

Comparative dynamics of suction feeding in marine and freshwater three-spined stickleback, *Gasterosteus aculeatus*: kinematics and geometric morphometrics

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Locomotion and feeding are key axes of diversity among fishes, and these are commonly integrated for successful prey capture. However, little is known about biomechanical variation among highly ecologically divergent populations of fishes. Three-spined stickleback, *Gasterosteus aculeatus*, is an ideal species for teasing apart the relationships between ecology, form and function, given the numerous independent invasions of freshwater lakes and streams from a marine ancestor. These natural replicates afford the opportunity to isolate convergence and/or many-to-one mapping of form to function. To explore the divergence between marine and freshwater stickleback, we investigated the differences in suction feeding kinematics and ram speed among individuals from replicates of both habitats. Feeding sequences were obtained using a high-speed camera, and microcomputed tomography was used to assess three-dimensional geometric morphometrics. Prey capture kinematics of marine and freshwater stickleback differed significantly, with marine individuals consistently exhibiting faster ram speeds, larger gapes, more jaw protrusion, and greater magnitudes and speeds of cranial rotation. These are consistent with an attack strategy aimed at evasive prey. In addition, populations exhibited significant morphological divergence, but not always along the marine–freshwater axis of divergence. Thus, there appears to be a mismatch between morphology and function. Together, our approach has revealed the dynamic kinematic divergence of marine and freshwater stickleback and how functional studies can be used to predict ecology.

ADDITIONAL KEYWORDS: biomechanics – fish – functional morphology – ram speed – swimming.

INTRODUCTION

Locomotion and feeding are key axes of diversity among fishes, and phenotypes associated with these activities are commonly integrated for successful prey capture (Rice & Westneat, 2005; Higham, 2007a; Rice & Hale, 2010; Kane & Higham, 2015). Consequently, aspects of predator–prey interactions may contribute to decreased fitness of hybrids and migrants in ecologically divergent populations, ultimately playing a role in ecological speciation (Higham *et al.*, 2016). However, the links between biomechanics and ecological and

evolutionary divergence remain largely unknown (but see Holzman *et al.*, 2012 for example).

Suction feeding in fishes involves the rapid expansion of the mouth cavity to generate a negative pressure, which drives the movement of water and the prey into the mouth (Day *et al.*, 2005, 2015; Higham, Day & Wainwright, 2006a). The relatively small area of water that is impacted around the fish's mouth necessitates a strong contribution from the locomotor system (Higham, 2007b; Kane & Higham, 2015), and thus, the speed at which the fish is moving at the time of the strike is critical for success. Although much is known about interspecific differences in feeding kinematics (Norton, 1991; Norton & Brainerd, 1993; Porter & Motta, 2004; Higham *et al.*, 2007; Longo *et al.*, 2016)

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and the detailed kinematics of single species (Sanford & Wainwright, 2002; Svanbäck, Wainwright & Ferry-Graham, 2002; Day *et al.*, 2005; Van Wassenbergh, Aerts & Herrel, 2005), much less is known about intraspecific variation in feeding kinematics in relation to ecological divergence among populations (McGee, Schluter & Wainwright, 2013). Intraspecific analyses are excellent for examining the consequences of ecological pressures and constraints (Garland & Losos, 1994), especially given that differences due to evolutionary history will likely be minimal. Therefore, this can reveal potential incipient speciation and, consequently, critical evolutionary patterns and processes (Sinervo & Losos, 1991; Herrel, Meyers & Vanhooydonck, 2001).

Three-spined stickleback, *Gasterosteus aculeatus*, is a widespread species that occupies many coastal marine and freshwater lakes and streams that formed after the last glacial recession (10–15 000 years ago) (Bell & Foster, 1994). The freshwater populations are thus descendants of the ancestrally marine lineage. Three-spined stickleback exhibit a number of phenotypic and genetic changes associated with freshwater environments, and these often parallel changes in behaviour (Di-Poi *et al.*, 2014), physiology and morphology (Bell & Foster, 1994; Walker & Bell, 2000; Peichel *et al.*, 2001; Rogers *et al.*, 2012) (reviewed in Rogers, Mee & Bowles, 2013). The multitude of transitions from the marine environment to independent freshwater systems makes three-spined stickleback a model system for studying contemporary evolution. That said, much attention has focused on the phenotypic divergence and speciation within a single freshwater location (e.g. benthic and limnetic species pairs), which is often linked to ecological conditions (Schluter, 1995). In this case, the open water stickleback tend to consume free-swimming plankton, whereas benthic macroinvertebrates are consumed by the benthic stickleback (Lavin & McPhail, 1986). In addition, recent work found that anadromous populations of three-spined stickleback exhibit lower suction index (epaxial muscle cross-sectional area divided by buccal area, which is then multiplied by the ratio of the in-lever to the out-lever in the suction feeding system) compared to benthic freshwater populations (McGee & Wainwright, 2013a). Within single lakes, limnetic stickleback strike faster and exhibit greater jaw protrusion than benthic fish (McGee *et al.*, 2013). Information regarding the functional outcome of transitioning from marine to freshwater habitats is lacking.

Here, we leverage the multiple independent freshwater invasions in three-spined stickleback to address key questions about the differences between distinct marine and freshwater populations. We use two freshwater and three marine populations to test the following questions: (1) Do freshwater and marine fish diverge in function? (2) Do differences in function

parallel differences in morphology? (3) Do the independent invasions of freshwater habitats result in convergent function and morphology?

METHODS

FISH COLLECTION

Stickleback were collected at several locations in British Columbia, Canada, including anadromous marine fish from the Little Campbell River (49°14'N, 122°5'W); marine fish from Bamfield Inlet (48°49'N, 125°8'W) and Roquefeuil Bay (48°51'N, 125°6'W); and freshwater fish from Black Lake (48°46'N, 125°5'W) and Frederick Lake (48°51'N, 125°1'W). Fish were collected using minnow traps and were transported to the Bamfield Marine Sciences Centre (BMSC) where they were acclimated for at least 21 days before the kinematic experiments. In addition to wild-caught individuals, we also used several F1 individuals from crosses and two hybrid individuals from a cross between Frederick Lake and Black Lake source populations. Our overall goal was to capture the potential variation in morphology in order to see how this might influence function. Fish were housed in 113-L aquaria and provided with flow through saltwater or freshwater, aeration and filtration. Temperatures were maintained at ~16 °C. Fish were fed chironomid worms until two days prior to experiments. During these normal feeding events, bright filming lights were used in order for the fish to get used to feeding under experimental conditions. Following experiments, fish were euthanized and fixed in a standardized position in 10% neutral buffered formalin for 24 h and then stored in 70% ethanol for microcomputed tomography (μ CT) scanning.

EXPERIMENTAL PROTOCOL: FEEDING KINEMATICS

All experimental procedures were approved by the Institutional Animal Care and Use Committees at the University of California, Riverside, the BMSC, and Canadian Council for Animal Care standards at the University of Calgary. Individual fish were presented with chironomid worms (~1.0 cm long), following standard suction feeding fish experimental protocols (Higham, Day & Wainwright, 2005). Worms were dropped at the opposite side of the aquarium, ~20 cm from the starting location of the stickleback. A 1 × 1 cm grid was placed directly behind the interaction in order to calibrate the distance and other variables. The space between the grid and the front of the filming tank was limited to 10 cm to avoid issues related to the distance between the fish and the calibration grid. A lateral view of the interaction was recorded using a high-speed camera (Phantom Miro M100) operating at 500 frames/s. To illuminate the field of view, a single

600-W floodlight was used. It was only turned on during the actual interaction to avoid heating the water. Only those approaches and strikes that were perpendicular to the camera angle were saved for subsequent analyses.

KINEMATIC ANALYSES

Video sequences were imported into MATLAB 2013 (The MathWorks, Inc., Natick, MA, USA), and a series of landmarks were digitized using DLTdv (Hedrick, 2008). Landmarks included the tip of the upper jaw, tip of the lower jaw, opercular lobe, point of dorsal fin insertion, the anterior margin of the eye and the ventral-most point of the hyoid. From these digitized points, the following displacement, timing, velocity and rotation variables were quantified: maximum gape (MG), time to peak gape (TTPG), maximum hyoid depression (HD), timing of HD relative to MG, maximum cranial rotation (CR), time of CR relative to MG, velocity of CR, ram speed, time of prey capture relative to MG and maximum jaw protrusion (JP). Ram speed (RS) was calculated (and averaged) over the final 40-ms immediately preceding mouth opening, and TTPG was defined as the time between 20 and 95% of maximum gape, which avoids the impact of the slow opening phase of mouth expansion (Day *et al.*, 2005; Higham *et al.*, 2006a). In addition, calculating TTPG this way facilitated comparisons to other kinematic studies of stickleback (McGee & Wainwright, 2013b). Body length was quantified from a video frame immediately prior to mouth opening and was digitized as the distance between the tip of the upper jaw to the base of the caudal fin. All displacement variables were expressed as a percentage of body length, and ram speed was expressed as body lengths per second. The narrow range in body length among the individuals in this study likely limited the impact of body size.

The ten kinematic variables (some size corrected) were analysed using principal components analysis. Both size-corrected (using values as a proportion of body length) and raw values were used, but both yielded the same result. The scores from the first two PC axes were extracted, and the differences among populations (all five) and between habitat (marine and freshwater) were assessed using an analysis of variance (ANOVA). If the scores differed significantly, we assumed that variables with loadings greater than 0.5 were significantly different between habitat types.

Using previous visualization experiments and modelling from centrarchid fishes (Higham *et al.*, 2006a; Kane & Higham, 2014), a generalist group of predators, we estimated the ingested volume of water (IVW) for the stickleback in our study. This modelling approach required only the 2D values of MG and ram speed from the stickleback. We assumed that the volume was an

ellipsoid (as in Kane & Higham, 2014), and we therefore assumed that the width of the volume was equal to the height. The height (H_{IVW}), length (L_{IVW}) and width (W_{IVW}) were predicted using the following equations from Kane & Higham (2014):

$$H_{IVW} = 0.670 + (0.018 \times \text{Ram}) + (1.311 \times \text{MG})$$

$$L_{IVW} = 0.927 + (0.037 \times \text{Ram}) + (0.611 \times \text{MG})$$

$$W_{IVW} = H_{IVW}$$

The IVW was the estimated using the ellipsoid volume equation:

$$\text{IVW} = \frac{4\pi}{3} \times (L_{IVW} \times W_{IVW} \times H_{IVW})$$

THREE-DIMENSIONAL GEOMETRIC MORPHOMETRIC ANALYSES

In order to determine if the integrated phenotype differed in a similar way to kinematics, preserved fish from the kinematic analyses were subjected to μ CT using a Scanco μ CT35 instrument (Scanco AG, Brütisellen, Switzerland). Specimens were placed in a standardized position and held in place with clear plastic wrap. Fish were scanned at a resolution of 20 μ m (70 kVp, 160 μ A, 500 projections). Raw scans were reconstructed into three-dimensional volumes, and these data were imported into Amira 5.4 (FEI Visualization Sciences Group, Burlington, MA, USA). A set of 30 bilateral 3D landmarks in the anterior skull (Fig. 1), encompassing known locations that are important for suction feeding, were collected and exported for geometric morphometric analyses.

Raw landmarks were corrected for scale, rotation and translation using a Procrustes superimposition (Dryden & Mardia, 1998). These corrected data were then subjected to Procrustes ANOVA to assess



Figure 1. μ CT image of a three-spined stickleback showing the digitized landmarks in the anterior region of the skull.

the effect of allometry on shape, where size was represented by the centroid size of each landmark configuration. Note that this technique, as implemented here, is robust to the challenges of high-dimensional data (Collyer, Sekora & Adams, 2015). This analysis revealed the presence of significant allometry in the data set (see Results for details), and that this relationship was similar among all populations examined. We therefore produced an allometry-free shape data set consisting of regression residuals with the consensus shape added back in for each individual. These data were then subjected to further shape analysis.

Procrustes ANOVA was used on the allometry-free data to test the hypothesis that shape varied between populations (Collyer *et al.*, 2015). PCA was used to visualize the major axes of shape variation in these populations, and deformation grids were produced using thin-plate splines.

All geometric morphometric analyses were performed in R version 3.3.1 (R Core Team, 2016), using the geomorph package version 3.0.3 (Adams & Otárola-Castillo, 2013).

RESULTS

KINEMATICS

Body size did not differ between the marine (50.2 ± 1.4 mm) and freshwater (47.9 ± 2.2 mm) stickleback (Fig. 2). Marine and freshwater fishes differed significantly in feeding kinematics, as indicated by significant differences in PC1 scores (Fig. 3). This was true for both the scaled variables and the raw variables, although only the former were used in analyses. Variables with strong loading (> 0.50 ; Table 1) included MG (freshwater: 3.5 ± 0.5 mm, marine: 5.3 ± 0.3 mm), JP (freshwater: 0.8 ± 0.05 mm, marine: 1.5 ± 0.11 mm), HD (freshwater: 1.2 ± 0.11 mm, marine: 1.5 ± 0.09 mm), ram speed (freshwater: 7.3 ± 0.9 cm/s, marine: 14.0 ± 1.5 cm/s), CR (freshwater: $7.8 \pm 0.8^\circ$, marine: $12.4 \pm 0.8^\circ$) and speed of CR (freshwater: $476.3 \pm 61.9^\circ/\text{s}$, marine: $652.0 \pm 46.7^\circ/\text{s}$). The timing of CR relative to MG (freshwater: $+7.1 \pm 0.5$ ms, marine: $+9.3 \pm 0.5$ ms) loaded somewhat strongly on PC1 as well (Table 1). Both marine and freshwater stickleback accelerated immediately prior to, and during, prey capture and then decelerated, using pectoral fin abduction, immediately following prey capture (Fig. 4). The estimated IVW of marine stickleback (19.6 ± 1.4 cm³) was significantly greater than that of freshwater stickleback (ANOVA, $P < 0.05$; 9.6 ± 0.8 cm³) (Fig. 2).

MORPHOLOGY

Morphology differed among the populations (e.g. Fig. 5), but not in the same way as function (Fig. 6). Size had a significant effect on shape ($F = 3.313$,

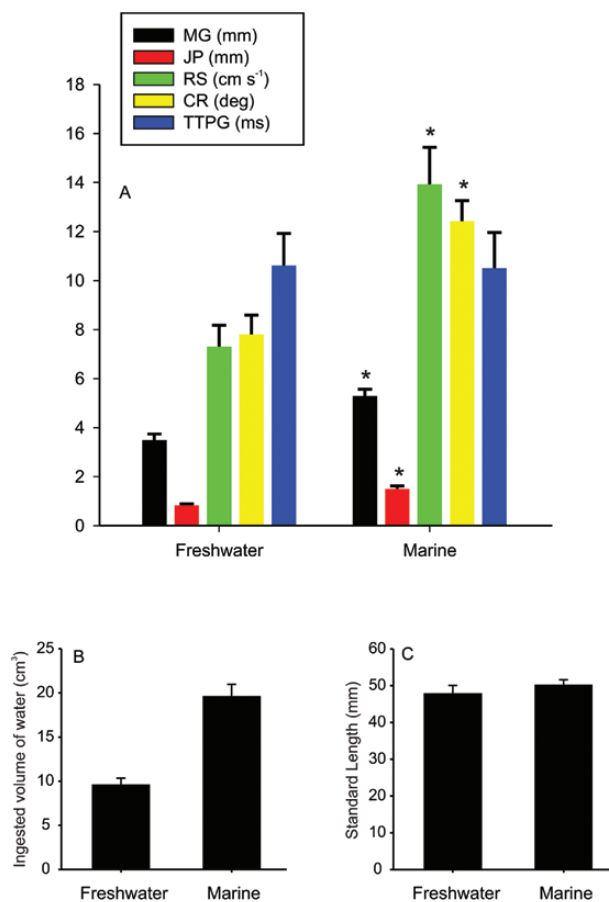


Figure 2. Mean values of several kinematics variables from marine and freshwater stickleback (A), where asterisks indicate that variable loaded strongly on the first PC axis of the kinematic PCA. This axis differentiated the marine and freshwater habitats, indicating that these variables are significantly different between the fish from the different habitat types. The y-axis encompasses multiple variables with different units (indicated in the legend). MG, maximum gape; JP, jaw protrusion; RS, ram speed; CR, cranial rotation; TTPG, time to peak gape. (B) The average estimated ingested IVW during suction feeding in marine and freshwater habitats. (C) Average body length of the marine and freshwater stickleback. Length did not differ significantly between habitats.

$Z = 2.859$, $P = 0.001$), and the relationship was similar for all groups examined. The marine and freshwater habitats differed significantly from each other in shape once the effects of allometry were removed from the data sets ($F = 2.294$, $Z = 2.037$, $P = 0.009$). Populations within the freshwater ($F = 2.589$, $Z = 2.022$, $P = 0.009$) and marine ($F = 2.438$, $Z = 1.906$, $P = 0.002$) habitats also differed from one another.

The first principal component (PC1; 21% of variance explained) separated the Little Campbell and Black Lake populations from the other three groups. These individuals, at the positive end of PC1, exhibit a deeper

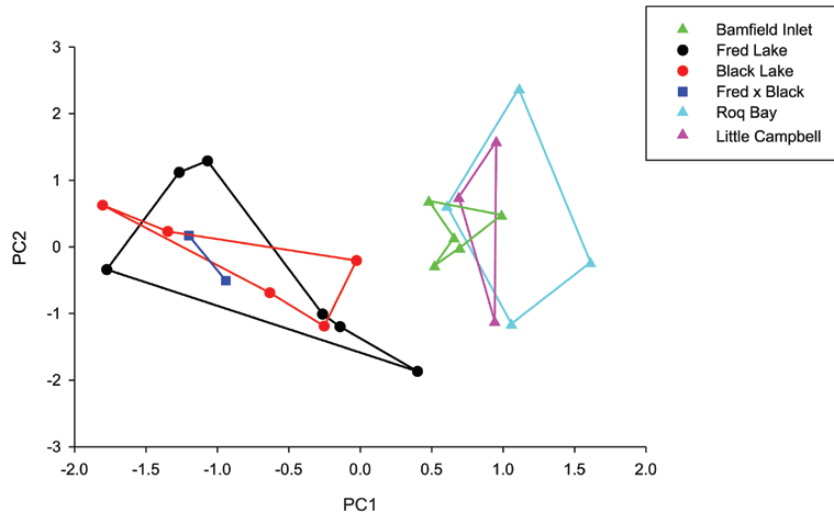


Figure 3. The results of principal components analyses on the kinematic data. The freshwater populations (red and black circles) and the freshwater hybrids (blue squares) are on the left, whereas the marine populations (triangles) are on the right. Note that the PC scores for marine and freshwater fish differed significantly along PC1, but not PC2.

Table 1. Component loadings for the PCA of kinematic variables

Variable	PC1	PC2
Maximum gape (MG)	0.850	-0.223
Hyoid timing	-0.090	-0.568
Jaw protrusion (JP)	0.636	0.006
Time of prey capture	-0.449	0.394
Cranial elevation timing	0.450	-0.496
Hyoid depression (HD)	0.533	0.517
Ram speed (RS)	0.611	-0.218
Time to peak gape (TTPG)	-0.028	-0.672
Cranial rotation (CR)	0.867	0.014
Velocity of cranial elevation	0.668	0.574

PC1 and PC2 explained 34.4 and 18.7% of the variation, respectively. Values in bold highlight numbers with an absolute value above 0.5.

and broader skull, elongated jaws and a larger orbit. The second principal component (PC2; 18% of variance explained) separated the Little Campbell population from the other four groups. These individuals, at the positive end of PC2, exhibit a broad anterior skull and a deeper and more rounded neurocranium. Finally, none of the morphological PC axes were significantly correlated with the kinematics PC axes.

DISCUSSION

Our study quantified and linked the kinematic and morphological differences between populations of marine and freshwater stickleback. Overall, the main axis of kinematic divergence is between habitat

types, and not among populations of a single habitat. Morphometric divergence was driven by one marine population, one freshwater population and a cluster of individuals from the remaining populations. Given that this did not parallel the kinematics differences, there may be a mismatch between functional and morphological disparity. Our novel results not only provide insight into differing ecological demands between marine and freshwater fishes but also highlight the extreme differences that can occur within a single species that occupies different habitats.

Marine habitats differ considerably from freshwater habitats in a number of ways, such as flow conditions, temperature, salinity, density, visibility, predation regime and prey availability. These differences have likely driven parallel shifts in morphology (Klepaker, 1993; Nosil & Reimchen, 2005; Rogers *et al.*, 2012; Jamniczky *et al.*, 2015), physiology (Barrett *et al.*, 2011; Dalziel, Ou & Schulte, 2012a; Dalziel, Vines & Schulte, 2012b) and behaviour (Di-Poi *et al.*, 2014; Greenwood *et al.*, 2015). For suction feeding, likely ecological drivers of the differences in our study are the difference in flow and diet. The former can influence predatory behaviour by potentially requiring faster speeds of attack to avoid the negative impacts of high flow (Higham, Stewart & Wainwright, 2015). The latter can induce a number of differences in attack and suction variables among fishes (Nyberg, 1971; Coughlin & Strickler, 1990; Norton, 1991; Norton & Brainerd, 1993).

The performance of marine stickleback was generally greater than freshwater stickleback, including higher ram speeds, a larger gape, increased cranial elevation and more jaw protrusion (Fig. 2). There was not, however, a difference in TTPG between the habitat types

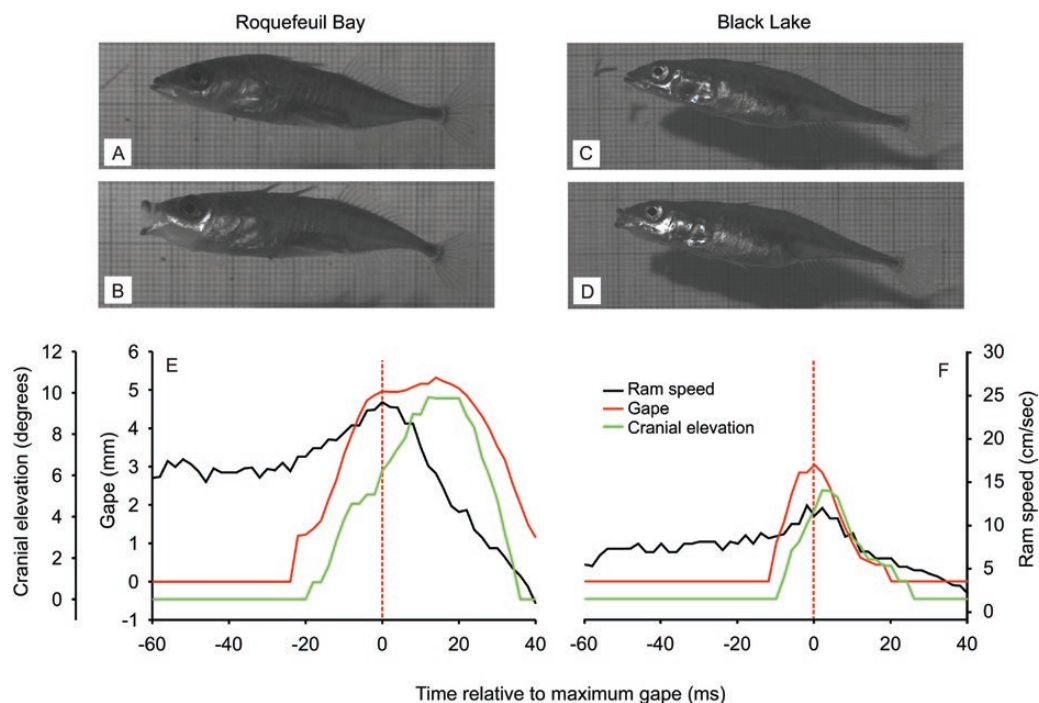


Figure 4. Example sequence for a marine (A, B, E) and freshwater (C, D, F) stickleback. The photos above the plot show the fish immediately prior to opening their mouths (A, C) and the point of maximum gape (B, D). The plots (E, F) show gape (red), cranial elevation (green) and ram speed (black) relative to maximum gape (vertical red line).

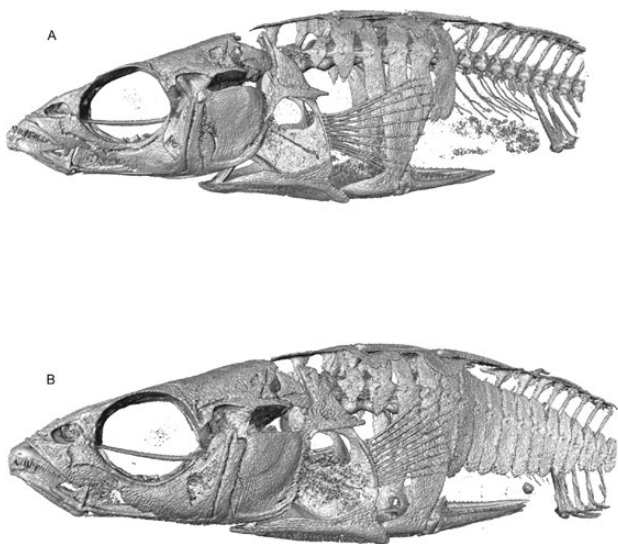


Figure 5. Representative μ CT scans for freshwater (Frederick Lake, A) and marine (Bamfield Inlet, B) stickleback.

(Fig. 2). The increased gape and ram speed, despite no difference in TTPG, resulted in an estimated IVW of approximately double in marine stickleback. Unlike in other studies that find an inverse relationship between ram speed and TTPG, we found no link (Norton &

Brainerd, 1993; Wainwright *et al.*, 2001). Along a continuum from suction to ram, fishes that specialize for suction tend to exhibit shorter TTPG (Norton, 1991; Higham *et al.*, 2006a). This will dramatically increase the subambient buccal pressure, resulting in higher velocities of water entering the mouth (Higham, Day & Wainwright, 2006b; Day, Higham & Wainwright, 2007; Wainwright *et al.*, 2007) and greater accelerations of the fluid (Holzman *et al.*, 2008a). However, it appears that marine stickleback have maximized ram speed while maintaining a comparable TTPG, suggesting that their particular strategy relies on both high fluid speeds and fast swimming.

Jaw protrusion is a mechanism for increasing the forces on prey by moving the source of the flow closer to the prey (Holzman *et al.*, 2008b). Marine stickleback exhibit greater jaw protrusion, after accounting for body size, suggesting that they benefit from elevated acceleration. Thus, we expect the acceleration of the fluid, and the forces exerted on prey, to be higher in marine stickleback. In addition, having increased jaw protrusion will effectively increase the ram speed of the stickleback (movement of the jaws relative to the prey), which has been identified as an adaptation for capturing evasive prey in Neotropical cichlids (Waltzek & Wainwright, 2003). In addition, the reduced jaw protrusion, and therefore reduced excursion of the premaxilla, of freshwater stickleback could enhance the

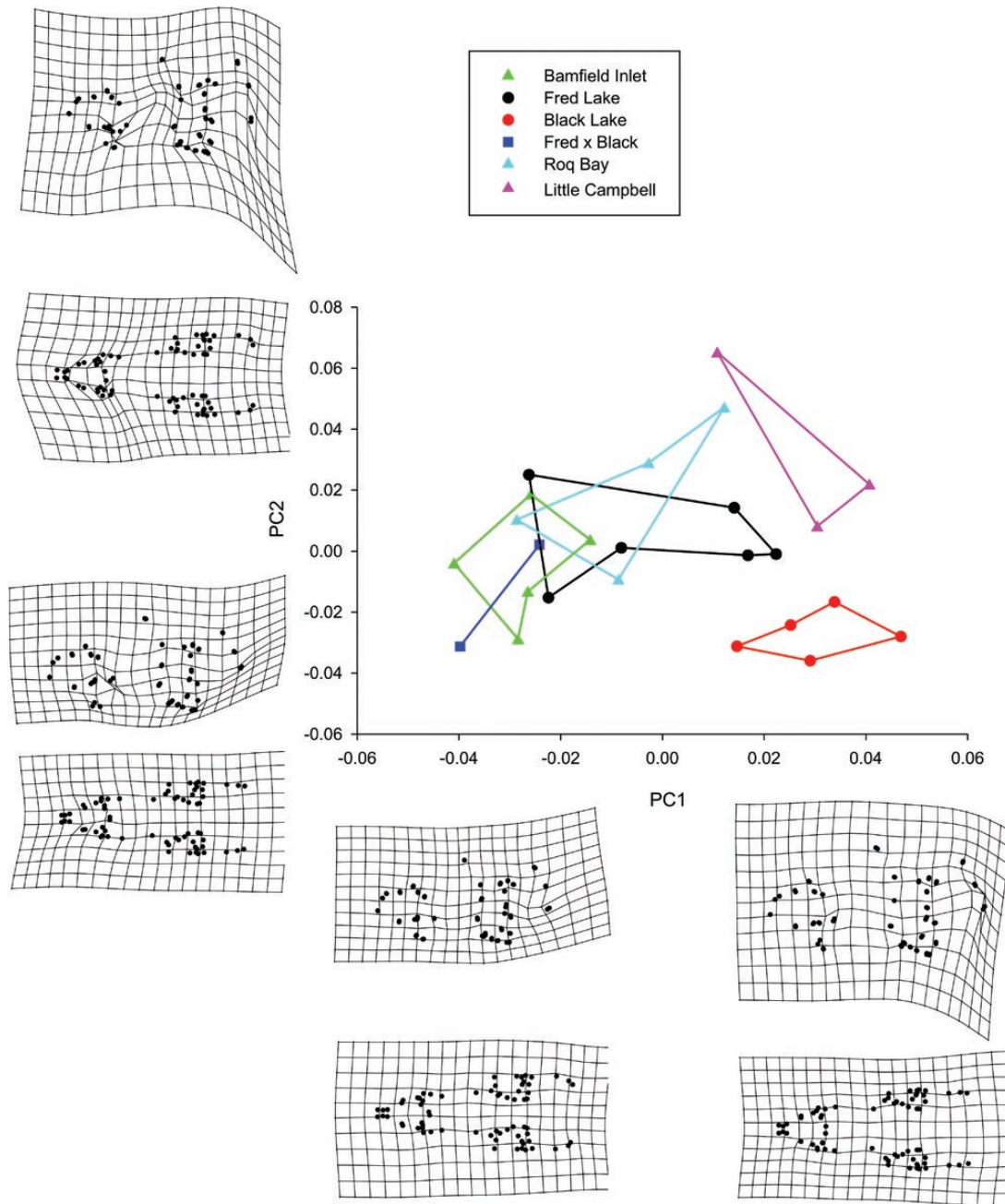


Figure 6. The results of principal components analyses on the morphometric data. Below the morphometric PCA plot are deformation grids showing the size-corrected shifts in shape in both left lateral (top grids) and ventral (bottom grids) views. Shapes differed significantly between habitats and between populations.

ability to manipulate and bite prey using the oral jaws, something that might be important for benthic-feeding stickleback (McGee & Wainwright, 2013b).

Marine stickleback exhibit a greater magnitude and angular velocity of cranial elevation. Higher cranial elevation without a concomitant decrease in TTPG is surprising given that short TTPG and increased CR are often linked, as both will contribute to a rapid change

in buccal volume in order to maximize water velocity entering the mouth (Van Wassenbergh *et al.*, 2015). For example, in a study of 14 species of teleost fishes, a PCA found that reduced TTPG and high CR loaded strongly together (Gibb & Ferry-Graham, 2005). In contrast, recent work has highlighted a positive correlation between cranial movements, such as cranial elevation, and ram speed in serranid fishes (Oufiero *et al.*, 2012).

This is thought to contribute to an increased buccal volume, which would be beneficial for a fast-swimming predator (see below, for more information).

We estimated the ingested IVW for stickleback using empirical measurements from centrarchid fishes (Higham *et al.*, 2006a; Kane & Higham, 2014). Marine stickleback appear to ingest almost twice the IVW compared to freshwater stickleback, again suggesting an evasive prey type that needs to be better characterized in nature. Although the exact values may not be accurate given the potential for variation in internal buccal dimensions, the relative differences among stickleback are not likely far off. Future work should quantify internal mouth shape and use fluid visualization to quantify fluid speeds and volumes during prey capture under a common garden framework, as well as characterizing diet differences between environments. Although we lack measurements of buccal pressure or fluid speed (from particle image velocimetry), we predict that marine stickleback will exhibit greater fluid speeds and acceleration when capturing prey.

Body ram is a key aspect of attack strategies among fishes (Rand & Lauder, 1981; Webb, 1984a, b; Domenici, 2001; Wainwright *et al.*, 2001; Higham, 2007a; Higham *et al.*, 2007; Kane & Higham, 2015), and is likely the main axis of divergence across feeding niches on evolutionary time scales (Longo *et al.*, 2016). In addition to the marine stickleback exhibiting higher ram speeds, we found that they have larger relative maximum gape distances, something commonly observed in other species (Higham, 2007b; Higham *et al.*, 2007; Oufiero *et al.*, 2012; Kane & Higham, 2015). The general idea is that faster-swimming speeds during prey capture may reduce the ability to accurately position the mouth relative to the prey item. This means that a larger gape and ingested IVW will benefit a fast-swimming predator by reducing the need for accuracy. This is corroborated by a parallel increase in cranial elevation, which will also contribute to an increased volume of ingested water. In contrast, a smaller mouth, as observed for the freshwater stickleback, may reflect a greater reliance on suction performance for capturing non-evasive prey (Norton, 1991; Wainwright *et al.*, 2007). Given the abundance of information regarding other species, we predict that marine stickleback will capture more evasive prey by employing an attack strategy that matches other evasive prey specialists (Nyberg, 1971; Norton, 1991) and generalist predators when feeding on evasive prey (Nemeth, 1997). If this is the case, future work should assess the ability of freshwater and marine fish to modulate their attack behaviour in response to prey type.

We observed a mismatch between changes in kinematics and morphology, in that the main kinematic, but not morphological, divergence was between freshwater and marine habitats. The source of this

mismatch is not known, but could emerge from a number of factors that require further investigation. First, the divergent attack kinematics observed between the marine and freshwater fishes might be driven primarily by behavioural modulation, rather than requiring a morphological shift. This was observed in a recent study of serranid fishes in which maximum gape differed among species during suction feeding, but did not differ anatomically (Oufiero *et al.*, 2012). Additionally, much of the variation is in ram speed during the attack, which means that a morphological difference might be found in the locomotor system. Finally, our experimental design could be improved by increasing the sample size of each population, and by looking at F2 individuals, when recombination occurs. Our current study incorporates a mixture of wild and F1 fish.

There are several cases where morphology and function are not directly linked (Koehl, 1996; Higham, Gamble & Russell, 2017). This could result from many-to-one mapping of form to function, in which several morphological or physiological solutions can result in comparable function (Wainwright *et al.*, 2005). The freshwater fish may be reaching their specific level of performance via multiple pathways that are not fully understood at this point. Finally, it may be possible that we are not capturing the aspect of shape that is most important for suction feeding. Regardless, it is clear that morphology differs strongly among stickleback populations, and future work should focus on quantifying differences in both cranial and postcranial features that directly or indirectly affect feeding performance. Such work will require larger sample sizes, which will help to better delimit subtle morphological differences that may be influencing function.

Previous studies have examined the kinematics of anadromous stickleback from Cheney Outlet in Bodega Bay, CA, USA (McGee & Wainwright, 2013b). Our values of cranial rotation, jaw protrusion, time to peak gape, maximum gape and hyoid depression all fell within the values reported for these anadromous stickleback. Ram speed was not reported, so it is unclear how this compares. Finally, live cladocerans (*Daphnia magna*) were used by McGee & Wainwright (2013b), whereas we used chironomid worms. This did not seem to impact performance given the overlap in TTPG. An additional study examined the kinematics of feeding in limnetic and benthic stickleback from Paxton Lake, Texada Island, BC, Canada (McGee *et al.*, 2013), although the size of those individuals were smaller than ours based on the values of maximum gape reported (~2.85 mm) compared to ours (3.49–5.29 mm). Despite this, the values of cranial rotation of the freshwater stickleback in our study (7.8°) closely matched the stickleback (all freshwater) in their study (7.12 and 8.72°). The marine stickleback in our study (12.4°) greatly exceeded those values.

We found dramatic differences among populations of three-spined stickleback, especially between marine and freshwater habitats. Future work should identify the main ecological variables that might be driving these differences, whether it is the prey type, predation pressure or abiotic factors in the environment. Regardless, we highlight that a single species can exhibit considerable variation and that ecological factors must be considered when asking general questions about a specific species. It is also important to determine the biomechanical consequences of integrated phenotypes when testing hypotheses about speciation. Three-spined stickleback, in particular, are a model system for examining such questions given their repeated isolation in freshwater environments (Bell & Foster, 1994), and their ability to rapidly diverge following this isolation (Klepaker, 1993; Kristjansson, Skulason & Noakes, 2002).

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