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Reduction of the pectoral spine and girdle in domesticated Channel Catfish is likely caused by changes in selection pressure

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Tables 1

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Locked pectoral spines of the Channel Catfish *Ictalurus punctatus* more than double the fish's width and complicate ingestion by gape-limited predators. The spine mates with the pectoral girdle, a robust structure that anchors the spine. This study demonstrates that both spine and girdle exhibit negative allometric growth and that pectoral spines and girdles are lighter in domesticated than in wild Channel Catfish. This finding could be explained by changes in 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 for 13 weeks. Spines and girdles grow isometrically in the fingerlings, and regression analysis indicates no difference in proportional pectoral growth between control and predator-exposed fish. Therefore a change in selection pressure likely accounts for smaller pectoral growth in domesticated Channel Catfish. Decreasing spine growth in older fish suggests anti-predator functions are most important in smaller fish. Additionally, growth of the appendicular and axial skeleton is controlled differentially, and mechanical properties of the spine and not just its length are an important component of this defensive adaptation.

Spines have been examined extensively as anti-predator adaptations in a number of aquatic species including *Daphnia* (Tollrian and Dodson 1999) and the Three-Spined Stickleback *Gasterosteus aculeatus* (Huntingford and Coyle 2007). A major adaptation of catfishes, one of the most successful teleost groups, is a hypertrophied first pectoral spine that can be bound and locked (Fine et al. 1997). Binding involves active muscular force that engages friction-locking surfaces between the spine base and the pectoral girdle. Locking requires full abduction of the spine, which traps a spine process within a recess in the coracoid preventing further movement (abduction or adduction). In addition the spine can be rubbed against the cleithrum to produce stridulatory sounds that can serve in distress, agonistic and courtship capacities (Fine and Ladich 2003; Kaatz et al. 2010; Parmentier et al. 2010). These specialized functions are mediated by rearranged pectoral muscles (Diogo et al. 2001; Miano et al. 2013) and derived processes (dorsal, anterior and ventral) on the spine base (Hubbs and Hibbard 1951) that mate with complimentary structures on the pectoral girdle (Diogo et al. 2001; Fine et al. 1997). The pectoral girdle (a fused cleithrum, coracoid and scapula) is a robust structure (Fine et al. 1997), providing rigid support for the spine (Schaefer 1984). When locked in a fully-abducted position, the stout pectoral spines of Channel Catfish, *Ictalurus punctatus* Rafinesque more than double the fish’s width (Sismour et al. 2013) and impede ingestion by gape-limited predators. Intact Channel Catfish fingerlings are three times less likely to be eaten by Largemouth bass, *Micropterus salmoides* Lacepede than comparably-sized individuals with clipped spines (Bosher et al. 2006), and Largemouth bass consume fewer Channel Catfish than Goldfish, *Carassius auratus* L. and Bluegills, *Lepomis macrochirus* Rafinesque in choice experiments, supporting Forbes’ dangerous prey hypothesis (Sismour et al. 2013).

Channel Catfish spines collected beneath eagle nests (Duvall 2007) exhibited subtle morphological differences from those of domesticated stocks (Fine et al. 1997) calling into question the effects of domestication on spine development. Domestication in Channel Catfishes has selected for more rapid growth rate than in natural populations and a reduction of genetic diversity although introduction of new fish has likely minimized inbreeding (Hallerman et al. 1986; Smitherman and Dunham 2003).

We compared pectoral spines of Channel Catfish from the James River, Virginia with domesticated fish purchased from an Arkansas stock. Smaller spines found in domesticated fish could result from changes in selection pressure over multiple generations. Alternately, exposure to predators in wild fish could induce an epigenetic effect that turns on genes that induce spine growth (phenotypic plasticity). We tested the epigenetic hypothesis by exposing domesticated Channel Catfish fingerlings to Largemouth Bass behind a mesh barrier for several months. Previous work with these individuals demonstrated that fingerlings exposed to Largemouth Bass move and eat less than controls and grow more slowly (Fine et al. 2011). The current study demonstrates that although control fingerlings were longer and heavier than experimental fish, the proportional pectoral spine and girdle weight are similar in control fish and experimental fish exposed to Largemouth Bass, indicating that predators did not induce increased growth of the pectoral skeleton.

Methods

Wild Channel Catfish *I. punctatus* were caught by electroshocking (Virginia Department of Game and Inland Fisheries and Virginia Commonwealth University VADGIF permit number 0444631 and IACUC AD20216) in the James River, Virginia. 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 in mm (TL). Frozen fish were thawed and boiled briefly to clear the skeletons. After drying, pectoral spines were measured for length with digital calipers, and spines and girdles were weighed in milligrams. Since spine tips often break, the longer and heavier spine was used for analysis. Measurements were linearized by log-log transformation, regressed against TL or weight, and regressions of domesticated and wild fish were compared by analysis of covariance. Adjusted means for domesticated and wild fish were calculated from regressions for 400 mm TL and 1,000 g Channel Catfish. Data were plotted on a linear scale to illustrate growth rate.

Experimental protocols for non-consumptive effects of Largemouth Bass *Micropterus salmoides* predators are provided in Fine et al. (2011) and will be summarized briefly. Juvenile Channel Catfish were measured for total length TL in mm and weighed to 0.1 g. Largemouth Bass (29 to 43 cm TL) were obtained by hook and line from a small impoundment at the Rice Center of Virginia Commonwealth University (Charles City County, Virginia). Fish were maintained in eight 300 L fiberglass tanks at 23 C under a 14:10 LD cycle. A black polyethylene plastic mesh barrier (6.5 mm square openings) was erected across the center of each tank separating them into halves, and a clay flowerpot was added to the catfish side for shelter. Ten Channel Catfish were acclimated in the right half of each tank for one week, after which a Largemouth Bass was added to four of the tanks. Largemouth bass are a generalized fish predator that commonly consumes Channel Catfish in rivers and ponds (Sismour et al. 2013). The other four tanks with no bass served as controls. There was no difference in mean size of control and experimental Channel Catfish at the beginning of the experiment (Fine et al. 2011). Largemouth bass were fed at least twice a week with a dead Channel Catfish from the same stock as the experimental catfish. Catfish were fed to satiation multiple days per week using a standard 32% protein floating-pellet catfish ration. The experiment was conducted for 13 weeks.

Based on data from wild and domesticated fish and our hypothesis that predators would increase spine growth, we compared means from control and experimental tanks with a one-tailed T-test. TL, fish weight, pectoral spine length and weight and girdle weight from the four control and four experimental tanks were averaged so that each tank was treated as a unit (N = 4 per treatment), and $p < 0.05$ was considered significant. Because of differences in size of control and experimental fish at the termination of the experiment (see results), tank means were not sufficient to determine if relative spine and girdle growth are affected by predators. Growth was therefore evaluated by linear regressions of spine length against fish TL and spine and girdle weight against fish weight. Regressions were compared by analysis of covariance. Because of possible tank effects, we first compared regressions across tanks within control and within experimental treatments. Slopes and intercepts were not significant for spine length, spine weight or girdle weight within either treatment. Since there were no tank effects, data from individual tanks were combined, and relative growth for spines and girdles was evaluated and regressions were compared between control and experimental tanks using individual fish.

Results

PECTORAL COMPARISONS BETWEEN WILD AND DOMESTICATED CHANNEL CATFISH

Spine length, spine weight and girdle weight increased continuously with fish size although at a decelerating rate in catfish that ranged from 87 to 562 mm TL (Fig. 1). Adjusted means for domesticated and wild fish respectively were 40.4 and 49.9 mm for spine length, 0.60 and 1.04 g for spine weight and 5.42 and 7.50 g for girdle weight. Wild catfish had longer and heavier spines and heavier girdles than domesticated individuals. Slopes for spine lengths did not differ between domesticated and wild fish ($F_{1,91}$=1.23, p =0.2669), but elevations were greater in wild fish ($F_{1,92}$=219.6, p<0.0001). Slope for spine weight against fish weight were so much greater in wild fish ($F_{1,91}$=10.7, p=0.0015) that intercepts could not be tested. Slopes for girdle weight were similarly greater in wild fish ($F_{1,86}$=8.10, p=0.0055).

PREDATOR EXPOSURE EXPERIMENT

Mean TL ranged from 16.40 to 17.59 mm for control and 14.38 to 16.06 for experimental tanks and weights from 37.24 to 45.35 and 21.20 to 31.65 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. 2a, b) indicating that a predator across the barrier retarded growth (Fine et al. 2011). The control fish had longer (T_6=2.420, p =0.0259) and heavier (T_6=2.078, p=0.0415) pectoral spines (Fig. 2c, d) as would be expected of larger individuals, but the difference in girdle weight did not reach significance (T_6=1.808, p=0.1206). In this size range spines and girdles of both control and experimental catfish grew linearly (Table 1; Fig 3). The r^2 values for linear regressions of control fish ranged from 0.75 to 0.93 for spine length against TL, spine weight against weight and girdle weight against weight except for control spine length against TL with an r^2 of 0.46 (Table 1). Data points for control and experimental fish co-scattered, and analysis of covariance indicated no significant difference between proportional pectoral spine length, weight or girdle weight of control fish and fish exposed to Largemouth Bass (Table 1, Fig 3). The largest individuals were all in control tanks, and these individuals were likely responsible for slight though not significant differences in intercepts of spine and girdle weight regressions. Similar experimental and control regressions indicate that exposure to predators did not increase pectoral growth.

Discussion

The most parsimonious hypothesis to account for the decreased pectoral spine and girdle weight we observed in domesticated fish is changes in selection pressure for this defensive adaptation rather than an epigenetic effect caused by exposure to predators. Domestication of Channel Catfish has involved selection for fast growth in ponds without fish predation, and the pectoral apparatus was not considered when choosing breeding stock (Dunham and Smitherman 1982; Hallerman et al. 1986; Smitherman and Dunham 1993). However, breeding for fast growth could have inadvertently selected for fish with smaller pectoral spines and girdles. We note that spines are not a concern in large commercial ventures since fish are rarely handled individually until processing when they are stunned with a weak electric current before decapitation (Marshall 2004). Additionally smaller spines in aquaculture fish are still an effective adaptation and deter predation by Largemouth Bass (Bosher et al. 2006 Sismour et al. 2013).

Relaxation of selection pressure during domestication and inadvertent selection for smaller spines are complimentary hypotheses to explain spine reduction although inadvertent selection might be unlikely in the presence of fish predators. Unraveling the course of selection on spine size will be complex since contemporary aquaculture stocks do not have a single population of origin (Smitherman and Dunham 1983). Therefore, comparisons between cultured stocks with a single wild population of origin would not be possible. Experimentation to confirm either hypothesis could involve developing a cultivated stock from a wild population and following spine development over several generations. Such an endeavor would require at least a decade given that Channel Catfish in Virginia require 4-6 years to mature (Hubert 2000). It should also be possible to compare spine size in populations of domesticated fish with different growth rates.

Selection pressure on spine length has been demonstrated in populations of *Gasterosteus aculeatus* under different predation regimes: populations dominated by fish predators have long spines whereas populations with invertebrate predators have shorter ones (Huntingford and Coyle 2007). Similarly, in Nine-Spined Sticklebacks, a common garden experiment demonstrated that predator density and food availability did not affect body shape or armor, indicating that anti-predator traits are constituitive rather than inducible (Välimäki et al. 2012). The classic example of a predator-induced defense in fishes is an increase in body depth in Crucian carp, *Carassius carassius* (Brönmark and Miner 1992). Similarly, the presence of predators caused morphological changes in perch and roach (Eklöv and Jönssön 2007) and in pumpkinseed sunfish (Januszkiewicz and Robinson 2007).

The 13-week experiment reported here resulted in significant growth differences but not in changes in pectoral development when normalized to fish size. Our results do not rule out the possibility of an epigenetic effect on smaller or wild individuals. The negative allometry of pectoral girdle growth suggests that the axial and appendicular skeletons are controlled by different genetic mechanisms. One would expect axial and appendicular skeletons to grow proportionately in most fishes in order to provide normal control of fine movement. Our finding therefore supports the importance of the fused pectoral girdle (Schaefer 1984) as a major component of the anti-predator adaptation provided by the pectoral spines. Furthermore, linear pectoral spine growth in young but not older fish emphasizes the importance of the adaptation in small fish that face increased predation risk. We caution that growth rate in domesticated and wild fish differ.

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 larger 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; Huntingford and Coyle 2007) other dimensions that contribute to the material properties of a defensive spine are important to its function.

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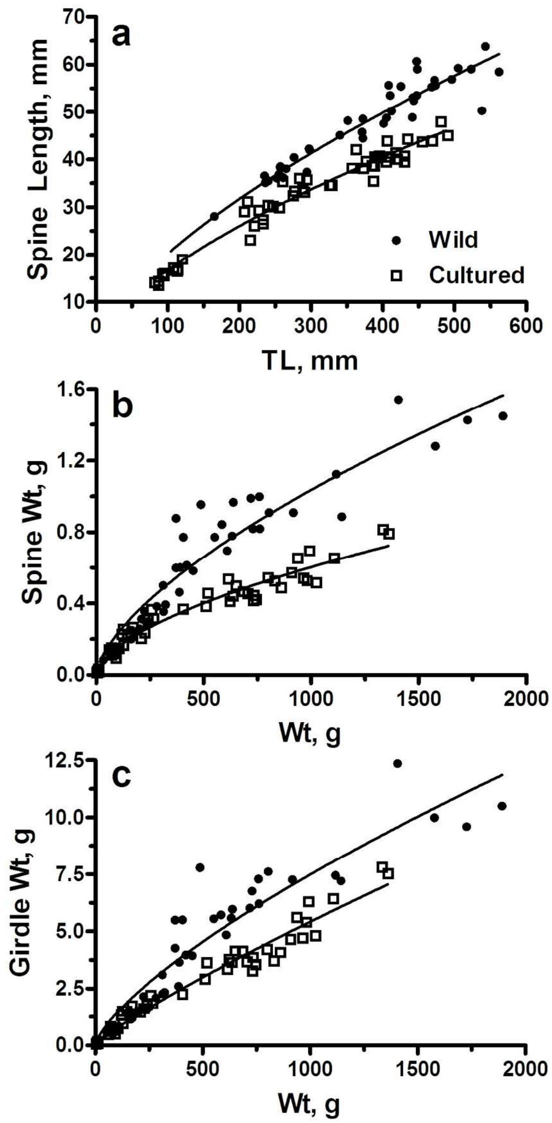
Figure Legends

Figure 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. Equations for James River fish: Log Spine L = 0.001654 + 0.6518 Log TL, r^2 = 0.92; Log Spine Wt = -1.928 + 0.6480 Log Wt, r^2 = 0.89; Log Girdle Wt = -1.284 + 0.7197 Log Wt, r^2 = 0.87. Equations for domesticated fish: Log Spine L = -0.0561 + 0.639, Log TL, r^2 = 0.96; Log Spine Wt = - 1.984 + 0.5885, r^2 =0.96 Log Wt; Log Girdle Wt = - 1.833 + 0.8558 Wt, r^2 = 0.96.

Figure 2. Mean ± SE total length, fish weight, spine length, spine weight, spine length for control and experimental Channel Catfish. * $p < 0.05$, ** $p < 0.01$.

Figure 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 Channel Catfish.



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. Equations for James River fish: Log Spine L = 0.001654 + 0.6518 Log TL, r2 = 0.92; Log Spine Wt = -1.928 + 0.6480 Log Wt, r2 = 0.89; Log Girdle Wt = -1.284 + 0.7197 Log Wt, r2 = 0.87. Equations for domesticated fish: Log Spine L = -0.0561 + 0.639, Log TL, r2 = 0.96; Log Spine Wt = - 1.984 + 0.5885, r2 =0.96 Log Wt; Log Girdle Wt = - 1.833 + 0.8558 Wt, r2 = 0.96.
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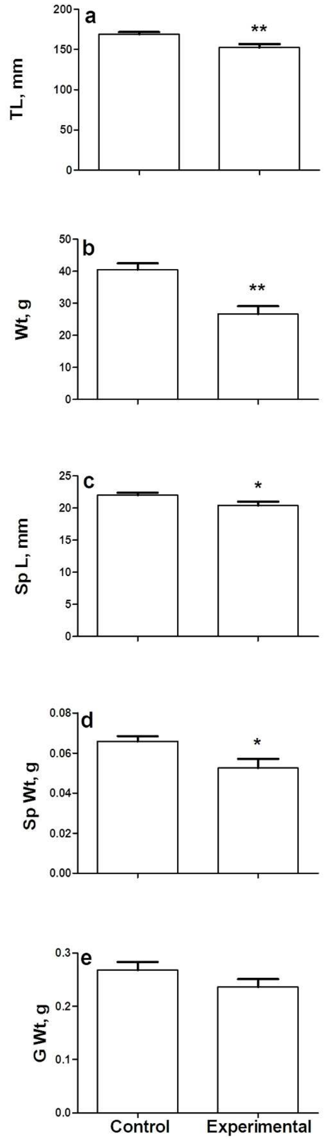

Figure 2. Mean ± SE total length, fish weight, spine length, spine weight, spine length for control and experimental Channel catfish. * $p < 0.05$, ** $p < 0.01$.
72x216mm (300 x 300 DPI)

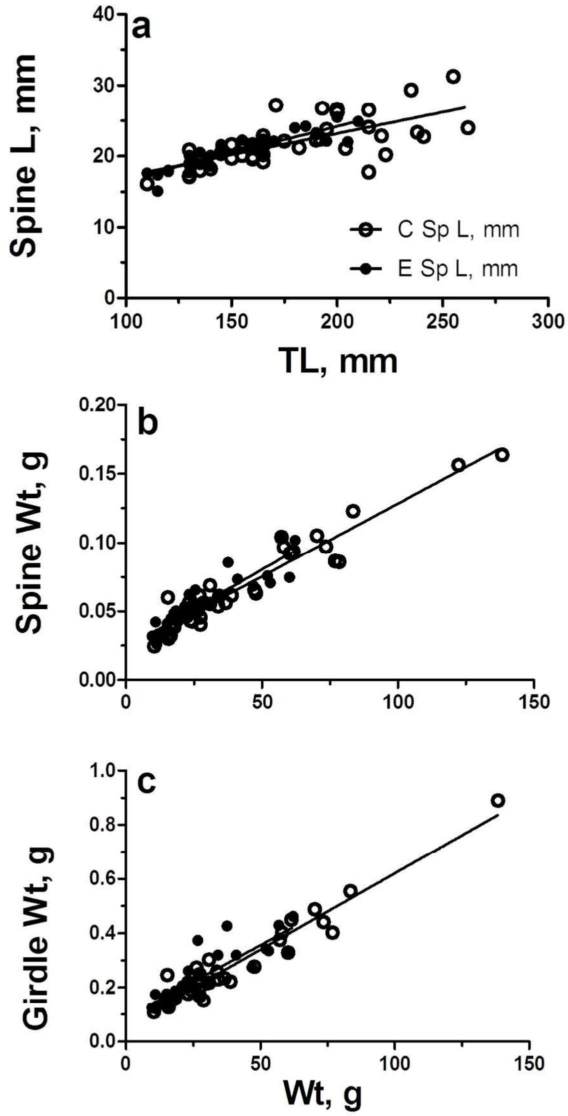

Figure 3. Relationship of spine length to total length (a), spine weight to total weight (b) and girdle weight to total weight (c) in control and experimental Channel catfish.
113x201mm (300 x 300 DPI)

Table 1 Regression equation 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 160 mm TL and 50 g weight Channel catfish

Regression Equations		Slope		Intercept		Adjusted Mean
	r^2	F	p	F	p	
Ctrl Spine L=0.0601 TL+11.18	0.46	$F_{1,67}$=0.97	0.32	$F_{1,68}$=0.68	0.41	20.80 mm
Exp Spine L=0.0752 TL+9.168	0.79					21.20 mm
Ctrl Spine Wt=0.0010 Wt+0.022	0.92	$F_{1,67}$=0.48	0.4	$F_{1,68}$=2.37	0.13	0.072 g
Exp Spine Wt=0.0011 Wt+0.023	0.83					0.078 g
Ctrl Girdle Wt= 0.0056 Wt+0.058	0.93	$F_{1,65}$=0.14	0.7	$F_{1,66}$=3.57	0.06	0.338 g
Exp Girdle Wt=0.0054 Wt+0.086	0.75					0.356 g