Studying the Force-Length Relationship in the Adductor Mandibulae of Pink and King Salmon

Elska Kaczmarek[1,2], Nicholas Gidmark[1]

Blinks-NSF REU-BEACON 2015
Summer 2015

[1]Friday Harbor Labs, University of Washington, Friday Harbor, WA 98250
[2] RSMAS, University of Miami, Coral Gables, FL 33124

Contact Information:
Elska Kaczmarek
Rosenstiel School of Marine and Atmospheric Sciences
University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149
ekaczmarek@umiami.edu

Keywords: salmonidae, biomechanics, functional morphology, muscle physiology

Abstract

Specialized feeding behavior is generally reflected not only in skeletal anatomy (as has been the major focus of functional morphology literature) but also in muscular morphology and physiology. We show that this is the case for salmon feeding mechanics of king and pink salmon. King salmon (*Oncorhynchus tshawytscha)* eat small, fast fish; and pink salmon (*Oncorhynchus gorbuscha)* primarily filter feed on planktonic organisms by keeping their mouths open while swimming. Salmon close their jaws using the adductor mandibulae, which, like all skeletal muscles, is constrained by a strict relationship between muscle length and force. Muscles that are over-stretched or over-shortened exert weaker forces than they do at optimal length, and muscle length corresponds to gape. We compared the force-length curves of king and pink salmon adductor mandibulae and demonstrated that the maximum bite force of king salmon is achieved closer to maximum gape (67% of max gape, n = 3). This may allow them to take advantage of optimal muscle length, and thus greater force production, when eating large or elusive prey. In pink salmon, the force-length curve is centered at a smaller relative gape, closer to mid-gape (43% of max gape, n = 6). This may facilitate filter feeding, allowing reasonably high forces at a range of medium gape sizes. The different feeding preferences of these species may have put different pressures on the evolution of jaw muscle physiology, resulting in distinct optimal solutions to the force-length constraint.

Introduction

Utilizing effective feeding strategies is paramount to the survival of a species. Food is often a limiting resource for reproduction and survival, and in nearly all ecological communities, a prey item is sought after by numerous competing species. Feeding morphology is critical, because head and jaw anatomy—form—determines how effectively a fish can capture its prey—function. Functional morphology is the study of the strong connection between a structure's form and function.

Historically, functional morphologists have focused on the skeletal system rather than the muscular system. Studies of feeding morphology and mechanics have quantified and modeled skeletal lever biomechanics of the jaw without studying the muscles that pull on those levers. The skeleton is easy to study and reveals a lot about diversity, but muscles, too, have a breadth of structural diversity and are just as integral to the function of the jaw. Functional morphology has been overlooking this half of the picture, and without an accurate measure of muscle force and performance, our biomechanical models are incomplete.

Studies of biting force have typically considered the single maximum bite force for an individual (Anderson, Mcbrayer, & Herrel 2008). However, this ignores the relationship between a muscle's length and the force that muscle can produce. During a static contraction, a muscle is kept at a fixed length, roughly determined by the gape of the jaw (Anapol & Herring 1989). Bite force depends on muscle length, so an individual has a series of gape-specific bite forces. When muscle length is near the extremes of the *in vivo* length range of the muscle, the muscle is weakest (Ramsey & Street 1940). At an individual's optimum muscle length, L_0, the maximum force, P_0, is attained. A force-length curve can be constructed by performing static (i.e. fixed-end) contractions at a range of gapes (Fig. 1A).

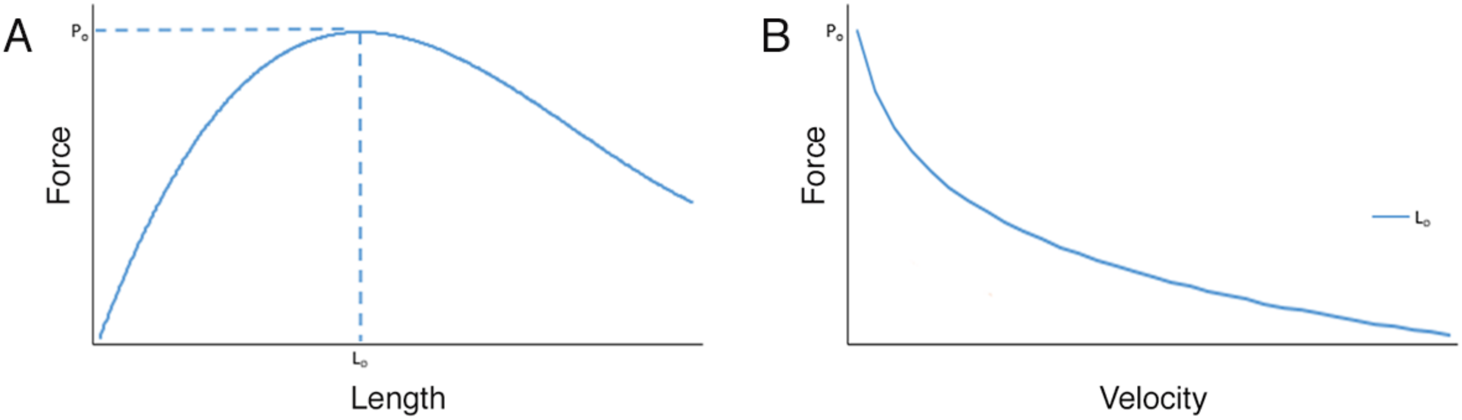

Figure 1. (A) Generalized force-length curve. During a static contraction, a muscle of length L exerts a force P. The muscle tenses but does not shorten (i.e. it contracts with zero velocity). A maximum force, P_0, is produced at the optimal muscle length, L_0. Muscles weaken at shorter or longer lengths. (B) Generalized force-velocity curve. Force production decays exponentially as the velocity of a contraction increases. It is assumed that the muscle contracts at a consistent initial gape size. If the initial gape size is L_0, as shown, the maximum force, P_0, can be achieved during a static contraction.

This force-length relationship has been analyzed in studies of muscle physiology and in locomotion biomechanics but has rarely been included in studies of feeding biomechanics. However, this relationship is important when considering the bite forces that an organism can produce and the prey it can consume (Huber et. al 2005, Gidmark et. al 2013). The force-length relationship of vertebrate skeletal muscle constrains peak force to a relatively small working range of muscle lengths, and these lengths are often exceeded when an organism executes its daily tasks. Beyond this optimal range, muscle force declines quickly, along with organismal performance.

Muscles are also constrained by a relationship between the velocity of a muscle contraction and the force produced (Wilkie 1949). Muscles can contract rapidly when exerting small forces, but will contract much more slowly when producing large forces (Fig. 1B). As contraction velocity increases, there is an exponential decay in the force that can be produced, so a single bite can be fast or strong, but not both. Understanding how this trade-off is balanced is important when considering the evolution of a species' jaws.

The five species of salmon coexisting in the Pacific Northwest have evolved to feed on different prey items to avoid competition. King and coho salmon (*Oncorhynchus tshanytscha*, and *Oncorhynchus kisutch* respectively) primarily feed on fish. Pink, sockeye, and chum salmon (*Oncorhynchus gorbuscha, Oncorhynchus nerka*, and *Oncorhynchus gorbuscha*, respectively) have more variable diets and tend to be more planktivorous than king and coho salmon (Brodeur et. Al 2007). These different diets require different feeding methods: king salmon need to catch fast-moving fish, while pink salmon must hold their mouths open at a wide gape to filter plankton from the water.

In salmon, the mouth is closed by a single muscle complex called the adductor mandibulae. In this simple system, there is a clear link between gape size and muscle length, and thus gape-specific bite forces will correspond to length-specific muscle forces. This makes salmon good model organisms for studying how differences in feeding behaviors are reflected in muscle physiology.

In this study, we explore how the specific force-length and force-velocity relationships of jaw muscles in pacific salmon may have evolved to optimize the performance when feeding on specific prey types. We expect that physiological diversity among species will be a product of functional variations. To test this, we quantify the force-length of the adductor mandibulae for both king and pink salmon specimens and compare the resulting force-length curves.

Materials and Methods

Specimens

Three king salmon and seven pink salmon were caught by hook-and-line in the waters around San Juan Island, Washington State, USA. While on the fishing boat, the salmon were kept in coolers that were periodically replenished with new seawater. At Friday Harbor Labs, they were kept in a large tanks until they were selected for surgery. All animal-related procedures were approved by the University of Washington's IACUC.

Surgery

The salmon were anesthetized with Metomidate (0.025g/L). We maintained anesthesia for the entire procedure and controlled the level of sedation by adding more seawater or more Metomidate to the water. An air pump kept the water oxygenated and a pump maintained water flow over the gills. We removed both eyes so we could locate the mandibular nerves. We looped the ends of two wires around each nerve bundle, then cuffed the attachments with tubes of flexible plastic. The wires were connected to an electrical stimulator (Grass S-48, Middleton, Wisconsin). After surgery, we continued to maintain anesthesia and the fish was held in a clamp, which was secured to the tank (Fig. 2). This prevented any translational or rotational motion of the neurocranium.

Force-Length Relationship

To measure the force-length relationship in the adductor mandibulae, we stimulated the muscles supramaximally at a range of gapes. We secured kevlar thread between the mandibular symphysis and a digital force gauge (Imada DS2-44, Toyohashi, Japan). The force gauge was fixed to a board that could be raised or lowered to adjust the gape of the mouth. During each stimulation, the electrical stimulator applied 10V pulses for 300ms with a pulse duration of 0.25ms and a train rate of 350 pulses per second. Electrical stimulation contracted the muscle, causing the jaw to pull on the force gauge, but gape (and thus muscle length) did not change significantly. We recorded the gape and the peak force for each contraction, which were performed at five minute intervals to prevent muscle fatigue. The initial contraction occurred at a closed gape at the zeroth minute. We increased the gape size incrementally for the following contractions (Series 1). This series will be referred to as a subject's '1st Series' curve. When we reached the maximum gape, we started a new series and incrementally decreased the gape size (Series 2). We repeated this process up to four times on each specimen (Fig. 3).

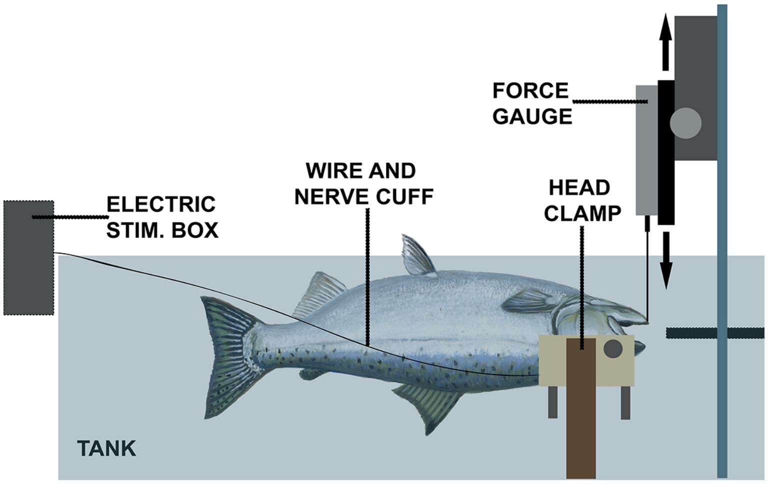

Figure 2. Experimental apparatus. The specimen is clamped in place. Kevlar thread connects the tip of the mandible to the force gauge, which can be translated vertically to change the specimen's gape between contractions. Wires wrap around the mandibular nerve bundles and connect to the electrical stimulator.

Data Analysis

We assessed the accuracy of the 1st Series curves by calculating the rate of muscle weakening. We plotted the decrease in force production between a 1st Series curve and a 2nd Series curve, as indicated by arrows in Figure 3, against the time of contraction (Fig. 4). The x-intercept of these lines are the times that the muscles began to fatigue.

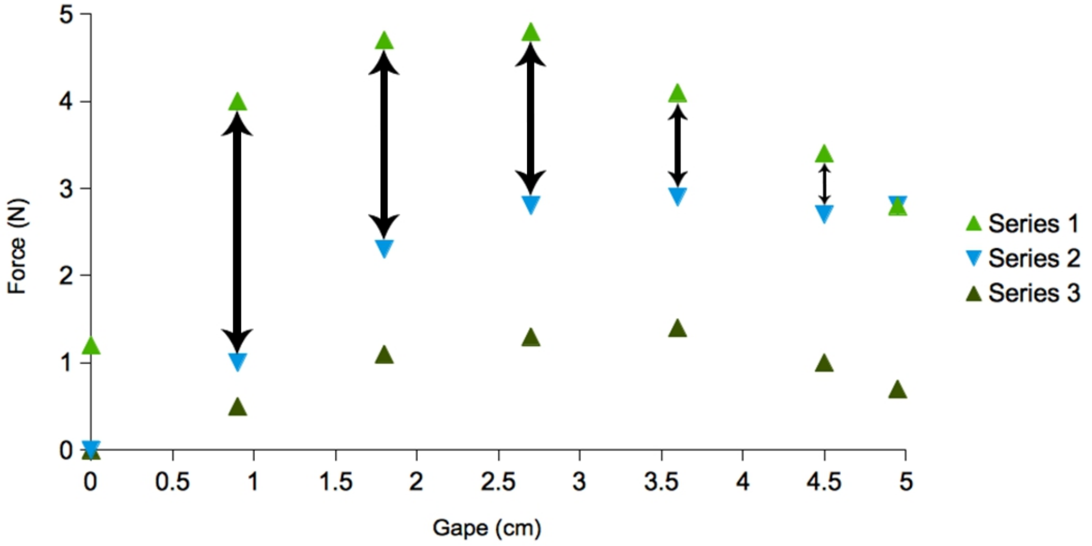

Figure 3. Data collected from a single specimen. Series 1 (and Series 3) begin at a closed gape and proceed to the right. Series 2 begins at the maximum gape and proceeds to the left. Over time, the muscles weakened and force production decreased, as indicated by the black arrows.

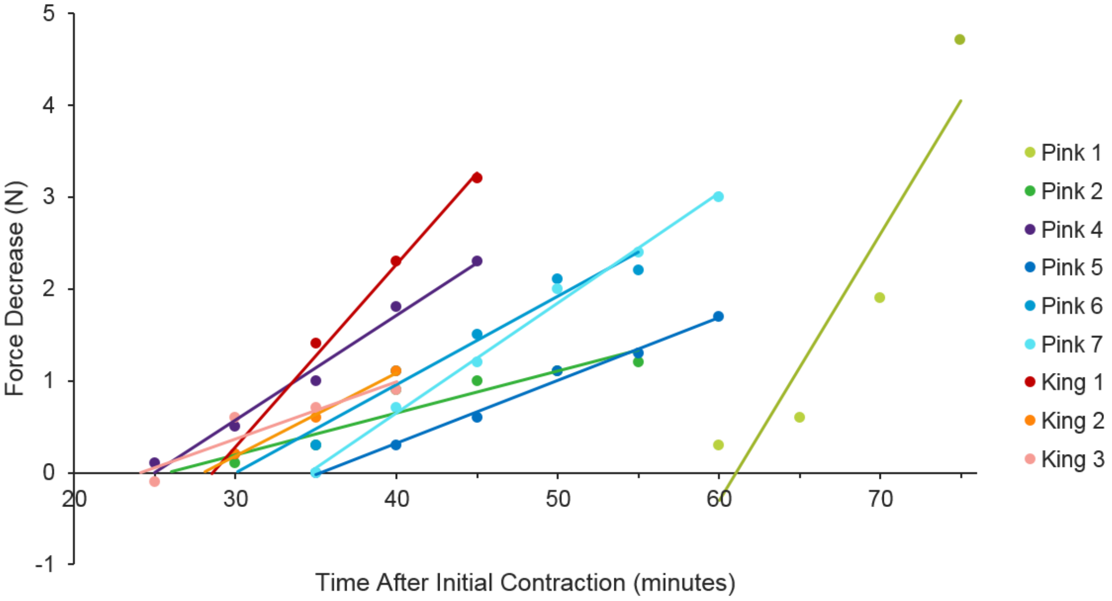

Figure 4. Rates of muscle weakening. The decrease in force production indicated by the black arrows in Fig. 3 was graphed over time to show the rate of fatigue for each specimen. The x-intercept of each line represents the time that fatigue began.

We normalized the 1^{st} Series data points to each specimen's maximum gape and maximum force and calculated best fit curves using JMP software (Cary, North Carolina). Using the best fit curves, we found the relative gape at which force production was highest. We also calculated the area under the best fit curves by integrating them from zero to one. This was used as an measure of force production across a wide range of gapes. A two-tailed, unpaired Wilcoxon-Mann-Whitney test was used to compare the optimal gapes and the areas under the force-length curves of king and pink salmon.

Results

The accuracy of the 1^{st} Series curves was not affected by muscle weakening. With the exception of Pink 2 and Pink 6, the 1^{st} Series data points had been collected before the specimen's muscle began weakening. In these two specimens, weakening began just one minute before the final 1^{st} Series contraction and had a negligible effect on that contraction force.

The scaled 1^{st} Series force-length curves for both species appear to fall into two groups, with king salmon generating peak forces at larger relative gapes than pink salmon (Fig. 5). (Specimen *Pink 6* is an exception; it follows the trajectory of the three king specimens before reaching its optimal gape, and then follows the path of the other pink specimens as it decreases.)

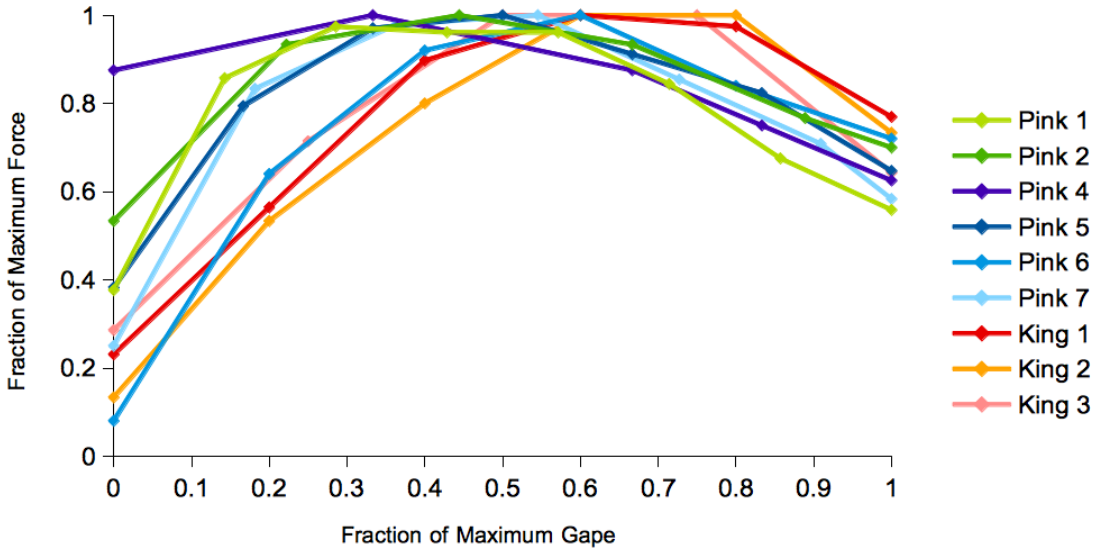

Figure 5. 1^{st} Series force-length curves, scaled by maximum gape and maximum force, for six pink salmon and three king salmon.

Eight of the nine best fit curves generated were good approximations of their respective data series (R-square value > 0.95). The best fit curve for Pink 3 was not a good approximation and was not included in our analysis. Pink salmon had a scaled optimal gape size (L_0/L_{Max}) of 0.43 ± 0.08 and the area under the force-length curve was 0.84 ± 0.04 (mean ± 1 s.d., n = 6). In king salmon, the scaled optimal gape size was 0.67 ± 0.03 and the area under the force-length curve was 0.79 ± 0.03 (mean ± 1 s.d., n = 3). Pink and king salmon have significantly different scaled mean optimal gapes ($p = 0.028$, Fig. 6A), but the definite integrals were not statistically different ($p = 0.095$, Fig. 6B).

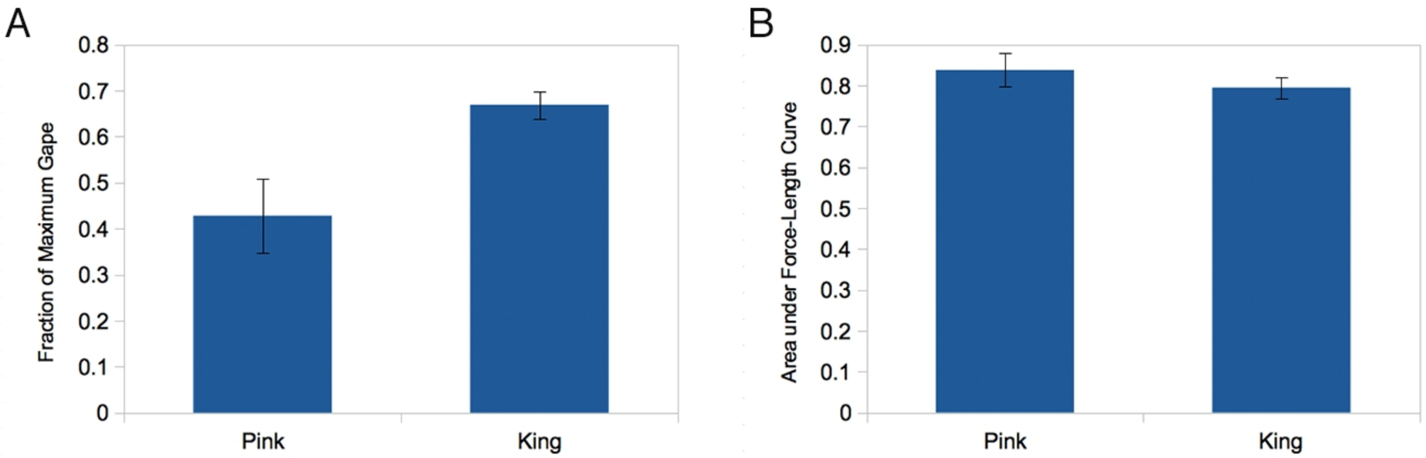

Figure 6. A comparison of optimal gape and area under the force-length curves between pink and king salmon. (A) Position of optimal gape size relative to maximum gape. Scaled optimal gape size was 0.43 ± 0.08 for pink salmon, and 0.67 ± 0.03 for king salmon (mean ± 1 s.d, pink: n = 6, king: n = 3, p-value = 0.0282). (B) Area under the force-length curves. These were calculated by integrating the best fit curves from zero to one. The area under the F-L curve was 0.84 ± 0.04 for pink salmon and 0.79 ± 0.03 for king salmon (mean ± 1 s.d, pink: n = 6, king: n = 3, p-value = 0.0952).

Discussion

Our comparisons show that the force-length relationship constrains king salmon and pink salmon adductor mandibulae in different ways. Namely, optimal muscle length is at a larger relative gape in king salmon than in pink salmon. The functional differences between the two species are mirrored by a difference in muscle physiology, and this variation should provide a feeding advantage to each species. King salmon need to be better at catching fish than pink salmon, which, because of their variable diet, are less concerned with the biting power of their adductor mandibulae.

One way to understand how the variation we observed could be beneficial, is by looking at the force velocity relationship that constrains muscles. A force-velocity curve shows an exponential decline in muscle force as contraction velocity increases. The shape of this curve is independent of the initial muscle length, but the absolute force production is not. A muscle starting closer to L_0 will be able to produce stronger forces, albeit limited by the contraction velocity. If the muscle starts at its optimal length, and has zero contraction velocity, then it can produce that maximum force P_0 that is seen in the force-length curve. If the muscle starts at any other gape, the muscles will produce weaker forces, and if the muscles contract with positive velocity, force production will also decline.

When a salmon is biting prey, it must move its muscles with a positive contraction velocity, so the best way to maximize its force production is to start with a muscle length close to L_0. Eating larger prey items requires a larger gape. Having L_0 shifted to a larger gape suggests that king salmon are effective at biting down hard on larger prey and at snapping its jaws shut quickly on smaller prey. Pink salmon have a more general force-length curve that is centered at mid-gape. A centered force-length curve would be useful for getting similar muscle performance at a range of medium gapes. Pink salmon have a more varied diet than king salmon, and having optimal muscle performance at mid-gape allows them to eat bigger or smaller prey without suffering large losses in muscle force production. To filter feed, they swim with their mouths open so that planktonic organisms will be caught on their gill rakers. This behavior is much less dependent on force production than biting so a peak at mid-gape may provide an advantage to the muscles during filter feeding.

The area under the force-length curves does not differ significantly between pink and king salmon. This parameter is a means of quantifying the width of the curve without selecting a specific fraction of maximum force at which to measure the width. A wide curve, with a larger area underneath it, indicates that the muscles are able to produce large forces across a broad range of gape sizes. A muscle with a narrow curve would be specialized to exert large forces at a limited range of gape sizes. Selecting a fraction of maximum force at which to measure the curve width would introduce a bias and the width would be less meaningful. King and pink salmon produce maximum forces at different gapes, but they are able to produce similar areas across the functional length range of the muscle (i.e. from closed gape to maximum gape).

Conclusions

Among fish species there is a huge variety of morphologies and behaviors. A wide range of morphologies exists to fill the breadth of ecological niches. Specialization limits the pool of competitors a species must outperform when finding resources. Specialized feeding behavior is generally reflected not only in skeletal anatomy (as has been the major focus of functional morphology literature) but also in muscular morphology and physiology.

We demonstrate that this is true for the feeding mechanics of king and pink salmon. In king salmon, maximum bite force is achieved at a larger relative gape than in pink salmon. This may allow king salmon to take advantage of optimal muscle length when eating large or elusive prey and may be beneficial to pink salmon by allowing reasonably high forces at a range of medium gapes. Differences in dietary preferences may have evolved with jaw muscle physiology, creating distinct solutions to the force-length constraint.

Future studies should quantify the force-length relationship in other salmon species in the Pacific Northwest. Collecting data from sockeye and chum salmon, which, like pink salmon, filter feed and have variable diets, would be especially helpful to understanding how force-length constraints are related to feeding behavior when diet is variable. It would also be interesting to explore the force-velocity relationship and test whether it supports our explanation of these findings.

Acknowledgements

Thank you to everyone who was involved in this research including Nicholas Gidmark, Alexus Roberts, Francesca Giammona, Katherine Corn, Carter Searles, Mo Turner, and the Blinks-NSF REU-BEACON Program.

References

Anapol, F., & Herring, S.W. (1989). Length–tension relationships of masseter and digastric muscles of miniature swine during ontogeny. *The Journal of Experimental Biology*, 143: 1-16.

Anderson, R.A., Mcbrayer, L.D., & Herrel, A. (2008). Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biological Journal of the Linnean Society*, 93: 709-720.

Brodeur, R.D., Daly, E.A., Sturdevant, M.V., Miller, T.W., Moss, J.H., Thiess, M.E., Trudel, M., Weitkamp, L.A., Armstrong, J., & Norton, E.C. (2007). Regional comparisons of juvenile salmon feeding in coastal marine waters off the west coast of North America. *American Fisheries Society Symposium*, 57: 183-203.

Gidmark, N.J., Konow, N., LoPresti, E., & Brainerd, E.L. (2013). Bite force is limited by the force-length relationship of skeletal muscle in black carp, *Mylopharyngodon piceus*. *Biology Letters*, 9.

Huber, D.R., Eason, T.G., Hueter, R.E., & Motta, P.J. (2005). Analysis of the bite force and mechanical design of the feeding mechanism of the durophagous horn shark, *Heterodontus francisci*. *The Journal of Experimental Biology*, 208: 3553-571.

Ramsey, R.W., & Street, S.F. (1940). The isometric length–tension diagram of isolated skeletal muscle fibers of the frog. *Journal of Cellular Physiology*, 15: 11-34.

Wilkie, D.R. (1949). The relation between force and velocity in human muscle. *The Journal of Physiology*, 110: 249-280.