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Development of Pectoral Apparatus in Ictalurid Catfish

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Development of Pectoral Apparatus in Ictalurid Catfish

General Abstract

The thesis contains two manuscripts- 1) Developmental changes in pectoral muscle fiber diameter and number in the Blue Catfish, *Ictalurus furcatus* and 2) Reduction in pectoral spine and girdle in domesticated Channel Catfish, *Ictalurus punctatus* is likely caused by the absence of fish predators. The first study showed that fiber diameter increased linearly with fish size, whereas fiber number increases non linearly with fish size in the Blue Catfish correlated with dietary shift. The second study showed shorter spines in domesticated Channel Catfish as compared to wild Channel Catfish was a result of reduction in selection pressure during domestication in the absence of fish predators.

General Introduction

The Fine lab has been working on catfish pectoral spines for many years. Catfish spine is an anti-predator adaptation and can be bound, locked and stridulated to produce sound (Fine et al. 1996, Fine et al. 1997). Work has been done on comparison of pectoral spines in Virginia catfish (Duvall 2007), effects of predators on Channel Catfish, *Ictalurus punctatus* (Nellis 2008), morphology and behavioral ecology of pectoral spine of Channel Catfish (Fine et al. 2011) scaling of pectoral muscles in Virginia catfish (Miano et al. 2013).

This thesis contains two manuscripts, which follow up work on the pectoral muscles and spines of the Blue and Channel Catfish.

In the first manuscript titled **Developmental changes in pectoral muscle fiber diameter and number in the Blue Catfish *I. furcatus***, I examined at the development of muscles weight, fiber diameter and fiber number.

In the second manuscript titled **Pectoral spine and girdle reduction in domesticated Channel Catfish is likely caused by the absence of fish predators**, some experiments were previously done, and I measured the length and weight of the spines and weight of the pectoral girdle of control and experimental channel catfish exposed to largemouth bass, and analyzed the data to determine there is any difference between them.

**Developmental changes in pectoral muscle fiber diameter and number
in the Blue Catfish *Ictalurus furcatus***

Abstract

Catfish have a complex pectoral spine that can be bound, locked or rubbed to produce stridulatory sounds and which functions in swimming and as an antipredator adaptation. There are 8 pectoral muscles including four for the first spine and four for the remaining rays. Pectoral muscles in the Blue Catfish *Ictalurus furcatus*, an invasive species in Chesapeake estuaries, are known to grow continuously. In this study I categorized growth of six pectoral muscles in young of the year to young mature adults of 5-6 years of age including muscle weight, fiber diameter and fiber number. The abductor muscles for the spine and ray are larger and have more fibers than the arrector ventralis and arrector dorsalis that mediate specialized spine functions (locking and stridulation). In this size range muscle weight and fiber diameter increase linearly, but fiber number exhibits a complex nonlinear increase. Fiber number barely increases in small fish and then jumps dramatically over two fold in fish of about 30 cm TL before stabilizing in larger fish. The fiber increase correlates with dietary shifts to piscivory.

Introduction

Catfishes include nearly 2700 species, comprising about one third of all freshwater fish making them among the most successful of fishes (Teugels 2003). They are important for commercial and recreational interest and for scientific research. A characteristic feature of catfishes is the pectoral spine that is formed by fusion and subsequent hypertrophy and modification of the first fin ray with the first distal radial (Reed 1924). The pectoral spine can be bound, locked or stridulated and used as an anti-predator device (Fine et al 1997; Fine et al 2011). The pectoral spine base possesses distinct apomorphic, dorsal, anterior and ventral processes (Hubbs and Hibbards 1951), forming complex articulations with the cleithrum and scapulacoracoid of the pectoral girdle (Brousseau 1976). The pectoral girdle is composed of a fused cleithrum and scapulacoracoid that provides a rigid base to anchor the pectoral spine (Schaefer, 1984). The cleithrum makes up the anterolateral portions of the pectoral girdle, which is an L- shaped bone that bears the spinal fossa, a curved chamber that surrounds the dorsal process of the pectoral spine (Miano et al. 2013).

In ictalurids, the anterior cleithrum runs obliquely forward parallel to the coracoid, and the paired bones articulate at the midline (Brousseau 1976). The posterior scapulacoracoid is medial to the cleithrum and forms a set of articulations with the anterior and ventral processes of the pectoral spine. The posterolateral scapulacoracoid bears a ring shaped channel called the scapular ring (Fine et al. 1997; Diogo et al. 2001).

Morphology of catfish pectoral musculature has been described in eight families by Diogo et al. (2001); however the family Ictaluridae, endemic to North America (Grande and Lundberg 1988),

was not included in his study. Few studies have focused on the morphological differences within the Ictaluridae (Brousseau 1976; Duvall 2007), and such morphological differences may have profound effect on behavior and biological activities (Maie et al. 2007). Miano et al. (2013) described muscle morphology and explored relationships in scaling between pectoral muscles and pectoral spines and girdles of ictalurid catfish within and among species. They noted that pectoral spines grow non-linearly with respect to fish and girdle weight, but girdles grow isometrically, linearly with fish weight. Pectoral muscle growth decreases in larger Blue and Channel Catfish, *Ictalurus furcatus* and *Ictalurus punctatus* respectively, but increases linearly with fish weight and girdle weight in other species. Spine width in all species increases linearly with total length, and in most species the spine base also grows linearly with total length. In all species except for wild Channel Catfish girdle depth increases linearly with total length (Duvall, 2007, Miano et al. 2013).

In ictalurid catfishes, pectoral spine movements are mediated by specialized muscles. A different nomenclature has been used to describe the pectoral musculature of catfishes by Miano (2013), Diogo (2001, 2007), and Brousseau (1976). The nomenclature of Miano (2013) is simple and based on the function.

There are four spine muscles: the spine abductor, the arrector dorsalis, the arrector ventralis and the spine adductor. The four muscles of the pectoral rays are the abductor and adductor superficialis, and the abductor and adductor profundus. Generally, the spine abductor and spine adductor are similar in weight, and both are larger than the arrector dorsalis and arrector ventralis (Miano et al. 2013). The abductor and adductor superficialis are similar, and both are

considerably larger than the profundal muscles that mediate fine movement. Abductors are larger than adductors and spine muscles are larger than ray muscles, except in Flathead Catfish, *Pylodictis olivaris* attesting their importance in binding, locking and stridulation.

The arrector dorsalis and ventralis appear to be involved in locking, binding, and stridulation whereas the spine abductor is thought to mediate locking, which occurs when the pectoral spine is fully abducted (Fine et al. 1997). During unlocking, the arrector ventralis contracts, unhooking the anterior process from the locking fossa and the spine adductor retract the anterior process from locking fossa. Contraction of the arrector ventralis during abduction causes stridulation. When the arrector dorsalis contracts, it moves the anterior process against the coracoid and wedges the dorsal process in spinal fossa (Fine et al. 1997). The arrector dorsalis and ventralis, and spine abductor and adductor, are pennate muscles that strongly attach to a tendon and are difficult to remove from the fish by surgery. On the other hand, ray muscles are parallel muscles. Pennate muscles can exert more force per unit area (Miano et al. 2013). They contract more powerfully and help in pivoting the spine forward. Fiber pattern following directions of Miano et al. (2013) are described in Table -1.

Table 1. Pectoral muscles and description of fiber orientation in the Blue Catfish *Ictalurus furcatus* (from Miano et al. 2013)

Name of Pectoral muscle	Fiber orientation
Spine Abductor	A convergent muscle in the distal half with a central tendon. Muscle fibers are parallel proximally and appear bipennate in the distally half as fibers converge upon the tendon.
Spine Adductor	An unipennate muscle. There are multiple small tendons proximally, which converge on the single broad tendon half way from the distal end.
Ray Abductor	A long parallel muscle and with several thin tendons of varying length.
Ray Adductor	A short and rectangular shaped muscle with multiple tendons.
Arrector Dorsalis	A bipennate muscle with a central tendon and 20% from the proximal end is formed of the central tendon.
Arrector Ventralis	A multipennate muscle.
Abductor profundus	A small parallel muscle with tendons at both ends.
Adductor profundus	A small parallel muscle.

Although pectoral spine and muscle growth have been described (Duvall 2007; Miano et al. 2013), sizes and numbers of muscle fibers in the pectoral muscles have never been described in ictalurid catfishes or seemingly any teleost. This information would be important in understanding muscle recruitment in binding, locking, and stridulation functions and the dynamics underlying muscle growth. The present study examines the relationship of number and diameter of the muscle fibers with total length and weight of different spine and ray muscles of Blue Catfish, *Ictalurus furcatus*.

Materials and Methods

Blue catfish, *Ictalurus furcatus* were collected from fresh-water regions of the James River near the VCU Rice Center. Wild fish were placed in 280 L tanks in the laboratory. To eliminate ectoparasites and minimize bacterial contamination, fish were treated with 10mg/L dose of potassium permanganate for 7 minutes prior to placement in tanks.

Fish were anesthetized in 200mg/L MS-222 (tricaine methanesulfonate), weighed and measured for total length (TL). Fish ranged from 13 cm to 44 cm and 18 gm to 865 gm. The muscles were exposed using a ventral approach (Miano et al. 2013). The head and girdle were severed from the body transversely. The apex of the pectoral girdle was separated from the pharyngeal cavity. The organs were removed by cutting through the connective tissue joining them to the pectoral girdle. The hypaxial muscles around the girdle were cut with scissors down the midline until the coracoids was reached. Then using fingers or a blunt probe, the hypaxial muscles were separated from skin to expose the spine and ray adductors. The small abductor and adductor profundus muscles were difficult to dissect out, particularly in small fish and hence, were excluded from the present study. The pectoral muscles were dissected in the following order: arrector ventralis, arrector dorsalis, ray abductor, spine adductor, ray adductor and spine abductor. The dissected muscles were fixed in 7% formalin for a day and then rinsed in 0.1 M phosphate buffer. Muscles were embedded in OCT embedding compound and sectioned at 12 μm in the transverse plane on a cryostat. Sections were mounted on subbed slides and stained in methylene blue. The number of fibers was determined with a Howard disc in the ocular of a compound microscope and average of greatest and least fiber diameters of at least 20 fibers were measured with a compound microscope, and the Element program. Fiber diameters were averaged for each fish, which was

treated as an N of 1. Muscle fiber number and diameter were compared among fish with a repeated measures ANOVA followed by Tukey's test (Graphpad Prism Software). Regressions of fiber number and diameter against fish TL and weight were calculated.

Results

Fish weight increased exponentially with total length (TL; exponential growth equation $Y = 8.013 * \exp 0.1X$; $r^2 = 0.88$). There was a 5 cm gap in fish size from 27 to 32 cm TL when weight increase accelerated (Fig. 1). Unpublished data recalculated from Miano et al. (2013) for muscle weight against fish weight indicated linear growth (r^2 ranged from 0.86 to 0.98 and $p < 0.0001$) in the six pectoral muscles in fish from 101 to 985 g (Table-1, Fig. 2), and the adductor and abductor muscles were larger than the arrector muscles. Similarly, fibers in abductors and adductors for ray and spine muscles were larger, and these muscles had more fibers than in the arrector dorsalis and ventralis (Fig. 3). For muscle fiber diameter $F_{5,90} = 26.1$; $p < 0.0001$, $r^2 = 0.59$; for muscle fiber number $F_{5,75} = 292.5$; $p < 0.0001$, $r^2 = 0.95$.

Muscle fiber diameter increased linearly with TL and weight (Fig-4A-L). For total length r^2 ranged from 0.4 to 0.7 (Table-2A) and for weight from 0.5 to 0.67 (Table-2B). Fiber number increased non-linearly with TL and weight (Fig-5A-L). Data were fit with a third order polynomial regression. Fiber number increased minimally in small fish from 13.1 to 21 cm in TL then increased somewhat before a big jump in 33 cm fish. Fiber number in larger fish (> 33 cm) remained constant. A similar pattern occurred with weight. Values of r^2 for fiber number to total length ranged from 0.93 to 0.98 (Table-3A) and for fiber number to weight from 0.88 to 0.96 (Table-3B).

Discussion

Unlike the six muscles in typical teleost, which may be further divided in some cases (Winterbottom 1974), catfishes have eight pectoral muscles, four for the pectoral spine and four for the remaining rays (Diogo et al.2001, Miano et al. 2013). The ray muscles namely ray adductor and abductor seem to be conserved in the catfish, though the muscles of the spine have changed considerably (Miano et al. 2013).

This study examined development in six of the eight muscles, the four spine muscles and the superficial ray abductor and adductor. The profundal ray abductor and adductor are small and difficult to dissect in small fish and were therefore excluded. Data from Miano et al. (2013) indicate the pectoral muscles grow linearly in fish of the size range utilized in the current study although there is a decrease in larger fish. However muscle weight doesn't indicate the relative contribution of fiber growth and the addition of new fibers.

Fiber diameter and the number of fibers of the ray muscles and spine abductor and adductor were found to be two times higher than in the arrector dorsalis and ventralis, in agreement with Miano et al. (2013). The fiber diameter grows linearly with total length and weight. However, fiber number is relatively static in smaller fish, increases rapidly at around 33 cm and then remains constant as fish grow. Fish in this study included young of the year (YOY) (< 30cm), juveniles and newly matured adults (> 130 cm) (Greenlee and Lim 2011). We have used polynomial equations to analyze the relationship of fiber number to total length and weight. The polynomial equation fits best for the size range used in the current study. The fiber number will attenuate when the fish is very large, and we use the regression to describe the data and do not consider it mechanistic.

Based on Greenlee and Lim's age and growth study, the largest fish in the study was 5-6 yrs old (Greenlee and Lim 2011). Blues Catfish according to Graham (1999) matures at 4-7 yrs, therefore increase in fiber number appears to precedes sexual maturity and correlates with the dietary shift. Blue catfish in the James River consume a variety of prey, and major diet constituents differ among size ranges. Fish smaller than 30 cm mostly feed on amphipods (42.1%) whereas fish ranging from 30-60 cm eat fewer amphipods (13.4%) and start to consume bony fish. Additionally, macroinvertebrates (56.3) are consumed in higher proportion than in small catfish (40.1%). The dietary shift from smaller to larger invertebrates and bony fish correlates with the rapid increase in fibers in fishes above 30 cm (Schloesser et al. 2011). In mammals approximately 100% of postnatal muscle growth is controlled by hypertrophy of a static number of fibers (Wirtz et al. 1983, Alnaqueeb & GoldSpindle 1987). Fishes however can add fibers postnatally. In Pyramid Butterflyfish, *Hemitaurichthys polylepis* sonic muscles fibers are highly variable in size and cylindrical in cross section (Boyle et al. 2012). In Rainbow Trout, *Oncorhynchus mykiss* early postnatal growth of axial is mostly from hyperplasia which is gradually replaced by hypertrophy (Weatherley et al. 1979; Weatherley and Gill 1981; Strickland 1983). In Oyster Toadfish, *Opsanus tau* sonic muscle the number of muscles fibers increases by 16 fold, and mean minimum diameter of the fiber increases by 3 fold as the fish grow (Fine et. al 1990). Muscle fibers increase at a decelerating rate in older fish. The Blue Catfish is of interest since it can grow to immense sizes, i.e. greater than 45 kg (Greenlee and Lim 2011, Schloesser et al. 2011). Most muscle growth is initially by fiber hypertrophy before a rapid increase in fiber number, which stabilizes at about 32 cm. Therefore muscle growth in larger fish appears to continue by hypertrophy, mirroring the mammalian pattern after a period when new fibers are added.

Table-1 Linear regression equation and r^2 for pectoral muscle weight against weight in six Blue Catfish *I. furcatus* (recalculated from unpublished data in Miano et al. 2013)

Regression Equations	r^2	p
Spine Abductor $W_t = 0.001 W_t + 0.019$	0.98	<0.0001
Spine Adductor $W_t = 0.0006 W_t + 0.024$	0.92	<0.0001
Ray Abductor $W_t = 0.001 W_t - 0.03$	0.98	<0.0001
Ray Adductor $W_t = 0.0004 W_t + 0.03$	0.86	<0.0001
Arr Dorsalis $W_t = 0.0003 W_t - 0.017$	0.87	<0.0001
Arr Ventralis $W_t = 0.0001 W_t - 0.005$	0.89	<0.0001

sTable-2 Linear regression equation and r^2 for fiber diameter against total length in six pectoral muscles in the Blue Catfish *I. furcatus*

Regression Equations	r^2	p
Spine Abductor Diameter= $0.45 TL + 30.93$	0.70	<0.0001
Spine Adductor Diameter = $0.5 TL + 31.44$	0.47	0.0008
Ray Abductor Diameter = $0.5 TL + 30.74$	0.40	0.0026
Ray Adductor Diameter = $0.33 TL + 34.04$	0.70	<0.0001
Arr Dorsalis Diameter = $0.134 TL + 35.75$	0.43	0.0015
Arr Ventralis Diameter = $0.122 TL + 32.27$	0.56	0.0002

Table-3 Linear regression equation and r^2 for fiber diameter against weight in six pectoral muscles in the Blue Catfish *I. furcatus*

Regression Equations	r^2	p
Spine Abductor Diameter =0.017 Wt+39.76	0.60	<0.0001
Spine Adductor Diameter =0.021 Wt+40.41	0.50	0.0005
Ray Abductor Diameter =0.03 Wt +38.35	0.61	< 0.0001
Ray Adductor Diameter =0.013 Wt + 40.63	0.68	<0.0001
Arr Dorsalis Diameter =0.006 Wt +37.92	0.60	<0.0001
Arr Ventralis Diameter =0.005 Wt +34.53	0.55	0.0002

Table-4 Polynomial regression equation and r^2 for fiber number against total length in six Blue Catfish *I. furcatus*

Regression equations	r^2
Spine Abductor No.= $1237-123.6TL+5.575TL^2-0.065TL^3$	0.97
Spine Adductor No. = $1335-140TL+6.3TL^2-0.075TL^3$	0.97
Ray Abductor No. = $1012-113.3TL+5.53TL^2-0.067 TL^3$	0.98
Ray Adductor No. = $1746-184.2 TL +7.68 TL^2-0.088 TL^3$	0.98
Arr Dorsalis No. = $958.1-118.4 TL +5.03 TL^2-0.057 TL^3$	0.93
Arr Ventralis No.= $948-110.9 TL +4.58 TL^2-0.051 TL^3$	0.94

Table-5 Polynomial regression equation and r^2 for fiber number against weight in six Blue Catfish *I. furcatus*

Regression Equations	r^2
Spine Abuctor No. = $307+3.48Wt-0.005Wt^2+ 0.000003Wt^3$	0.95
Spine Adductor No.= $286.7+3.53Wt-0.0056Wt^2+0.000003Wt^3$	0.96
Ray Abductor No.= $206.3+4.2Wt-0.007Wt^2+ 0.000004 Wt^3$	0.96
Ray Adductor No.= $250.6+3.57Wt-0.005Wt^2+ 0.000003 Wt^3$	0.96
Arr Dorsalis No.= $55.21+1.93Wt-0.0017Wt^2+0.0000002 Wt^3$	0.93
Arr Ventralis No. = $66.32+2.03Wt-0.0028Wt^2+ 0.000001Wt^3$	0.88

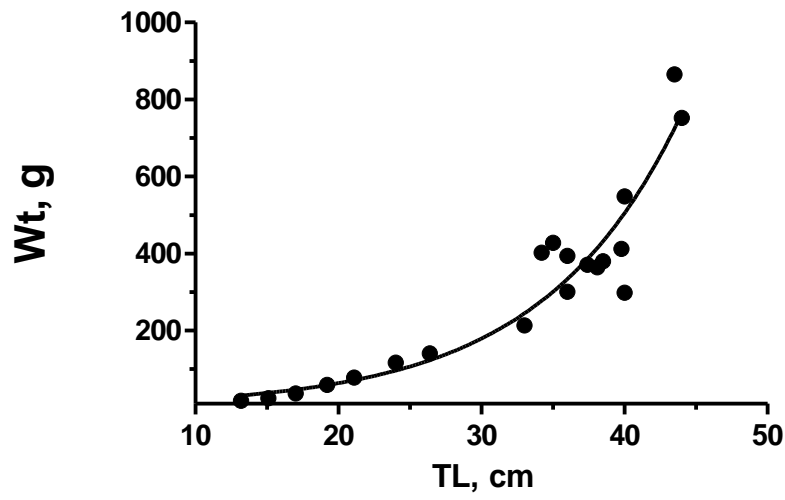


Fig. 1- Relationship of fish weight to total length in Blue Catfish *Ictalurus furcatus*.

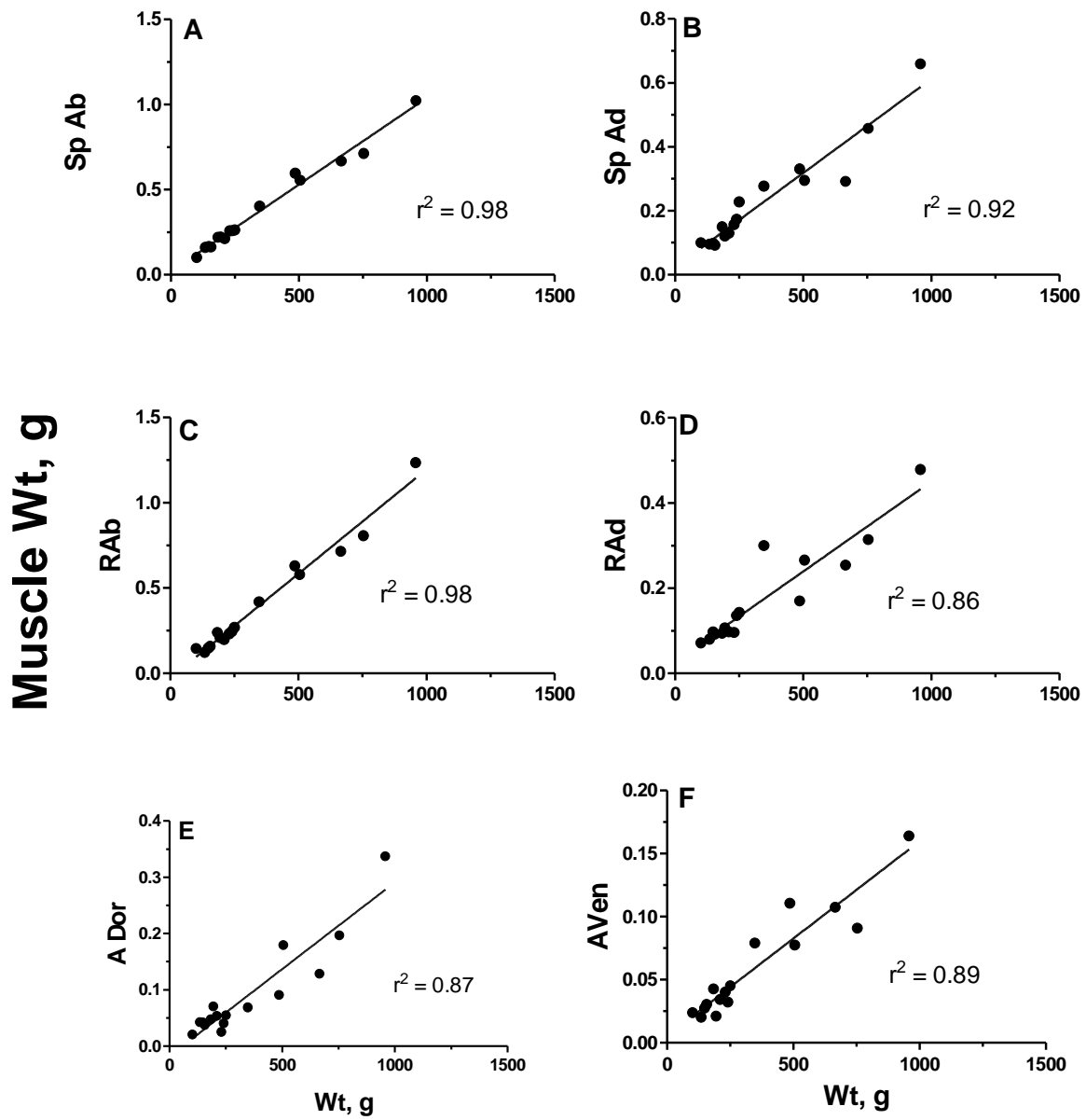


Fig. 2- Relationship of muscle weight to fish weight for six pectoral muscles in Blue Catfish *I. furcatus*. For all muscles $p < 0.0001$ (Recalculated from Miano et al. 2013)

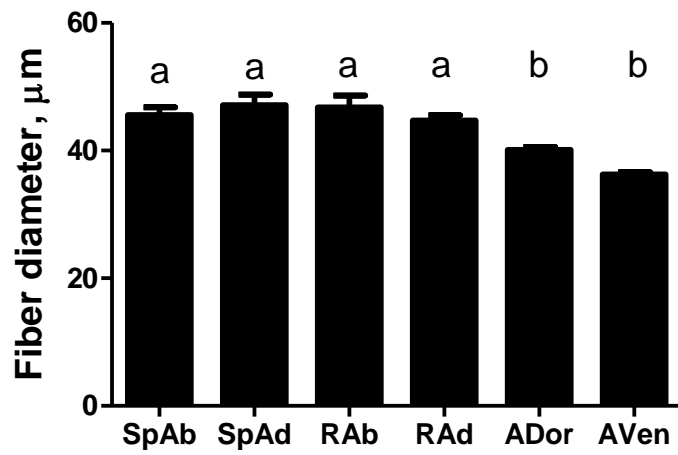


Fig. 3 Fiber diameter in six pectoral muscles of the Blue Catfish *I. furcatus* ($F_{5,90} = 26.21$, $P < 0.0001$).

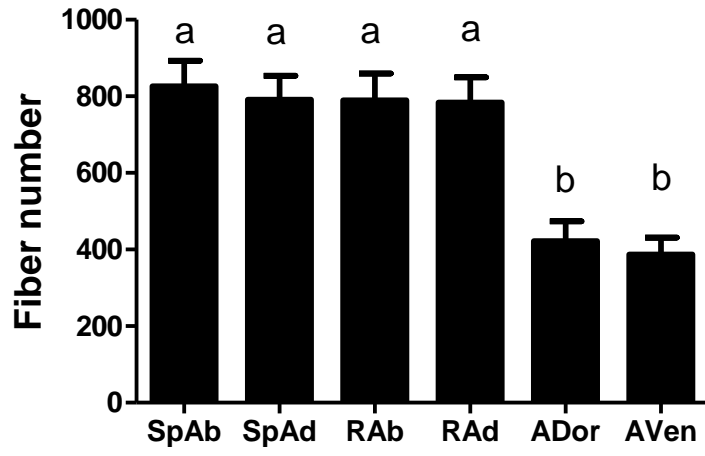


Fig. 4 Fiber number in six pectoral muscles of the Blue Catfish *I. furcatus* ($F_{5,75} = 292.5$, $P < 0.0001$).

Fiber diameter, μm

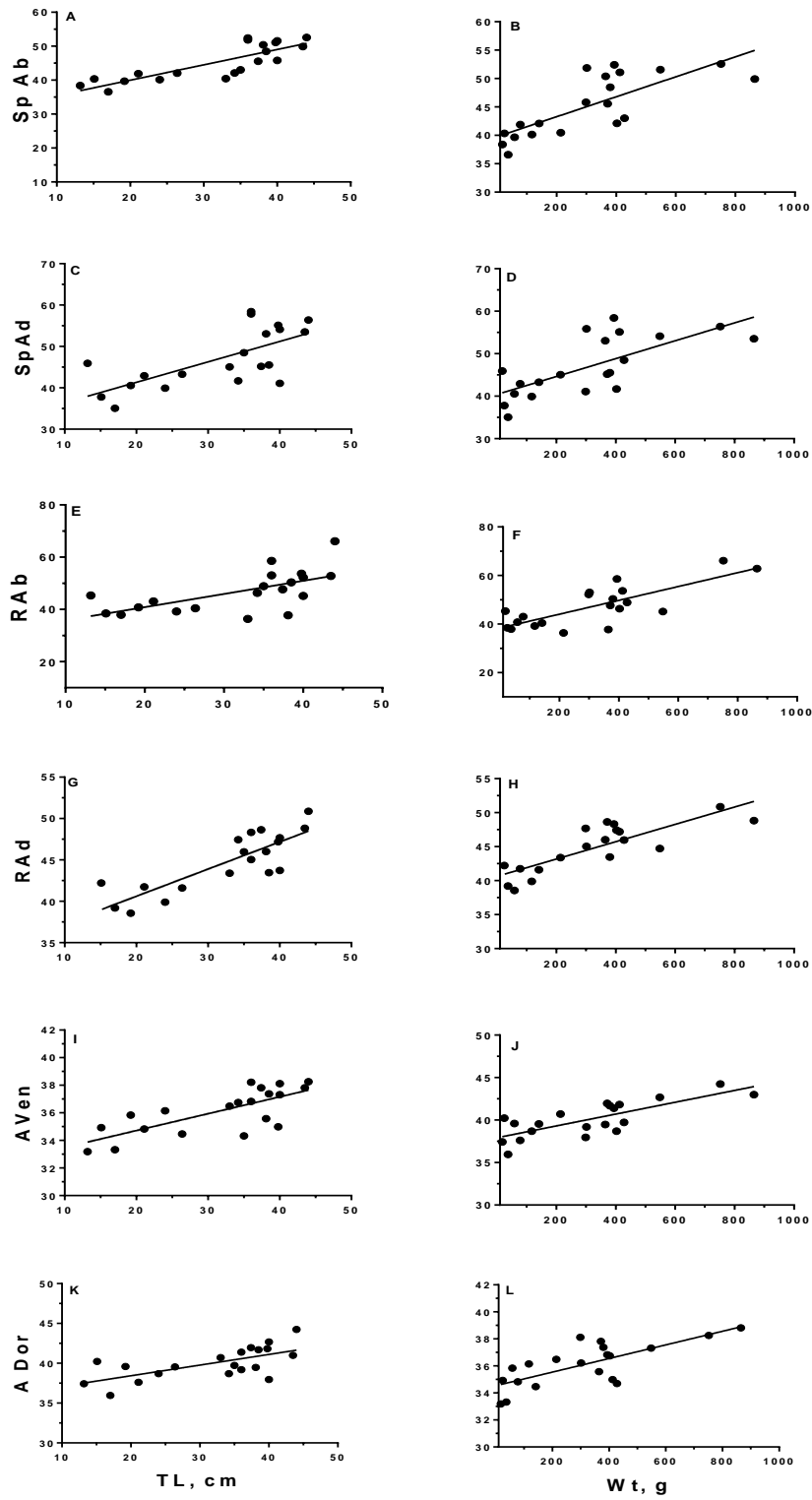


Fig.5 - Relationship of fiber diameter of six pectoral muscles to total length (A,C,E,G,I,K) and weight (B,D,F,H,J,L) in the Blue Catfish *I.furcatus*.

Fiber Number

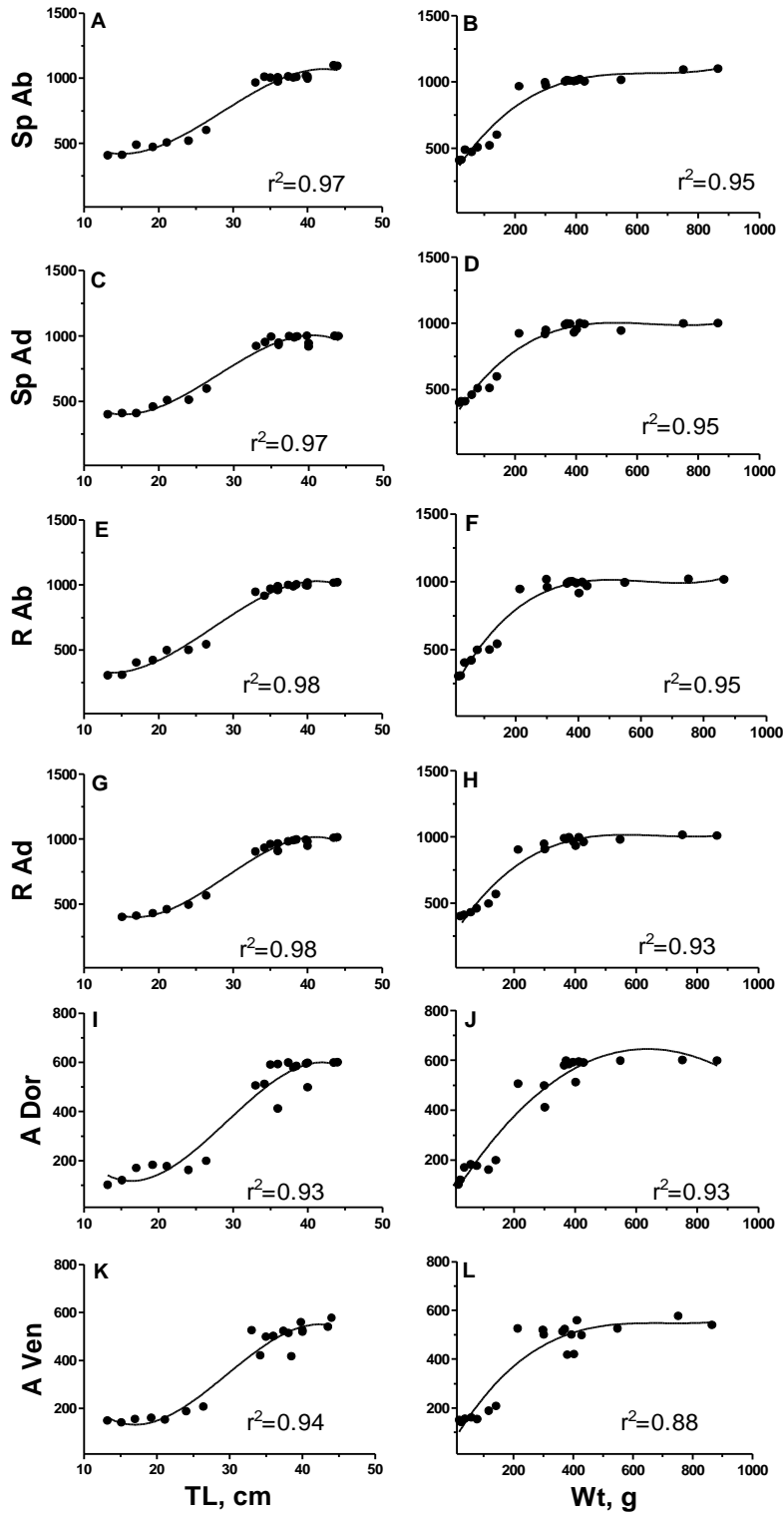


Fig.6- Relationship of fiber number to total length (A,C,E,G,I,K) and weight (B,D,F,H,J,L) in the Blue Catfish.

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Pectoral spine and girdle reduction in domesticated Channel catfish is

likely caused by the absence of fish predators

Abstract

When locked in a forward position, the stout pectoral spines of the Channel Catfish *Ictalurus punctatus* more than double the fish's width and complicate ingestion by gape-limited predators. Processes on the spine base mate with complimentary features on the pectoral girdle, a robust and fused structure that anchors the locked spine. This study demonstrates that the pectoral spines and girdle are lighter in domesticated than in wild Channel Catfish and that both spine and girdle exhibit negative allometric growth. This finding could be explained by relaxation of selection pressure for spine growth during domestication or by an epigenetic effect in which exposure to predators in wild fish stimulates pectoral growth. We tested the epigenetic hypothesis by exposing domesticated Channel Catfish fingerlings to Largemouth Bass (*Micropterus salmoides*) predators. Spines and girdles grew isometrically in these fingerlings, and control fish, which grow larger, have longer and heavier spines and heavier girdles than predator-exposed fish. However, regression analysis indicates no difference in proportional pectoral growth between control and predator-exposed fish. We therefore suggest a decrease in selection pressure accounts for smaller pectoral growth in domesticated Channel Catfish. Additionally, mechanical properties of the spine and not just its length are an important component of this adaptation.

Introduction

In a number of aquatic species, the spines have been examined as an antipredator adaptation. Laforsch and Tollrian (2004) found that *Chionochloa flavicans*, *Cyclops sp.* release chemical cues that induces longer tail spine and helmet in water flea *Daphnia*, and these morphological changes protect *Daphnia* from predators. Recher and Recher (1968) found fish with spines escape from herons more often than spineless fish. The catfish spines are an antipredator adaptation which when locked in fully abducted position doubles the fish width and thereby hinders ingestion by gape limited predator (Sismour et al. 2013). In fact, Largemouth Bass, *Micropterus salmoides* are reported to eat three times as many Channel Catfish, *Ictalurus punctatus* fingerlings with clipped than with intact spines in comparably sized fish (Bosher et al. 2006) and in choice situation Largemouth Bass, will eat fewer channel catfish as compared to Bluegills, *Lepomis macrochirus* and Goldfish, *Carassius auratus auratus* (Sismour et al. 2013). There are subtle morphological differences between channel catfish spines collected beneath eagle nests by Bryan Watts of VCU Center of Conservation of Biology and domesticated stocks (Fine et al. 1997). Domesticated catfish may have smaller spines, which could result from relaxation in the selection pressure in absence of predators or exposure to predators in wild fish could result in spine growth due to an epigenetic effect. The epigenetic hypothesis was tested by exposing channel catfish fingerlings to largemouth bass behind a mesh barrier for several months. Previous work shows that when channel catfish are exposed to largemouth bass, they eat less, grow more slowly and move more slowly than control fish (Fine et al. 2011).

Materials and Methods

Domesticated fish were obtained from the aquaculture facility of Virginia State University from stocks purchased from Arkansas. Fish were weighed in grams and measured for total length (TL). Frozen fish were thawed and boiled briefly to clear the skeletons. After drying in air, pectoral spines were measured for length with digital calipers, and spines and girdles were weighed. Because spine tips often break during handling, the longer and heavier spines were used for study. Measurements were linearized by log-log transform and regressed against TL or weight. Transformed regressions of domesticated and wild fish were compared by analysis of covariance.

Largemouth Bass *Micropterus salmoides* (29-43 cm LT) were obtained by hook and line from a small impoundment at the Rice Center of Virginia Commonwealth University (IACUC permit number AD20042), and work was carried out using methods approved by the Virginia Commonwealth Animal Care and Use Committee (AM10047). Fish were maintained in eight fiberglass tanks at 23°C. To separate each tank into halves, a black polyethylene plastic mesh barriers was erected across the center of each tank. Ten channel catfish were introduced in the right half of each tank and acclimated for one week; largemouth bass was added to four of the tanks. The other four tanks in which no bass was added served as controls. The experiment was conducted for 13 weeks. Fish were fed multiple times a week.

Based on data from wild and domestic fish and our hypothesis that predators would increase spine growth, we compared means from control and experimental tanks with a one tailed t test (Duvall, 2006). The TL, fish weight, pectoral spine length, pectoral spine weight and girdle

weight from the four control and four from experimental tanks were averaged so that each tank was treated as a single unit for analysis ($N = 4$ per treatment), and $p < 0.05$ was considered significant. Because differences in size of control and experimental fish tank means were not sufficient to determine if relative spine and girdle growth are affected by predators, growth was categorized by linear regressions of spine length against TL and spine and girdle weight against total weight. Regressions across the tanks for control and experimental treatments were compared with analysis of covariance to see if there were any tank effects. Since slopes and intercepts were not significant for spine length, spine or girdle weight with either treatment (Table-1), data from individual tanks were combined and relative growth for spines and girdles was categorized and compared using individual fish.

Result

In catfish that ranged from 87 mm to 562 mm, girdle weight, spine length and spine weight increased with fish size at a decelerating rate. Adjusted means for domesticated and wild were 40.4 and 49.9 mm for spine length, 0.6 and 1.04 g for spine weight and 5.42 and 7.5 g for girdle weight respectively. Domesticated catfish had shorter and lighter spines and lighter girdles than the wild catfish (Fig. 1). Slope for spine weight against fish weight were so high in wild fish ($F_{1,91} = 10.7$, $p < 0.0015$) that intercept couldn't be tested. Slopes for spine lengths didn't differ between domesticated and wild fish ($F_{1,91} = 1.23$, $p = 0.2669$) but elevations were higher in wild fish ($F_{1,92} = 219.6$, $p < 0.0001$). Slopes for girdle weight were similarly higher in wild fish ($F_{1,86} = 8.1$, $p = 0.0055$).

In the Largemouth Bass experiment, mean TL ranged from 16.4-17.59 cm and 14.38-16.06 cm and weights from 37.2-45.4 and 21.2-31.7 for control and experimental respectively. Control Channel Catfish were longer ($T_6 = 3.229$, $p = 0.0179$) and heavier ($T_6 = 4.384$, $p = 0.0046$) than experimental fish at the termination of the experiment (Fig. 2) indicating that the presence of the predator across the barrier retarded growth [13]. The control fish had longer ($T_6 = 2.420$, $p = 0.0259$) and heavier ($T_6 = 2.078$, $p = 0.0415$) pectoral spines as would be expected of larger individuals (Fig. 2), but the difference in girdle weight was not significant ($T_6 = 1.808$, $p = 0.1206$). Spines and control and girdles of both control and experimental catfish grew linearly, in this size range (Table-2, Fig 3). The r^2 values for linear regression of control fish ranged from 0.82-0.95 for spine length against TL, spine weight against weight and girdle weight against weight. Analysis of covariance indicated no significant difference between pectoral spine lengths, weight or girdle weight of control and experimental fish (Table-2; Fig 3). Analysis of covariance

indicated no significant difference between relative pectoral spine length, weight or girdle weight of control fish exposed to largemouth bass (Table-2).

Discussion

Domestication in Channel Catfish has involved selection for fast growing stocks (Dunham and Smitherman 1982) in ponds without fish predation, and selection on the pectoral apparatus was not considered. The decrease in pectoral spine and girdle weight in domesticated fish was likely caused by relaxed selection pressure for growth of this defensive adaptation rather than an epigenetic affect caused by exposure to predators. Selection pressure on spine length has been demonstrated in populations of sticklebacks under different predation regimes: populations dominated by fish predators have long spines whereas populations with invertebrate predators have shorter ones (Huntingford 2007). The classic example of a predator-induced defense in fishes is an increase in body depth in Crucian Carp (Bronmark and Miner 1992). The 13 week experiment reported here resulted in significant growth differences but not changes in pectoral development. The negative allometry of pectoral girdle growth suggests that the axial and appendicular skeletons are controlled differentially. Furthermore, linear pectoral growth in young but not older fish suggests the importance of the adaptation when fish are small and face increased predation risk. We caution that growth patterns in domesticated and wild fish could differ. Domesticated fish might have to move less, which could result in slower growth in them.

The pectoral spine of Channel Catfish is an enlarged flattened fin ray that tapers toward the tip (Fine et al. 1997). The enlarged horizontal profile will increase resistance in the horizontal plane, which opposes forces caused by passage through a predator's mouth or underwater obstructions. Spine and girdle growth is isometric in small juveniles used in this study, and the negative allometry in spine length and weight is due to decreasing growth in larger individuals who would be less vulnerable to predation. Additionally, breakage of spine tips occurs commonly in both domestic and wild individuals. Decreasing girdle weight supports a growth

effect and not just breakage. The increase in spine weight would be determined by linear dimensions, particularly near the wider spine base, and spine weight per millimeter of spine length increases exponentially with TL (Duvall 2007). Wild Channel Catfish have wider spines (Duvall 2007), which increases the moment of inertia (a greater cross sectional area further from the midline) and therefore breakage resistance of the structure. The increased mass of the pectoral girdle in wild fish is striking since it is a major component of the fish's girth. Finally, the increased spine mass suggests that in addition to spine length (Tollrian and Dodson 1999) other dimensions that contribute to the material properties of a defensive spine are important to its function.

Table 1- Analysis of covariance for the four control and the four experimental tanks.

	Slope		Intercepts	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Ctrl SpW/TW	$F_{3,32}=0.7308$	0.541	$F_{3,35}=0.6068$	0.615
Exp SpW/TW	$F_{3,23}=0.6628$	0.583	$F_{1,68}=1.4039$	0.264
Ctrl SpL/TL	$F_{3,31}=1.320$	0.285	$F_{3,34}=0.9993$	0.405
Exp SpL/TL	$F_{3,23}=0.1801$	0.908	$F_{3,26}=1.3763$	0.272
Ctrl GW/TW	$F_{3,29}=2.324$	0.095	$F_{3,32}=1.4156$	0.256
Exp GW/TW	$F_{3,24}=1.5915$	0.217	$F_{3,27}=1.694$	0.191

Table-2 Relationship and coefficient of determination for spine length against total length, spine weight against fish weight and girdle weight against fish weight, analysis of covariance for slopes and intercepts and adjusted means calculated for 16 cm TL and 50 g weight

Regression Equations		r ²	Slope		Intercept		Adjusted Mean
			F	p	F	p	
Ctrl Spine Length=0.6TL+11.18	Exp Spine Length=0.75TL+9.16	0.46	F _{1,67} =0.97	0.32	F _{1,68} =0.68	0.41	20.78 cm
Ctrl SpineWt=0.001Wt+0.022	Exp Spine Wt=0.001Wt+0.023	0.79	F _{1,67} =0.48	0.4	F _{1,68} =2.37	0.13	21.16 cm
Ctrl Girdle Wt= 0.005Wt+0.058	Exp Girdle Wt=0.005Wt+0.086	0.92	F _{1,65} =0.14	0.7	F _{1,66} =3.57	0.06	0.072 g
		0.83					0.073 g
							0.308
							0.336

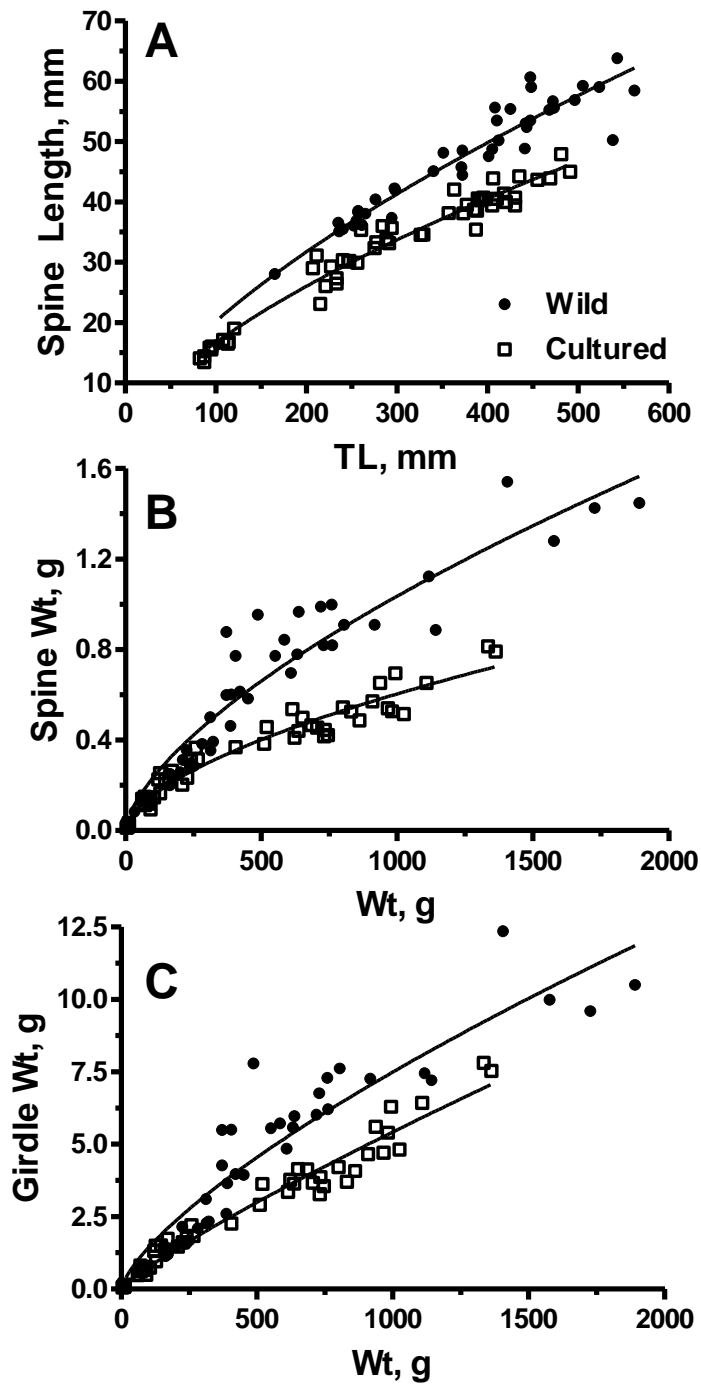


Fig. 1 Relationship of spine length to total length (A), spine weight to fish weight (B) and pectoral girdle weight to fish weight (C) for wild and domesticated catfish.

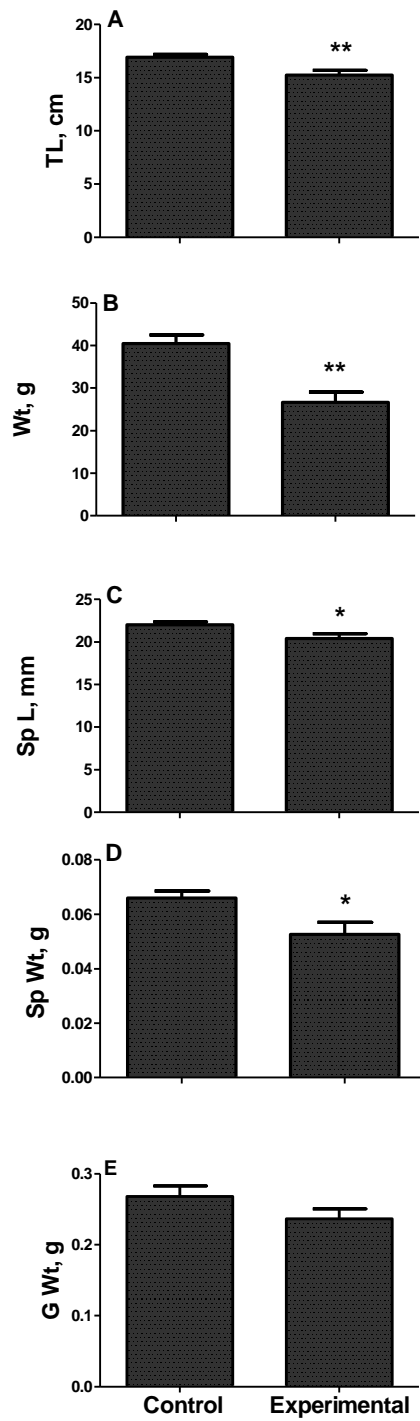


Fig. 2 Fish total length, fish weight, spine length, spine weight and girdle weight in control and experimental fish. * p< 0.05, **p<0.01

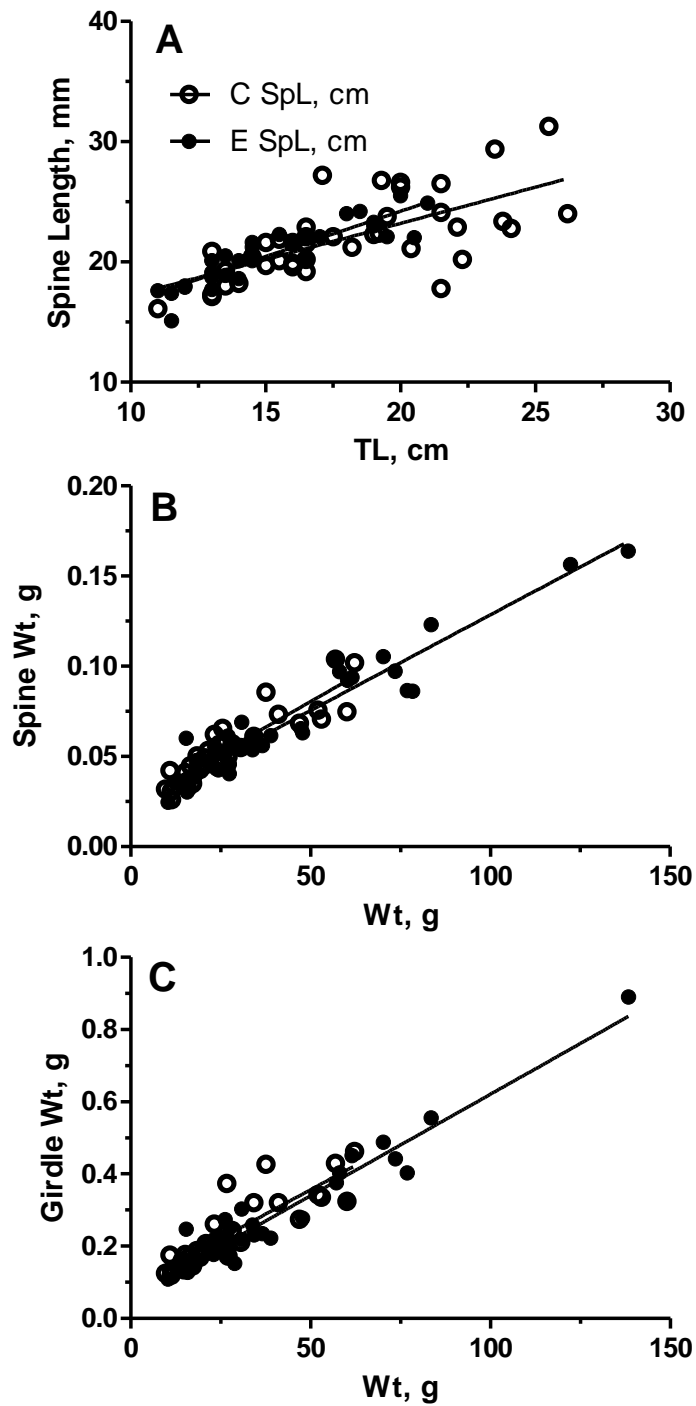


Fig. 3 Relationship of spine length to total length (A), spine weight to total weight (B) and girdle weight to total weight (C) in control and experimental fish.

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