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Myosin heavy chain and parvalbumin expression in swimming and feeding muscles of *centrarchid* fishes: The molecular basis of the scaling of contractile properties

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ABSTRACT

In centrarchid fishes, such as bluegill (*Lepomis macrochirus*, Rafinesque) and largemouth bass (*Micropterus salmoides*, Lacepède), the contractile properties of feeding and swimming muscles show different scaling patterns. While the maximum shortening velocity (V_{max}) and rate of relaxation from tetanus of swimming or myotomal muscle slow with growth, the feeding muscle shows distinctive scaling patterns. Cranial epaxial muscle, which is used to elevate the head during feeding strikes, retains fast contractile properties across a range of fish sizes in both species. In bass, the sternohyoideous muscle, which depresses the floor of the mouth during feeding strikes, shows faster contractile properties with growth. The objective of this study was to determine the molecular basis of these different scaling patterns. We examined the expression of two muscle proteins, myosin heavy chain (MyHC) and parvalbumin (PV), that affect contractile properties. We hypothesized that the relative contribution of slow and fast MyHC isoforms will modulate V_{max} in these fishes, while the presence of PV in muscle will enhance rates of muscle relaxation. Myotomal muscle displays an increase in sMyHC expression with growth, in agreement with its physiological properties. Feeding muscles such as epaxial and sternohyoideus show no change or a decrease in sMyHC expression with growth, again as predicted from contractile properties. PV expression in myotomal muscle decreases with growth in both species, as has been seen in other fishes. The feeding muscles again show no change or an increase in PV expression with growth, contributing to faster contractile properties in these fishes. Both MyHC and PV appear to play important roles in modulating muscle contractile properties of swimming and feeding muscles in centrarchid fishes.

1. Introduction

Many fishes, including sunfish of the family Centrarchidae, employ suction feeding to capture prey (Lauder, 1980; Ferry-Graham and Lauder, 2001). In this feeding mode, rapid buccal expansion creates negative pressure within the buccal cavity, drawing water and prey into the mouth. To successfully capture evasive prey, suction feeding fish must employ high-speed kinematics. The cranial musculature muscle must therefore be capable of relatively fast kinetics to power these rapid feeding strikes. Indeed, the muscles that power suction feeding in sunfish are capable of relatively fast contractile properties (e.g. Carroll et al., 2009). Buccal expansion during suction feeding is due to contraction of the cranial epaxial musculature and ventral sternohyoideus and hypaxial musculature, at least in some fish species (Lauder et al., 1986; Carroll, 2004). In sunfishes, the epaxial muscle rotates the neurocranium upward, expanding the buccal cavity dorsally while the sternohyoideus muscle contributes to jaw opening as well as hyoid depression, which also increases the volume of the buccal cavity (Carroll, 2004).

Given the importance of successful feeding on survival, we previously examined the contractile properties of the feeding muscles of two species of sunfish (Carroll et al., 2009). The focus was on how the contractile properties of feeding muscles changed with growth relative to myotomal or swimming muscle. The two species, largemouth bass (*Micropterus salmoides*, Lacepède) and bluegill (*Lepomis macrochirus*, Rafinesque), differ in terms of the scaling of the cranial feeding morphology. Largemouth bass scale nearly isometrically—jaw morphology maintains constant proportions with growth, while bluegill scale allometrically—the length of in-levers of jaw opening and closing increase faster than fish length (Richard and Wainwright, 1995; Wainwright and Shaw, 1999). Contractile properties, such as maximum shortening velocity (V_{max}), of locomotory muscle typically slow with growth (James et al., 1998; Coughlin et al., 2001; Coughlin, 2002; Carroll et al., 2009). The same pattern for locomotory muscle is observed across a wide size range of phylogenetically-related animals (Rome, 1992). The feeding muscles of some fish species also show this pattern of slowing contraction kinetics with growth (e.g. African catfish, Van Wassenbergh et al., 2007).

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However, the scaling of feeding muscles of sunfish differed from this pattern. The contractile properties of the cranial epaxial muscle showed no scaling response in maximum shortening velocity growth in either species studied, maintaining a constant V_{max} across a five-fold increase in length (Carroll et al., 2009). Further, the epaxial muscle actually showed an increase in maximum steady state power output (W_{max}) with growth, while the myotomal muscle shows no change in W_{max} with growth (Carroll et al., 2009). Carroll and Wainwright (2011) recently demonstrated that the *in vivo* shortening velocity of the epaxial muscle during feeding strikes by largemouth bass also does not show a scaling response. Using sonomicrometry across a similar size range of bass feeding on evasive prey, they found little change in the shortening of velocity of muscle during the strikes (or strain rate). Successful feeding strikes appear to require high speed contractile properties at all sizes of bass and bluegill. A different scaling pattern was observed for the contractile properties of sternohyoideus muscle of the two sunfish species under study. Bluegill showed slowing contractile properties with growth, while bass showed a significant increase in shortening velocity with growth (Carroll et al., 2009). The difference in scaling response of this muscle between the two species may be associated with the variation in the scaling of cranial morphology seen in bass and bluegill.

The examination of the contractile properties in sunfish suggests that, in the same individual, the contractile properties of swimming muscles slow with growth while those of the feeding muscles generally do not. The focus of the present study is the molecular basis of different scaling properties of swimming vs. feeding muscles in sunfish. Differential shifts in muscle contractile properties suggest that different developmental programs of gene expression occur in myotomal vs. epaxial muscle, for instance. Although both epaxial and the myotomal muscle under study here are "white" or "fast-twitch" muscle, they apparently differ in the composition of the myofibrillar proteins. For instance, shifts in V_{max} are most directly dependent on myosin heavy chain (MyHC) content of a given muscle (Moss et al., 1995). Myosin molecules in muscle are hexamers, with two MyHC molecules and four myosin light chain (two regulatory light chains, two essential light chains). MyHC is responsible for cross-bridge formation and the generation of force and movement. In rainbow trout, the slowing of red or slow-twitch myotomal muscle with growth is associated with shifts in MyHC and regulatory light chain expression (Weaver et al., 2001; Donato et al., 2008).

Other proteins can vary in expression with growth. For instance, trout myotomal muscle (both red and white) displays a decrease in parvalbumin (PV) expression with growth (Coughlin et al., 2007). Parvalbumin is a low molecular weight myoplasmic protein. Greater parvalbumin content is found in fast-twitch muscle of various vertebrates—muscle with high rates of relaxation (Heizmann et al., 1982; Hou et al., 1991; Berchtold et al., 2000). PV binds myoplasmic Ca^{2+} during contractions, lowering Ca^{2+} concentrations in the myoplasm and thereby enhancing muscle relaxation. Variations in PV content are associated with shifts in contractile properties such as relaxation rate in different regions of the myotomal muscle of various fishes (Wilwert et al., 2006; Coughlin et al., 2007; Schoenman et al., 2010). Collectively these studies showed that decreases in parvalbumin content with growth are associated with the slowing of the relaxation rate in myotomal muscle as fish grow.

We hypothesize that the MyHC and PV content of sunfish muscle will modulate contractile properties. Specifically, we predict that for muscles that slow with growth, such as the white myotomal muscle in bass and bluegill, there will be an increase in the relative contribution of slow myosin heavy chain (sMyHC) to the myofibrillar structure. Similarly, we predict that the same muscles will also show a decrease in total PV content with growth. Alternatively, no change in MyHC and PV content would be expected in cranial epaxial muscle that shows little change in contractile properties with growth. The goal of this work is to uncover the molecular basis of variations in the scaling of muscle.

2. Materials and methods

2.1. Animals

Largemouth bass (*M. salmoides*) and bluegill (*L. macrochirus*) were obtained from Kurtz Fish Farm, Chester County, PA, USA. The fish were maintained in re-circulating aquaria at 25 °C and fed live fish during a brief holding period. Data are reported here for five fish from two size classes for each species of fish (Table 1). These size classes were at the extremes of the size ranges of bass and bluegill from our previous scaling study (Carroll et al., 2009). All handling of experimental animals was reviewed by the Widener University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council.

To test the hypothesis that changes in MyHC and PV expression explain the variations in V_{max} with growth in feeding and swimming muscles of sunfish, parvalbumin content of muscle sample was assessed via SDS–PAGE and myosin heavy chain composition of muscle was assessed via immunohistochemistry. The five bass or bluegill from each of two size groups (20 fish in total) were euthanized. Two muscle samples were taken for each of the three muscle types (cranial epaxial, myotomal, sternohyoideus muscles): approximately 0.25 g of muscle tissue for protein extraction and PAGE and ~5 mm cube of tissue for histological analysis. For the small fish, the sternohyoideus sample for histological analysis included the entire muscle. The goal of histological analysis was to collect muscle similar to that used in previous physiological analysis of sunfish muscle (Carroll et al., 2009), not necessarily the entire cranial epaxial muscle or myotomal cross-section.

2.2. SDS–PAGE and Western blotting

Prior to PV expression analysis, PV isoforms in sunfish muscle were identified via SDS–PAGE and Western blotting via a monoclonal PV antibody (Parv-19, Sigma-Aldrich, P3088). Muscle samples collected from myotomal muscle in addition to the samples referenced above were homogenized using a protocol previously described (e.g. Coughlin et al., 2007). Muscle samples were homogenized in homogenization solution (250 mM sucrose, 100 mM KCl, 20 mM Tris-base, 5 mM EDTA, with protease inhibitors) in a 7.0 mL glass grinder at a 4:1 ratio of solution to muscle sample mass. The homogenized samples were centrifuged at 10,000*g* for 10 min, after which the PV-rich supernatant was removed. This supernatant was then partially purified for parvalbumin by raising the temperature to 95 °C for 5 min, after which it was centrifuged for 10 min at 10,000*g*. The PV-rich supernatant was retained.

PAGE running samples were prepared using Tricine Buffer (BioRad 161-0760) for a final protein concentration of approximately 0.025 mg mL^{-1}. Western blots were prepared by loading 20 μL of sample onto a 16.5% Tris–Tricine/Peptide SDS–PAGE precast gel (BioRad 161-1107) and running at 50 V for 30 min and 125 V for 120 min at 4 °C. PV was transferred from the gel to a PVDF membrane in the presence of Towbin buffer using a Trans-Blot Semi-Dry Transfer Cell (BioRad). The blot was developed using the BioRad protocol provided with the transfer cell. The monoclonal antibody Parv-19 was

Table 1
Fish sizes for scaling studies of muscle protein expression.

Species	Size	Body mass (g)	Total length (cm)	Standard length (cm)
Largemouth Bass	Small	3.2 ± 0.5	6.0 ± 0.3	5.1 ± 0.4
	Large	167.9 ± 12.0	24.0 ± 0.4	20.8 ± 0.6
Bluegill	Small	3.4 ± 0.7	6.1 ± 0.3	5.1 ± 0.3
	Large	84.9 ± 24.3	16.2 ± 1.6	13.6 ± 1.4

Mean ± SD of body mass, total length, and standard length are given for two size class of the two species under study: largemouth bass and bluegill.