Five Fishes, Five Faces:

Comparative Functional Morphology of the Feeding Apparatus in Sculpins (Cottoidea)

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Abstract

By studying variation in feeding apparatus morphology across similar sympatric species, we can better understand the evolutionary relationships and ecological niches of these species. The most common feeding technique among vertebrates is suction feeding, in which an animal rapidly expands its buccal cavity to create negative pressure and suck in prey. Suction feeders not only open their jaws quickly; they must also close them rapidly to prevent elusive prey from escaping. In this study, we compared jaw morphology and feeding kinematics of five species of Salish Sea sculpin. We used anatomical dissection to measure differences in jaw adductor morphology and jaw leverage, and we used Sonometric crystal implantation to measure gape change and muscle strain during feeding. Although we found high conservation of body length to muscle mass ratio among species, visual inspection of the head and jaw revealed important differences. We found that the red Irish lord (*Hemilepidotus hemilepidotus*) possessed the fastest jaw, as demonstrated by anatomical measurement of a small lever ratio, kinematic measurement of a large gape-change to muscle-strain ratio, and behavioral observation of the red Irish lord's ambush hunting strategy. This study highlights the importance of including behavior and ecology in analyses of organismal morphology.

Introduction

Variation in jaw morphology and feeding kinematics across similar sympatric species can help us understand the evolutionary history and ecological niches of a community. The most common feeding technique among vertebrates is suction feeding, in which an animal rapidly expands its buccal cavity to create negative pressure and suck in prey. Suction feeders not only open their jaws quickly; those which hunt elusive prey must also close their mouths rapidly to prevent prey from escaping (Norton 1991).

In most teleost fishes, jaw closure is controlled by the *adductor mandibulae* muscle complex which consists of three major components: the *partes malaris*, *rictalis* and *stegalis*. To close the mouth these muscles pull dorsally and posteriorly on the coronoid process, a bony protrusion on the lower jaw, to rotate the lower jaw around its joint. Morphological traits such as jaw force and speed are influenced by the lever ratio: the relative distance of the jaw joint from the coronoid process and the jaw tip. By the equation $F_{in} * L_{in} = F_{out} * L_{out}$, as lever ratio increases, jaw force increases and jaw speed decreases. This tradeoff between speed and force is central to the study of comparative functional morphology, and to the evolution and maintenance of diversity within ecosystems.

Sculpin (Perciformes: Cottoidea) are an ideal study organism for comparative feeding apparatus functional morphology due to their simple jaw lever system and eagerness to feed in the laboratory. Furthermore, sculpin are a highly diverse group of benthic fishes with more than 825 species worldwide and over 50 species coexisting in the Salish Sea alone (Smith and Busby 2014, Eschmeyer et al 1983). The key to understanding how this immense diversity of spiny bottom-fishes can coexist in one place may lie in the study of feeding apparatus morphology.

Feeding apparatus morphology has been shown to correlate with diet and feeding habits in numerous teleost fishes including sculpins, wrasses, cyprinids, centrarchids and chichlids (Norton 1991, Wainright 1988, Gidmark et al 2013, Gidmark et al 2014, Lauder 1983, Higham et al 2006). For example, sculpin jaw size influences prey capture success;

small-mouthed sculpin species have lower success at hunting shrimp than do large-mouthed species, but both large- and small-mouthed sculpins have high success at hunting crabs (Norton 1991). In some cases, specialized jaw morphology allows a species to exploit a dietary niche which is unavailable to competitors. The redear sunfish (*Lepomis microlophus*) uses a unique pattern of pharyngeal muscle contractions to crack the shells of freshwater snails, thereby utilizing a prey source which is unavailable to the rest of its genus (Lauder 1983).

Understanding the morphological basis of feeding performance across fishes is a critical step toward interpreting the diversity present in the ocean (Wainright, Carroll et al 2007). By studying the variation in feeding apparatuses across five species of Salish Sea sculpin, we hope to shed light on the principles of morphological, functional and ecological diversity which allow numerous similar species to coexist as members of the same community.

Materials and Methods

Specimens Used

The thirteen adult sculpin examined in this study comprised five species: red Irish lord (*Hemilepidotus hemilepidotus*), buffalo sculpin (*Enophrys bison*), staghorn sculpin (*Leptocottus armatus*), spinyhead sculpin (*Dasycottus setiger*) and great sculpin (*Myoxocephalus polyacanthocephalus*). Specimen body length ranged from 157 to 417 mm, and head width from 31.9 to 83.9 mm. Specimens were collected using beach seine, otter trawl and SCUBA diving at multiple locations in the Salish Sea. The specimens were fed a diet of live and frozen shrimp, primarily California spot prawn (*Pandalus platyceros*) collected from the waters around San Juan Island. Specimens were separated by size into 500-gallon concrete tanks and 50-gallon plastic tanks with flow-through seawater. At least two days prior to surgery, each specimen was acclimated to a 10-gallon isolation tank while food was withheld. All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Surgical Technique

Animals were anesthetized in 10 gallons of buffered 0.1 g/L MS-222 seawater solution. Anesthesia was maintained at 0.05 g/L and adjusted throughout surgery to maintain good animal condition (i.e. regular breathing but no bodily movements). Sterile technique was used to implant six 2 mm Sonometric crystals using 0.7 metric 18-inch Ethilon black monofilament nylon suture. Crystals were inserted into the upper and lower lips, and within either end of the two superficial components of the *adductor mandibulae* muscle complex, the *pars malaris* and *pars rictalis* (Figure 1). During surgery an Aquatic Gardens Powerhead 601 was used to pump water through the specimen's mouth and out the gills. Each surgery, from induction of anesthesia to replacement of the specimen into pure seawater, typically lasted under two hours. Specimens were allowed to recover from surgery in 10-gallon isolation tanks.

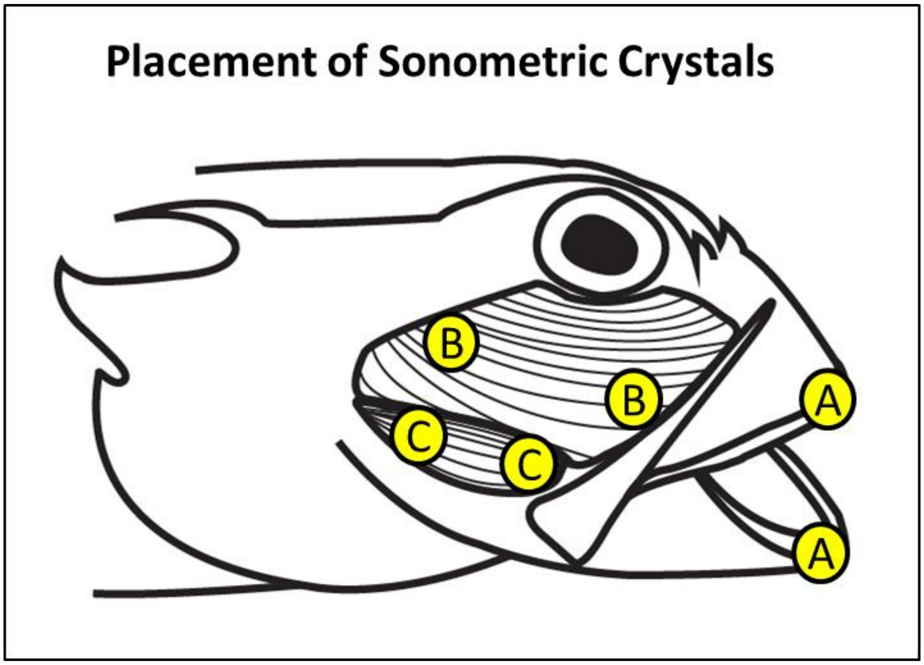

Figure 1: Surgical placement of Sonometric crystals within the lips (A) and the two superficial components of the *adductor mandibulae* muscle complex, the *pars malaris* (B) and the *pars rictalis* (C).

Feeding Trials

Each Sonometric crystal transmitted and received sound waves, allowing us to monitor the distance between any two crystals at all times using SonoLabDS3 Version 1.0.0.55. Feeding trials were conducted by offering pieces of shrimp to a sculpin while simultaneously recording Sonometric data of gape and muscle length before, during and after each feeding event. Specimens were tested for several days until a minimum of five successful feeding trials had been completed.

Sonometric Data Analysis

Sonometric data were viewed in SonoVIEW Version 3.4.60 RC1, and artifacts from background noise and echo were removed manually. Gape and muscle-length change during mouth closure were measured from the peak (i.e. maximum gape and muscle-length) to the following trough (i.e. minimum gape and muscle-length). Gape change was determined by the equation (max gape - min gape) * 100%, and muscle strain by the equation ([min length - max length] / min length) * 100%. Data were analyzed in Microsoft Excel and MatLab. Statistical significance was determined by an ANOVA test, and pairwise significance by a Tuky Posthoc test.

Anatomical Dissection

Specimens were euthanized by either a 10-minute submersion in 0.1 g/L buffered MS-222 solution or by an injection of 3 mg/mL Metomedate solution into the brain cavity. Both methods of euthanasia were followed by spinal cord and aortic severance. Anatomical measurements, taken immediately after euthanasia, included body mass, gape length and angle, head and body length, and in-lever and out-lever length. The *adductor mandibulae partes malaris*, *rictalis* and *stegalis* were measured to determine fiber length, total length, angle in relation to jaw, and mass.

Results

Anatomy

Across all five species of studied sculpins, general morphological conservation was observed. The body-mass to body-length relationship (Figure 2) and head-length to jaw-length relationship (Figure 3) followed similar positive linear correlations for all five species.

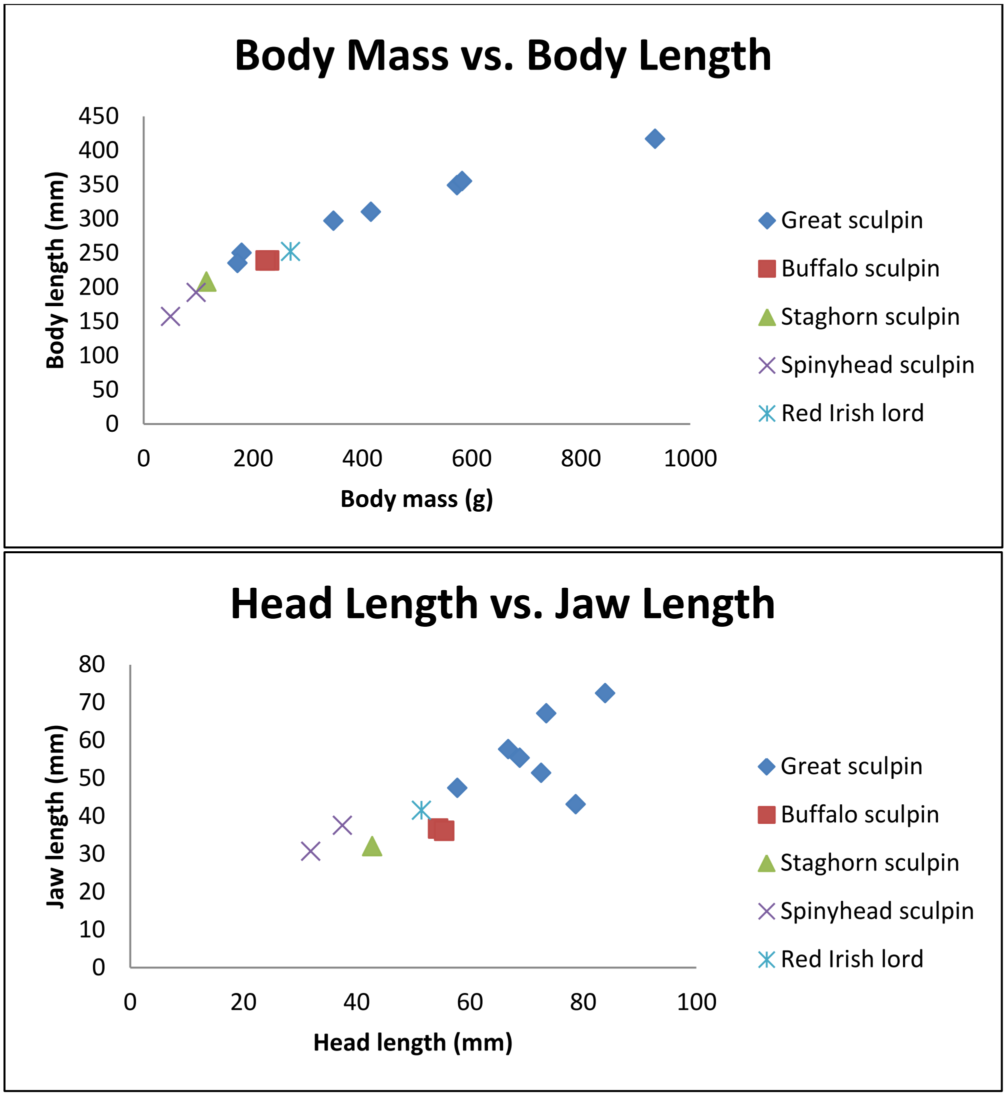

Figure 2: Body mass and length were positively correlated along approximately the same slope in all five species of sculpin. Head and jaw length showed a similar positive correlation.

However, detailed visual inspection and anatomical dissection revealed important differences in head shape and muscle conformation (Figure 3). Exteriorly, red Irish lords have round, lumpy heads with protruding eyes. Buffalo sculpin have short and wide heads with a squat appearance. Staghorn sculpin have distinctly streamlined heads with a flat, narrow shape and no protruding spines. Spinyhead sculpin have small, triangular heads with numerous sharp spines. Finally, great sculpin have long, wide heads with large mouth volumes.

Removal of the skin, subocular bone and *levator* muscle revealed further morphological diversity (Figures 3 and 4). Each species displayed a unique arrangement of *adductor mandibulae* muscles and facial bones. Red Irish lords have a bulging *malaris* which partially overlaps the *rictalis* and equals it in mass. Buffalo sculpin have prominent preopercular spines and a *malaris* which is about 1.5 times the size of the *rictalis*. The *malaris*, distorted by the large *levator* muscle superficial to it, increases in size from a thin posterior edge to a thick anterior edge. Staghorn sculpin have a large but narrow *malaris* and a bulging yet small *rictalis*. Unlike the muscle fibers of the other four species, the staghorn sculpin's *malaris* and *rictalis* muscle fibers are parallel. In the spinyhead sculpin, intricate subdermal bony plates and spines surround the eyes and cover the preopercle. The ventral section of the *malaris* becomes a thin sheet overlapping the dorsal half of the *rictalis*. The spinyhead sculpin was the only species to exhibit a smaller *malaris* than *rictalis*. Great sculpin have the largest *malaris* to *rictalis* ratio, a finding which is supported visually by the appearance of a large, wide, flat *malaris* and a small, tubular *rictalis*.

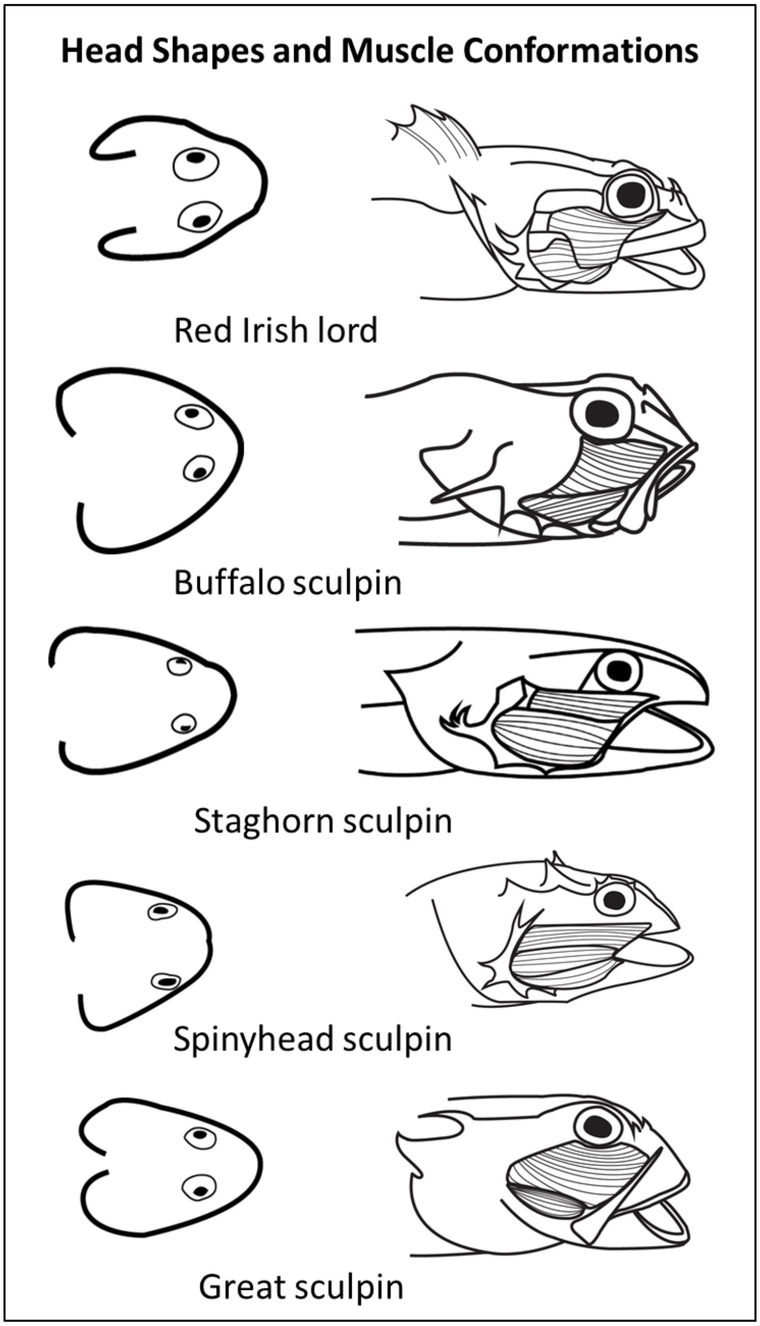

Figure 3: Visual inspection and anatomical dissection revealed important morphological differences in head shape and muscle conformation of the five studied species of sculpin.

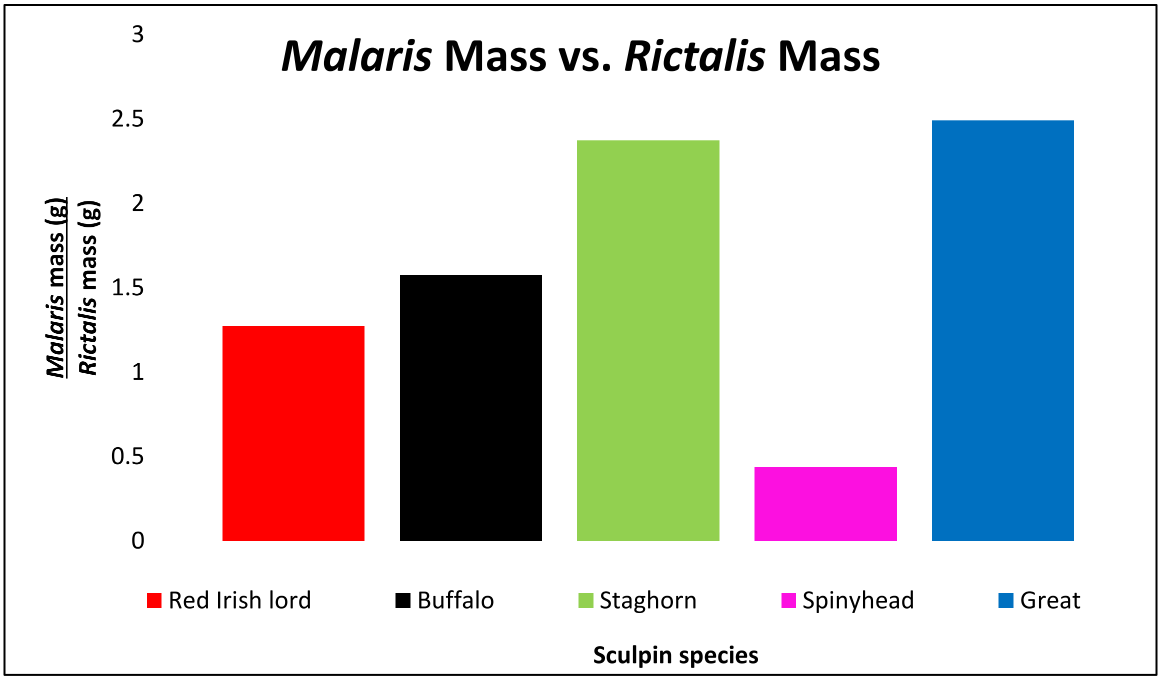

Figure 4: The relative masses of the *adductor mandibulae partes malaris* and *rictalis* among five species of sculpin illustrate diversity in facial muscle morphology.

Quantitative anatomical measurements of facial structures reinforced these qualitative observations. Of particular note were the variable lever ratios (in lever/out lever) which ranged individually from 0.15 in a great sculpin to 0.40 in a staghorn sculpin (Figure 5). Great sculpin, the only species for which we obtained more than two specimens, showed a positive linear correlation between body mass and lever ratio. When we excluded juvenile great sculpin, the red Irish lord became the species with the lowest lever ratio, at 0.26.

Interestingly the adult great sculpins, with large body masses, had lever ratios similar to those of the other four species, despite their smaller body masses. To study groups with comparable jaw biomechanics, we chose to exclude small juveniles from our comparative analyses and include only adult great sculpin.

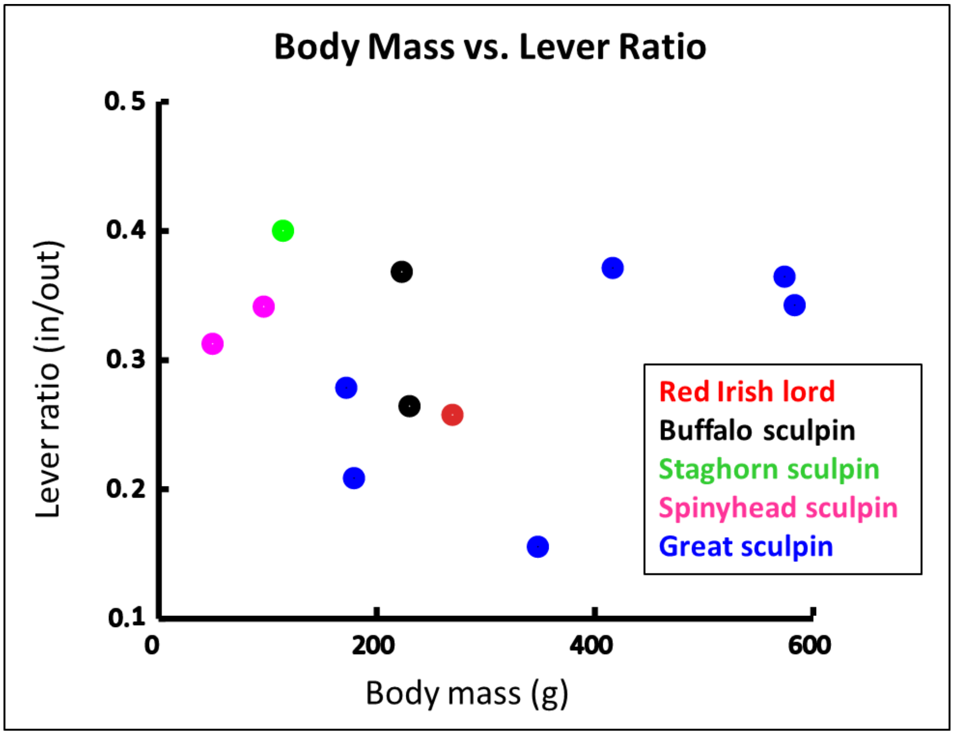

Figure 5: Lever ratio and body mass showed a positive linear correlation in great sculpin, the only species for which we studied more than two specimens. Because the adult (larger) great sculpin have similar jaw biomechanics to the other four species, we will exclude the juvenile (smaller) great sculpin from further analyses.

Feeding Behavior

Of the five species studied, red Irish lords and staghorn sculpins were typically the most eager feeders, while buffalo sculpins were the least interested in food. All sculpin species were ambush predators in the laboratory. When a piece of shrimp was introduced to a group tank, multiple sculpin would focus their eyes on the shrimp and rotate their bodies to face it for several seconds. Suddenly, one sculpin would swim, suck the shrimp into its mouth, and float back to the tank floor. Red Irish lords were the most cautious hunters, preferring to wait in PVC tubes on the tank floor before swimming and striking with more apparent urgency than the other species. Red Irish lords swam immediately back to their tubes rather than floating slowly to the floor. The red Irish lord’s tendency to sit in PVC tubes was similar to its wild behavior of hiding in rock crevices, which we observed while SCUBA diving.

Feeding Kinematics (Sonometrics)

Using both visual observation and Sonometric data collection, we found that each sculpin progressed through five stages of motion while feeding: normal breathing, striking, clenching, chewing, and finally a return to normal breathing (Figure 6). In the Sonometric data, a feeding strike is visible as a peak in gape and muscle length. The steep upward slope leading to the peak is expansion of the buccal cavity to create suction; the steep downward slope following the peak is rapid closure of the mouth to capture elusive prey before it can escape. Directly subsequent to the feeding strike, a dip is visible in both gape and muscle length, indicating that the jaw is clenched. After a successful feeding event, the sculpin usually chews several times before returning to normal breathing.

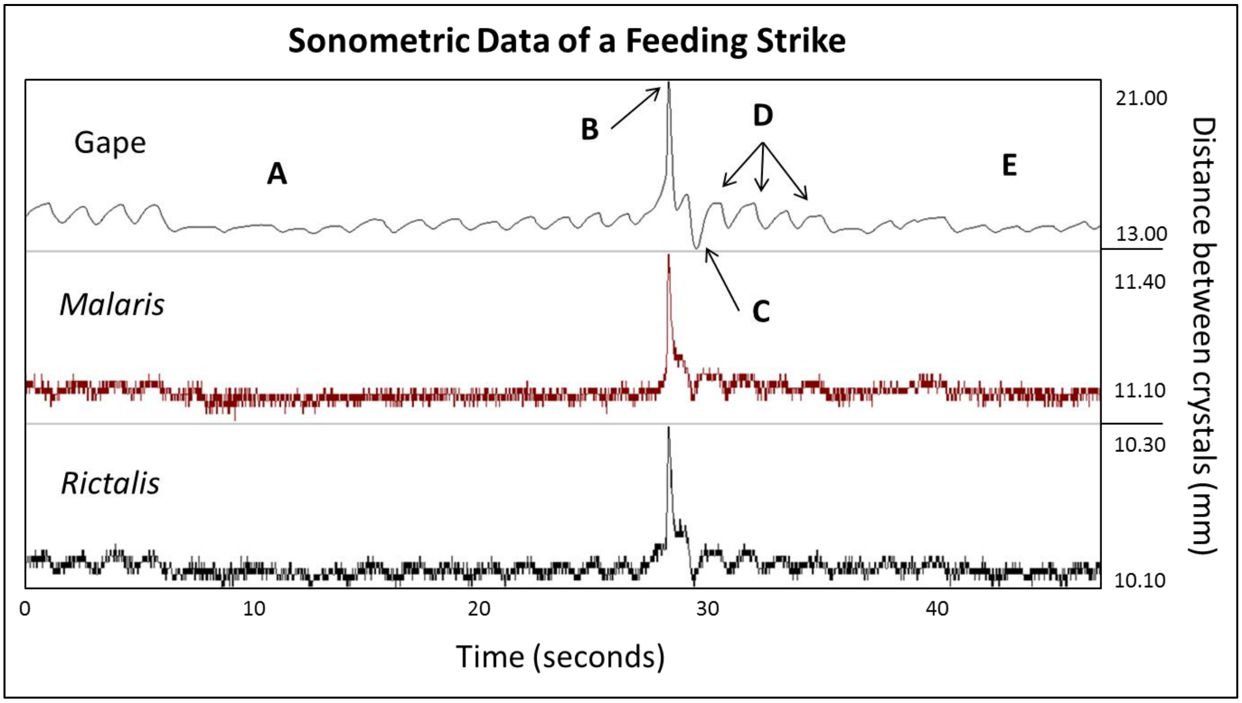

Figure 6: Sonometric data from a feeding buffalo sculpin. The sculpin passed through five stages of motion: normal breathing (A), striking (B), clenching (C), chewing (D), and return to normal breathing (E). Four chews are visible in this trial.

Analysis of the Sonometric data revealed interspecific differences in the relationship between gape change and muscle strain. The gape-change to *rictalis*-strain ratio was significantly higher in the red Irish lord than in all other species ($p > 0.05$) except the staghorn sculpin, which showed too much error to determine significant difference (Figure 7). None of the species other than the red Irish lord differed significantly from one another.

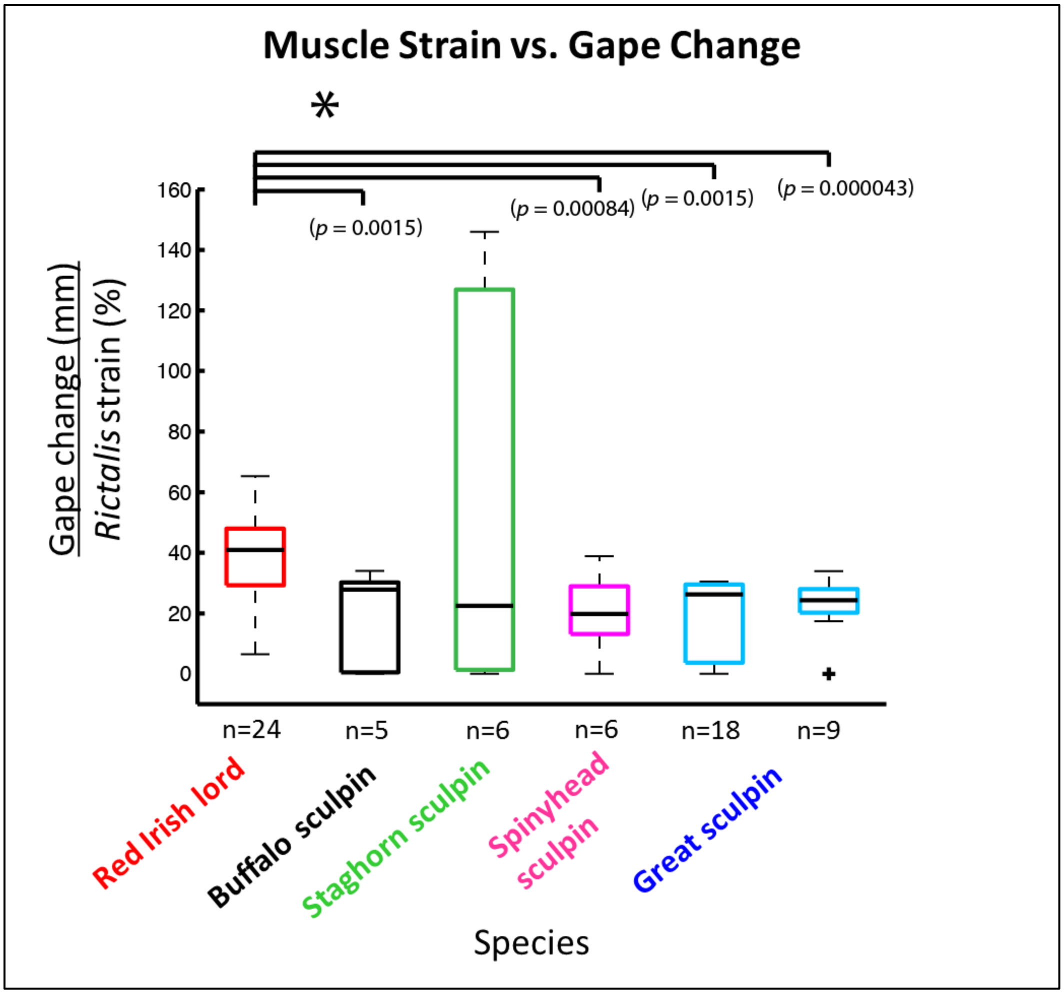

Figure 7: The ratio of gape change to muscle strain was highest in the red Irish lord, signifying a faster jaw-closing motion. Each box-and-whisker represents an individual sculpin; each color represents a species.

Discussion

We found overall morphological conservation among five species of spiny Salish Sea bottom-fish, but important anatomical, kinematic and behavioral differences were present. Interestingly, all three lines of data revealed an identical pattern: the jaw of the red Irish lord is faster than that of the other species.

Anatomically, the red Irish lord's jaw had a relatively short in-lever and long out-lever. Although we did not have enough individuals to show a significant trend for lever ratios within species, our individual red Irish lord had the smallest lever ratio when compared to the other four species (excluding juvenile great sculpin). Due to its small lever ratio, we expect a given amount of muscle force to result in a greater amount of gape change for the red Irish lord. Simply put, we expect a small lever ratio to result in a faster jaw.

Kinematically, the predicted result was observed in sculpins during feeding. Sonometric analysis revealed the red Irish lord's high gape-change to muscle-strain ratio. Because the gape-change per muscle-strain relationship reveals how much a muscle must shorten in order to move the jaw a certain distance, it is a measure of jaw-closing speed in which a higher ratio indicates a faster jaw. Due to the relationship between force and length, we expect that the red Irish lord must make a biomechanical sacrifice of strength to achieve its fast jaw.

Recent phylogenies present the red Irish lord as the most basal of all five studied species of sculpin (Figure 8). The red Irish lord's distinctively fast jaw may have a genetic basis which evolved within the *Hemilepidotus* lineage after the other Cottoids diverged. Alternatively, the clade of non-*Hemilepidotus* species may have evolved a slower, higher-force lever system after diverging from their *Hemilepidotus* ancestors. In the future, we plan to phylogenetically correct our data in order to make more robust inferences about the evolution of feeding apparatus morphology across Cottoidea.

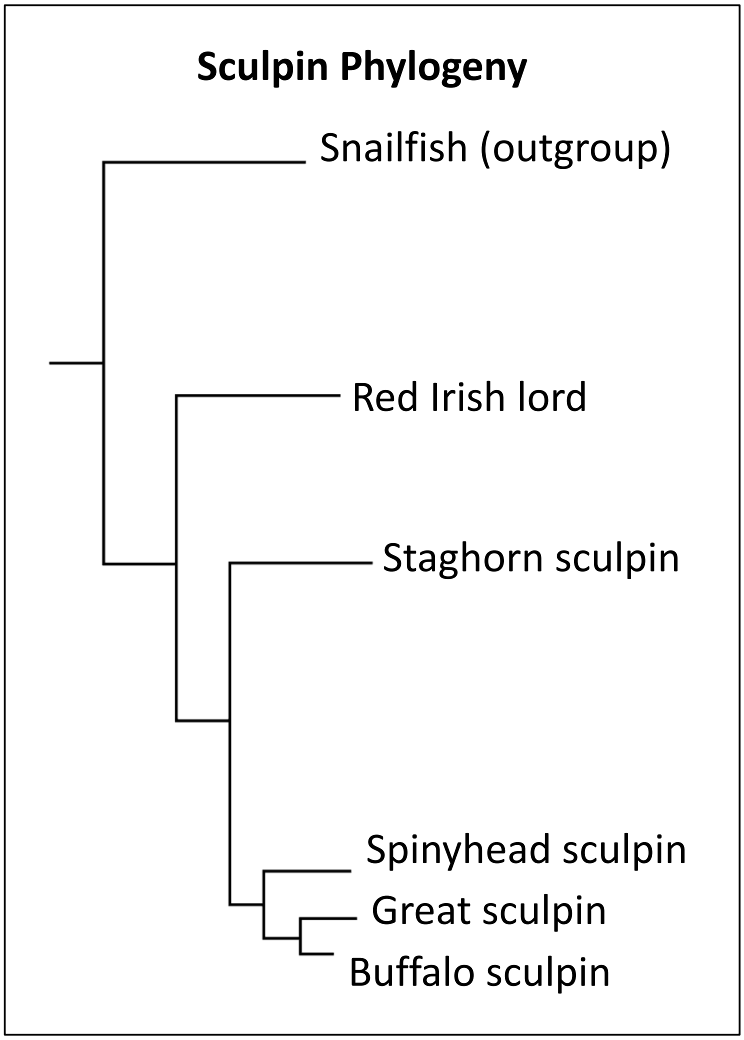

Figure 8: The red Irish lord is basal to the other four studied sculpins. Source: Smith and Busby 2014.

Although the red Irish lord possesses the fastest jaw out of the five studied species of sculpin, it does not appear to prefer the fastest prey (Table 1). Three of the five studied sculpins prefer highly elusive prey items: great and staghorn sculpin are piscivores, and spinyhead sculpin are shrimp specialists. In contrast, the red Irish lord's diet is composed primarily of crabs, a slow-moving prey. There are several possible explanations for the puzzling notion that the sculpin with the fastest jaw does not hunt the fastest prey. First, the data in Figure 1 are species-specific in all cases except that of the red Irish lord, for which genus-specific data were used (from congeneric species *H. jordani* and *H. gilberti*). Second, the red Irish lord might rely on its highly elusive secondary prey item, fish, during a certain season or life stage. Third, and most interestingly, the red Irish lord's distinctive feeding behavior might provide the context necessary to understand this sculpin's speed-focused jaw morphology.

Species	**Primary food**	**Secondary food**
Red Irish Lord	Crabs (45% weight)	Fish (10% weight)
Great	Fish (52% weight)	Crabs (34% weight)
Staghorn	Fish (40% volume)	Crabs (30% volume)
Spinyhead	Shrimp (64% weight)	Mysids (3% weight)
Buffalo	Ulva (56% volume)	Shrimp (10% volume)

Table 1: Each species of sculpin prefers a different diet: one species prefers crabs but accepts fish; two prefer fish but accept crabs; one specializes in shrimp; and one is primarily herbivorous. Sources: Napazkov 2009, Johnson 1968, Mace 1975, Isakson et al 1971, Tokranov 1995, Jewett et al. 1989

Like the other sculpins, red Irish lords are sit-and-wait predators, but their habitat choice and hunting strategy are distinctive. In the wild, red Irish lords tend to hide deep within rock crevices, striking out for food and quickly returning (Eschmeyer et al 1983, Isakson et al 1971). Other sculpin species prefer open habitats: great and spinyhead sculpin are found on soft bottoms, buffalo sculpin in eelgrass beds, and staghorn sculpin on sand-gravel substrate (Eschmeyer et al 1983, Johnson 1968, Isakson et al 1971). In the laboratory, these differences in habitat preference were apparent in the red Irish lords' reluctance to remain outside of their PVC tubes for any amount of time. While feeding, red Irish lords made the fastest prey-capture strikes, both with their swimming speed (qualitative observation) and jaw-closing speed (implied by the biomechanical results in this paper but not measured directly). The red Irish lord's fast jaw-closing mechanism might be an adaptation which complements its habitat and feeding behavior, allowing it to capture prey rapidly while minimizing time exposed from the protection of its rock crevice. This study highlights the importance of incorporating ecology and behavior into analyses of morphological traits in order to provide complete organismal context.

Overall, our results indicate that while many anatomical features are conserved across Cottoids, distinct interspecific differences in head shape and jaw adductor morphology are present. In the future, we plan to phylogentically correct our data to determine the relationship between evolutionary changes in fiber length and lever ratio. Together, this study's results and future analyses will help us untangle the evolutionary relationships and ecological roles of a massively abundant and diverse group of teleost fishes, the sculpins, so that we may better understand the morphological bases underlying the ability of dozens of similar species to coexist sympatrically.

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