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Cite this article: Gidmark NJ, Konow N, LoPresti E, Brainerd EL. 2013 Bite force is limited by the force–length relationship of skeletal muscle in black carp, *Mylopharyngodon piceus*. Biol Lett 20121181.
http://dx.doi.org/10.1098/rsbl.2012.1181

Received: 18 December 2012
Accepted: 23 January 2013

Subject Areas:
biomechanics

Keywords:
XROMM, bite force, Cyprinidae, pharyngeal, jaw

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2012.1181 or via http://rsbl.royalsocietypublishing.org.

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Biomechanics

Bite force is limited by the force–length relationship of skeletal muscle in black carp, *Mylopharyngodon piceus*

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Bite force is critical to feeding success, especially in animals that crush strong, brittle foods. Maximum bite force is typically measured as one value per individual, but the force–length relationship of skeletal muscle suggests that each individual should possess a range of gape height-specific, and, therefore, prey size-specific, bite forces. We characterized the influence of prey size on pharyngeal jaw bite force in the snail-eating black carp (*Mylopharyngodon piceus*, family Cyprinidae), using feeding trials on artificial prey that varied independently in size and strength. We then measured jaw-closing muscle lengths *in vivo* for each prey size, and then determined the force–length relationship of the same muscle *in situ* using tetanic stimulations. Maximum bite force was surprisingly high: the largest individual produced nearly 700 N at optimal muscle length. Bite force decreased on large and small prey, which elicited long and short muscle lengths, respectively, demonstrating that the force–length relationship of skeletal muscle results in prey size-specific bite force.

1. Introduction

Durophagous vertebrates (those that eat strong foods like snails or nuts) use high bite forces to counter prey defences. The potential crushing ability of a predator is typically characterized as a single, maximum bite force [1]. This characterization ignores the force–length relationship of vertebrate skeletal muscle: maximum force is produced at intermediate instantaneous muscle lengths and declines when stretched or shortened [2]. Because jaw-closing muscles change length across gapes, bite force should vary with gape size [3]. Whereas bite force is expected to be nonlinear (optimum at intermediate gape sizes), prey force (defensive strength) often increases linearly with size [4,5]. Prey size dictates predator gape during biting, and these different force–size patterns can result in nonlinear predation pressure across prey sizes [6].

Predator bite force can be gape-specific [3,7,8], but general patterns and determinants of gape-specific bite force are not well known. The relationship between predator gape and jaw muscle length has been examined post-mortem [9], but the complex architecture, dynamic moment arms and multiple jaw-closing muscles of many vertebrate feeding systems obscure the relationship between length-specific muscle force and gape-specific bite force.

To link length-specific muscle force with gape-specific bite force in live animals, we explore the pharyngeal jaw apparatus of black carp (*Mylopharyngodon piceus*, a snail-eating fish) as a model system. This model is ideal because it is anatomically and mechanically simple: a single jaw-closing muscle with relatively simple architecture and a static moment arm that elevates the pharyngeal jaw into occlusion. Moreover, the pharyngeal jaws are the only structures in this system capable of mechanical food breakdown. This simple anatomy functionally links length-specific muscle force explicitly to gape-specific bite force.

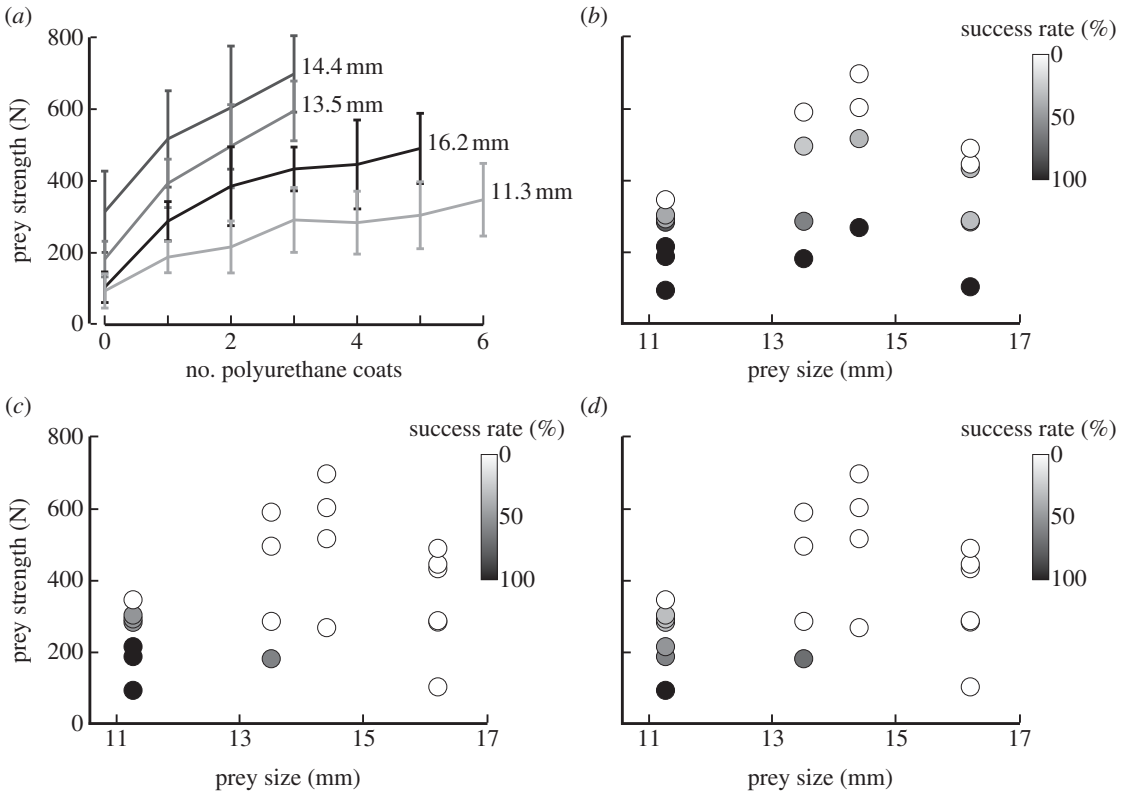

Figure 1. Prey strength, prey size and individual success. (*a*) Size and strength (mean ± 1 s.d.) of manufactured prey items. Grey level reflects tube size. (*b–d*) Prey crushing performance by each of the three individuals: 106, 81 and 75 mm head length, respectively. Crushing success spans 100% (black) to 0% (white).

We first test gape-specific crushing performance in black carp using feeding trials with manufactured prey (decoupling prey size from strength) to probe peak bite force across a range of gapes. Second, we use X-ray reconstruction of moving morphology (XROMM) to measure muscle lengths used *in vivo* for each prey size. Third, we characterize the force–length relationship for the same muscles *in situ* (immediately post-mortem, with the muscle still alive). Finally, we superimpose our *in vivo* and *in situ* muscle length measurements to test whether the force–length relationship of the jaw-closing muscle, driven by variable gape *in vivo*, limits bite performance in a gape-specific way.

2. Material and methods

(a) Specimens

Three farm-raised adult black carp, 650, 550 and 500 mm total length (106, 81 and 75 mm head length), were trained to feed on ceramic tubes filled with pellet food.

(b) Feeding trials

We simulated prey using ceramic tubes from aquarium filter media of four diameters (11.3–16.2 mm). These tubes were selected after surveying available brands for appropriate size (non-gape limiting to our experimental individuals) and acceptable strength consistency. We increased strength within each size by coating the tubes in layers of polyurethane (M-coat, Vishay). We tested tube fracture strength using an MTS MiniBionix858 and steel compression platens (jaw-mounted crushing tests could not be aligned consistently) with force directed perpendicular to the long axis of the tube (cineradiography revealed that black carp always crushed tubes in this orientation) and a loading rate of 1 mm s^{-1}. We fractured 5–20 of each prey size/number of coats combination for a total of 317 force-testing trials. We used a 2-way ANOVA with Tukey post hoc corrections to explore differences between specific size/number of coats combinations.

To test feeding performance, we conducted feeding trials with tubes of the same type described above packed with moistened pellet food. Typically, the tube was immediately sucked into the pharyngeal cavity, followed by a crushing attempt. Successful trials involved an audible crack, followed by the fish expelling broken ceramic pieces. We scored the trial unsuccessful if this did not occur within 5 min. We performed less than 10 trials per day with greater than 5 min between trials to avoid satiation and fatigue. Each day, we tested a random mix of the four sizes, proceeding from weak to strong within a size, to probe maximum bite force across sizes. Tests of a given size/strength combination were discontinued if the first five trials were entirely successful or unsuccessful. Otherwise, five additional tests were conducted.

(c) Radio-opaque marker implantation

Following feeding trials, tantalum spheres (1–2 mm, Bal-Tec) were surgically implanted [10] into the neurocranium and both pharyngeal jaws of anaesthetized animals (MS-222, Argent, 0.05–0.1 g l^{-1}).

(d) Video and force recording

Once normal behaviour resumed, feeding was imaged at 125 fps with biplanar X-ray video (OEC-9400 fluoroscopes, Photron1024PCI cameras) at 80–90 kVp and 20 mA. We analysed 31 feeding trials on 2–3 tube sizes that bracketed the successful crushing range for each individual.

Following *in vivo* data collection, we constructed muscle force–length curves for each individual. Immediately post-mortem (Metomidate, 0.1 g l^{-1} followed by cervical dislocation), all musculoskeletal elements were removed from the neurocranium, except for one pharyngeal jaw and its jaw-closing

muscle (*m. Levator Arcus Branchialis V;* LABV). The preparation was mounted in-series with a force transducer and submerged in oxygenated Ringer's solution (see the electronic supplementary material, figure S1). The transducer was anchored to a threaded rod which permitted lengthening between contractions. We endeavoured to orient the jaw *in situ* in a similar posture to that observed *in vivo*, and our reconstructions showed a similar position of the muscle insertion site. The muscle was whole-field stimulated using a Grass s48H stimulator (10 V, 0.2 ms pulses, 250 pps, 300 ms train) via platinum paddle electrodes to elicit fused tetani. Stimulations started at a short length and successive stimulations (5 min intervals) were completed after 1–2 mm lengthening. We continued contractions until force declined below 5 per cent of P_0, resulting in 13–20 stimulations/individual. Because the bilateral muscles can act simultaneously, force was doubled. Imaging was identical to *in vivo* technique. Herein, we refer to these data as *in situ*, as they were collected from an electrochemically viable muscle, in its anatomical position, operating in an anatomically relevant way.

(e) X-ray reconstruction of moving morphology

We processed X-ray videos using XrayProject software ([10]; www.xromm.org) and calculated three-dimensional rigid body movements in MATLAB (The Mathworks). Polygonal mesh models from laser scans of cleaned bones (Microscan head, Microscribe MLX articulated arm) were processed in GeoMagic and re-animated in Autodesk Maya using rigid body movements from the X-ray videos [11].

We mapped LABV muscle attachment sites onto bone models to measure *in vivo* and *in situ* muscle length. We marked origins and insertions of five fascicles with locators in Autodesk Maya, and took straight-line distances between those attachment points. We used the mean of the five distances as a proxy for overall muscle length (in three dimensions) for each time point. We animated the bone models with *in vivo* data to identify operating lengths during feeding, and *in situ* data to determine force–length relationships. We used the identical bone model (complete with fascicle positions) for animation of both the *in vivo* and *in situ* data, so that fascicle length measurements would be directly comparable. Ultimately, only these muscle fascicle length measures were used for further analysis. All data for this study are available at xmaportal.org.

3. Results

The strength of our artificial prey (ceramic tubes) increased with successive polyurethane coatings, although repeated measurements of each size-coat combination showed substantial variability (figure 1*a*). Tube strength varied by tube size and coat number, and these variables interacted significantly (ANOVA, tube type $p < 0.001$, coat number $p < 0.001$ and interaction $p < 0.001$).

In vivo prey crushing trials demonstrated decreased crushing performance (lower success rate) at extreme prey sizes (figure 1) and muscle lengths (figure 2). Tukey-corrected post hoc tests revealed that, for the largest individual, successfully crushed intermediate-sized prey were stronger than larger and smaller prey that were uncrushable (both $p < 0.001$), indicating a performance optimum at intermediate prey sizes (figure 1*b*). Tubes of a given size are effectively larger for smaller animals, and indeed the two smaller individuals performed optimally on the smallest tubes, and could only crush uncoated 13.5 mm tubes (figure 1*c*,*d*). Larger tubes (14.4 and 16.2 mm) were uncrushable, regardless of coating, despite the fact that they would fit between the pharyngeal jaws.

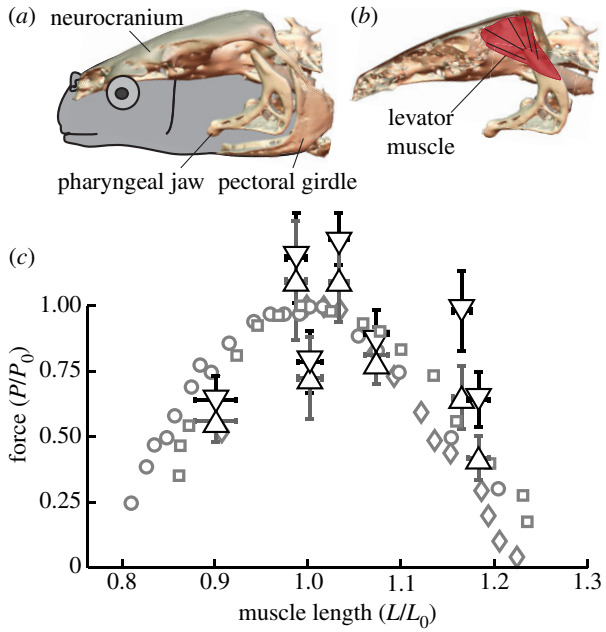

Figure 2. Anatomy and performance of black carp pharyngeal jaws. (*a*) Left lateral view of the bones associated with the pharyngeal jaw. (*b*) Lateral view, parasagittal cut-away, showing a schematic of the jaw-closing muscle and individual fibres tracked using XROMM. Note that muscle fibre orientations converge near the bite point, so little or no musculoskeletal leverage amplifies muscle force. (*c*) *In situ* force–length relationships (light grey) and *in vivo* tube-crushing performance (black triangles), normalized to L/L_0 and P/P_0. Each grey symbol represents a single *in situ* contraction in one individual (head lengths: circle, 106 mm; square, 81 mm; diamond, 75 mm). Up-pointing triangles indicate strongest crushable prey item *in vivo*, and down-pointing triangles indicate weakest uncrushable prey item; *x*-axis error bars denote ± 1 s.d. of *in vivo* muscle lengths, *y*-axis error bars denote ± 1 s.d. of prey strength determined via materials testing. All *in vivo* data are pooled in this figure; see electronic supplementary material, figure S1 for *in vivo* muscle length and performance data by individual.

We determined *in vivo* muscle operating lengths, followed by *in situ* characterization of the muscle's force–length relationship (figure 2). The jaw-closing muscle of two smaller individuals operated on the descending limb, involving a 50 per cent decrease in peak muscle force (see the electronic supplementary material, figure S1). The largest individual did not operate at similarly long muscle lengths, probably because we could not offer sufficiently large prey to drive its muscle to descending limb lengths. When crushing the smallest prey, the jaw closing muscle of the largest individual operated at ascending limb lengths, corresponding with approximately 50 per cent of P_0, and bite performance (prey strength) declined to a similar degree. Our three-individual dataset describes *in vivo* performance and *in situ* forces on the ascending limb, descending limb, and plateau regions for the force–length curve, with at least one individual in each region (figure 2*c*). Our *in vivo* estimates of bite force fell within 20 per cent of our *in situ* estimates, and our *in situ* data always fell within the confidence intervals of the *in vivo* tube strength data (figure 2*c*).

4. Discussion

Our two independent empirical estimates of gape-specific bite force (*in vivo* maximum-strength tube crushing and

in situ maximally stimulated tetanic muscle force) show that black carp produce high bite forces (350–700 N, electronic supplementary material, figure S1) that vary across gape (figure 2*c*). By linking these two datasets using identical measures of muscle length from XROMM, we show that length-specific muscle force limits gape-specific bite force.

Although the force–length relationship of skeletal muscle has been known for over 70 years [2], it was relatively recently proposed as a factor limiting bite performance [3,8]. All fish in this study voluntarily operated their jaw-closing muscles on the descending limb of their force–length curves, incurring a penalty in muscle force when crushing large prey items (figure 2*c*). While data from the wild on active selection for prey size are unavailable, we suspect that wild black carp behave similarly.

Large size is thought to provide an ecological refuge for prey, due either to the increased strength associated with increased size, or to anatomical constraints on predator mouth opening capability [4–6]. Our study suggests that the ecological refuge for large prey is based not only on increased strength, but also on the requirement of the predator to operate its jaw closing muscles at longer lengths, thus sacrificing muscle force. This could explain why intermediate-sized bivalves experience higher predation pressure than small or large individuals [6]. This trade-off has ecological ramifications on prey defence mechanisms and life-history strategies: prey could defensively focus exclusively on getting stronger, exclusively on getting larger or both. Future studies of wild predator–prey interactions could benefit from considering gape-specific bite force.

Our study is, to the best of our knowledge, the first relating *in vivo* measurements of bite performance and jaw muscle length to *in situ* measurements of the force–length relationship for the same muscle. This method allowed us to attribute bite performance as a function of prey size to force production as a function of muscle length. We found that black carp do not possess a single maximum bite force, but rather a range of gape-specific bite forces, shaped by intrinsic muscle properties.

All animal-related procedures were approved by Brown University's IACUC.

We thank Ariel Camp, James Tarrant, Henry Astley, Thomas Roberts and Brown University's Morphology group for intellectual input. Charles Vickers, Jr. and Edward Mullen provided logistical help. Supported by the American Society of Ichthyology and Herpetology, Sigma Xi, the W. M. Keck Foundation, the US National Science Foundation (0840950) and the Bushnell Graduate Research and Education Fund.

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