant differences in allometry within this group (Tables S5 and S6), unlike in the *Simoperca-Oligocephalus* species. Although some species from this clade (*E. crossopterum*) do show significant allometry when examined individually, the majority of this clade does not (Table S3). Divergence in overall head shape appears more important to the *Gemmaperca* and *Goneaperca* subgenera than shifts in allometry. The grouping of *N. rufilineatum* with this clade and its overall isometric pattern of head growth (though see comment above) may be indicative of the ancestral state of the *Etheostoma-Nothonotus* clade within the Etheostomatinae.

TABLE 1 Results of modularity analyses before and after correction for head shape allometry

Species	CR (prior to correction)	*p*-Value	CR (post-allometry correction)	*p*-Value
Etheostoma blennioides	**0.868**	**.046**	**0.852**	**.020**
Etheostoma blennius	0.915	.060	**0.906**	**.030**
Etheostoma caeruleum	**0.884**	**.017**	**0.847**	**.008**
Etheostoma crossopterum	**0.800**	**.004**	**0.857**	**.030**
Etheostoma duryi	0.927	.077	0.922	.074
Etheostoma flabellare	**0.799**	**.005**	**0.807**	**.009**
Etheostoma simoterum	**0.777**	**.007**	**0.854**	**.032**
Etheostoma spectabile	**0.887**	**.004**	**0.884**	**.002**
Etheostoma stigmaeum	**0.834**	**.001**	**0.834**	**.002**
Nothonotus rufilineatum	0.987	.096	**0.954**	**.027**

Note: The CR is calculated for a comparison of the pre- and post-orbital modules. Bolded values are significant.
Abbreviation: CR, covariance ratio.

3.3 | Darter head shape modularity

When examined across all specimens, we did not find significant modularity in darter head shape based on a pre-orbital and post-orbital modules ($CR = 1.01$, $p = .18$). However, when species were examined independently, seven showed intraspecific head shape modularity. The snubnose darter, *E. simoterum*, possessed the strongest modular signal (Table 1), while *N. rufilineatum* and *E. spectabile* did not show significant modularity.

Following size correction, darter head shape was shown to be modular ($CR = 0.74$, $p = .003$), and nine of 10 were found to have significant modularity. Thus, in general, head shape allometry promoted integration of the pre- and post-orbital regions of the head. Interestingly, however, the species (*E. simoterum*), with the lowest CR value (strongest modularity) prior to size correction, also showed the largest increase in CR value (0.78–0.85).

4 | DISCUSSION

Overall, we find significant variation in both allometry and modularity in the head shape across the 10 focal darter species in our study. Ontogenetic allometry showed a weaker impact on head shape than overall interspecific shifts in mean head shape. However, we found that following major shifts in overall shape (e.g., toward blunt snouts in the *Simoperca* species examined), allometry appears to have contributed to diverse head shapes in adults within different "morphotypes," particularly within the *Simoperca* subgenus. We interpret the results of the plotAllometry figure (Figure 4) as indicating ontogenetic divergence in head shape as a result of the diverging trajectories of head shape, following Esquerré et al. (2017). We note that these plots are produced via a PCA of the fitted values and not as part of the original analyses. Other aspects of shape not represented on these most important axes may show converging patterns of head shape. Overall, with these caveats in place, we interpret these findings to suggest the extent to which head shape allometry contributes to darter morphological diversity appears to have an overall impact of enhancing shape diversity among adult specimens in darters. We caution, however, that this analysis was based on relatively low sample sizes (12–26 per species) and only a limited sample of species (10 of the >200). Further sampling both taxonomically, accounting for any possible cases of sexual dimorphism, and greater intraspecific sampling would be critical to future explorations. This initial study does, however, reveal some potentially useful mechanisms contributing to darter head shape diversity.

Darters are a species rich clade within the North American ichthyofauna, second only to the Cyprinids (Etnier & Starnes, 1993; Near et al., 2011; Page, 1983). Numerous species of darters, and many species represented here, occur in sympatry (Etnier & Starnes, 1993; Page, 1983), predominantly within streams and rivers (though a few lentic species may be found). Divergence in microhabitat use and foraging ecology have both been suggested to contribute to the high rates of sympatry in this clade (Van Snik et al., 1997). While most species are aquatic invertebrate feeders, species specialize on feeding in different regions of the benthos—some species like *Etheostoma flabellare* (fantail darter) or *Percina squamata* (olive darter) feed within rocky crevices, others feed over the top of substrate or rocks, some bury in sand and ambush prey, and some, like the logperch (*Percina caprodes*), flip rocks over to find food (Carlson & Wainwright, 2010; Turner, 1921). Divergent allometric trajectories in head shape may enhance partitioning of foraging zones and may have helped to facilitate high overlap in species geographic range.

Across the 10 species examined, blunt-head shapes appeared to be associated with stronger allometric trends (although *N. rufilineatum* examined alone has strong allometry, Table S3). Where significant differences in allometries were found, they tended to produce adults with more diverse head shapes compared with juveniles. Adult darter diets vary but generally comprise aquatic insect larvae (most commonly Chironomidae and Ephemeroptera), and sometimes snails, fish eggs, larger aquatic invertebrates like stonefly nymphs, or vegetation/detritus in some amounts (Orr, 1989; Turner, 1921). Comparatively, darter diets tend to be more uniform as juveniles and are limited to very small prey (e.g., copepods and cladocerans), in most (but not all) species diversifying as they grow larger and can take on larger food items (Turner, 1921). Such a pattern would be benefitted by diverging head shape allometries, allowing greater specialization on adult food types while retaining the more uniform characteristics needed for early feeding. Interestingly, the *Simoperca* clade shows some of the highest functional diversity in feeding characteristics among the major lineages of darters (Carlson & Wainwright, 2010). Considerable restructuring and repositioning of the nasal, maxilla, and premaxilla bones have occurred in the evolution of the "blunt snout" phenotype, which likely has contributed to variation in cranial kinesis and are also likely to produce stronger bites than many other darter species (Carlson & Wainwright, 2010). Is such developmental flexibility an innovation linked to this functional diversity?

Functional demands associated with extremely blunt faces differ from *Goneaperca* species with pointed, terminal faces (including *E. flabellare* and *E. crossopterum*), some of which are reported to take disproportionately large prey compared to other species, even at small sizes (Turner, 1921). Only *E. crossopertum* showed significant allometry in head shape (Table S3), though, and overall the slopes of these two species did not differ from each other (Table S4–5). Long, pointed faces with large jaws may help to overcome gape limitations at small sizes and facilitate the taking of comparatively large prey (Claverie & Wainwright, 2014; Wainwright & Richard, 1995) but limit possible variability in allometric growth trajectories. The so-called prober species (*Percina squamata* and *Etheostoma sagitta*; Carlson & Wainwright, 2010) may be a helpful point of comparison in this regard, as these species show comparable fixation of the pre-maxilla, in contrast with the high mobility of the *Simoperca* crania.

The 10 darter species examined also varied in the extent of shape partitioning in the pre-orbital versus post-orbital modules. Seven of 10 species showed significant modularity but varied substantially in the strength of head shape modularity. The relative impact of allometry on head shape partitioning was also variable. Most species showed a small increase in modularity after correcting for the impact of head shape variation, suggesting that allometric trends promoted integrated shape changes. However, in one species (*E. simoterum*), allometry appears to have considerably promoted pre-orbital modularity (correcting for allometry resulted in a more integrated head). Such lability in head shape modularity has been previously observed in groups like *Anolis* lizards (Sanger et al., 2011) and may reflect differing functional demands across the skull. Indeed, among the species observed here, the role of suction and biting feeding modes are likely to vary (Carlson & Wainwright, 2010; Turner, 1921).

Within the darters sampled, several species show both moderate head shapes and isometry in head shape, including *E. spectabile*, *E. stigmaeum*, and *N. rufilineatum*. While our taxonomic sampling was too low to quantitatively reconstruct ancestral character states (i.e., ancestral mean head shape or allometric trends), the diverse species (including the only non-*Etheostoma* species) represented here could suggest that shifts in head shape and allometric trends are “derived” states among the darter clade. Most significant differences in allometry (as indicated by pairwise analyses) included members of the *Simoperca* clade, while *Goneaperca* and *Gemmaperca* species showed fewer significant contrasts and fewer species with significant allometries. This pattern does suggest that the blunt snouts and divergent allometric trajectories represent a derived condition among this subgenus. Indeed, many more “basal” members of the next largest genus of darters (*Percina*, *Atlantis* subclade) are also fairly generalized in their head shape, while other members like the logperches (*P. caprodes*) and the olive darter (*P. squamata*) represent more specialized feeders with derived feeding morphology (Carlson et al., 2009; Carlson & Wainwright, 2010). Inclusion of *Percina* species, especially more basal members of the clade, as well as improved sampling of the various darter subgenera, would be helpful in determining if darters ancestrally show isometric head shapes.

5 | CONCLUSIONS

Our study demonstrated that both allometry and modularity contribute to head shape variation in darters and that both factors were evolutionarily labile. Furthermore, patterns of ontogenetic allometry were divergent (adults develop more diverse head shapes than juveniles, as opposed to a pattern of isometry or convergent allometry) and appear to have promoted adult head shape diversity in some species, particularly within the *Simoperca* clade. A shift to variable allometries may be an innovation within this darter clade, though greater taxonomic sampling is needed to explore this. The pattern of allometric divergence, particularly in *Simoperca*, was similar to the divergent allometry trajectories observed in head shape in pythons (Esquerré et al., 2017). Juvenile darters across many species tend to be found in less “challenging habitats” (i.e., pools and regions of lower current), with large adults and riffle specialists (e.g., in *Nothonotus*) moving into swifter waters (Etnier & Starnes, 1993; Kuehnem & Barbour, 1983; Page, 1983). It is possible that body shape may also show significant allometry relationships. A previous study of simple linear morphometrics of three species of darters did also suggest that allometric slopes varied significantly (Paine, 1986).

It is also important to note that our analyses represented 2D landmark data, but the species observed here show variation in lateral width. The redline darter and other *Nothonotus* species are laterally compressed, while species like *E. blennioides* and *E. flabellare* are more rounded in profile. Previous studies have shown that the exclusion of lateral shape information may bias analyses (Buser et al., 2018). Incorporation of both greater taxonomic sampling and more comprehensive geometric morphometric data would allow for greater testing of whether allometry is a significant factor in driving the rapid morphological diversification across darters, or if it is largely representative of the *Simoperca* group. However, this preliminary work does strongly suggest that evolutionary variation in both allometry and modularity has contributed to the variation of head shapes observed in this diverse clade.

AUTHOR CONTRIBUTIONS

Maris Goodwin: Data curation (equal); formal analysis (equal); software (equal); visualization (equal); writing—original draft (supporting); writing—review and editing (supporting). **Jessica Arbour:** Conceptualization (lead); data curation (equal); formal analysis (equal); methodology (lead); project administration (lead); software (equal); supervision (lead); visualization (equal); writing—original draft (lead); writing—review and editing (lead).

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DATA AVAILABILITY STATEMENT

Geometric morphometric data are available in the supplementary materials.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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