Bigger, Stronger but Not Faster: ontogenetic change in the jaw biomechanics of the great sculpin, *Myoxocephalus polyacanthocephalus*

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Abstract

Suction feeding is the most common vertebrate feeding mode. Fishes suction feed by rapidly expanding the buccal cavity, creating a subambient pressure inside the mouth that causes water (and, ideally, a prey item) to rush in. The predator's ability to close the mouth around evasive prey determines feeding success. As a fish grows, the volume it engulfs should scale with length to the third power (volume $\propto$ length^3). This becomes a burden on larger fishes, as muscle force (which drives mouth closing) should scale with length squared (force $\propto$ muscle cross-sectional area $\propto$ length^2). Since suction volume increases faster with size than muscle force, a force deficit results as fish grow larger. Two ways to counteract this deficit are to increase muscle mass or increase skeletal leverage within the jaw. In this study, we examined musculoskeletal variation in anatomy and kinematics across an ontogenetic series in the suction-feeding great sculpin, *Myoxocephalus polyacanthocephalus*. Our results show that great sculpin mandibles change shape as they grow, increasing jaw-closing muscle leverage, which counters the force deficit ($N = 6$, $p = 0.0456$). Kinematic results agree: a given amount of muscle strain produces less jaw displacement in larger fish ($N = 6$, $p > 0.00015$). We did not find disproportionate changes in muscle mass with size ($N = 7$, $p=.514$). Smaller fish, therefore, rely on high-velocity jaw closing whereas larger fish rely more on high forces to close the jaw. We hypothesize that a smaller fish needs high speed to reduce the risk of prey escape from a small suction volume, whereas a large fish needs high forces to move the disproportionately large volume of water.

INTRODUCTION

Throughout nature, small animals experience a drastically different environment than those of large animals (Bonner, 2006). Because the animal's size dictates how they interact with their environment, morphology greatly differs across ontogeny (Schmidt-Nielson 1984). Evolution of ontogenetic scaling can drive the evolution of morphological scaling: changes in relative growth rates of different elements produces evolved changes over time in adults, resulting in overall morphological traits (Shingleton, 2010). Morphological traits reflect responses to different pressures between larval and adult stages. As an animal reaches adulthood, the physical laws of their environment provide possibilities for their growth, but create morphological limitations (Levinton and Allen, 2005). One challenge encountered is the difference in scaling rate between area and volume in relation to length. Volume scales to the third power (length^3), while area scales to the second power (length^2) (Schmidt-Nielson, 1984). In animals, area is proportional to cross-sectional area of muscle, and thus limits the amount of muscle force produced. (Levinton and Allen, 2005). For each increase in length, muscle force increases at a slower rate than volume.

This disproportionate relationship influences animals that engage in suction feeding. It used used often by fishes such as the lionfish (Muller *et al*, 1981). Our study species, the great sculpin (*Myoxocephalus polyacanthocephalus),* possess massive mouths with potent suction power. To utilize this suction, the sculpin approaches by moving in slow bursts toward the prey. Once close enough to the prey, the maxilla protrudes rapidly; the buccal cavity expands, causing an instant increase in volume and decline in pressure, sucking the water and prey into its mouth. To close its mouth after a suction feeding event, the great sculpin relies on the contraction of its main jaw adductor muscle system, the *adductor* *mandibulae*. The cooperation of muscle force and volume determine if prey is captured. Even if a prey item is captured, the fish's ability to keep evasive prey trapped inside the mouth links jaw-closing to overall feeding success.

The feeding success of the great sculpin is also likely to be impeded by the limitations of the volume vs. force correlation in relation to length. As fish length increases, the suction volume in the buccal cavity of the sculpin increases at a faster rate than the muscle force can keep up with (Figure 5). The muscle must overcome this force deficit by heavily increasing in strength at larger fish lengths. A larger fish produces more pressure for suction because of its higher force output, thus limiting the suction power of fish with less muscle force (Alexander, 1969).

Muscle force vs. volume is only one of many factors limiting great sculpin feeding success. Their feeding success also depends on the adaptions of the musculoskeletal elements of their jaw. This muscle system is divided into two main parts, the *pars malaris* and the *pars rictalis* (Datovo and Vari, 2013). These muscles attach to the jaw at the coronoid process (Figure 1). The contraction rotates the jaw around the jaw joint in a basic lever system. The length between the coronoid process and the jaw joint determines in-lever length, while the length between the jaw joint and the most anterior tip of the mouth determines out-lever length (Figure 2) (Westneat, 1994). The amount of force a jaw can produce (Levinton and Allen, 2005) and the distance it can move with a given amount of muscle force and contraction is governed by the lever ratio (L_{in}/L_{out}). Altering the leverage of the jaw is another way to counteract the muscle force deficit.

In this study, we examined how musculoskeletal variation in anatomy and kinematics across an ontogenetic series alters morphology in the suction-feeding great sculpin, *Myoxocephalus polyacanthocephalus*.

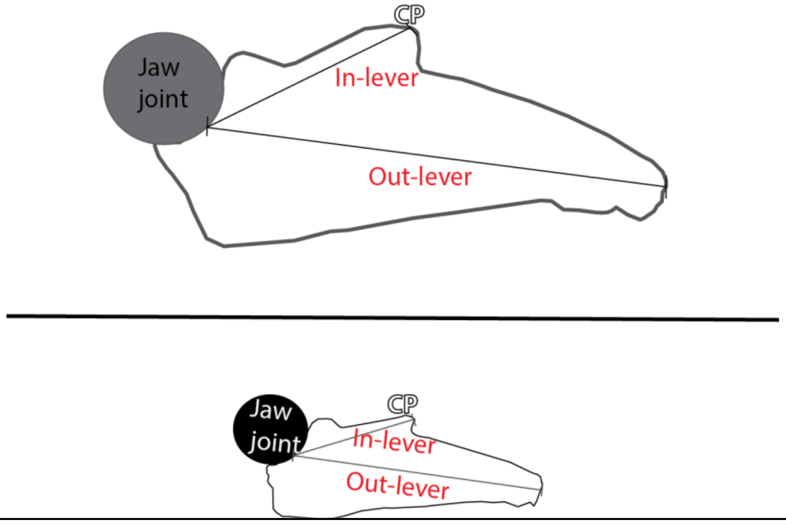


Figure 2. Large and small jaw models showing how in-lever and out-lever were measured.

METHODS

Specimens

The great sculpin (N = 7) examined in this study were obtained from the waters around San Juan Island by either trawl, seine, or captured by hand while diving. Fish were maintained in a flow-through system at all times at 11-16 °C. The sculpin were sorted into size categories to prevent cannibalism. No fish smaller than 100 mm were used since *in vivo* data collection was difficult at such sizes. All animals were used for *in vivo* data collection if they recovered sufficiently from surgery. Insufficient recovery was measured as repeated refusal to feed or death during/shortly after surgery. In preparation for surgery, animals were housed individually in 10 gallon tanks. Animals were starved for at least 2 days prior to surgery. Animals were fed spot prawn (caught in Friday Harbor, WA) as needed. All animal housing, maintenance and experimental procedures were approved by the University of Washington's Institutional Animal Care and Use Committee.

In vivo Data Collection and Analysis

Fish were anesthetized using ethyl 3-aminobenzoate methanesulfonate salt (MS-222, Sigma-Aldrich, St. Louis, MO) at an induction concentration of .1 gram per liter and maintained at .05-.075 gram per liter during surgery. Fresh sea water was added as needed to maintain a proper anesthesia plane. Sodium bicarbonate was used as a buffer in a concentration of .1 gram per liter. Flow over the gills was maintained by an aquarium pump (Shanghai Luby Pet Industries Co., Ltd, Shanghai, China).

Sonomicrometer transducers, hereafter referred to as "crystals" (Sonometrics Corp., Ontario, Canada), measuring 2 mm in diameter, were implanted in **1)** the tissue of the lower and upper lip, **2)** the *Adductor mandibulae pars malaris*, and **3)** the *Adductor mandibulae pars rictalis*. One incision each was made for crystals in the skin above *malaris* and *rictalis*, with the upper and lower jaw requiring one incision apiece. Each location (jaw, *malaris, rictalis*) contained two crystals each. Crystals were tucked into the muscle along the same muscle fiber and positioned to face the neighboring crystal at a minimum of 2 mm apart. Crystals were sutured (Ethilon nylon, 18'', Ethicon Inc, Somerville, NJ, USA) in the muscle. All incisions were closed with suture. Crystal wires were suspended above the fish with suture to prevent tangling of wires and reduce stress. Surgery was performed on one individual at a time.

The animals were allowed to recover after surgery in a 10 gallon tank for at least 6 hours. Feeding trials began after taking at least one breathing trial to determine soundness of sonometric signals. Breathing trials consist of data collection as the fish breathed normally with no food offered to the fish. During each attempted feeding trial, sonomteric transducer data was collected. Feeding trials lasted until the fish fed or until it was determined that the fish was uninterested in feeding. At least three feeding trials on each fish were attempted. To prevent satiation, feeding trials were attempted with small pieces of shrimp for no more than 2 hours at a time for a period of 1-2 days.

The Sonometric transducer was mounted above the 10 gallon tanks on a large piece of plastic and protected from water splash with a plastic box. Data was collected from the sonometric transducer at a 100.15 Hz sampling rate, transmit pulse = 625 ns, inhibit delay = 8.15 mm. These numbers were tweaked to improve the quality of crystal signals as needed.

Sonolab DS3 (Sonometrics Corp., Ontario, Canada) was used to collect data from the sonomicrometer crystals during breathing and feeding events. Using the corresponding data analysis software Sonoview (Sonometrics Corp, Ontario, Canada), the traces were measured from each pair of crystals for gape and the rictalis, focusing particularly on the spikes corresponding to a feeding event. Malaris data was thrown out because the malaris crystals worked inconsistently. Data was collected and analyzed from 57 feeding bouts from 6 fish. Peripheral points were removed in the collected data that only appeared because of poor tuning or background noise during data collection. From the smoothed data, gape and rictalis length change were calculated by measuring their peak during a feeding event and comparing it to the low point nearest to the right of that peak. This low point corresponded to the jaw being fully closed (gape=0).

Anatomy Measurements

Once at least one successful feeding trial was recorded, individuals were anesthetized with either .15 g/L MS-222, or 3 mg/mL metomidate injection to the brain. Anesthetized fish were euthanized via cervical dislocation and exsanguination. Anatomical data was measured using digital calipers (Mitutoyo Corp., Japan). The malaris, rictalis and stegalis muscles were all measured. Stegalis was not used during *in vivo* experiments because of its location medial to the malaris and rictalis, making survival surgery too difficult. Measurements included: total mass, maximum gape angle, maximum gape, total head length, total body length, muscle fiber lengths (5 measurements going ventral to dorsal), muscle angle, muscle total length, individual muscle mass, total muscle mass, in-lever and out-lever length.

Data collected in both anatomy and *in vivo* experiments was processed using MATLAB (MathWorks, Natick, Massachusetts, USA) and tested for significance using a linear regression test.

RESULTS

Using the in-lever/out-lever lengths, the lever ratio (L_{in}/L_{out}) was calculated. The data collected showed increased lever ratio with increase in size (Figure 3). In individuals 200 grams or less, the lever ratio stayed below .3 (with the exception of Myox 1).

Hypothetical muscle force output and muscle displacement output was calculated by solving the force-length equation $L_{in} * F_{in} = L_{out} * F_{out}$ for the desired variable. To solve, a hypothetical amount of force input (10 N) and muscle displacement input (2 mm) was used for fish of varying sizes.

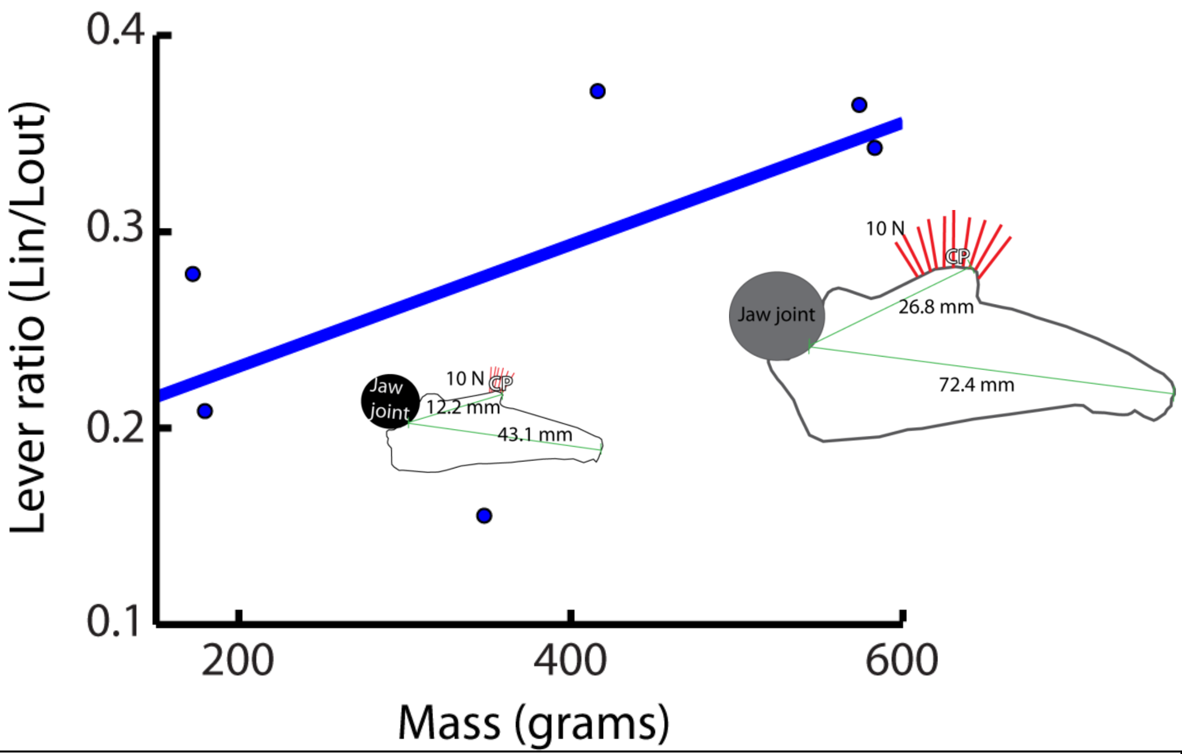


Figure 3. The relationship between lever ratio (L_{in}/L_{out}) and increase in mass in grams. Jaw models show actual in-lever/out-lever measurements from smallest and largest individual in this study. (N = 6, p = 0.0456).

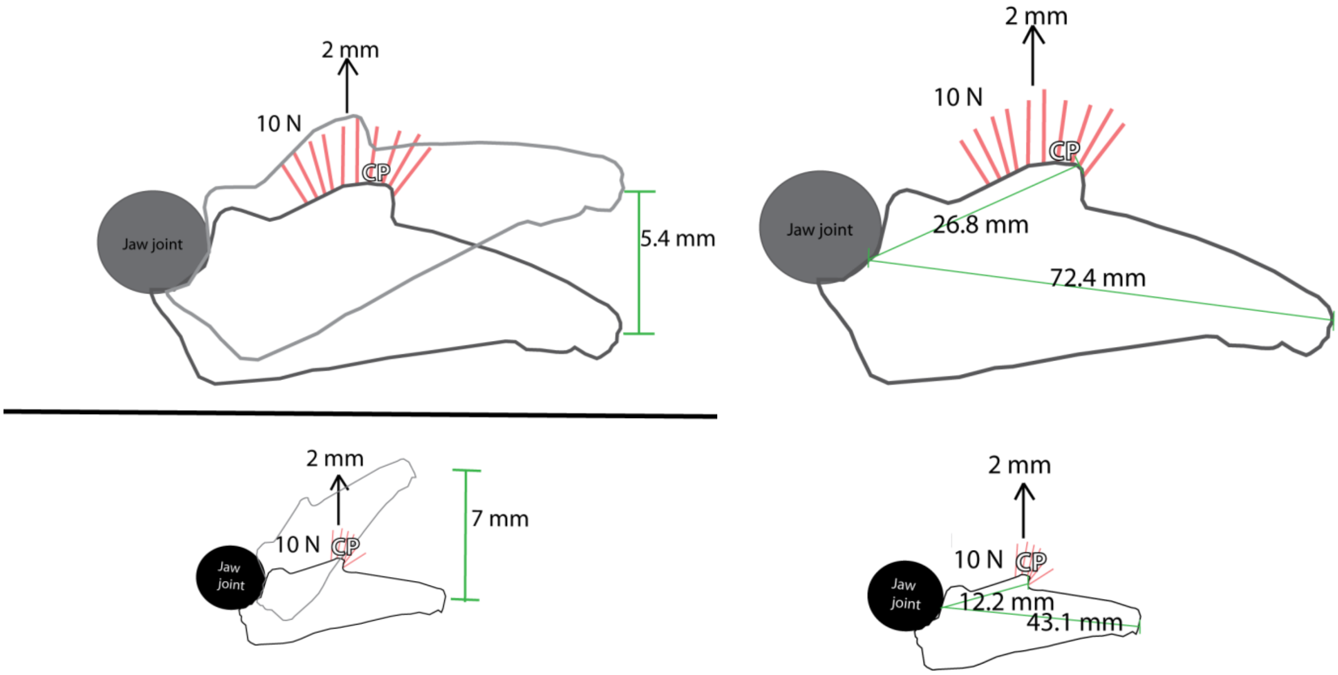
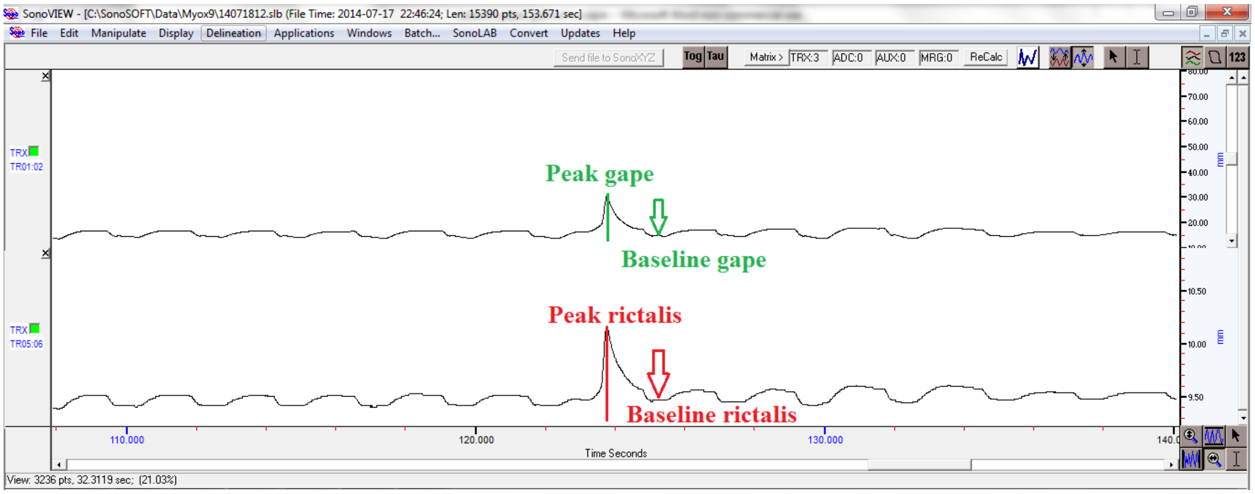


Figure 4. Hypothetical jaw movement and force output in a small and large jaw model.

Larger fish jaws produced a larger relative force at the jaw tip (3.702 N) compared to smaller fish jaws (2.831 N). The larger fish conserves a higher fraction of the force put into the system, whereas a smaller fish loses much of that initial force. With the same amount of force and muscle displacement, smaller fish jaws moved a farther relative distance (7 mm) than those of large fish (5.4 mm).

Gape change was calculated by measuring ((maximum gape - baseline gape)/baseline gape)), while muscle strain was calculated by measuring ((peak rictalis length – baseline rictalis length)/baseline rictalis length) x 100% (Figure 5A). The sonomicrometer data demonstrated that as length increased, the amount of gape change achieved from a given amount of rictalis muscle strain decreased (Figure 5B). A larger fish could input the same amount of muscle strain as a smaller fish, and still achieve a lower amount of gape change.



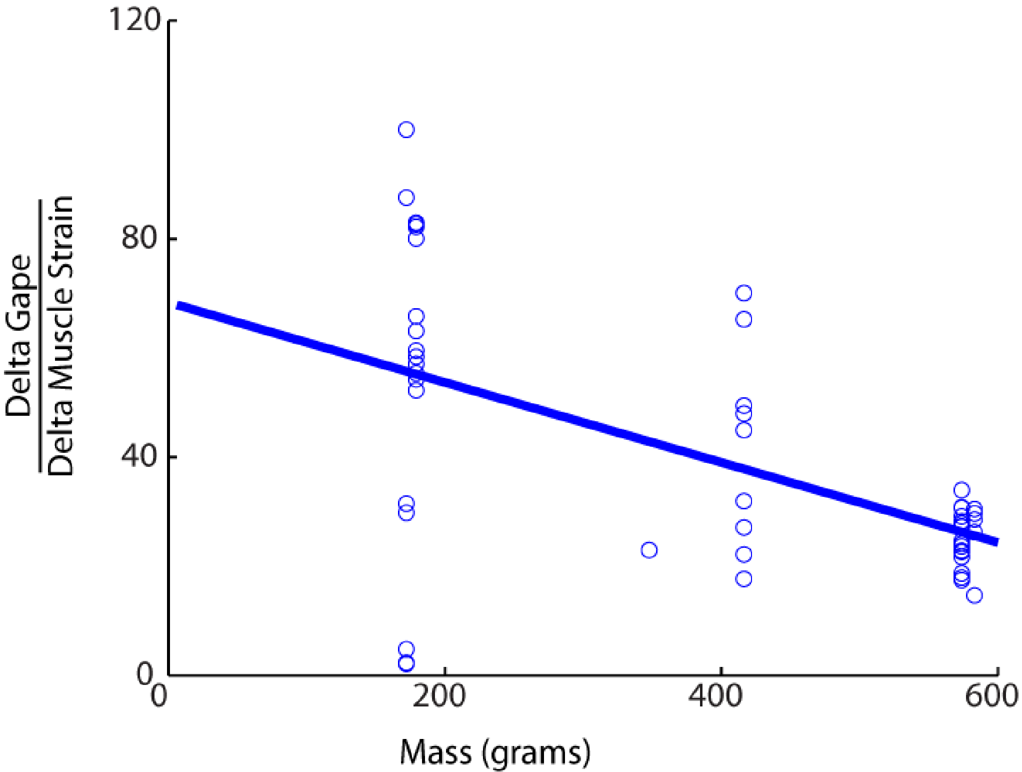


Figure 5. A) Gape change/muscle strain change as mass increases. N= 57 bouts from 6 individuals. R-squared = .315, p-value = 0.000151 B) Sonoview data showing the measurements taken to calculate length change in gape and muscle.

While anatomy measurements showed a positive trend with size and *in vivo* measurements showed a negative trend with size, it is important to note that both experiments demonstrated the same relationship. Anatomical data predicted that larger fish jaws would be strong but slow, and smaller fish jaws would be fast but weak. These predictions were supported by the recorded *in vivo* measurements.

DISCUSSION

Scaling theory dictates that with increasing size, volume will increase more rapidly than area. This relationship is biomechanically important for suction-feeding fishes, since the engulfed amount of water scales with volume (i.e. length^3) while muscle force will scale with area (i.e. length^2). This creates a force deficit for larger fishes, as the rate of force increase will be outpaced by the rate of volume increases. The muscle must find a way to overcome this force deficit. To compensate for the force deficit, larger sculpin change the leverage of their jaw-closing muscles (Figure 3), which is also apparent in the biomechanics of their jaws (Figure 5). In the jaw of both fish, speed or force dominated, but it was impossible to maximize both (Levinton and Allen, 2005). Based on what is known about levers, a higher lever ratio corresponds typically to a higher output of force (Westneat, 1994). The higher lever ratio of larger sculpin corresponds to higher force production, as well as a higher fraction of force conserved in the system. In maximizing force, larger sculpin sacrifice speed in jaw closure. These findings indicate that force is more of a limiting factor for large great sculpins whereas speed limits smaller sculpins.

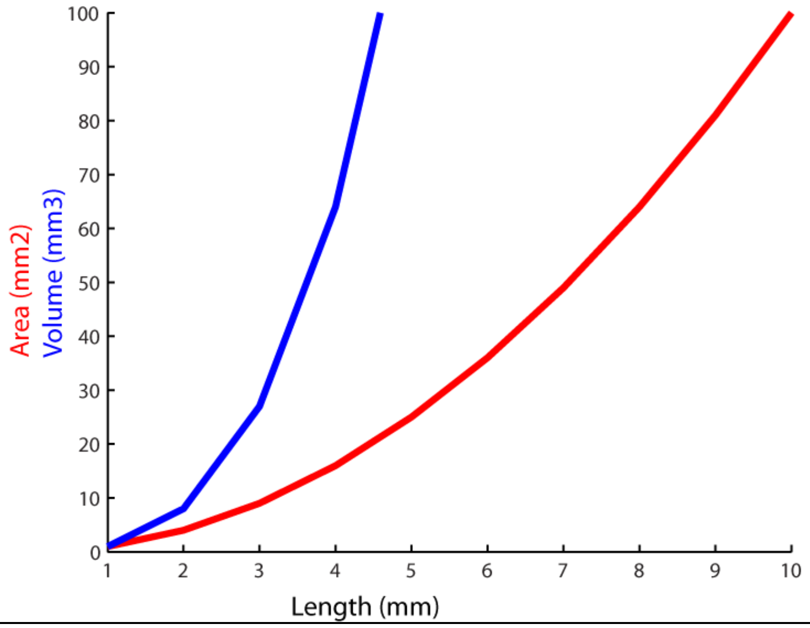


Figure 5. Disproportionate increase in volume compared to area for a given change in length. Area is proportional to muscle force in fish.

However, in smaller sculpin, speed is critical. At a small size, the volume-muscle force relationship is almost linear. The smaller fish's muscles do not experience a muscle force deficit, but encounter different challenges. The smaller fish's suction feeding requires it to produce a large enough volume to engulf its prey, while closing its mouth in time to prevent the prey from fleeing this smaller relative volume. To prevent prey escape, smaller sculpin rely on faster jaw closure. In depending on speed, smaller fish sacrifice strength of jaw closure.

Because this study regards muscle strain, the length-tension relationship also comes into play. This relationship states that a muscle will create the highest active force when at an optimum fiber length (P_0). The muscle decreases in force below and above that ideal fiber length. Each individual fish in this study reaches P_0 at some point, and thus cannot achieve any higher force before or after that. When a fiber is stretched longer, fibers are pulled farther apart from surrounding fibers (less overlap), causing a greater effort to go into the muscle crucial. Ideally the best ontogenetic series would have 3 to 5 individuals from small, medium and large size classes. Acquiring smaller crystals (1 mm or less) to implant in individuals smaller than 100 mm would allow for a more complete ontogenetic series.

CONCLUDING REMARKS

Our study reinforces the known properties of levers and the length-tension relationship, and applies both to an ideal biological model. The great sculpin's suction feeding, their large, easily assessable jaw closing muscles, their size variability, and voracious feeding behavior made them valuable to this study. Most notably, their ability to overcome the muscle force deficit at a larger size by an increase in lever ratio is a prime example of how morphology reflects the environmental and physical pressures acting on an animal.

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