

# Interactions among multiple selective pressures on the form–function relationship in insular stream fishes

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Relationships between body shape and escape performance are well established for many species. However, organisms can face multiple selection pressures that might impose competing demands. Many fishes use fast starts for escaping predator attacks, whereas some species of gobiid fishes have evolved the ability to climb waterfalls out of predator-dense habitats. The ancestral ‘powerburst’ climbing mechanism uses lateral body undulations to move up waterfalls, whereas a derived ‘inching’ mechanism uses rectilinear locomotion. We examined whether fast-start performance is impacted by selection imposed from the new functional demands of climbing. We predicted that non-climbing species would show morphology and fast-start performance that facilitate predator evasion, because these fish live consistently with predators and are not constrained by the demands of climbing. We also predicted that, by using lateral undulations, powerburst climbers would show escape performance superior to that of inchers. We compared fast starts and body shape across six goby species. As predicted, non-climbing fish exhibited distinct morphology and responded more frequently to an attack stimulus than climbing species. Contrary to our predictions, we found no differences in escape performance among climbing styles. These results indicate that selection for a competing pressure need not limit the ability of prey to escape predator attacks.

ADDITIONAL KEYWORDS: biomechanics – ecomorphology – escape response – geometric morphometrics – locomotion.

## INTRODUCTION

Form can aid or hinder organismal function in different contexts and environments (Koehl, 1996;

Patek *et al.*, 2007; Tytell *et al.*, 2010; Kane & Higham, 2015; Bellwood *et al.*, 2017). When faced with similar selection pressures, organisms with differing morphology can produce equivalent performance, a concept referred to as many-to-one mapping (Alfaro *et al.*, 2005; Wainwright *et al.*, 2005; Wainwright, 2007; Moody *et al.*, 2017). In other circumstances, strong selection on performance can lead to predictable associations of particular morphologies (Blob *et al.*, 2010; Kawano *et al.*, 2013). Such associations can,

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however, change through time as new functions evolve with competing demands (Walker, 2007; Blob *et al.*, 2020). In this study, we examine how form–function relationships are impacted when new locomotor functions evolve, using waterfall climbing in gobiid fishes as a model system.

Many environmental links between form and function are tied to obtaining resources or avoiding becoming a resource for other organisms. In prey species, the anti-predator strategies that are used will depend on (1) the form–function relationships of the prey itself, (2) the form–function relationships of predators, and (3) the environmental context (Abrahams *et al.*, 2007; Ingleby *et al.*, 2016). Owing to their stereotypical attack and escape behaviours, fishes have become a model system for studying how environmental factors influence predator–prey interactions (Abrahams *et al.*, 2007; Kane & Higham, 2015; Domenici & Hale, 2019). Fish fast-start escape responses, in particular, have been well characterized, with lateral bending that allows fish to swim away quickly from a predator attack (Domenici & Blake, 1997; Hale, 1999; Domenici & Hale, 2019). However, features that promote performance of this behaviour can be detrimental to the performance of other functions. For example, fish that cohabit with predators often possess deeper bodies that enable greater thrust production during escape, but these morphologies can impede actions such as swimming ability and manoeuvrability in other contexts (Brönmark & Miner, 1992; Domenici *et al.*, 2008). Thus, the emergence of new selection pressures could upset existing form–function linkages.

A striking system, in which multiple selection pressures have been implicated as drivers of form–function relationships, is composed of the amphidromous goby fishes. In these species, adults live in the freshwater streams of many circumtropical oceanic islands (Kinzie, 1988; Watanabe *et al.*, 2014). As part of their life cycle, larvae are swept downstream to the ocean, and juveniles migrate back to freshwater habitats that are often punctuated by waterfalls (Radtke *et al.*, 1988). Before reaching the first waterfall, all species of amphidromous goby encounter picivorous predators (Schoenfuss & Blob, 2007; Maie *et al.*, 2014). Some goby species can climb waterfalls and, thereby, reduce pressure from predation (Fitzsimons *et al.*, 2002). Gobies use one of two methods to climb waterfalls. The ancestral form of climbing is termed ‘powerburst’ and uses a suction disc, formed from fused pelvic fins, to attach to the substrate between bursts of lateral undulation that propel the fish up the waterfall (Schoenfuss & Blob, 2003; Blob *et al.*, 2019). A second, derived form of climbing is found in the genus *Sicyopterus* and is termed ‘inch’ (Schoenfuss & Blob, 2003; Cullen *et al.*, 2013; Blob *et al.*, 2019). In this style, fish also use their mouths as a point of attachment and inch up waterfalls by alternately attaching and moving their mouths and suction discs up the substrate

(Schoenfuss & Blob, 2003). Studies of juvenile *Sicyopterus stimpsoni* from Hawai‘i have measured the selection imposed on morphology by both predation and climbing and found that fish that survived predation trials had deeper bodies than control individuals, whereas individuals that climbed successfully had more streamlined body shapes (Blob *et al.*, 2008, 2010). These patterns are reflected in collections of adult individuals; for example, despite high gene flow among the Hawaiian islands, adult fish that had the opportunity to climb typically have larger heads and pelvic suckers than fish that did not have the opportunity to climb (Moody *et al.*, 2015). These studies provide a context in which comparisons of predator escape performance can be conducted across taxa to test for potential impacts on performance resulting from the acquisition of new functions, such as different styles of climbing.

In this study, we test for consequences of selection for waterfall climbing on anti-predator performance in gobiid fish species from the islands of Hawai‘i and La Réunion. We predict that non-climbing species will have the best escape performance, because they live consistently with predators and are not constrained in their morphology, behaviour or performance by potentially countervailing demands imposed by waterfall climbing. Given that the ancestral powerburst style of climbing uses lateral bending similar to that used in fast-start escape responses, we further predict that fishes using powerburst climbing will respond more frequently and have better escape performance compared with species that use the derived inching form of climbing. To test these predictions, we compare measurements of escape behaviour (response frequency), performance (escape angle, peak velocity and peak acceleration), and morphology (geometric morphometrics of body shape) among non-climbing, powerburst climbing and inching stream goby species.

## MATERIAL AND METHODS

### SPECIMEN COLLECTION

In March 2015–2017, adult gobies were collected on the Island of Hawai‘i from five localities (details in Supporting Information, Table S1). We collected individuals of the non-climbing amphidromous goby *Stenogobius hawaiiensis* from localities below or without waterfalls. All three climbing species, *Awaous stamineus* [powerburst climber (PB)], *Lentipes concolor* (PB) and *Sicyopterus stimpsoni* [inching climber (IN)] were collected from localities above waterfalls to ensure that we obtained measurements from individuals that had successfully evaded predators and climbed waterfalls. We also collected *A. stamineus* and *Sicyopterus stimpsoni* individuals from localities where they either had not climbed waterfalls or where were no waterfalls were present, in order to compare

populations that had and had not climbed within a species. We were not able to find any *L. concolor* below waterfalls or in localities that did not have a waterfall to climb. In April 2016, adult *Cotylopus acutipinnis* (PB) and *Sicyopterus lagocephalus* (IN) were collected on La Réunion from upstream reaches (Supporting Information, Table S1). In accordance with local collection permits, fish were caught with dip nets in Hawai'i and by electrofishing in La Réunion. Fish were housed in aerated stream water overnight to minimize effects of collection (Mitton & McDonald, 1994), and trials were conducted within 48 h of capture.

#### DATA COLLECTION

Escape trials ( $N = 282$ ) were conducted across the six goby species (*Stenogobius hawaiiensis*,  $N = 64$ ; *A. stamineus*,  $N = 40$ ; *L. concolor*,  $N = 23$ ; *Sicyopterus stimpsoni*,  $N = 118$ ; *C. acutipinnis*,  $N = 114$ ; *Sicyopterus lagocephalus*,  $N = 116$ ) using previously published protocols (Diamond *et al.*, 2019). Trials were filmed with a high-speed video camera (Fastec Highspec 2G, 1000 Hz) in ventral view and still water, using a jet pulse stimulus (Diamond *et al.*, 2016, 2019). We aimed to collect 20 escape response trials from each of our three general stimulus directions relative to the initial position of the fish (cranial, lateral and caudal) for a total of 60 escape response trials per species (and each location for *A. stamineus* and *Sicyopterus stimpsoni*). Categorical stimulus directions were separated by  $\geq 10^\circ$  (Supporting Information, Table S2). Owing to permit limits on the number of fishes we were allowed to collect, adults from *A. stamineus*, *L. concolor* and *Sicyopterus stimpsoni* have fewer trials. Only one trial (from the cranial, lateral or caudal direction, selected at random) was conducted per individual. After escape response trials, fish were lightly anaesthetized, photographed in lateral view, and released at their site of capture.

From videos, we first classified trial behaviour as either 'responding' or 'not responding' to stimuli (Diamond *et al.*, 2016, 2019). For all trials that elicited escape responses, the position of the stimulus and 15 points along the midline of each fish were digitized using DLTDATAVIEWER (Hedrick, 2008). The relative position of the centre of mass for each species was calculated using a hang test (Webb, 1976), and a quintic spline interpolation of the 15 points was used to estimate the coordinates of this relative position in each frame. The smoothed position, velocity and acceleration of the centre of mass were calculated using a quintic spline following previously published methods (Diamond *et al.*, 2016, 2019). We used geometric morphometrics to examine differences in body shape among species. For each species, ten

photographs of individuals that were all angled neutrally relative to the substrate were selected, and the same researcher digitized 12 landmarks on the left lateral view of the fish (Supporting Information, Fig. S1) using the program TPSDIG2 (Rohlf, 2006).

#### STATISTICAL ANALYSES

All analyses were run using R (v.3.6.1; R Core Team, 2019). To compare the probability of response among populations (the interaction between species and location) and climbing styles, we used a generalized linear mixed effects model with binomial family and logistic link, with climbing style and stimulus direction as fixed effects and species and location as random effects, using the lme4 package (Bates *et al.*, 2019). To compare escape performance measures (escape angle, maximum velocity and acceleration) among climbing styles, we used linear mixed effects models, with climbing style and stimulus direction as fixed effects and species and location as random effects, using the lme4 package (Bates *et al.*, 2019; Supporting Information, Tables S6, S7). We visualized the effects of these models using Harrell plots. We also performed pairwise comparisons between each climbing style for behavior and performance variables (Supporting Information, Table S3). For the two Hawaiian species in which we were able to collect individuals above and below waterfalls (*Sicyopterus stimpsoni* and *A. guamensis*), we also conducted ANOVAs to look for differences in each response variable (response frequency, escape angle, peak velocity and peak acceleration) among localities within each species. Full descriptive statistics can be found in Supporting Information, Table S4.

For morphometric data, we first ran a generalized Procrustes analysis using the gpaen function of geomorph (Adams & Otárola-Castillo, 2013) to rotate and translate data and to scale data to a unit centroid size. We performed a Procrustes ANOVA to test whether aligned coordinates from the generalized Procrustes analysis varied among climbing styles, using the procD.lm function from geomorph (Adams & Otárola-Castillo, 2013). To explore further how shape varies among climbing styles, we performed a principal components analysis on aligned coordinates using the gm.prcomp function in geomorph (Adams & Otárola-Castillo, 2013). We used the broken stick method to choose which principal components (PCs) to retain for analyses (Frontier, 1976). For PCs above the threshold, we performed Procrustes MANOVA, with climbing style, location and species as response variables, using the procD.lm function from geomorph (Adams & Otárola-Castillo, 2013). For variables that differed significantly, we also performed Tukey's HSD *post hoc* tests (Supporting Information, Table S5).

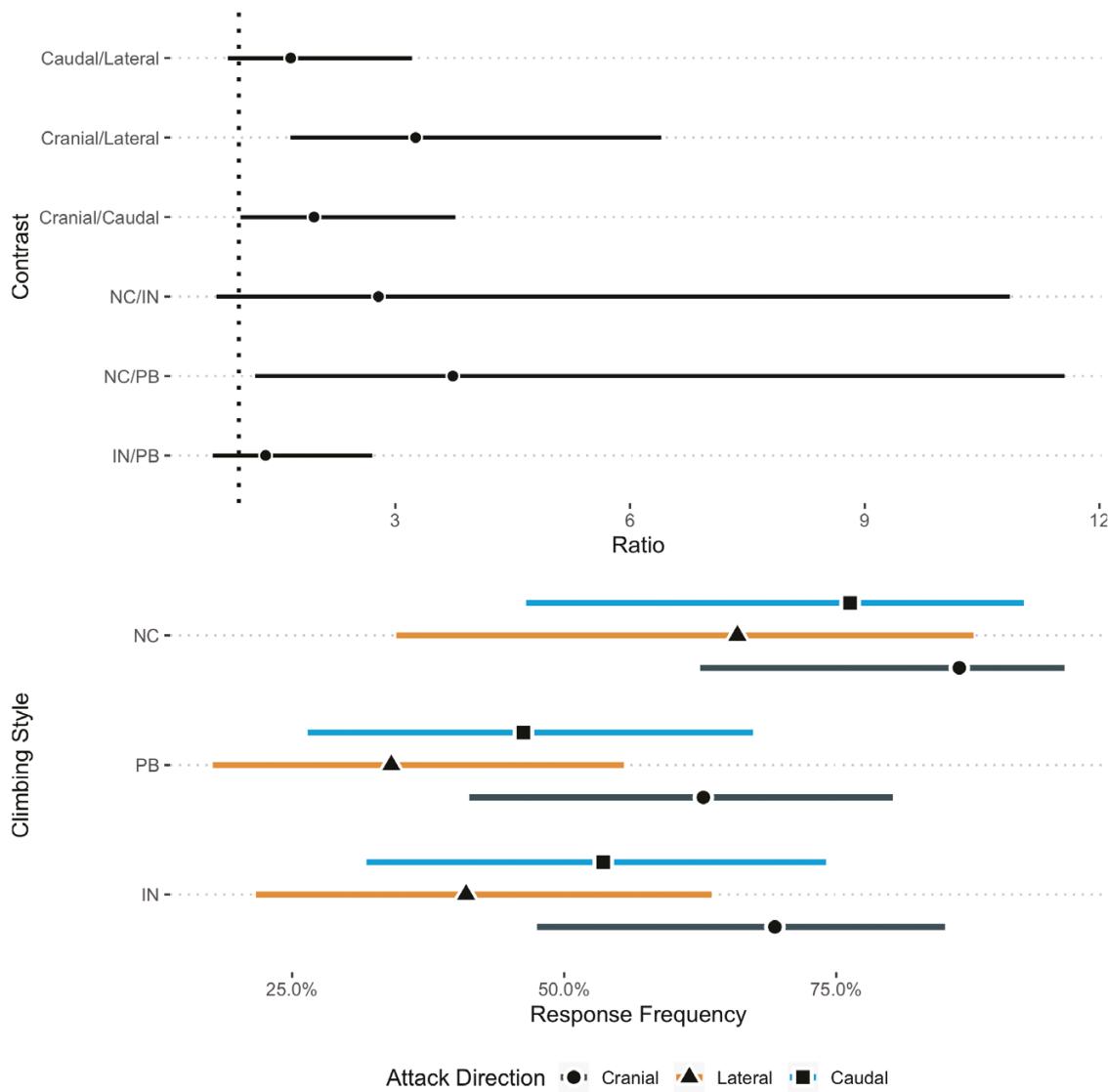
## RESULTS AND DISCUSSION

Our results suggest that selection for climbing and predation can influence patterns of morphological divergence between goby species. By exploring all three components of kinematic escape responses across species exposed to different levels of predation pressure, we are able to demonstrate how individuals might compensate behaviourally for the 'mismatch' of form and function for escape performance caused

by the selection on form imposed by more recent waterfall-climbing pressures.

## BEHAVIOUR

Overall, we found that response frequency was similar among climbing styles [ $\chi^2(2) = 4.928, P = 0.085$ ; Fig. 1]. However, gobies that are unable to climb (*Stenogobius hawaiiensis*) responded on average 3.7 times more frequently than the powerburst climbers



**Figure 1.** Harrell plot of response frequency among climbing styles and stimulus directions. The upper portion of the graph shows the odds ratios of the different stimulus directions and climbing styles (IN, inching; NC, non-climbing; PB, powerburst), with a vertical dotted line indicating an effect size of one. Bars in the upper portion of the graph are the 95% confidence intervals of the odds ratio. The lower portion of the plot shows the estimated marginal mean and the 95% confidence interval for this value based on the generalized linear mixed effects model: response frequency ~ climbing style + stimulus direction + (1 | species) + (1 | collection location). Colours and shapes represent different stimulus directions: black+circle, cranial; orange+triangle, lateral; blue+square, caudal.

[95% confidence interval (CI): 1.21, 11.55; Fig. 1]. *Stenogobius hawaiiensis* also responded 2.8 times more frequently than the inching climbers (95% CI: 0.71, 10.86; Fig. 1), although the 95% CI for this comparison overlapped with one, implying non-significance. These patterns were generally consistent with our predictions. We expected fish that cannot climb to respond more frequently because they must evade predator attacks consistently, whereas climbing either reduces or eliminates the threat of predation in climbing species.

Regardless of climbing style, response frequency differed among the stimulus directions in our study [ $\chi^2(2) = 12.479, P = 0.002$ ; Fig. 1]. Across all species, gobies stimulated from the cranial direction responded 3.3 times more frequently (95% CI: 1.66, 6.40) than fish stimulated from the lateral direction and 2.0 times more frequently (95% CI: 1.02, 3.77) when stimulated from the caudal direction. Gobies also responded slightly more frequently when stimulated from the caudal direction than from the lateral direction (odds ratio = 1.7; 95% CI: 0.86, 3.21), although the CI for this comparison implied statistical non-significance. These differences across stimulus directions were unexpected, because other picivorous fish predators attack prey more frequently from the lateral direction to maximize the surface area of the target that is available for attack (Webb, 1980). The stimulus used in the present study represented only the mechanosensory aspect of predator attacks, and these prey fish might receive input from visual cues when attacked by predators *in situ*. However, a recent *in situ* study of Hawaiian stream fishes found that predators in the Hawaiian gobioid system are more successful at capturing juvenile gobies when they attack prey from either a lateral or a caudal direction (Schneider *et al.*, 2021). This result might be facilitated by the lower frequency of escape responses from lateral stimuli or could be because prey items are larger targets when approached laterally.

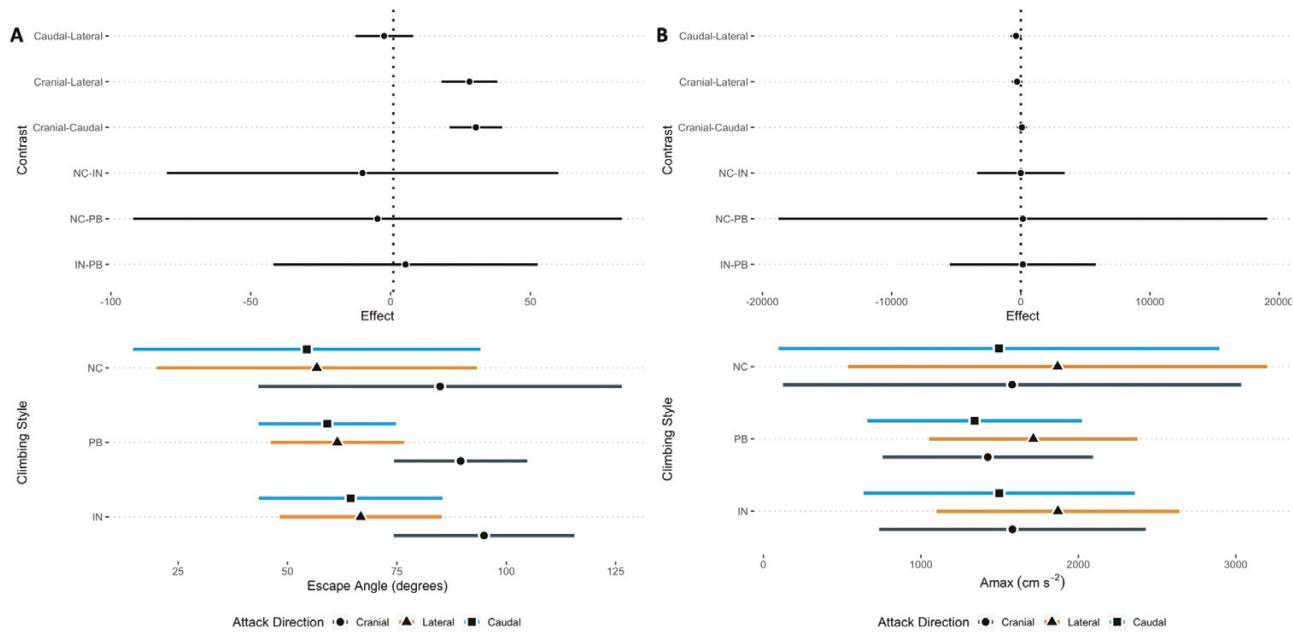
We were able to collect individuals from above and below waterfalls for two species, one inching climber (*Sicyopterus stimpsoni*) and one powerburst climber (*Awaous stamineus*). For the powerburst climber, individuals from below the waterfall responded less frequently on average [below (mean  $\pm$  SE), 55  $\pm$  11.1%; above, 80  $\pm$  8.9%] than individuals from above the waterfall [ $F(1,38) = 2.914, P = 0.096$ ; Supporting Information, Fig. S2]. In addition, for our inching climber, individuals below the waterfalls had the lowest average response frequency (50.0  $\pm$  11.2%), gobies above the first waterfall had the highest average response frequency (69.6  $\pm$  9.6%), and gobies above the second waterfall had an intermediate average response frequency (55.6  $\pm$  16.6%). These elevation patterns across inching *Sicyopterus stimpsoni* were

not clearly distinct [ $F(2,49) = 0.866, P = 0.427$ ; Supporting Information, Fig. S2], but did parallel previous comparisons of features related to climbing, which indicated that individuals from intermediate elevations, rather than the highest elevations, might have superior performance (Schoenfuss *et al.*, 2013). Although our sample sizes were relatively low and we did not find clear statistical differences among the locations we compared, for both species the lowest average response frequency was found in fish at the lowest elevation, below the waterfall. Given that populations of these species above waterfalls no longer face predators, we had expected them to respond less frequently than individuals from populations below waterfalls that overlap with predators. Although we tested adult gobies to minimize the effect of current predator-induced selection, it is possible that individuals above waterfalls were already selected positively for a higher response rate that might have contributed to their success in reaching upstream habitats, whereas gobies below waterfalls are still undergoing this selection process. If this is the case, we could envision climbing as a selection pressure that separates superior athletes (with higher overall performance for climbing and escape) from gobies that do not climb. Alternatively, individuals of climbing species that live below waterfalls might use an alternative predator-avoidance strategy, such as avoidance of detection by hiding or blending in with their surroundings, instead of using kinematic escape responses.

## PERFORMANCE

Owing to their range overlap with predators, we predicted that non-climbing gobies would exhibit escape angles more opposite to the direction of stimulation and have higher escape velocity and acceleration than climbing gobies. We also predicted that, owing to the lateral body movements involved in their climbing style, powerburst climbers would have greater escape performance than inching climbers. We found no significant relationships between climbing style and escape angle [ $\chi^2(2) = 3.133, P = 0.209$ ; Fig. 2A]. However, we did find differences in escape angle among stimulus directions [ $\chi^2(2) = 45.450, P < 0.001$ ; Fig. 2A], with gobies stimulated from the cranial direction having larger escape angles than gobies stimulated from either the lateral (28.2°; 95% CI: 18.21, 38.17) or caudal (30.5°; 95% CI: 21.10, 39.87) direction. This result suggests that fish attacked from all stimulus directions have similar escape performance, regardless of climbing ability.

We did not find any clear relationships between peak escape velocity and climbing style [ $\chi^2(2) = 0.002, P = 0.999$ ] or stimulus direction [ $\chi^2(2) = 3.004$ ,



**Figure 2.** Harrell plot of escape angle (A) and escape velocity (B) among climbing styles and stimulus directions. The upper portion of the graph shows the effect of the different stimulus directions and climbing style (IN, inching; NC, non-climbing; PB, powerburst), with a vertical dotted line indicating an effect size of one. Bars in the upper portion of the graph are the 95% confidence intervals of the effect. The lower portion of the plot shows the estimated marginal mean and the 95% confidence interval for this measurement based on the linear mixed effects model: response ~ climbing style + stimulus direction + (1 | species) + (1 | collection location). Colours and shapes represent different stimulus directions.

$P = 0.223$ ]. However, we did observe more variation in velocity for non-climbers than for inching or powerburst gobies (Supporting Information, Fig. S3). On average, inching climbers escaped with higher accelerations than powerburst climbers ( $156.6 \text{ cm s}^{-2}$ ; 95% CI:  $-5497.017, 5810.270$ ) and non-climbing gobies ( $2.056 \text{ cm s}^{-2}$ ; 95% CI:  $-3390.289, 3394.402$ ), although relationships between peak acceleration and climbing style were not statistically significant [ $\chi^2(2) = 0.752$ ,  $P = 0.687$ ; Fig. 2B]. On average, gobies stimulated from the lateral direction had higher accelerations than gobies stimulated from the cranial ( $289.0 \text{ cm s}^{-2}$ ; 95% CI:  $-122.680, 700.596$ ) or caudal direction ( $372.767 \text{ cm s}^{-2}$ ; 95% CI:  $-50.617, 796.150$ ), although confidence intervals overlapped with each other and with zero [ $\chi^2(2) = 3.338$ ,  $P = 0.188$ ; Fig. 2B]. We also did not find clear differences among localities for *Sicyopterus stimpsoni* or *A. stamineus* with respect to escape angle [*Sicyopterus stimpsoni*,  $F(2,28) = 2.666$ ,  $P = 0.087$ ; *A. stamineus*,  $F(1,25) = 0.417$ ,  $P = 0.524$ ], peak velocity [*Sicyopterus stimpsoni*,  $F(2,26) = 1.373$ ,  $P = 0.271$ ; *A. stamineus*,  $F(1,25) = 3.954$ ,  $P = 0.058$ ] or acceleration [*Sicyopterus stimpsoni*,  $F(2,26) = 0.396$ ,  $P = 0.677$ ; *A. stamineus*,  $F(1,25) = 3.966$ ,  $P = 0.058$ ].

One explanation for our results could be that all gobies are sufficiently fast to evade predators and might already be near the top of a fitness peak for

escape performance (Kawano *et al.*, 2013). This is suggested by the fact that both peak velocities and peak accelerations were similar among both climbing and non-climbing taxa. Moreover, we measured performance in adults, a stage in which there are smaller differences between powerburst and inching species in the proportions of axial muscle fibre types that power fast starts (Cediel *et al.*, 2008).

## MORPHOLOGY

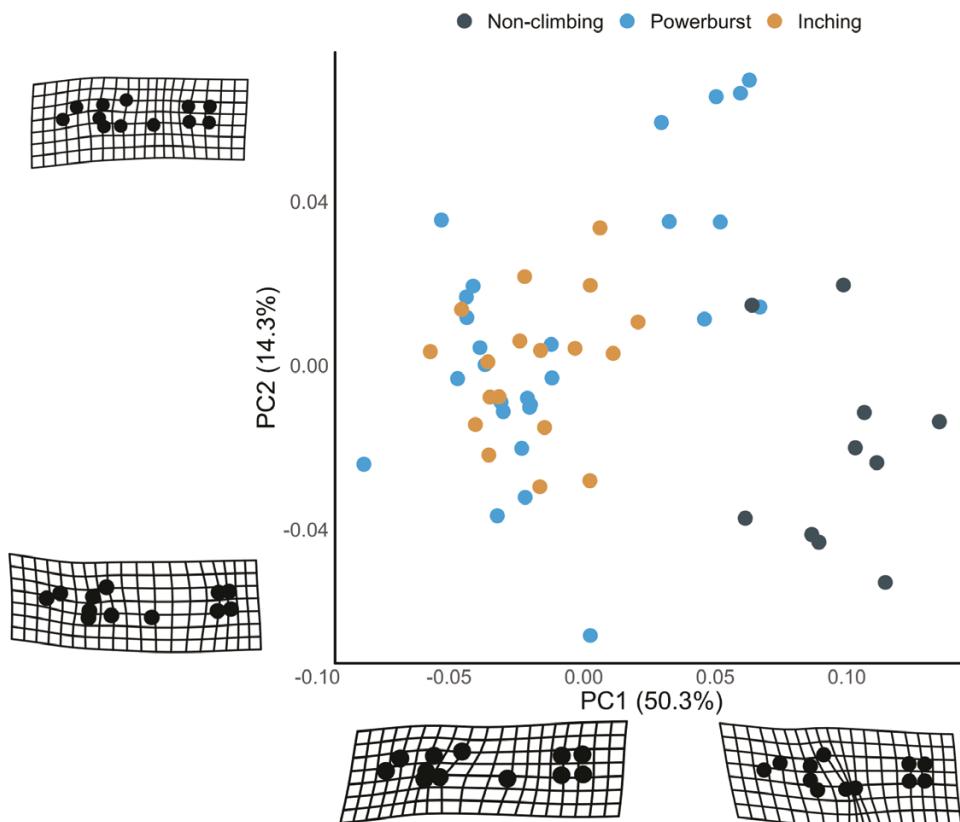
In our comparison across goby species, we found that overall shape does vary among climbing styles ( $F = 14.260$ ,  $Z = 4.950$ ,  $P = 0.010$ ). Past work focused on multiple populations within an inching species, *Sicyopterus stimpsoni*, suggested that predation pressure was a stronger selection pressure on morphology than waterfall climbing (Moody *et al.*, 2019; Blob *et al.*, 2020). These studies examined linear measurements of body shape (Moody *et al.*, 2019) and compared the proportion of red and white muscle (Blob *et al.*, 2020) among populations of *Sicyopterus stimpsoni* exposed to different levels of predation. Our results suggest that climbing is likely to exert a strong selection on body shape for species that climb, whereas further divergence is observed within species and populations exposed to different predation pressures.

Our first three PCs described 76.6% of the shape variation in our dataset and were analysed further. Principal component 1 explained 50.3% of the total variation in shape and PC2 explained 14.3% of total variation in shape, and both varied among climbing styles, localities and species (Table 1). Along PC1, our non-climbing species had deeper anterior bodies and had pelvic and anal fins that are proportionally closer together (Fig. 3). Differences in pelvic fin (i.e.

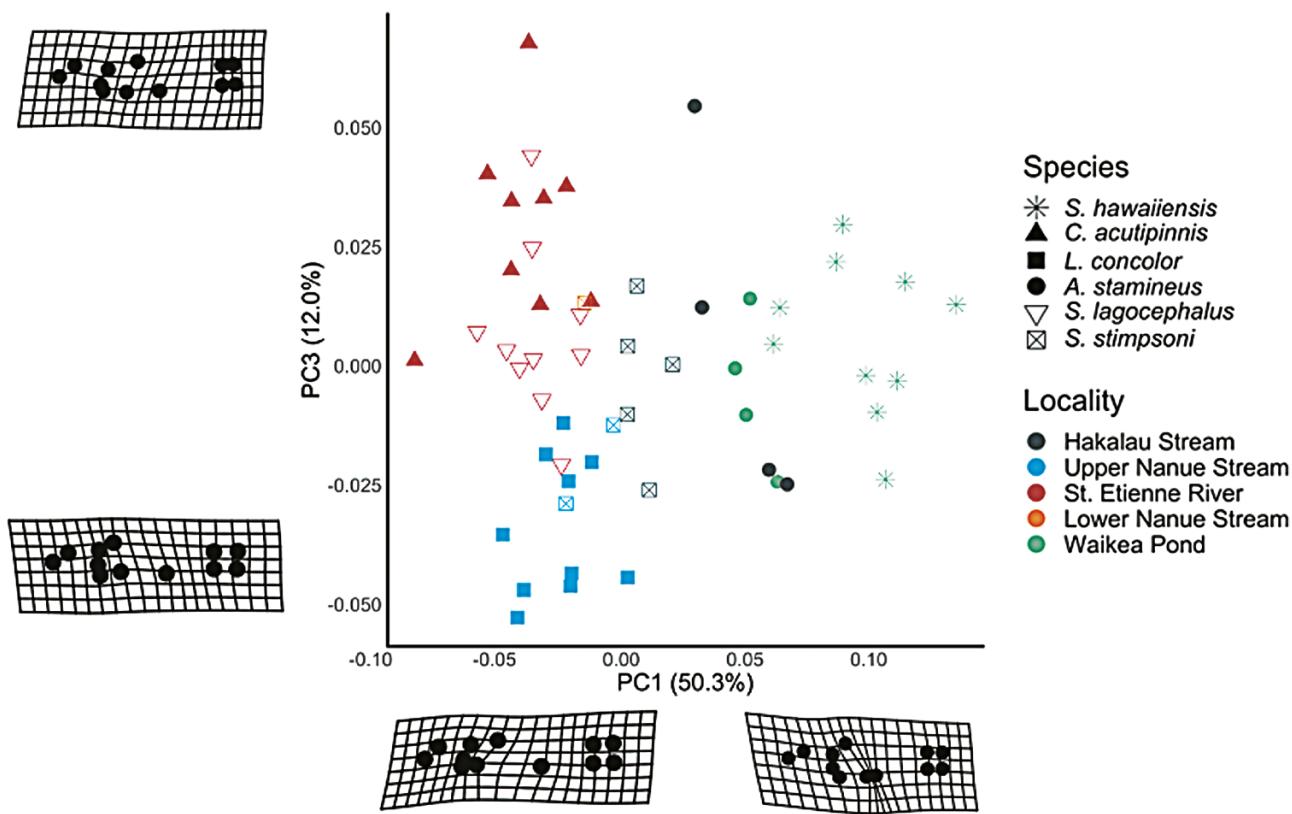
sucker) placement between non-climbing and climbing species along PC1 are likely to be related to the fact that climbing species use these fins for climbing (Blob *et al.*, 2007), and more anterior placement might be advantageous for resisting the flow of oncoming water while attached to a steep surface. We note here that morphological patterns in the present study were restricted to the lateral side of the body, and future geometric morphometric studies of ventral aspects

**Table 1.** Procrustes analysis of variance for shape among species, locality and climbing style for first three principal components of geometric principal components analysis

Parameter	Principal component 1			Principal component 2			Principal component 3		
	F	Z	P-value	F	Z	P-value	F	Z	P-value
Species	6.251	1.879	0.005	3.700	1.428	0.032	2.391	1.059	0.108
Locality	36.681	4.816	0.001	6.768	2.449	0.002	13.70	3.406	0.001
Climbing style	173.405	4.349	0.001	6.343	1.843	0.004	0.903	0.350	0.416



**Figure 3.** Shape variation among goby climbing styles, as indicated by the first two principal components (PCs) of the geometric morphometrics analysis. Colours represent climbing styles (black, non-climbing; blue, powerburst; orange, inching). Partial warps of the extremes of each PC axis relative to average body shape are plotted along each axis. Dots in warps represent landmarks chosen for this study, and warps show the difference between shape at the edge of the axis and the consensus shape. The PC1 score is associated with flank length and anterior body depth. The PC2 score is associated with posterior body length.



**Figure 4.** Shape variation among goby species and localities. Colours represent localities, and shapes represent different species. Partial warps of the extremes of each principal component (PC) axis relative to average body shape are plotted along each axis. Dots in warps represent landmarks chosen for this study, and warps show the difference between shape at the edge of the axis and the consensus shape. The PC1 score is associated with flank length and anterior body depth. The PC3 score is associated with the relative position of the dorsal fin.

of goby morphology, such as the suction discs that aid in climbing, might show additional variation among species and climbing styles. The deeper bodies observed in our non-climbing species could help prey to avoid consumption by becoming too tall for predators to consume (Domenici *et al.*, 2008). For example, body depths of *Stenogobius hawaiiensis* in the present study were similar to measures of peak gape from the fish *Eleotris sandwicensis*, the main predator of Hawaiian gobies (Maie *et al.*, 2014).

Principal component 2 was defined mainly by the length of the posterior body. Inching climbers were more condensed in morphospace along these first two PCs compared with powerburst climbers (Fig. 3). This might reflect a sampling bias, because the study included one more powerburst species than inching species. However, it is also possible that the more recently evolved, inching form of climbing constrains the possible body shapes that adults can expand into, or that this style has evolved too recently for morphological diversity comparable to that of powerburst climbers to have emerged (Blob *et al.*, 2019).

Principal component 3 explained 12.0% of total shape variation and did not show any clear patterns with climbing style or among species but did vary among localities (Table 1). Principal component 3 separated fish by the relative length of the posterior body and caudal peduncle (Fig. 4). To examine relationships of morphology further, we added species and locality labels to our geometric morphometric plots and found that gobies from La Réunion tended to have longer posterior bodies and shorter caudal peduncles than gobies from Hawai'i, regardless of climbing style (Fig. 4). Future research should explore how ecological differences between these localities, such as differences in stream topography or predator abundance, might be driving the morphological differences suggested by this result.

## CONCLUSIONS

Studies of form–function relationships between fish body shape and fast-start performance have a long history that provides a foundation for the predictions made in the present study (Webb, 1976; Walker, 1997; Hale, 1999; Domenici *et al.*, 2008; Domenici & Hale, 2019). Our

comparisons of amphidromous gobies have shown that the emergence of waterfall climbing as a new, strong selective pressure (Blob *et al.*, 2010; Moody *et al.*, 2017) can lead to patterns that run counter to such expectations. Under the pressure of this new function, streamlined body shapes in the present study, which might have been expected to show lower fast-start performance, in fact showed no difference in performance from non-climbing taxa. Behavioural compensation might also contribute to the unexpected patterns we identified (Moody *et al.*, 2019). Thus, even in the face of potential functional trade-offs (Walker, 2007; Blob *et al.*, 2010), strong selection promoting the performance of novel behaviours need not reduce other aspects of performance.

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### DATA AVAILABILITY

Data and code for the analyses are available from the GitHub repository: <https://github.com/DiamondKMG/GobyAdultComp>

### REFERENCES

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Sampling locality details. The number of fish collected from each locality ( $N$ ) is reported, in addition to the average standard length (SL)  $\pm$  SE. The waterfall column indicates whether fish were collected above or below a waterfall in the given locality, with NA indicating that Waiākea Pond does not have a waterfall to climb.

**Table S2.** Ranges of attack angles, listed by species, for categorical attack directions for all individuals that responded to our stimulus. Note that no *Cotylopus acutipinnis* attacked from the caudal direction responded to our stimulus.

**Table S3.** Pairwise comparisons among climbing styles for behaviour and performance variables. The odds ratio (odds), lower and upper confidence limits (LCL and UCL), Z ratio (Z) for response frequency,  $t$  ratio ( $t$ ) for escape angle, peak velocity and peak acceleration, and  $P$ -values ( $P$ ) for all possible pairs are listed.

**Table S4.** Descriptive statistics for all species. LO is the locality of each species, including St. Etienne River (SE), Hakalau Stream (HS), Waiākea Pond (WP) and Nānue Stream below (LN) and above (UN) the first waterfall. SD represents stimulus directions, cranial (Cr), lateral (La) and caudal (Ca).  $N$  for each response variable indicates the number of fish tested. Response % represents the percentage of fish tested that displayed an escape response. Each measurement is given  $\pm$  SE.

**Table S5.** Tukey's HSD *post hoc* tests for morphological differences among species, locality and climbing style for the first three principal components of geometric morphometric analysis. The difference in means (Diff), lower and upper confidence limits (LCL and UCL), and the adjusted  $P$ -values (Adj  $P$ ) for all possible pairs are listed. Species abbreviations: A.s., *Awaous stamineus*; C.a., *Cotylopus acutipinnis*; L.c., *Lentipes concolor*; S.h., *Stenogobius hawaiiensis*; S.l., *Sicyopterus lagocephalus*; S.s., *Sicyopterus stimpsoni*. Locality abbreviations: LN, lower Nānue Stream; HS, Hakalau Stream; SE, St. Etienne River; UN, upper Nānue Stream; WP, Waiākea Pond. Climbing style abbreviations: IN, inching climber; NC, non-climbing; PB, powerburst climber.

**Table S6.** Random effects from (generalized) linear mixed effects models.

**Table S7.** Fixed effects estimates from (generalized) linear fixed effects models.

**Figure S1.** Example of digitized points for shape analysis of left lateral view of *Sicyopterus lagocephalus*. Points represent: (1) centre of eye; (2) anterior insertion of first dorsal fin; (3) posterior insertion of second dorsal fin; (4) dorsal insertion of caudal fin; (5) ventral insertion of caudal fin; (6) posterior insertion of anal fin; (7) anterior insertion of anal fin; (8) posterior margin of pelvic sucker; (9) anterior insertion of pelvic sucker; (10) rostrum; (11) dorsal insertion of pectoral fin; (12) ventral insertion of pectoral fin.

**Figure S2.** Comparison of response frequency among collection locations for *Awaous stamineus* (PB) and *Sicyopterus stimpsoni* (IN). Locations are indicated as below the waterfall (BW) and above the waterfall (AW) for the powerburst climber *A. stamineus*. For the inching climber, *Sicyopterus stimpsoni*, we had two localities where we were able to collect above the waterfall, indicated as above the waterfall 1 and 2 (AW1 and AW2). Points represent the average response frequency for each locality. Error bars represent the SE from the general linear mixed effects model: response frequency ~ climbing style + stimulus direction + (1 | species) + (1 | collection location).

**Figure S3.** Harrell plot of peak velocity among climbing styles and stimulus directions. The upper portion of the graph shows the effect of the different attack directions and climbing styles. Bars in the upper portion of the graph are the 95% confidence intervals of the effect. The lower part of the plot shows the estimated marginal mean and the 95% confidence interval for this measurement based on the linear mixed effects model: peak velocity ~ climbing style + stimulus direction + (1 | species) + (1 | collection location). Colours and shapes represent different attack directions.