

Feeding Mechanics and Functional Morphology in the Jaws of Sculpins

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Abstract

Species with overlapping geographic ranges, similar morphology, and ecological roles often vie for the same resources and therefore face competitive exclusion. This competition can be reduced if species vary in skeletal and muscular anatomy, because it changes biomechanical performance across species. We examined biomechanical variation of feeding structures in a group of nineteen sculpins (Cottoidea) that co-occur in the marine habitat around the San Juan Islands. We quantified evolutionary correlations of skeletal morphology and muscle morphology by conducting phylogenetic independent contrasts using a phylogeny constructed from published molecular data. We hypothesized that skeletal leverage (mechanical advantage) and muscle architecture (gearing) could either display a positive evolutionary correlation (changing over evolutionary time to perform inversely of each other), or the features could display a negative correlation (changing over evolutionary time to perform in the same way). We found a positive correlation between evolutionary shifts of out-lever length and adductor muscle length, but no correlation between evolutionary shifts of in-lever length and adductor muscle length or adductor muscle length and lever ratio. Our results demonstrate that skeletal leverage and muscle architecture evolve independently in individual species of sculpins. These results also suggest that these two functional units (skeletal morphology and muscle morphology) both contribute to biomechanical diversity in closely related, geographically co-occurring sculpin species, indicating their importance as metrics of ecomorphological diversity.

Introduction

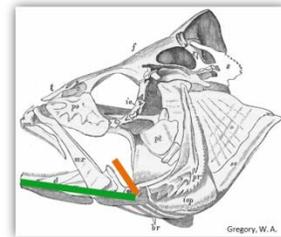
The fundamental purpose of ecology is to examine interactions between organisms and their surrounding environment. During ecological interactions species with overlapping geographic ranges, similar morphology, and ecological roles often vie for the same resources. Species competing for the same resource in the same way inevitably end up

with one organism competitively excluding the other. To avoid this competition, species must separate either how they gather a given resource or what resources are being attained. Species can reduce competition through obtaining resources in different ways by varying skeletal and muscular anatomy, thus changing biomechanical performance. By biomechanically modelling these types of variation, data collected on the structure and movement of an organism can be quantified in order to make predictions about the function and functional relationships of skeletal and muscular anatomy within its biological system.

One of the most significant pieces of information that can be obtained through biomechanical modelling is the amount of force that a system can produce given the



$$\text{Force in} \times (\text{in lever/out lever}) = \text{FORCE OUT}$$



$$F(\text{muscle}) \text{ in} \times (\text{in lever/out lever}) = \text{FORCE OUT}$$

Figure 1- The skeletal lever system of an organism closely resembles that of a crowbar system where there is a long arm where force is input and a short arm where force is output.

situation. The skeletal lever system of an organism closely resembles that of a crowbar system where there is one arm where force is input and another arm where force is output. In fish systems, the short arm of the lever, the in-lever, allows the amount of output force from the long arm, the out-lever, to be divided and applied to a specific point when capturing prey. Once the lever ratio of a system is ascertained, the output force of a system can be calculated when the force put into the system is accounted for. In the jaw system of fishes, the in-lever is the distance between the jaw joint and the coronoid

process, which is where the jaw muscles attach to the jaw, and the out-lever is the distance between the jaw joint and the tip of the jaw (Figure 1).

Given certain jaw morphologies, a fish can bite quickly or with a lot of force. This ability is typically determined by taking into account three traits that play into a characteristic bite. First, the jaw's skeletal morphology, more specifically the location of the coronoid process, must be examined. When the coronoid process is a shorter distance from the tip of the jaw, the jaw becomes stronger due to the increased lever ratio; as the process is closer in proximity to the jaw joint, the jaw becomes weaker due to the decreased lever ratio. Second, the jaw muscle morphology, more precisely the length of the jaw closing muscles must be studied. Given two of the same jaw muscles from different organisms have the same mass, the muscle with shorter-length fibers is composed of a larger number of fibers and it takes more absolute length change to open that organism's jaw a given amount. Due to this, that organism's jaw is stronger, but slower. With muscles that have longer-length fibers, there are also fewer fibers, but because it takes less absolute length change to open the jaw a given amount, that organism's jaw is faster and weaker. Finally, the muscle physiology needs to be inspected to predict whether an organism can bite quickly or forcefully. Typically, bite force has been measured and assigned as one value per individual, but the force-length relationship of a muscle suggests that an individual should have a range of gape height-specific bite forces (Gidmark 2013). The force-length relationship of muscle implies that a muscle has a maximum force output at intermediate lengths and that the force output decreases as the muscle is stretched or shortened from that intermediate position (Ramsey 1940).

By implementing studies of skeletal morphology, muscle morphology, and muscle physiology in fish jaws, this study aimed to understand evolutionary relationships between skeletal and jaw muscle morphology among sculpins around Washington's San Juan Islands. Sculpins, similar to the majority of other bony fishes, utilize suction feeding to capture prey. In this method of feeding, the fish rapidly expands the buccal cavity which then pulls water and prey inward due to the formation of a pressure gradient (Lauder 1980). In addition to rapidly opening the mouth, the fish must quickly close its jaws in order to retain the captured prey (Norton 1991). The muscle complex controlling this movement, called the *adductor mandibulae*, is composed of three major muscles termed the *pars malaris*, *pars rictalis*, and *pars stegalis*. In this study a two-pronged approach was utilized to aide in understanding the biomechanical diversity in these closely related, geographically co-occurring sculpin species in order to determine if there is functional diversity among the group of fishes and if that functional diversity involves co-evolution of muscles and the skeleton.

Materials and Methods

Specimens

The sculpins utilized in the *in vivo* portion of this study included four species: great sculpin (*Myoxocephalus polyacanthocephalus*), red Irish lord (*Hemilepidotus hemilepidotus*), staghorn sculpin (*Leptocottus armatus*), and spinyhead sculpin (*Dasycottus setiger*). The specimens were fed live and frozen spot prawns every other day. Prior to undergoing surgery, specimens were placed in isolation tanks in order to acclimate to the environment. At least two days prior to surgery, food was withheld. All

animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Surgical Procedure

The fish were anesthetized in 10 liters of 0.1 g/L MS-222 seawater solution. Anesthesia was maintained at 0.05 g/L and monitored during the surgery to ensure the specimen maintained regular ventilation, but remained immobile. Sterile technique was used to implant four 2 mm Sonometrics crystals (Sonometrics corporation, London, Ontario, Canada) using 6-0 monofilament. A single

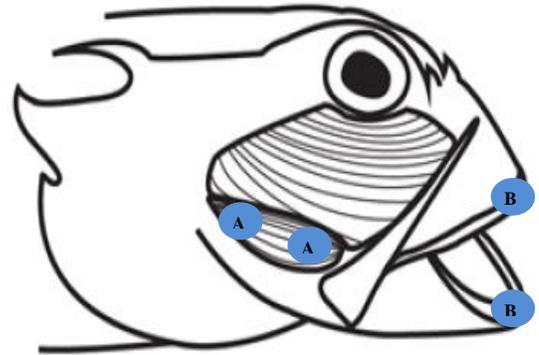


Figure 2- Placement of sonometric crystals. Crystals A were placed in the *pars rictalis* and crystals B were placed in the upper tip of the premaxilla and another in the anterior tip of the mandible. (modified from Finley, 2014)

crystal was placed in each end of the right *pars rictalis* (one of the *adductor mandibulae* muscles), along a single fiber to measure muscle length. Another crystal pair was placed to measure gape: one at the upper tip of the premaxilla and another in the anterior tip of the mandible (Figure 2). During surgery, a Powerhead 601 was utilized to pump water through the fish's mouth and out of the gills. The duration of each surgery, from placing the fish under anesthesia to placing it back into pure seawater, was kept to two hours or less. Specimens were allowed to recover from surgery in isolation tanks.

Feeding Trials

Each Sonometrics crystal can transmit and receive sound waves which enables the distance between any two crystals to be measured at all times using SonoLabDS3 version

1.0.0.55. Feeding trials were conducted by placing pieces of shrimp in the isolation tank while simultaneously recording time-series Sonometrics data of gape and muscle length before, during and after each feeding event. Several feeding trials were completed with each specimen over several days until a minimum of five successful feeding trials were completed.

In situ Muscle Stimulation

Specimens were euthanized by an injecting an overdose of Metomidate hydrochloride solution into the brain cavity. This was followed by spinal cord and aortic severance. Immediately following this, the portion of the *pars rictalis* containing the Sonometrics crystals was removed from the specimen in order to complete an *in situ* muscle stimulation. The section of *pars rictalis* removed was kept attached to connective tissue and the preorbital bone in order to anchor the muscle on to the force transducer apparatus. Non-compliant Kevlar was tied around the connective tissue and the Imada force transducer; zip ties were placed around the bone and the base of the apparatus to anchor the muscle. An electrical current of 10 volts was used stimulate a singular muscle contraction, which pulled on the thread that was connected to the force transducer thus producing a force reading. Other stimulus parameters were as follows: pulse duration of 2 milliseconds, no delay, stimulation rate 200 pulses per second, train duration of 300 milliseconds, and only a single train was elicited per stimulus event. The peak amount of pounds of force produced by the muscle at different lengths was measured and recorded for every stimulus. Stimulus events were elicited no more than once every five minutes to prevent muscle fatigue.

Sonometrics Data Analysis

Sonometrics data was viewed in SonoVIEW Version 3.4.60 RC1. Each individual feeding and force trial was inspected for peaks and troughs. In the feeding trials, the timing of peaks and troughs in muscle length recordings were compared to those in the gape size recordings. The difference between muscle length change and gape change were also calculated for the feeding trials. For each force trial, the peak or trough produced by the muscle stimulation at a certain length was compared to the force peak or trough produced at the other various muscle lengths. In both feeding and force trials, the difference in muscle length at the peak gape and resting muscle length was calculated. Due to the need for further analysis, this data will not be discussed any further.

Examination of Preserved Specimen

There were nineteen species of sculpin examined for this portion of the study all of which are from the Burke Museum of Natural History and Culture in Seattle Washington (Table 1). One of the *pars rictalis* and half of the lower jaw were dissected from each of the preserved specimens. The *pars rictalis* was digested in a 33% nitric acid solution. Fifty fibers from throughout each muscle were measured; the average of the fiber lengths was calculated and recorded as the *rictalis* length. Half of the lower jaw of each specimen was cleared and stained using procedures adapted from *Clearing and staining techniques* (Potthoff, T., 1984) and *Enzyme clearing of Alcian blue stained whole small vertebrates for demonstration of cartilage* (Dingerkus, G. and Uhler, L. D., 1977). The in-lever and out-lever distances on each of the jaws were measured. The body length, the distance from the tip of the snout to the beginning of the caudal fin, as well as

the width of the head was measured. The measurements of the jaw muscle fibers and the in-lever were statistically analyzed to determine if those traits are correlated in evolution.

Specimen Number	Scientific Name	Common name
1	<i>Blepsias cirrhosus</i>	Silverspotted sculpin
2	<i>Chitonotus pugetensis</i>	Roughback sculpin
3	<i>Artedius fenestralis</i>	Padded sculpin
4	<i>Artedius harringtoni</i>	Scalyhead sculpin
5	<i>Oligocottus maculosus</i>	Tidepool sculpin
6	<i>Artedius lateralis</i>	Smoothhead sculpin
7	<i>Clinocottus analis</i>	Wooly sculpin
8	<i>Jordania zonope</i>	Longfin sculpin
9	<i>Rhamphocottus richarsoni</i>	Grunt sculpin
10	<i>Myoxocephalus polyacanthocephalus</i>	Great sculpin
11	<i>Hemilepidotus hemilepidotus</i>	Red Irish lord
12	<i>Leptocottus armatus</i>	Staghorn sculpin
13	<i>Dasycottus setiger</i>	Spinyhead sculpin
14	<i>Enophrys bison</i>	Buffalo sculpin
15	<i>Hemilepidotus spinosus</i>	Brown Irish lord
16	<i>Nautichthys oculifasciatus</i>	Sailfin sculpin
17	<i>Psychrolutes phrictus</i>	Tadpole sculpin
18	<i>Icelinus borealis</i>	Northern sculpin
19	<i>Scorpaenichthys marmoratus</i>	Cabezón

Table 1- Museum specimen utilized for the evolutionary inference.

Statistical and Phylogenetic Correction

To analyze the data collected from the preserved specimen, RStudio was utilized to phylogenetically correct the data. It was then linearly regressed in order to determine if there is an evolutionary correlation between the jaw and jaw muscle morphology. Due to the unavailability of *Psychrolutes paradoxus* in the tree that was utilized for this inference, *Psychrolutes phrictus* was used in its place.

Results

Anatomy

Upon visual investigation of the specimens listed in Table 1, it was observed that there was varying morphology across the group. The *rictalis* of each specimen was in a slightly different position in regards to the overall position of the *adductor mandibulae*. Measurements revealed that each *rictalis* was also a different average length (Table 2). The jaws extracted from each specimen appeared to have very similar morphologies, but the in-lever and out-lever measurements revealed key morphological differences (Table 2). All data collected was corrected for body length (Table 3).

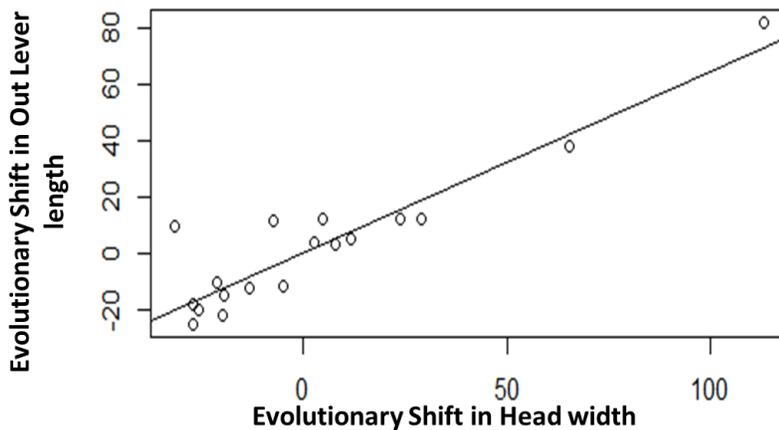
Species Name	Body Length (mm)	Head Width (mm)	Ave. Rictalis Fiber Length (mm)	In lever (mm)	Out lever (mm)
1-Blepsias cirrhosus	134	25.6	5.556	3.4	17.9
2-Chitonotus pugetensis	89	18.5	4.796	3.4	14.9
3-Arteidius fenestralis	78.9	19.9	4.196	3.9	13
4-Arteidius harringtoni	59.5	13.9	3.128	3.6	12
5-Oligocottus maculosus	62	13.1	3.032	2.4	10.2
6-Arteidius lateralis	74	18.1	3.628	2.7	14.8
7-Clinocottus analis	86.5	18	3.766	2.5	11
8-Jordania zonope	68.9	11	4.116	1.5	7.7
9-Rhamphocotus richarsoni	51.5	16.9	2.722	1.1	7.8
10-Myoxocephalus polyacanthocephalus	89.3	25.7	5.084	1.9	16.6
11-Hemilepidotus hemilepidotus	89.1	22	6.492	3.8	14.9
12-Leptocottus armatus	108.5	32.8	7.738	6.2	28.1
13-Dasycottus setiger	127.1	44.5	6.578	7	24.5
14-Enophrys bison	112	31.1	6.578	8.1	22.1
15-Hemilepidotus spinosus	164.9	45.9	9.802	6.8	31.2
16-Nautichthys oculfasciatus	107	21.5	4.654	3.9	13.6
17-Psychrolutes paradoxus	38.8	12	1.753	2.2	8.4
18-Icelinus borealis	73.8	19	4.024	4.5	16
19-Scorpaenichthys marmoratus	99.9	24	4.52	5.8	17.7

Table 2- Measurements of each specimen. Body length was taken as tip of the snout to right before the caudal fin. Head width was measured as the distance between the very outer side of the branchiostegals. Fiber length was determined by nitric acid digestion. In-lever and out-lever distances were measured after staining and clearing the jaw.

Species Name	Out lever/body length	In Lever/Body Length	In/out lever/ Body length	Rictalis/Body Length
1-Blepsias cirrhosus	0.13358209	0.025373134	0.001417494	0.041462687
2-Chitonotus pugetensis	0.16741573	0.038202247	0.002563909	0.05388764
3-Arteidius fenestralis	0.164765526	0.049429658	0.003802281	0.053181242
4-Arteidius harringtoni	0.201680672	0.060504202	0.005042017	0.052571429
5-Oligocottus maculosus	0.164516129	0.038709677	0.003795066	0.048903226
6-Arteidius lateralis	0.2	0.036486486	0.002465303	0.049027027
7-Clinocottus analis	0.12716763	0.028901734	0.00262743	0.043537572
8-Jordania zonope	0.111756168	0.021770682	0.002827361	0.059738752
9-Rhamphocotus richarsoni	0.151456311	0.021359223	0.002738362	0.052854369
10-Myoxocephalus polyacanthocephalus	0.185890258	0.021276596	0.001281723	0.056931691
11-Hemilepidotus hemilepidotus	0.167227834	0.042648709	0.002862329	0.072861953
12-Leptocottus armatus	0.258986175	0.057142857	0.002033554	0.071317972
13-Dasycottus setiger	0.192761605	0.055074744	0.002247949	0.051754524
14-Enophrys bison	0.197321429	0.072321429	0.003272463	0.058732143
15-Hemilepidotus spinosus	0.189205579	0.041237113	0.001321702	0.059442086
16-Nautichthys oculfasciatus	0.127102804	0.036448598	0.002680044	0.043495327
17-Psychrolutes paradoxus	0.216494845	0.056701031	0.006750123	0.045180412
18-Icelinus borealis	0.216802168	0.06097561	0.003810976	0.054525745
19-Scorpaenichthys marmoratus	0.177177177	0.058058058	0.003280116	0.045245245

Table 3- Measurements of each specimen corrected for body length.

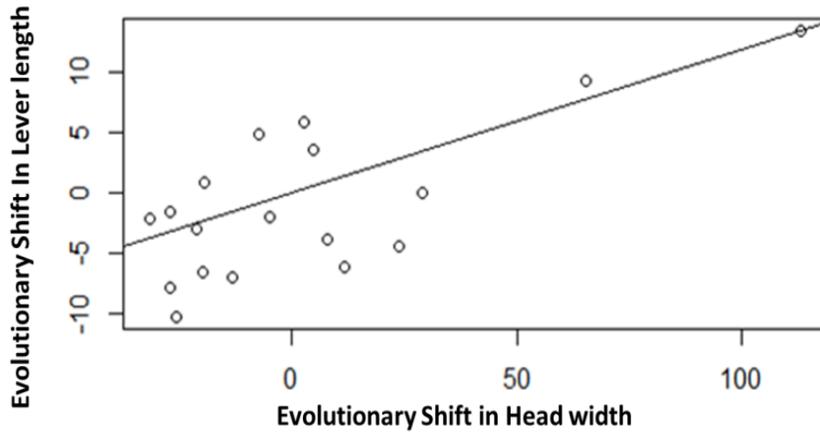
Experimental relationship between the Out lever and Head width



Slope: 0.64112
P- value: 1.619e-08
Adjusted R² : 0.8453

Figure 3- Experimental relationship between the out-lever and head width.

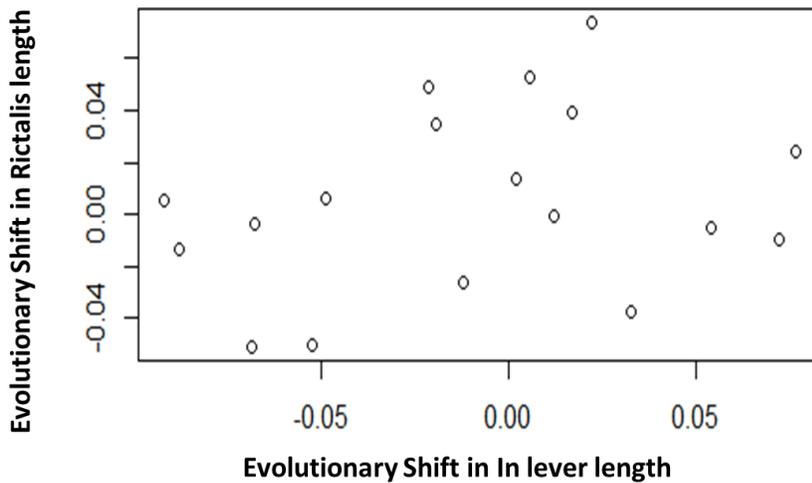
Experimental relationship between the In lever and Head width



Slope: 0.11931
P- value: 0.0009637
Adjusted R² : 0.4522

Figure 4- Experimental relationship between the in-lever and head width.

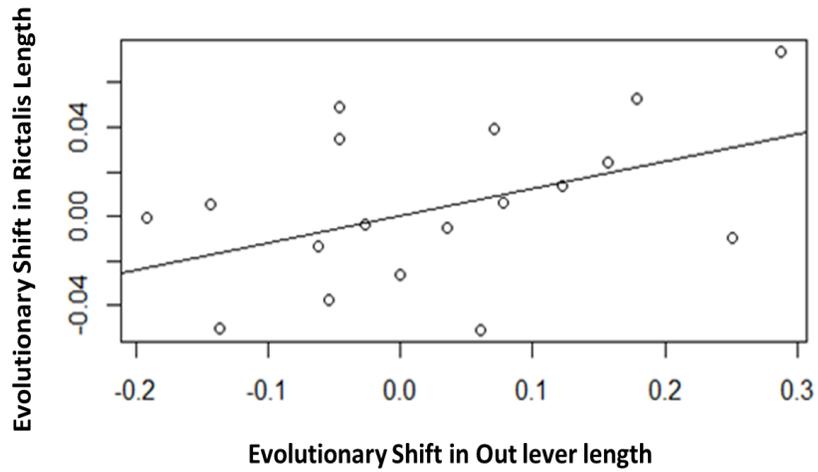
Experimental relationship between the Muscle length and In-lever



Slope: 0.161
P- value: 0.3262
Adjusted R² : 0.001229

Figure 5- Experimental relationship between the *rictalis* length and out-lever.

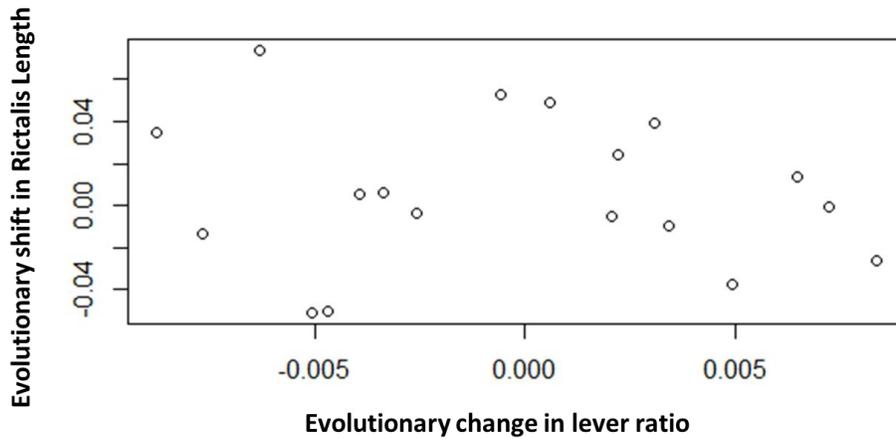
Experimental relationship between the Muscle length and Out Lever



Slope: 0.12174
P- value: 0.0435
Adjusted R² : 0.1727

Figure 6- Experimental relationship between *rictalis* and in-lever.

Experimental evolutionary relationship between the muscle length and lever ratio



Slope: -0.8387
P- value: 0.641
Adjusted R² : -0.04

Figure 7- Experimental relationship between *rictalis* length and lever ratio.

Discussion

Evolutionary Inference

We found several well-defined evolutionary correlations between jaw and jaw muscle morphology. For example, evolutionary shifts in head width were positively correlated with shifts in in-lever and shifts in out-lever. Shifts in the length of *rictalis* muscle fibers were also positively correlated with shifts in out-lever length. In each of these graphs, the best-fit line, all of which have an adjusted R^2 value close to 1, shows that the data patterns significantly represent what is occurring in nature. With the data from these graphs, a few things can be concluded: First, in regards to the in and out-lever versus head width graphs, the positive correlation implies that as the width of a fish's head increases or decreases over evolutionary time, the in-lever and the out-lever will both increase or decrease as well. Due to this correlation, it can be concluded that as a fish's head increases in size and the lever ratio gets larger, a fish's jaw gets stronger and slower; if the head width and lever ratio decrease over evolutionary time, a fish's jaw would get faster and weaker. The second conclusion that can be drawn in regards to the graph of *rictalis* length versus out-lever length, is that as *rictalis* length increases or decreases the out-lever increases or decreases as well. Hypothetically, if the out-lever in a fish's jaw system is increasing and the in-lever is remaining the same, the lever ratio in this system would be getting smaller. In contrast, if the system's out-lever is decreasing in length while the in-lever is remaining the same, the lever ratio would be getting larger. Due to this, it can be concluded that as these two traits are evolving over time the jaw itself would be getting stronger while the muscle is getting faster or the jaw would be getting faster while the muscle is getting stronger.

In the last two figures displaying the evolutionary shift in *rictalis* length versus in-lever length, as well as, the evolutionary shift in *rictalis* length versus that of the lever ratio, there is no clear pattern that can be seen and/or assigned to the data and significance is not supported by the p-value. It has been determined that *rictalis* length and in-lever length, as well as, the evolutionary shift in *rictalis* length and the evolutionary shift in the lever ratio have no correlation meaning the jaw and the jaw muscles have evolved and independently of one another in any way that is best for a particular species.

Conclusion

Our results demonstrate that skeletal leverage and muscle architecture evolve independently in individual species of sculpins. These results also suggest that these two functional units (skeletal morphology and muscle morphology) both contribute to biomechanical diversity in closely related, geographically co-occurring sculpin species, indicating their importance as metrics of ecomorphological diversity. While sculpins native to waters surrounding the San Juan Islands clearly exhibited evolutionary correlations between the skeletal morphology and the muscle morphology of the jaw, there are innumerable fishes that these correlations may or may not occur similarly in. In future studies we plan to further test these correlations by including multiple sculpin outgroups as well as freshwater sculpins to determine if jaw biomechanics function in similar ways. This comparison will allow us to investigate how ecomorphological diversity differs in various species and between saltwater and freshwater species.

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