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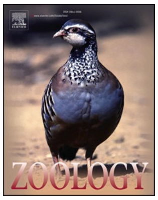

The fast and the tendinous? Locomotor modifications of the caudal peduncle in *Gila* spp. from the American Southwest

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ABSTRACT

In the American Southwest, the fishes within the genus *Gila* evolved in an environment with seasonal rainstorms that caused stochastic flooding. Some species within this genus, such as bonytail (*Gila elegans*), possess locomotor morphologies that are similar to those seen in high-performance swimmers such as tuna and lamnid sharks. These shared features include a shallow caudal peduncle, lunate tail, and mechanisms to transmit force from the anterior musculature to the tail fin. We compared the skeletal anatomy of the caudal region of bonytail to roundtail chub (*Gila robusta*) and humpback chub (*Gila cypha*) to determine which vertebral elements have been modified to create a shallow peduncle. We also tested the tensile strength of the red (slow oxidative) axial muscle by performing a standard stress test. If the muscle can withstand a large load, this suggests it may play a tendon-like role in transmitting force from the anterior muscle to the hypural plate of the tail. Lastly, we measured the collagen content of the red axial muscle (visualized using serial sections and Masson's trichrome stain) to determine if increased tensile strength is associated with increased collagen content. We found bonytail caudal peduncles are characterized by acute vertebral spines and have red axial muscle that can resist tearing under tension. Roundtail chub peduncles are characterized by relatively more obtuse angles and the red muscle tears easily under tension. Humpback chub possess an intermediate morphology, with relatively obtuse vertebral spine angles and the red muscle can resist tearing under tension. Bonytail have increased collagen content in posterior red axial muscle compared to the anterior musculature also suggesting a tendon-like role of the posterior red muscle. In combination with previous studies of swimming performance, our findings suggest that the axial musculature of bonytail may play a role in transmitting force directly to the shallow peduncle in a manner similar to that of the great lateral tendon of scombrids.

1. Introduction

Bony fishes (Osteichthyes) provide an excellent model to study the link between anatomy (shape, integument, muscles, and skeleton) and performance because there is a large diversity in anatomical form. Bony fishes demonstrate a variety of swimming modes — from caudal-fin and axial-body-based (anguilliform to thunniform), median-fin-based (triggerfishes and knifefishes), and pectoral-fin-based (skates, rays and labrids), each with characteristic profiles to match the behavior (Sfakiotakis et al., 1999). Caudal fin propulsion is the most commonly seen mode of locomotion in fishes and there is variation in both the anatomy and behavior of caudal-fin swimmers. One type of caudal-fin-based locomotion of particular interest has been thunniform (tuna-type) locomotion because fishes that employ this swimming mode can sustain high swimming speeds for long periods of time, while at the same time experiencing relatively low metabolic costs (Sfakiotakis et al., 1999).

Over the past 400 million years, scombrid fishes and lamnid sharks have converged on similar morphologies to accomplish thunniform locomotion (Carroll, 1988). Thunniform swimmers are characterized by a narrow caudal peduncle that is largely amuscular (Bernal et al., 2001), which reduces form drag (drag from body shape) and also contributes to reduction of induced drag (drag produced when fluid flow is redirected, Sfakiotakis et al. (1999)). In tuna, a shallow peduncle is achieved by both shortening and reducing the spine length of the neural and hemal spines from the vertebrae in the peduncle (Moran et al., 2016).

Because slow oxidative (red) muscle is the main muscle group that powers sustained swimming in fishes, thunniform swimmers have

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modified this muscle group extensively, while fast glycolytic (white) muscle remains relatively unchanged (Shadwick, 2005). Both tuna and lamnid sharks have increased the percentage of red muscle composition (Dickson, 1995) and internalized their red muscle, such that it is located more medially, closer to the vertebral column. In contrast, in most fishes, the red muscle is located at the lateral margin of the body (Shadwick, 2005). The red muscle in tuna interdigitates with tendons that transmit force from anterior musculature to the posterior hypural plate; these tendons arise from the anterior and posterior cones of each muscle and span multiple vertebrae before converging along the midline to become the great lateral tendon (Shadwick et al., 2002). The main component of tendons is collagen, a protein that is strong under tension and serves to increase the tensile strength of the tendon and their ability to transmit force from the anterior musculature to the peduncle (Shadwick et al., 2002). Lamnid sharks possess similar modifications of the red muscle but display a very different mechanism to transmit the force from the anteriorized red muscle to the caudal fin. Lamnids have short, but strong, tendinous sheaths that arise from each muscle and insert on the their skin; this enables the muscle to transmit the force to the tail at a greater mechanical advantage than transmitting than would occur if forces were transferred through the vertebrae (Wainwright et al., 1978).

Most studies involving high-performance swimmers have focused on marine fishes (tunas and sharks), and only a few freshwater fishes have been examined in this context. One group of interest for locomotor studies is the *Gila* complex, a group of 20 chubs (family Cyprinidae), native to the American Southwest. Because of extensive habitat modification, many of these fishes are listed as threatened or endangered species. Swimming performance is of interest for this group, especially for bonytail (*Gila elegans*), as they share key anatomical characteristics with high-performance swimmers. Bonytail possess a high aspect ratio tail and a shallow caudal peduncle (Moran et al., 2018). These anatomical characteristics are associated with reduced metabolic demand during high-speed swimming in bonytail, relative to other cyprinid fishes (Moran et al., 2016). Given the anatomical convergence between bonytail and high-performance marine swimmers, we asked the following questions. (1) When compared to other *Gila* species, are bonytail distinct in the anatomy of the series of vertebrae that comprise the posterior axial skeleton? (2) Is bonytail's red axial muscle stronger under tension, relative to other *Gila* species? (3) If bonytail muscles are stronger under tension than other *Gila* species, what enables the red axial musculature to be stronger under tension? We hypothesized that bonytail would have shorter and more acute vertebral spine angles compared to the other *Gila* species; we made this prediction because these are the mechanisms by which scombrids produce a narrow caudal peduncle. We also hypothesized that bonytail would have stronger (under tension) red axial musculature when compared to other *Gila* species because their musculature may be transmitting force to the caudal peduncle, in a manner similar to the great lateral tendon of scombrids. We hypothesize that muscle that is stronger under tension will have an increased amount of collagen in the muscle tissue, as collagen is the main protein in the connective tissue of muscles and the main protein in tendons.

To answer these questions, we compared the morphology of bonytail to that of roundtail chub (*Gila robusta)*; we used roundtail chub for comparison because they are thought to possess a more "generalized" swimming morphology (Moran et al., 2016; Moran et al., 2018). We also compared bonytail to humpback chub (*Gila cypha*) because the morphology of humpback chub appears to be an intermediate between roundtail chub and bonytail. To assess our predictions, we measured the vertebral spine angles and lengths of the posterior-most twelve vertebrae to assess how the vertebral elements of bonytail and the other *Gila* species form the anatomy of the peduncle. Because thunniform swimmers evolved elaborate tendon system to transmit force, we expect that bonytail also have a mechanism to transmit force from their anterior musculature directly to their caudal peduncle (Egginton and Sidell, 1989). Thus, we anticipate that there are modifications to the red muscle that accommodate increased force passed along the muscle. To assess this, we compared the tensile strength and collagen content of the anterior (non-peduncle) musculature to the posterior (peduncle) musculature in each species and also compared the collagen content of bonytail axial red muscle to the other *Gila* species. Collagen content was examined because it is a significant protein in the connective tissue of muscle and the main protein of tendons in tunas and sharks (Shadwick et al., 2002); thus, it is likely that increased collagen is responsible for differences in tensile strength in the red axial muscle among *Gila* species.

2. Methods

2.1. Fish acquisition

Specimens were acquired from the United States Geological Survey (USGS, David Ward). All fish were euthanized at one to two years of age. Specimens were obtained dead and frozen (for skeletal analysis) or obtained alive and then euthanized to be used in muscle studies at Northern Arizona University (NAU). Endangered species work was conducted in collaboration with USGS under David Ward's Federal threatened and endangered species permit (TE821356-0). All maintenance and euthanasia of fishes for these experiments was conducted under Northern Arizona University's IACUC protocol, 19-003.

2.2. Skeletal and soft tissue anatomy

Vertebral anatomy was visualized by clearing and staining for bone using a protocol modified from Song and Parenti (1995), using individuals of bonytail (n = 9), humpback chub (n = 10), and roundtail chub (n = 8). Cleared and stained individuals were photographed, and measurements of vertebral anatomy were made using NIH ImageJ (Rasband, 2011). Length and angle (relative to the vertebral column) of the neural and hemal spines of the last 12 vertebrae were measured for each individual (Fig. 1). Length measurements were divided by the standard length of each individual to standardize these measurements for body size. To quantify how spine angle is different in anterior vs. posterior vertebrae, we calculated the change in vertebral angle from one vertebra to the next. The change in angle was determined by plotting spine angle (y-axis) and vertebral number (x-axis) of the first nine of the last twelve vertebrae. A line of best fit was imposed through these data and the slope of this line was used as the change in vertebral angle — this is a measurement that quantifies how many degrees a vertebral spine angle changes from one vertebra to the next. The angle and relative spine length for vertebra number nine was used when comparing the shallowest portion of the peduncle across species because the more posterior vertebrae are modified to construct the hypural plate (Minicozzi et al., 2019).

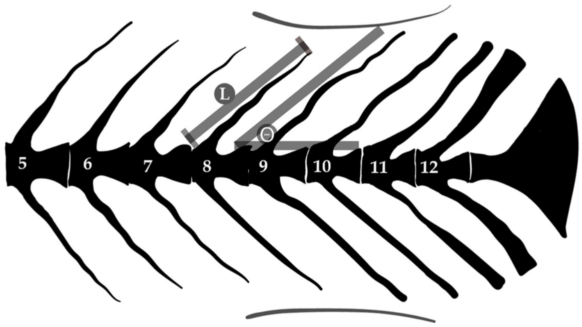

Fig. 1. Schematic representation of axial skeleton of fishes illustrating how morphological data were gathered. Vertebrae were numbered with the last (posterior-most) numbered as vertebra 12. Angles (theta) and lengths were measured from vertebrae 1 – 9, as shown for both neural (dorsal) and hemal (ventral) spines.

2.3. Dissections and material testing

Additional individuals for each species (n = 6 per species) were acquired, euthanized and used for soft-tissue comparisons. Following euthanasia (buffered MS-222), a section of red (slow oxidative) axial musculature was dissected and removed from each individual; red muscle was removed from the caudal peduncle to the base of the dorsal fin (Fig. 2). Red muscle was removed from both the right and left side of the fish. Fascia and white muscle were subsequently removed from each sample by careful dissection and the section of red muscle was then separated into “anterior” and “posterior” portions by sectioning it into two halves (Fig. 2). A small sample of each muscle was removed for histological analysis before material testing was performed. The muscle was imaged (to allow size measurements to be taken) and then transferred to a Discovery Series Hybrid Rheometer (HR2).

The Rheometer was used to perform a standard tension test, where the muscle was mechanically lengthened until it failed by tearing. Anterior and posterior sections of muscle were tested on the rheometer in random order. During the rheometer testing, the muscle was stretched at a strain rate of 1 mm per second for 20 seconds, for a maximum extension of 20 mm (Wren et al., 2001). The force necessary to cause failure by tearing for a given muscle was divided by the cross-sectional area (width multiplied by the depth) of that muscle segment to calculate stress for each muscle. The width was measured with images of each muscle prior to the tension test. However, each section of red muscle was relatively thin and difficult to measure photographically because the muscle could not be propped on its lateral axis. Because each individual for the muscle tests were similar in sizes, 2.0 mm was used as the estimated depth for all muscle samples.

2.4. Histology

Prior to sectioning, the anterior and posterior sections of the red axial muscle were fixed and embedded in paraffin wax using a Shandon Citadel 2000 tissue processor. Each muscle sample was sliced at 10 microns sagittally and then stained using Masson’s Trichrome (Foot, 1933). Slides were examined using a Lecia DM6 B and Leica Application Suite X (LASX) and images were taken at 40x magnification. The percent area of collagen (Calvi et al., 2012) was calculated from these images by measuring the area of collagen (blue pixels) and comparing that to the total area of the muscle (everything in the section that was not blue). One representative image was used per section of each muscle.

2.5. Statistical Analyses

All analyses were conducted using IBM’s SPSS, version 26. For skeletal variables (angle, length ratio, and change in vertebral angle), the tension test, and percentage collagen content, multiple one-way analysis of variance (ANOVA) tests with Tukey’s post hoc tests were used to determine if these variables differed among species. When comparing the anterior red muscle to the posterior red muscle from the peduncle (for both tension and collagen content), a paired t-test was used to assess potential differences between the muscles. Specifically, the anterior variable (tension or collagen content) was subtracted from the posterior variable for each individual. This number was then compared to zero (the null hypothesis).

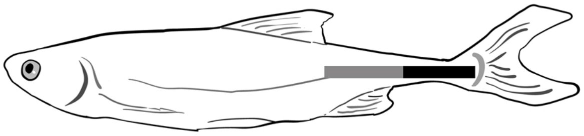

Fig. 2. Illustration of a bonytail documenting the location of the red axial muscle tested in the study. After the muscle was removed, it was separated into an anterior portion (grey) and a posterior portion (black) before being used in a stress test or for histological analyses.

3. Results

3.1. Skeletal anatomy

To assess differences in skeletal anatomy across the *Gila* species, we cleared and stained individuals from each species and captured images of the peduncle region (Fig. 3). Each individual demonstrated a change in vertebral spine angle, such that posterior vertebral spines were consistently more acute when compared to the more anterior spines.

ANOVA was used to quantify the association between caudal peduncle anatomy and vertebral morphology in the three *Gila* species.

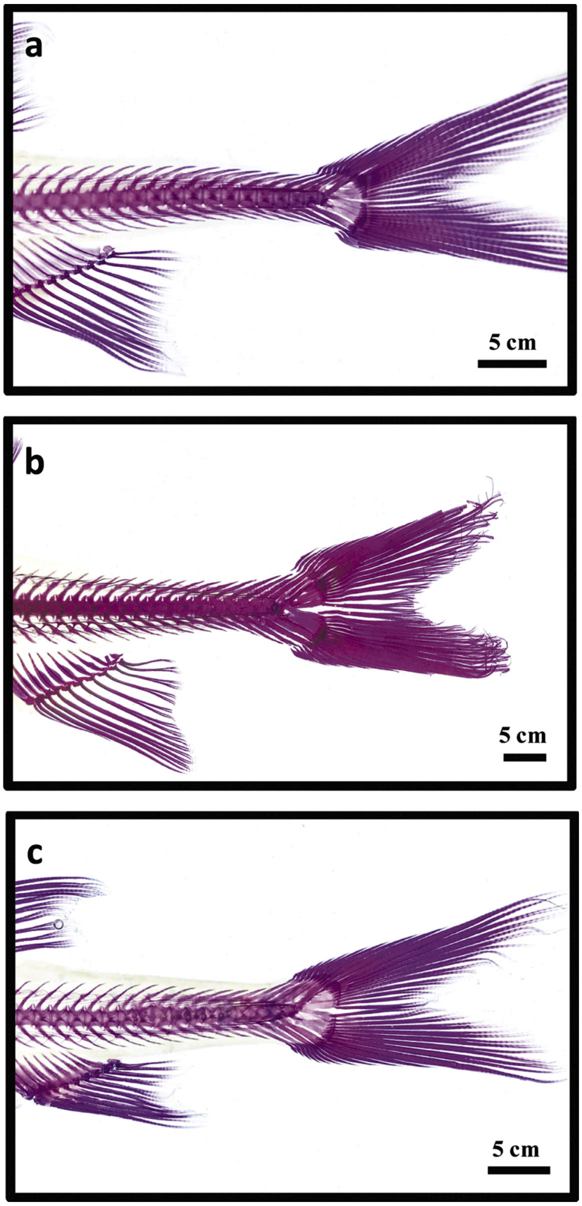

Fig. 3. Cleared and single stained (bone, alizarin red) peduncle region images of representative individuals of each species. Bonytail (panel a) have a similar vertebral angle in both the anterior and posterior vertebrae. However, bonytail vertebrate have more acute angles when compared to other species (panel b or c). Humpback chub (panel b) and roundtail chub (panel c) display relatively more obtuse vertebral spines in the anterior that become more acute in the posterior region of the vertebral column. Spine length does not change from the anterior to the posterior in any of these three species. See also Fig. 4.

Bonytail display more acute neural and hemal spine angles (ANOVA $F = 7.152$, $df_1 = 24$, $df_2 = 2$, $p = 0.004$ and ANOVA, $F = 30.252$, $df_1 = 24$, $df_2 = 2$, $p < 0.001$ respectively), when compared to roundtail chub (Tukey post hoc, $p = 0.007$ and < 0.001 respectively) and humpback chub (Tukey post hoc, $p = 0.012$ and $p < 0.001$ respectively, Fig. 4). Bonytail also displayed acute hemal spine angles, when compared to neural spine angles (t-test, $p = 0.018$, Fig. 4a). The three species did not display differences in spine-length-ratio (spine length divided by the total length) for either the neural or hemal spines (ANOVA, $F = 1.273$, $df_1 = 23$, $df_2 = 2$, $p = 0.299$ and ANOVA, $F = 1.130$, $df_1 = 24$, $df_2 = 2$, $p = 0.340$ respectively, Fig. 4b).

Changes in spine angle were used to document skeletal modifications from anterior to posterior and variation among the three species. There was less of a change in spine angle from anterior to posterior for bonytail (for both neural and hemal spines), ANOVA, $F = 7.704$, $df_1 = 23$, $df_2 = 2$, $p = 0.003$ and ANOVA $F = 13.507$, $df_1 = 24$, $df_2 = 2$, $p < 0.001$ respectively), when compared to humpback chub (Tukey post hoc, $p = 0.024$ and $p < 0.001$) or roundtail chub (Tukey post hoc, $p = 0.003$ and $p = 0.001$, Fig. 4c). To put this more simply, bonytail have more acute spine angles in the anterior compared to the other *Gila* species and the spine angle is similar among all vertebrae measured. When neural and hemal spines are compared within a species, humpback chub and roundtail chub show similar patterns in the dorsal and ventral vertebral spines (t-test, $p > 0.05$); however, bonytail show asymmetry when neural and hemal spine angles are compared within a specimen (t-test, $p = 0.001$, Fig. 4c). Hemal spine angles become more acute from anterior-to-posterior, relative to neural spine angles. However, these angle changes are still small, relative to the pattern demonstrated by other *Gila* species.

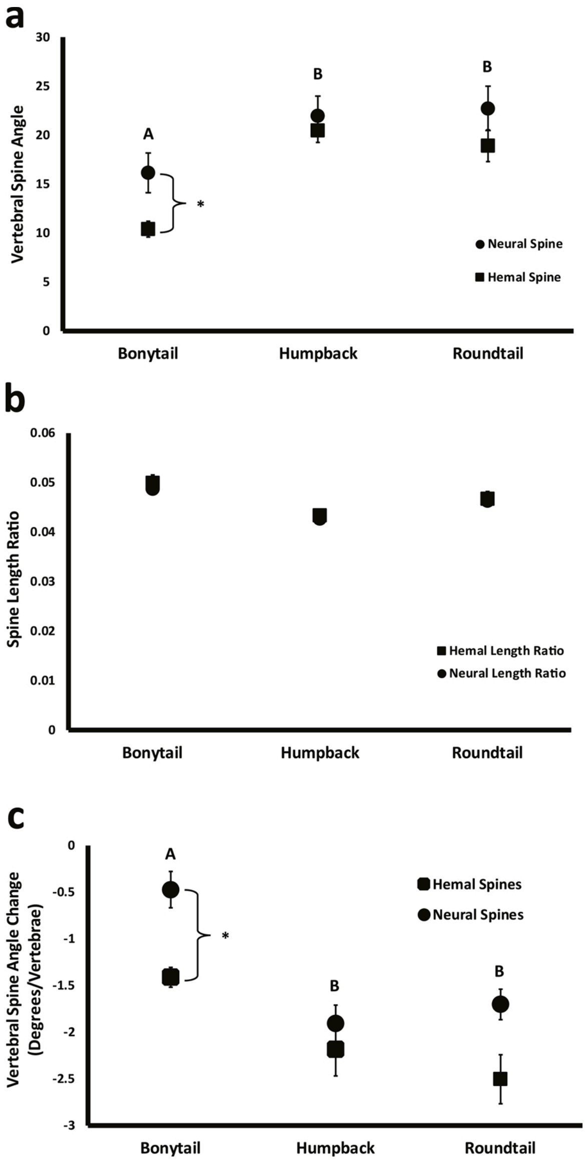

Fig. 4. Bonytail ($n = 9$) display more acute vertebral spine angles (a) and very little change in vertebral spine angles from anterior to posterior (c), when compared to humpback chub ($n = 10$) and roundtail chub ($n = 8$). There are no differences in the relative (size adjusted) lengths (b) of the vertebral spines among the three species. Letters denote significant differences across species for both neural and hemal spines. Asterisk denotes differences between neural and hemal spines within a species. Error bars represent +/- 1 S.E.M.

3.2. Material Testing

To determine if bonytail muscles have a greater ability to resist tensile forces, relative to the other *Gila* species, we performed tension tests on red axial muscle. There were no differences between the right and left sides of the body (t-test, $p = 0.34$ and $p = 0.67$) for the anterior and posterior regions respectively; thus, the muscles from the right and left sides of the body were pooled in future analyses. Roundtail chub showed “weaker” posterior red muscle (i.e., the muscle failed at lower stress values), when compared to the posterior muscle of bonytail (ANOVA, $F = 8.468$, $df_1 = 32$, $df_2 = 2$, $p = 0.001$, Tukey post hoc $p < 0.001$) and humpback chub (Tukey post hoc $p = 0.031$, Fig. 5a, squares). All three species showed similar ability to withstand stress in the anterior portion of the red muscle (ANOVA, $F = 0.883$, $df_1 = 30$, $df_2 = 2$, $p = 0.424$, Fig. 5a, circles). Bonytail and humpback chub also possess “stronger” red muscle in the posterior region of the red muscle when compared to the anterior portion (Table 1, Fig. 5a). Roundtail chub’s red muscle does not display any stress differences between the anterior and posterior portions of their body Table 1, Fig. 5a).

3.3. Collagen

Although there was a trend for bonytail and humpback chub to have more collagen in their posterior muscle than roundtail chub (Fig. 6), these differences were not significant (ANOVA, $F = 0.216$, $df_1 = 30$, $df_2 = 2$, $p = 0.807$). Bonytail had 20.3% collagen in their posterior red muscle, humpback chub had 19.2 % collagen, and roundtail chub had 15.5 % (Fig. 5b). The anterior muscle also showed no difference in collagen content across the three species (ANOVA, $F = 1.338$, $df_1 = 30$, $df_2 = 2$, $p = 0.278$, Fig. 5b). However, when comparing anterior to posterior muscle within a species, only bonytail had greater collagen content in their posterior muscle when compared to their anterior muscle; neither of the other fishes showed differences in collagen content in anterior vs. posterior muscle (Table 1, Fig. 5b).

4. Discussion

This paper provides support for the hypothesis that bonytail are converging on a similar morphology to that of scombrids (Moran et al., 2016). When compared with their close relatives, both bonytail and scombrids have more acute angles in the neural and hemal spines and these modified spines form shallow caudal peduncle. Although it has not yet been measured, it seems likely that scombrids reduce the neural and hemal angles symmetrically, while bonytail reduce the hemal angles more than the neural angle. However, scombrids also reduce the *length* of their neural and hemal spines to create a shallower peduncle, whereas bonytail do not modify spine length along the caudal body, when their spines are compared to those of close relatives. Data presented here suggest that fishes can modify a variety of anatomical parameters to produce similar gross anatomical outcomes. However, Scombrids are true thunniform locomotors, while bonytail demonstrate some locomotor characteristics that are more similar to those of fishes that use

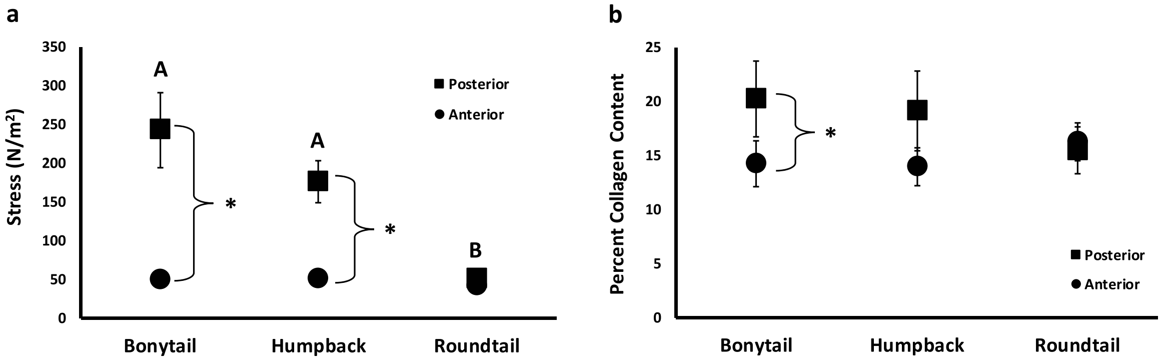

Fig. 5. The posterior-most muscle of bonytail (n = 12) and humpback chub (n = 10) is stronger under tension (a) than the muscle of roundtail chub (n = 10). There is no difference in the collagen content (b) among the three species, but bonytail have more collagen in their posterior red muscle when it is compared to their anterior red muscle. Letters denote a statistical difference between the species. Asterisk denotes difference between the anterior and posterior muscles as per a paired t-test. Error bars represent +/- 1 S.E.M.

Table 1
Summary of statistics comparing the anterior muscle to the posterior muscle in *Gila spp*. Values represent the difference between the anterior and posterior muscle within one species. The differences were compared to zero and considered significant if $p < 0.05$. Asterisk denotes a difference between the anterior and posterior muscle within each species.

Species	Variable	Difference	t	p-value
Bonytail	Anterior - Posterior Stress	−16.93 N/m^2	−4.166	0.002*
	Anterior - Posterior Collagen	−6.90%	−3.315	0.007*
Humpback chub	Anterior - Posterior Stress	−14.39 N/m^2	−4.744	0.001*
	Anterior - Posterior Collagen	−4.70%	−1.491	0.17
Roundtail chub	Anterior - Posterior Stress	−1.16 N/m^2	−0.881	0.399
	Anterior - Posterior Collagen	2.30%	0.342	0.74

carangiform locomotion (Moran et al., 2018). Indeed, the anatomy of bonytail appears to mirror this intermediate locomotor mode — their peduncle is not as shallow (Moran et al., 2016) and their skeletal anatomy is not as highly modified as that of scombrid fishes.

Lamnid sharks and scombrids have developed specialized mechanisms to transfer force from the anterior musculature to the caudal fin (Bernal, 2001). Tunas evolved an elaborate musculotendon system, while Lamnid sharks transmit force through their skin, both achieving a similar outcome in drastically different ways (Shadwick et al., 2002; Wainwright et al., 1978). Regardless of how these fishes transmit force, they have both evolved elongate red musculature (myomeres that span multiple vertebrae) that reduces axial bending of the anterior body (Donley et al., 2004). Bonytail may transmit force directly through the posterior region of the superficial red axial muscle, which is rich in collagen fibers compared to the anterior region of the same muscle. Future work could examine the myomeres and locomotor kinematics to see if the myomeres span multiple vertebral segments and if bonytail swim with reduced anterior bending, relative to less specialized roundtail chub.

Gila spp. appear to provide an excellent model in which to study the evolution of tendons. Scombrids have evolved an elaborate tendon system (Shadwick et al., 2002), but *Gila* may be using increased collagen within the muscle fibers to strengthen the muscle. The purpose of lateral tendons in scombrids is to transmit force from anterior musculature to the hypural plate. Therefore, the increased tensile strength in posterior musculature observed in *Gila spp*. may indicate that red muscle is transmitting force from the anterior region of body to the hypural plate. However, we did not find significant differences in collagen content in the red muscle across species. We note that our statistical power is low because of our limited ability to obtain specimens of imperiled species. However, it is possible that fascia around the muscle or other attachments to the skin may influence the tensile strength of this muscle, beyond any strength improvement provided by collagen. Other factors that can influence tensile strength are the number of protein-to-protein cross linkages and orientation of the collagen fibers (Reddy, 2004). An increase in protein-to-protein cross linkages will increase the strength of collagen (Vogel, 1991) and tendons (Bernal et al., 2001; Shadwick et al., 2002) and allow the fibers to resist large forces. If tensile forces are applied along the same axis as the collagen fibers, this will enable tendons to resist greater forces than if the collagen fibers were randomly arranged (Reddy, 2004). Although we did not find a statistical difference in collagen composition between species, we did find a difference in tensile strength and collagen content between anterior and posterior portions of bonytail's red muscle. This suggests that collagen may play a role in increasing the tensile strength of the red muscle of *Gila spp.*, possibly conferring increased swimming ability in high flow environments.

The historic riverine environment and seasonal flooding regime of the Colorado River system likely served as a strong selection pressure for *Gila* fishes to evolve new morphologies or behaviors that allowed them to avoid being swept downstream during stochastic, high-flow events. Bonytail have a shallow peduncle compared to roundtail chub (Moran et al., 2016), and this morphology is achieved by a modification of the skeletal anatomy. Specifically, bonytail create shallow peduncle by reducing their vertebral spine angles (Fig. 4). Surprisingly, the spine angle changes little from anterior to posterior (approximately one degree per vertebrae). This is a different pattern than has been observed in cyprinodontiformes (Minicozzi et al., 2019) and scombrids (Moran et al., 2016), where vertebral angles are more obtuse (larger angle) in the anterior, but become more acute along the body, toward the caudal peduncle. Bonytail have relatively acute angles in the anterior and there is little to no change toward the caudal peduncle, especially for the hemal spines. This relationship is also dependent on the number of vertebrae in the peduncle region, where fewer vertebrae may have to display a greater change in angle to create a shallow peduncle. However, the total number of vertebrae in the peduncle was not considered for our comparisons with scombrids.

Somewhat surprising, bonytail do not display symmetry between the

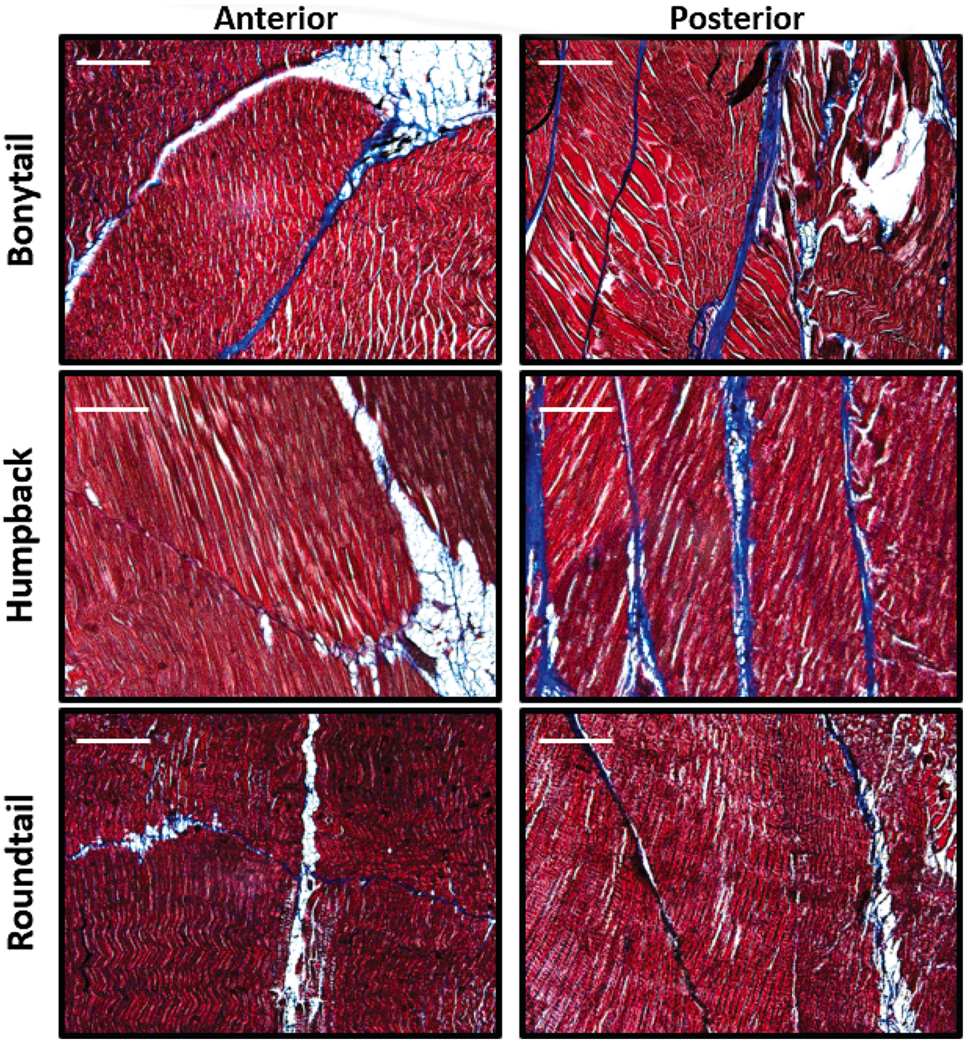

Fig. 6. Representative images of histological sections of the red (slow oxidative) axial muscle of three *Gila* species. Sections were stained using Mason's trichrome to show muscle tissue in red and collagen fibers in blue. The left column represents the anterior red muscle (grey bar illustrated in Fig. 2), while the right displays the posterior (black bar illustrated in Fig. 2) for bonytail, humpback chub, and roundtail chub. Scale bar represents 500 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

neural and hemal spines; fishes are thought to typically display symmetrical vertebral-spine anatomy (Ford, 1937; Minicozzi et al., 2019). In bonytail, the hemal-spine angle is more acute than the neural-spine angle (Fig. 4a) — thus contributing more to creating a shallow peduncle. The other *Gila* species displayed spine angle symmetry, that is, they had similar neural and hemal spine angles. However, it is not yet clear to what extent the skin and connective tissues contribute to forming the overall peduncle shape.

The *Gila* complex represent a *continuum* of morphologies and behaviors that have evolved for their specific historic range and seasonal flow regimes. Both humpback chub and roundtail chub have more acute spine angles when compared to other cyprinids (Moran et al., 2016) and cyprinodontids (Minicozzi et al., 2019) and humpback chub have stronger (tensile strength) red axial muscle than roundtail chub. These morphologies may also prove to be accurate predictors of *how* species within this genus maintain position within high flow. Although additional work on humpback chub is required to elucidate these patterns, previous work has shown that bonytail swim at high speeds with relatively lower oxygen consumption, while roundtail chub brace themselves on the bottom when confronted with high flows (Moran et al., 2018). We predict that humpback chub would exhibit intermediate behaviors, such as, swimming against the water current similar but would brace themselves at the bottom at a slower current than bonytail. If this is true, the *Gila spp*. complex is a fascinating model system in which to examine trade-offs in the evolution of anatomy, behavior, and performance.

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Declaration of Competing Interest

The authors report no declarations of interest.

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