SYMPOSIUM

Morphological Selection and the Evaluation of Potential Tradeoffs Between Escape from Predators and the Climbing of Waterfalls in the Hawaiian Stream Goby *Sicyopterus stimpsoni*

Richard W. Blob,[1,]* Sandy M. Kawano,* Kristine N. Moody,* William C. Bridges,[†] Takashi Maie,*
Margaret B. Ptacek,* Matthew L. Julius[‡] and Heiko L. Schoenfuss[‡]

*Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA; [†]Department of Applied Economics and Statistics, Clemson University, Clemson, SC 29634, USA; [‡]Aquatic Toxicology Laboratory, St Cloud State University, St Cloud, MN 56301, USA

From the symposium "Contemporary Approaches to the Study of the Evolution of Fish Body Plan and Fin Shape" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2010, at Seattle, Washington.

[1]E-mail: rblob@clemson.edu

Synopsis Environmental pressures may vary over the geographic range of a species, exposing subpopulations to divergent functional demands. How does exposure to competing demands shape the morphology of species and influence the divergence of populations? We explored these questions by performing selection experiments on juveniles of the Hawaiian goby *Sicyopterus stimpsoni*, an amphidromous fish that exhibits morphological differences across portions of its geographic range where different environmental pressures predominate. Juvenile *S. stimpsoni* face two primary and potentially opposing selective pressures on body shape as they return from the ocean to freshwater streams on islands: (1) avoiding predators in the lower reaches of a stream; and (2) climbing waterfalls to reach the habitats occupied by adults. These pressures differ in importance across the Hawaiian Islands. On the youngest island, Hawai'i, waterfalls are close to shore, thereby minimizing exposure to predators and placing a premium on climbing performance. In contrast, on the oldest major island, Kaua'i, waterfalls have eroded further inland, lengthening the exposure of juveniles to predators before migrating juveniles begin climbing. Both juvenile and adult fish show differences in body shape between these islands that would be predicted to improve evasion of predators by fish from Kaua'i (e.g., taller bodies that improve thrust) and climbing performance for fish from Hawai'i (e.g., narrower bodies that reduce drag), matching the prevailing environmental demand on each island. To evaluate how competing selection pressures and functional tradeoffs contribute to the divergence in body shape observed in *S. stimpsoni*, we compared selection imposed on juvenile body shape by (1) predation by the native fish *Eleotris sandwicensis* versus (2) climbing an artificial waterfall (~100 body lengths). Some variables showed opposing patterns of selection that matched predictions: for example, survivors of predation had lower fineness ratios than did control fish (i.e., greater body depth for a given length), whereas successful climbers had higher fineness ratios (reducing drag) than did fish that failed. However, most morphological variables showed significant selection in only one treatment rather than opposing selection across both. Thus, functional tradeoffs between evasion of predators and minimizing drag during climbing might influence divergence in body shape across subpopulations, but even when selection is an important contributing mechanism, directly opposite patterns of selection across environmental demands are not required to generate morphological divergence.

Introduction

Efforts to understand how the morphology of animals relates to variation in the environments where they live have been a prominent focus of many evolutionary, ecological, and functional studies (Webb 1984; Losos 1990; Liem 1993; Wainwright and Reilly 1994; Walker 1997; Domenici 2003; Langerhans et al. 2003; McGuigan et al. 2003;

C-133

TRIAL EXHIBIT AB-139

Langerhans 2008; Rivera 2008). Fishes have served as a critical model in this research because of the wide range of functional studies that have helped to identify correlations between body shape and performance (Webb 1975, 1978, 1986; Westneat 1994; Drucker and Jensen 1997; Pettersson and Hedenström 2000; Walker 2000; Ferry-Graham et al. 2001; Rosenberger 2001; Wainwright et al. 2002; Walker and Westneat 2002; Lauder et al. 2003; Blake 2004, 2006; Carroll et al. 2004; Higham et al. 2005; Domenici et al. 2008; Blake et al. 2009). These functional studies provide a foundation for predicting how different body shapes should succeed or fail under pressures from different environments, and whether particular morphologies may entail functional tradeoffs, improving performance of some tasks at the expense of others (Ghalambor et al. 2003, 2004; Walker 2007; Langerhans 2009a). Because environmental pressures may vary over the range of a species, subpopulations may be exposed to divergent functional demands. How does exposure to competing demands shape fish morphology and influence divergence of populations? Understanding the potential for functional tradeoffs to contribute to population divergence is significant, because such divergence can be one of the first steps leading to speciation (Schluter 2001; Coyne and Orr 2004).

The stream gobies of the Hawaiian Islands provide a system that is particularly well suited for examining the roles that varying environmental conditions and resultant functional tradeoffs play in shaping fish morphology because of the distinctive life history of these fishes and the specific pressures exerted by their native habitats. All five native species of gobioid fishes inhabiting Hawaiian streams share an amphidromous life cycle: adults live and lay eggs in freshwater, but when larvae hatch they are swept downstream to the ocean, where they develop for several months before returning to habitats used by adults in island streams (Nishimoto and Fitzsimons 1999; Keith 2003; McDowall 2003, 2004; Schoenfuss and Blob 2007). Evidence indicating that these fishes home to their natal stream is lacking, and population-genetic data have suggested that fish hatched on one island may recruit to other islands (Chubb et al. 1998). Upon arriving at a stream, however, juvenile gobies returning from the ocean encounter two major, contrasting environmental challenges (Fig. 1). First, the lower reaches of Hawaiian streams are patrolled by a non-climbing, piscivorous member of the sleeper family, *Eleotris sandwicensis* (Fitzsimons et al. 1997; Schoenfuss and Blob 2007). Second, because of the volcanic origin of the islands (Carson and Clague 1995), Hawaiian

streams typically descend from steep mountains and are punctuated by substantial waterfalls. For three goby species (*Awaous guamensis*, *Lentipes concolor*, and *Sicyopterus stimpsoni*), the habitats used by adults are upstream of waterfalls that can be thousands of body lengths high (Fitzsimons and Nishimoto 1995). Thus, to reach their destination, juveniles of these three species must first avoid being eaten by predators, and then climb up massive falls in the streams. One key to the waterfall-climbing ability of these fish is that, as members of the goby clade, they possess a distinctive ventral sucker formed from the fusion of the pelvic fins (Nishimoto and Fitzsimons 1999; Schoenfuss and Blob 2003). Moreover, gobies are free from native predators once above the falls, as *E. sandwicensis* does not climb and no birds or invertebrates are known to exert substantial predatory pressure on fish in upstream habitats of the Hawaiian Islands (Schoenfuss and Blob 2007).

Based on experimental studies of fish performance and hydrodynamic principles, body shapes that should enhance the ability of gobies to evade predators are expected to differ substantially from those that should promote successful climbing of waterfalls. Escape from predators would be aided by increased production of evasive thrust (Walker et al. 2005), which can be facilitated through increases in the depth of the posterior body and caudal peduncle relative to anterior regions like the head, and increases in the size of median fins (Walker 1997; Domenici et al. 2008). In contrast, climbing rock surfaces through flowing water should be aided by body shapes that improve adhesion and minimize drag (Blob et al. 2008). Successful climbers, therefore, might have larger suckers (Maie et al. 2007) as well as narrower, flatter bodies than those of unsuccessful climbers. This shape of the body would reduce the area exposed to flow (Hoerner 1958; Webb 1989; Vogel 1994; Domenici 2003) and contribute to values of morphological indices (e.g., fineness, lengthening, and flattening ratios) that are closer to optimal values for the reduction of drag in benthic fish (Arnold and Weihs 1978; Webb 1989; Blake 2006).

In addition to the contrasting demands presented to gobies by predation and climbing, differences in stream habitats across the Hawaiian Islands have the potential to cause one or the other of these pressures to predominate, so that divergent morphological phenotypes might be advantageous on different islands (Blob et al. 2008). On Kaua'i (the oldest major island), erosion has caused many waterfalls to recede far inland, creating streams with long,
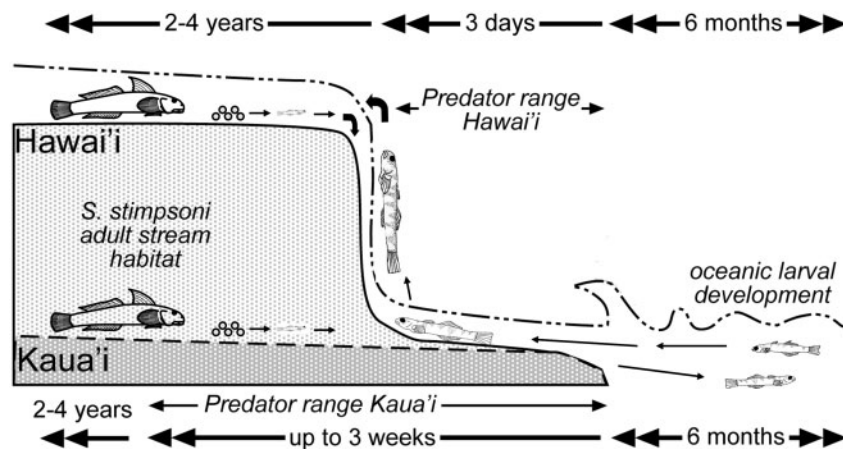
Fig. 1 Schematic illustration of the life cycle of *Sicyopterus stimpsoni* on the islands of Hawai'i (top) and Kaua'i (bottom), reproduced from Blob et al. (2008). Adults live upstream (left side of figure) on both islands. Larvae hatching from eggs (circles) are swept by stream currents to the ocean (right side of figure). Juveniles returning to freshwater and migrating upstream encounter predatory, non-climbing *Eleotris sandwicensis* before reaching waterfalls in the streams. The inland erosion of waterfalls on Kaua'i extends the exposure of juvenile *S. stimpsoni* to predators for up to 3 weeks (time scale at bottom of figure), whereas near-shore waterfalls on Hawai'i shorten the range of juveniles' exposure to predators to ~3 days (time scale at top of figure) but place an early premium on waterfall-climbing performance.

meandering stretches extending upstream from the ocean, thereby forcing juvenile gobies to swim through an extended gauntlet of non-climbing predators during their upstream migration (Fig. 1; Fitzsimons et al. 1997; Schoenfuss and Blob 2007; Blob et al. 2008). In contrast, on the Island of Hawai'i (the youngest island), waterfalls are typically close to shore, subjecting juvenile gobies to the challenge of extreme flow shortly after entering streams, but minimizing exposure to predators (Fig. 1; Fitzsimons et al. 1997; Schoenfuss and Blob 2007; Blob et al. 2008). Because of this difference in the predominant early selective pressures presented to gobies on each island, body shapes suited to improve evasion of predators might be favored on Kaua'i, whereas body shapes suited to improve climbing performance might be favored on Hawai'i. Differences in body shape among fish from these islands support this prediction: morphometric analyses of adult *S. stimpsoni* collected in 2005 indicated that, regardless of sex, fish from Kaua'i tended to have tall bodies advantageous for generating evasive thrust, whereas fish from Hawai'i tended to have low, narrow bodies better for reducing drag (Blob et al. 2008). However, direct comparisons of the potential for the competing selection pressures of predation and climbing to contribute to morphological divergence between subpopulations of stream gobies have not been performed.

To evaluate how competing selection pressures and functional tradeoffs might contribute to divergence in the body shapes of stream gobies across the Hawaiian Islands, we conducted two sets of laboratory-based selection experiments (Garland 2003; Fuller et al. 2005) on juvenile fish to compare how body shape might specifically be influenced by predation by the native fish *E. sandwicensis* versus climbing an artificial waterfall (~100 body lengths). Because different body shapes are thought to improve performance during evasive swimming versus climbing, we might expect such functional tradeoffs to be reflected by predation and climbing both exhibiting opposite patterns of selection for many morphological traits. In addition, we compared the body shapes of juvenile fish collected on Kaua'i and Hawai'i to assess how selection might be acting in the wild. If juvenile fish, as well as adults, differ in shape across the islands, then selection may have been contributing to divergence of subpopulations over many generations of recruits, and the possibility that divergence in the body shape of adults between islands is strictly a plastic response to the environment (DeWitt et al. 1998; Domenici et al. 2008) seems unlikely. For these analyses, we focused on *S. stimpsoni* as a model species. Juvenile *S. stimpsoni* average just over 2 cm in length and "inch up" surfaces by alternating advancement of the pelvic sucker and a second, oral sucker associated with the upper jaw (Schoenfuss et al. 1997; Nishimoto and Fitzsimons 1999; Schoenfuss and Blob 2003). Focus on juvenile *S. stimpsoni* simplifies expectations for the types of body shapes that minimize drag

(Walker et al. 2010), because its mode of climbing involves almost no lateral bending of the body axis for propulsion (Schoenfuss and Blob 2003; Blob et al. 2007). In addition, because previous studies by our group had established that adult *S. stimpsoni* from Kaua'i and Hawai'i differ in body shape, and had measured morphological selection imposed on juvenile *S. stimpsoni* by the demands of climbing (Blob et al. 2008, 2009), focus on the same species for our new analyses of morphological divergence among juveniles and selection imposed by predators could build from a substantial context in evaluating the influence of selection and tradeoffs in the evolution of body shape in Hawaiian waterfall-climbing gobies.

Materials and methods

Evaluation of selection imposed on gobies by predators

Juvenile *S. stimpsoni* for both sets of selection experiments (predation and climbing) were collected with dip nets from the lower reaches of Hakalau stream on the Island of Hawai'i, downstream from a 1-m vertical concrete embankment (same location as used by Schoenfuss and Blob 2003; Blob et al. 2006). Transparency of the fish indicated all were fresh recruits into the stream (Schoenfuss and Blob 2003, Blob et al. 2006), limiting previous in-stream exposure to selective pressures. Fish were transferred in stream water to laboratory facilities (provided by the Department of Land and Natural Resources, Division of Aquatic Resources in Hilo, Hawai'i) within 2 h of capture.

Experiments on predation were performed in 2007 following protocols based on those described by Swain (1992). To help ensure that all fish in our sample were similar in developmental stage, collections for each predation trial were limited to the number of fish we could collect in a single day, and Swain's (1992) protocols were modified accordingly. Each stock group of gobies collected for a predation trial was divided into "treatment" and "control" groups by drawing batches of juveniles from a stock tank with a dip net and alternately distributing these fish to separate treatment and control tanks (400-l cattle tanks half filled with aerated stream water). Dip-net withdrawals for treatment tanks contained twice as many fish (∼20) as those for control tanks (∼10), and the distribution of fish to tanks was repeated until each treatment tank approached a target of 100 fish and each control tank approached a target of 50 fish. This protocol helped to ensure that only chance differences should exist between fish in treatment and control groups, even if dip-net withdrawal is selective (Swain 1992). After distribution, juvenile gobies were allowed to acclimate for 1 day before 10 predatory *E. sandwicensis* (which had been starved for the prior 24 h) were added to the treatment tank. Experiments were run until approximately one-half of the juvenile *S. stimpsoni* in the treatment tank were eaten, which was consistently close to 36 h across all trials. At this point, predators were removed from the tanks and all remaining gobies (∼50 survivors and 50 controls) were collected and preserved in 40% ethanol. Each fish was digitally photographed in lateral and ventral views and weighed to the closest 0.01 g. Linear measurements of anatomical variables (Fig. 2) hypothesized to be relevant to locomotor performance during either evasion of predators or climbing waterfalls (Blob et al. 2008) were obtained using NIH Image 1.62 or Image J v1.41.

The consumption of juvenile gobies by the predators forced us to make our comparisons for predation trials between surviving fish and control fish that had not been exposed to selection, rather than between surviving fish and fish that failed under selection. To accommodate this design, we evaluated the direction and strength of directional selection on morphological traits following the procedures outlined by Lande and Arnold (1983) for cross-sectional data. Prior to analysis, fish with a body condition index (BCI = body length^3/body mass) > ±2 SD from their population mean were excluded, and all variables for remaining fish were standardized for body size using the transformation [ln(variable) − ln(total length)] (Mosimann and James 1979). Newly recruited *S. stimpsoni* show a very limited range of body lengths, minimizing any potential effects of differences in allometric growth on our analyses (Blob et al. 2008). Because the rather small sample sizes we had available for individual predation trials limited our ability to detect significant results (Kingsolver et al. 2001), we focused our analyses of selection imposed by predation on data pooled across all five trials, thereby providing a total of 228 survivors of predation and 167 control fish.

We evaluated the significance of both direct and indirect directional selection by calculating and comparing two parameters: the selection differential and the selection gradient. Selection differentials (*s*) represent change in a character's mean due to both direct and indirect directional selection, and were calculated for each character as the difference between the mean value for surviving fish and the
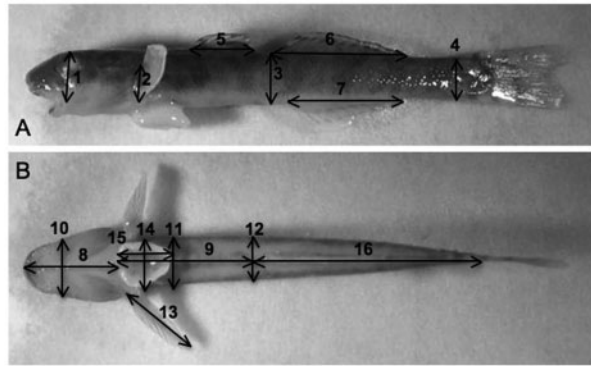
Fig. 2 Photographs of lateral (A) and ventral (B) views illustrating linear anatomical variables measured on juvenile *S. stimpsoni*. Note that standard length also was measured to enable standardization of other linear variables, and that tail length was included as a variable only in analyses comparing the morphology of fish from Kaua'i versus Hawai'i (not selection analyses).
1, Head height; 2, Basal length of pectoral fin; 3, Height at midbody; 4, Height of caudal peduncle; 5, Basal length of first dorsal fin; 6, Basal length of second dorsal fin; 7, Basal length of anal fin; 8, Head length; 9, Trunk length; 10, Head width; 11, Trunk width; 12, Width of the body at the location of the anal pore; 13, Length of the margin of the pectoral fin; 14, Sucker width; 15, Sucker length; 16, Tail length.

mean value for all fish in the trial; each value of s was then divided by the standard deviation of the character to produce the standardized parameter s' (Lande and Arnold 1983). Assigning surviving fish a fitness (W) of 1 and control fish a W of 0, we transformed W to relative fitness (w) by dividing W by mean population fitness, and then tested the significance of s' values with Spearman rank correlations of relative fitness with transformed values for each character. Directional selection gradients (β) indicate the strength of only direct directional selection on each character, and standardized gradients (β') were calculated from the product $P^{-1} s'$, where P is the phenotypic variance–covariance matrix calculated from standardized measurements of control fish (representing the pre-selection or "young cohort" indicated by Lande and Arnold [1983]). Standard errors and significance tests for the gradients were performed by bootstrap methods (Efron and Gong 1983). This procedure used 1000 replicate samples from the data set to create an empirical sampling distribution of gradient values, and standard errors and significance tests were developed from this distribution. All gradient and Spearman rank analyses were conducted in SAS (Cary, NC, USA, Version 9.1).

To complement our analyses of linear measurements, we also evaluated selection on functional ratios associated with hydrodynamic performance,

including fineness ratio (length/maximum height), flattening ratio (maximum width/maximum height), and lengthening ratio (length from rostrum to location of maximum height/length) (Arnold and Weihs 1978; Webb 1989; Blake 2006). Because these ratios are based on combinations of the linear morphological variables under study, they could not be included in calculations of selection gradients with the variables from which they were derived. However, we did calculate s' for these three ratios between survivors of predation and control fish, and used Spearman rank correlations to test if selection by predators favored taller bodies that could increase thrust, rather than values closer to optima for reduction of drag (10 for fineness ratio, 2 for flattening ratio, 0.3 for lengthening ratio [Blake 2006]).

Evaluation of selection imposed on gobies by waterfalls

Experiments to evaluate selection imposed on the morphology of juvenile *S. stimpsoni* by the demands of waterfall climbing were conducted in 2006; details of our procedures and results were reported previously (Blob et al. 2008), with the results corrected in a subsequent report (Blob et al. 2009). Two different experimental setups were implemented in our original study of selection related to climbing, but for comparison to selection parameters calculated for predation we considered only the results from experiments conducted on the longer of two climbing arenas in which fish were tested in groups of 20, thereby better simulating the tendency of juvenile *S. stimpsoni* to climb in large groups (Nishimoto and Fitzsimons 1999). To summarize these experiments, groups of juveniles were introduced into a basin containing stream water and challenged with a 240-cm climbing chute extending at $\sim 70°$ from the horizontal. A siphon directed acclimated stream water down the chute at a rate of $\sim 200 \, ml \, min^{-1}$ and a depth of 1–1.5 mm, stimulating the positively rheotactic fish to climb. Climbing performance was evaluated as either "success" for fish reaching the top of a chute, or "failure" for fish that failed to climb or that attempted climbing but failed to reach the top. Fish were collected upon completion of trials, preserved in 40% ethanol, and photographed and measured as described for predation trials.

Because individual fish could be distinguished as successful or failed, the strength of directional selection imposed on each morphological character by climbing was evaluated using procedures outlined by Lande and Arnold (1983) for longitudinal data. Procedures for the exclusion of outliers (leaving a

sample of 224 fish), standardization of measurements for differences in body size, and calculation of s' (for hydrodynamic ratios as well as linear measurements) were the same as those used in the study of predation. For the assessment of fitness, successful climbers were assigned an absolute fitness (W) of 1 and unsuccessful climbers a W of 0, with all-or-nothing designations reflecting an assumption that partial ascension of a waterfall still indicates failure to reach the habitat of reproductive adults. To calculate directional selection gradients (β), we followed the modifications of Lande and Arnold's (1983) methods recommended by Janzen and Stern (1998) and calculated a selection gradient vector ($\beta_{avggrad}$) from logistic multiple regression of relative fitness on all characters. Logistic regressions and Spearman rank correlations were calculated using SAS (Cary, NC, USA, Version 9.1).

Comparisons between selection imposed by predators and waterfalls and the evaluation of functional tradeoffs

We conducted several comparisons of the selection imposed by predation and waterfalls. First, for each variable, the signs and significance of s' and β were compared between selection regimes (DeWitt and Langerhans 2003). This allowed us to evaluate whether patterns of selection were direct or indirect, matched *a priori* expectations based on functional principles, and were opposing (i.e., different in sign) between regimes, similar (i.e., same sign) between regimes, or simply different (i.e., significant in one regime but not the other).

In addition, we more formally evaluated the nature of tradeoffs between the demands of avoidance of predators and climbing, using two approaches. First, we used our vectors of selection gradients for each regime to represent performance gradients (i.e., coefficients of partial regressions of morphology on functional performance, rather than on fitness) (Arnold 1983) and then constructed an **F** matrix showing the relationships between morphology and performance (Ghalambor et al. 2003; Walker 2007), in which predation gradients for all 16 morphological variables were in the first column, and climbing gradients for all 16 variables were in the second column. There are complications with this approach to **F**-matrix construction: for instance, our measure of performance in predation trials was simply survival, rather than a variable such as maximal fast start acceleration (Walker et al. 2005) that might mediate the relationship between morphology and survival (Arnold 1983), which is commonly

considered a measure of fitness (Langerhans 2009b). Nonetheless, because both the ascension of a waterfall and the escape from a predator also could be considered a type of performance, we felt our simplification of the morphology–performance–fitness relationship (Arnold 1983) was appropriate to facilitate exploration of potential tradeoffs between the primary functional pressures in our system. After constructing **F**, we calculated the matrix $\mathbf{F^T F}$, allowing us to evaluate if there was a net tradeoff (negative off diagonals) or facilitation (positive off diagonals) between the functions of predator evasion and waterfall climbing (Ghalambor et al. 2003; Walker 2007). Further multiplication of $\mathbf{F^T F}$ by a column vector of ones produced the vector **c**, which provided ranks of the relative constraints on the evolution of predator evasion and climbing performance in *S. stimpsoni* (Ghalambor et al. 2003). In a complementary analysis, we also calculated the correlation (r) between the selection coefficients for predation and climbing to further assess the nature of the relationship between these functional pressures.

Morphometric comparisons of juvenile S. stimpsoni from Kaua'i and Hawai'i

Evaluations of morphological differentiation between juvenile *S. stimpsoni* recruiting to Kaua'i versus Hawai'i were performed using new collections made during February and March 2009. Fish were collected by dip nets near the mouths of streams on both islands (Waimea and Wailua on Kaua'i, Hakalau and Honoli'i/Maili on Hawai'i) before major exposure to predators or waterfalls had occurred. Fish were anesthetized with MS-222 and photographed in lateral and ventral views to allow the measurement of linear anatomical variables (Fig. 2) using Image J v1.41. Measurements were transformed and standardized for differences in body length as in the morphometric analyses for studies of selection. Morphological distinction of fish between the two islands was evaluated using principal components (PCs) analysis (based on the covariance matrix) (Neff and Marcus 1980) performed in JMP v.8.0.1 for Macintosh (SAS Institute, Cary, NC, USA).

Results

Selection imposed by predation on juvenile S. stimpsoni

Juvenile *S. stimpsoni* that survived predation by *E. sandwicensis* differed significantly in morphology from fish from control tanks. Of the 16 variables, 11 we evaluated showed significant s' values, but of

Table 1 Comparison of selection differentials and gradients for anatomical variables of juvenile *S. stimpsoni* assessed in predation and waterfall climbing experiments

Anatomical variable	Predation		Climbing[a]	
	s'	$\beta' \pm SE$	s'	$\beta_{avggrad} \pm SE$
Head height	0.069	0.005 ± 0.056	0.299**	0.102 ± 0.026**
Pectoral fin base length	0.115**	0.217 ± 0.063**	0.129**	0.054 ± 0.022**
Mid-body height	0.282**	0.129 ± 0.132	−0.204**	-0.047 ± 0.040
Caudal peduncle height	0.272**	0.254 ± 0.121**	−0.041	-0.011 ± 0.028
First dorsal fin base length	0.242**	-0.017 ± 0.081	0.060	-0.011 ± 0.021
Second dorsal fin base length	0.138**	-0.066 ± 0.048**	0.103**	0.027 ± 0.027
Anal fin base length	0.111**	0.055 ± 0.051	0.192**	0.111 ± 0.029**
Head length	0.191**	-0.056 ± 0.055	0.000	0.005 ± 0.026
Trunk length	0.065**	0.040 ± 0.055	−0.036	0.014 ± 0.025
Head width	0.053	-0.152 ± 0.079**	0.037	0.027 ± 0.025
Trunk width	0.181**	-0.020 ± 0.091	−0.106**	0.110 ± 0.048**
Anal width	0.258**	0.381 ± 0.113**	−0.205**	-0.171 ± 0.058**
Pectoral fin margin length	−0.028	-0.073 ± 0.052	−0.199**	-0.088 ± 0.025**
Sucker width	0.058	0.126 ± 0.058**	0.105*	0.055 ± 0.024**
Sucker length	−0.105**	-0.127 ± 0.048**	−0.014	-0.057 ± 0.025**
Mass	−0.044	-0.519 ± 0.118**	−0.113**	-0.017 ± 0.032

s', standardized directional selection differential (s/character SD); β', standardized directional selection gradient for cross-sectional data; $\beta_{avggrad}$, standardized directional selection gradient for longitudinal data (Janzen and Stern 1998).
[a]Revised values reported by Blob et al. (2009) for group climbing trials on 240-cm chute; *$P < 0.10$; **$P < 0.05$.

those 11 only 1, sucker length, was significantly smaller in survivors (Table 1). The remaining 10 variables were significantly larger in survivors of predation, and included several (e.g., mid-body height, caudal peduncle height, sizes of the dorsal and anal fins) that reflected greater depth and lateral area of the body, matching our expectations for features in which larger size would be predicted to improve the ability of fish to generate thrust and evade predators (Walker 1997; Domenici et al. 2008). The greater values of trunk width and anal width measurements among survivors compared to controls (Table 1) might reflect greater propulsive musculature or better body condition that could also aid in escape from predators, but the higher values of features such as head length and trunk length among survivors do not clearly follow hydrodynamic predictions. In addition to linear variables, all three hydrodynamic ratios showed s' values indicating a significant difference between control fish and those surviving predation (Table 2). Both fineness ratio and flattening ratio were significantly smaller among survivors of predation (Table 2), but because the height of the body is in the denominator of both of these indices, this comparison indicates that surviving fish actually have taller bodies than do controls, as would be expected to improve generation of evasive thrust (Walker 1997; Domenici et al. 2008).

Lengthening ratio was significantly greater (Table 2) and closer to the optimum value for minimizing drag (Blake 2006) among survivors of predation versus control fish, potentially reducing drag without compromising production of thrust for surviving *S. stimpsoni*.

Selection gradients for the predation experiment were less uniform in pattern than were selection differentials (Table 1). Eight of the 16 variables showed significant gradients: 4 were positive and indicated direct directional selection for larger values (length of the base of the pectoral fin, height of the caudal peduncle, and widths of the sucker and the body at the location of the anal pore), and 4 were negative and indicated direct directional selection for smaller values (width of the head, lengths of the second dorsal fin and sucker, and body mass). The gradient for body mass in particular had a large (Kingsolver et al. 2001) negative magnitude (-0.519 ± 0.118), indicating a strong tendency of predatory *E. sandwicensis* to eat heavier juvenile gobies.

Comparisons of selection differentials with selection gradients reveal complexities in the pattern of selection imposed on juvenile *S. stimpsoni* by predators. For example, of the two primary body features for which greater height was predicted to improve production of thrust and which exhibited positive differentials (height at mid-body and height of the

Table 2 Comparison of directional selection differentials for hydrodynamic ratios assessed from predation and waterfall-climbing experiments for juvenile *S. stimpsoni* from the Island of Hawai'i

	Predation (N = 228 successful, 167 controls)			Climbing (N = 159 successful, 65 failed)		
	Fineness ratio	Flattening ratio	Lengthening ratio	Fineness ratio	Flattening ratio	Lengthening ratio
Optimal value for low drag	10	2	0.3	10	2	0.3
Trait mean ± SD (success)	8.403 ± 0.744	0.886 ± 0.048	0.213 ± 0.008	8.356 ± 0.451	0.919 ± 0.044	0.205 ± 0.008
Trait mean ± SD (all fish)	8.637 ± 0.836	0.897 ± 0.055	0.211 ± 0.011	8.272 ± 0.453	0.917 ± 0.045	0.205 ± 0.009
s'	−0.281**	−0.203**	0.180**	0.185**	0.044	0

Fineness ratio, (length/height); flattening ratio, (width/height); lengthening ratio, (length from rostrum to location of maximum height/length); s', standardized directional selection differential (s/character SD); data for climbing from Blob et al. (2009) for long-chute (240 cm) experiment; optimal values for low drag from Blake (2006); **$P < 0.05$.

caudal peduncle), only caudal peduncle height also showed a significant positive selection gradient (Table 1). This indicates that of these two traits only the height of the caudal peduncle is under direct directional selection, and that the response of mid-body height to selection by predators must be an indirect effect due to selection on other traits. Moreover, with the large selection gradient favoring smaller body mass in juvenile *S. stimpsoni*, it might be expected that many other traits, even those with positive β' values (indicating direct selection favoring larger size), might show negative s' values indicating smaller size among survivors of predation due to indirect selection correlated with the negative selection observed for body mass. Instead, three of four traits that showed significant positive selection gradients also showed significant positive differentials (length of the base of the pectoral fin, height of the caudal peduncle, and width of the body at the location of the anal pore), indicating direct directional selection for larger size despite negative selection on body mass. In addition, seven other traits (height at mid-body; lengths of the bases of the first and second dorsal fins and anal fin; length of the head; and length and width of the trunk) showed significant positive s' values either without significant β' values or, for basal length of the second dorsal fin, despite a significant "negative" selection gradient. These patterns of indirect directional selection indicate that the potential for smaller sizes of these structures to be favored due to correlated selection for smaller body mass was superseded by selection on other correlated characters favoring larger size. In fact, even the very large negative selection gradient for body mass was masked by indirect selection, as the s' value for body mass was not significant. In other words, there was no difference in body mass between survivors and controls, even though body mass was the trait under the greatest direct selection in the entire experiment.

Comparisons between selection patterns imposed by predation and by climbing: evaluation of tradeoffs

Although the difference in body shapes expected to optimize predator evasion versus climbing performance had led us to predict that many traits would show opposing patterns of selection (i.e., opposite in sign) between these two regimes, this was true for very few of the linear variables (Table 1). Only one trait, width of the body at the location of the anal pore, showed a significant positive differential and gradient for predation, but a significant negative differential and gradient for climbing (directions consistent with functional expectations). A second trait, height at mid-body, showed a significant positive differential under predation and a significant negative differential under climbing—also consistent with functional expectations—but because gradients were not significant for mid-body height under either regime, these patterns must have resulted from indirect selection (Table 1).

Much more common than opposing patterns of selection between predation and climbing were instances in which selection for a trait was significant under one functional demand, but not the other. This was true for seven traits, of which four (basal length of first dorsal fin, head length, trunk length, and head width) showed only a significant differential or gradient, but not both, under one regime (Table 1). Of the remaining three traits, height of the caudal peduncle showed a significant positive differential and gradient under predation (matching functional expectations), head height showed a significant positive differential and gradient under climbing, and the length of the margin of the pectoral fin showed a significant negative differential and gradient under climbing. Somewhat surprisingly, also more common than opposing patterns of selection were instances in which significant selection on a trait was imposed in the same direction under both regimes. This occurred for five traits (body mass,

basal length of the anal fin, width and length of the sucker, and basal length of the pectoral fin), of which the latter three showed significant gradients (i.e., direct selection) under both predation and climbing (Table 1). Thus, both predation and climbing favored juveniles with wider, shorter suckers and longer bases of the pectoral fins.

Because patterns of selection for individual linear variables under both predation and climbing are complicated (Table 1), it is difficult to evaluate their correspondence with expectations based on functional assessments of the body shapes best suited to successfully navigate these pressures. However, the responses of hydrodynamic ratios to selection imposed by both climbing and predation show a prominent contrast, consistent with the expected demands of these functions. Of the three hydrodynamic ratios compared, only fineness ratio showed a significant differential under climbing (Table 2). This differential was positive, indicating greater body length for a given height in successful climbers and a closer approach to the optimum value for reducing drag in benthic fish (Blake 2006). It also directly opposes selection patterns for fineness ratio under predation, in which negative differentials reflected lower values for survivors (taller bodies better for generating evasive thrust) (Table 2).

Assessment of the tradeoffs between the functional demands of predation and of waterfall climbing in *S. stimpsoni* led to calculation of the $\mathbf{F^{T}F}$ matrix:

$$\begin{matrix} 0.6160 & -0.0499 \\ -0.0499 & 0.1576 \end{matrix}$$

The negative values of the off-diagonals for this matrix support the prediction of a functional tradeoff between predator-evasion and climbing. Further calculation of the **c** matrix:

$$0.5661 \text{ (Predator-evasion)}$$
$$0.1077 \text{ (Climbing)}$$

suggests that the evolution of predator-evasion performance, with the greater value, is less resisted than that of climbing performance. However, while calculation of the correlation (r) between the performance gradients for predator evasion and for climbing shows a negative value (-0.177), the slope of this relationship (Fig. 3) is not significant ($P = 0.512$).

Morphometric comparisons among juvenile *S. stimpsoni* from Kaua'i and Hawai'i

MANOVA of the 16 original morphological variables (Fig. 2) for juvenile *S. stimpsoni* collected in 2009 indicated significant morphological differentiation

($F = 63.193$, $P < 0.001$) between fish from Kaua'i ($N = 403$) versus fish from Hawai'i ($N = 318$), as did MANOVA of PCs calculated from the 16 original morphological variables ($F = 64.120$, $P < 0.001$). ANOVAs conducted on single PCs indicated significant differences between fish from Kaua'i and those from Hawai'i for two of the PCs (Fig. 4): PC1, accounting for 23.2% of sample variation ($F = 651.633$, $P < 0.001$), and PC3, accounting for 10.5% of sample variation ($F = 46.037$, $P < 0.001$). On PC1 fish from Hawai'i tended to have positive scores (mean $\pm$ SE $= 1.496 \pm 0.080$) whereas fish from Kaua'i tended to have negative scores (mean $\pm$ SE $= -1.180 \pm 0.068$), but on PC3 this pattern was reversed (Kaua'i mean $\pm$ SE $= 0.282 \pm 0.068$; Hawai'i mean $\pm$ SE $= -0.358 \pm 0.062$). The difference in mean PC score between fish from the two islands is smaller for PC3 than for PC1, but one factor contributing to this pattern is a group of about 30 fish from Kaua'i ($\sim$7% of the sample) with high-negative scores on PC3 that plot outside the main cluster for this island (Fig. 4).

PC loadings (Table 3) indicate that all of the original morphometric variables measured from juvenile *S. stimpsoni* were significantly correlated with PC1, but that large measurements of width and length of the sucker, and height and width of the head, have the strongest correlations with high-positive PC1 scores (i.e., fish from Hawai'i), whereas large measurements of height at mid-body and width of the body at the location of the anal pore had the strongest correlations with high-negative PC1 scores (i.e., fish from Kaua'i). For PC3, large measurements of the length of the bases of the dorsal and anal fins, length of the margin of the pectoral fin, height at mid-body, height of the caudal peduncle, and width of the body at the location of the anal pore had the strongest correlations with high-positive scores (i.e., fish from Kaua'i), whereas large measurements of basal length of the pectoral fin, length and width of the sucker, and length of the trunk had the strongest correlations with high-negative scores (i.e., fish from Hawai'i).

Discussion

Correspondence of results from selection experiments with functional expectations, and tradeoffs between the evasion of predators and the climbing of waterfalls

Juvenile *S. stimpsoni* re-entering freshwater streams face two primary functional pressures: evading predators and scaling waterfalls. Based on empirical data and models of fish locomotor performance, these
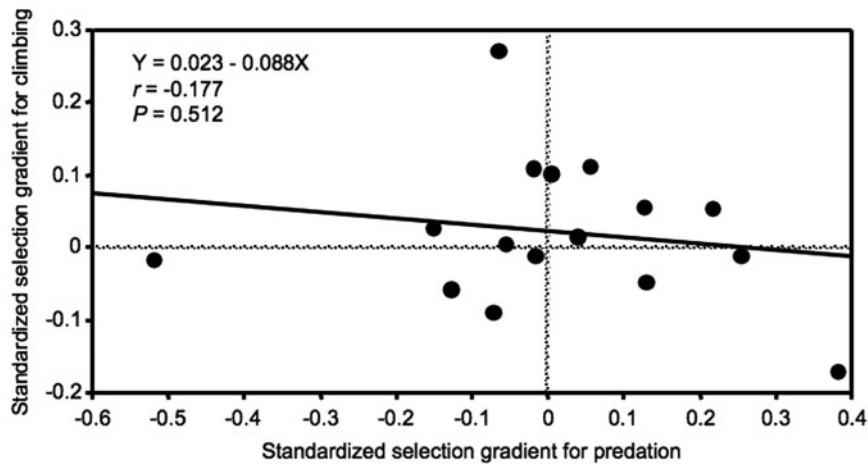
Fig. 3 Bivariate plot illustrating correlation of standardized selection gradients for juvenile *S. stimpsoni* derived from experiments on predation (*x*-axis) versus experiments on climbing (*y*-axis).
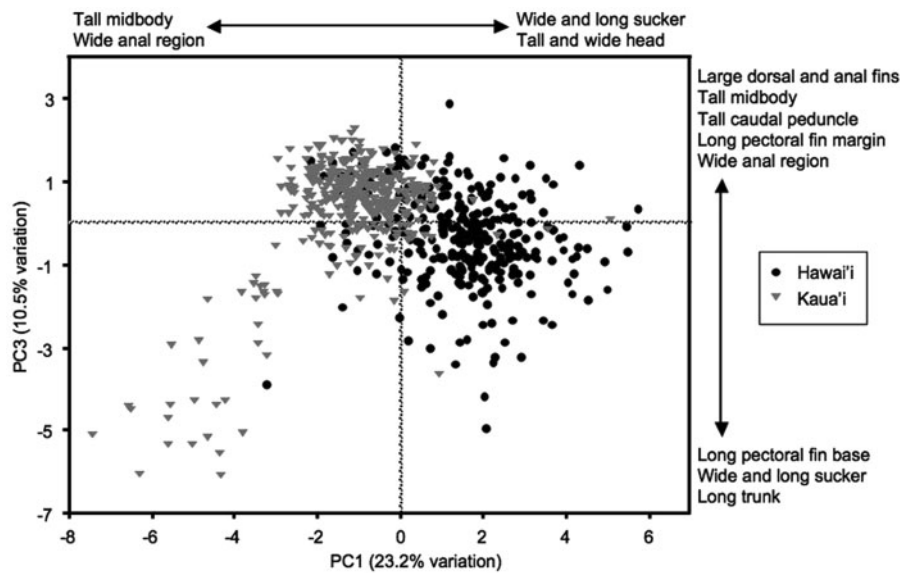

Fig. 4 Bivariate plot of PC1 versus PC3 for juvenile *S. stimpsoni* from Hawai'i (black circles) versus those from Kaua'i (grey triangles). The primary original variables contributing to dispersion along each axis are labeled (Table 3).

two pressures were expected to entail functional tradeoffs, such that their demands would be best met by contrasting body shapes. While the evasion of predators was expected to be aided by taller bodies and larger median fins that improve production of thrust (Walker 1997; Domenici et al. 2008), successful climbing of waterfalls was expected to be aided by body shapes with lower profiles that minimize drag, and larger suckers that improve adhesion (Blob et al. 2008). How closely do the body shapes favored in our selection experiments match these functional expectations?

Although patterns of selection for both evasion of predators and climbing are complicated, for many traits there was close correspondence in at least

one selection regime between the direction of selection and the body shape that would be expected to improve performance under that regime. For example, in spite of strong countervailing pressure for smaller body mass, predation still imposed direct directional selection for taller caudal peduncles and indirect directional selection for a taller mid-body and larger median fins in survivors (Table 1), all features expected to improve generation of evasive thrust (Walker 1997). With regard to climbing, direct directional selection favored wider suckers in successful climbers that would improve adhesion, and direct (for width of the body at the location of the anal pore) or indirect (for trunk width and for height at mid-body) directional selection favored

Table 3 Correlations of measured anatomical variables with principal components calculated for juvenile *S. stimpsoni* from Hawai'i ($N = 318$) and Kaua'i ($N = 403$)

	Anatomical variable	PC1 (23.2% variation)	PC3 (10.5% variation)
1	Head height	0.739**	0.078**
2	Pectoral fin base length	0.703**	−0.262**
3	Mid-body height	−0.455**	0.380**
4	Caudal peduncle height	0.102**	0.281**
5	First dorsal fin base length	0.229**	0.519**
6	Second dorsal fin base length	0.467**	0.553**
7	Anal fin base length	0.429**	0.623**
8	Head length	0.287**	−0.018
9	Trunk length	0.214**	−0.214**
10	Head width	0.710**	−0.089**
11	Trunk width	0.264**	0.233**
12	Anal width	−0.198**	0.275**
13	Pectoral fin margin length	0.243**	0.332**
14	Sucker width	0.786**	−0.168**
15	Sucker length	0.757**	−0.239**
16	Tail length	−0.118**	0.199**

Numbers in first column correspond to measurements labeled in Fig. 2; note that tail length was not measured for individuals used in selection trials. **For df = 720, correlations greater than 0.077 are significant at $P < 0.05$ (Rohlf and Sokal 1995).

lower, narrower bodies that would be expected to reduce drag (Table 1; Blob et al. 2009). Direct directional selection for shorter lengths of the margins of the pectoral fins is also consistent with fin shapes previously reported for fish in high flow (Peres-Neto and Magnan 2004), although this result is somewhat surprising because these fins are folded against the body when juvenile *S. stimpsoni* climb (Schoenfuss and Blob 2003) and would have been expected to contribute little additional drag that might be selected against. Selection for hydrodynamic ratios also showed good correspondence with functional expectations. Successful climbers had fineness ratios closer to the optimal value for low drag (Blake 2006) than did unsuccessful climbers (Table 2; Blob et al. 2008), and survivors of predation showed fineness and flattening ratios that reflected taller bodies than those of control fish, potentially aiding production of evasive thrust, as well as lengthening ratios closer to the optimum than those of control fish, potentially reducing drag with limited reduction of thrust (Table 2). Although some results from selection experiments ran counter to functional expectations (e.g., larger heads among successful climbers) (Table 1; Blob et al. 2009), and other significant

results were difficult to interpret in a functional context (e.g., direct directional selection for larger bases to the anal fin in climbing, which might provide increased friction against backsliding), such exceptions were generally more common for the climbing experiment, and overall correspondence between the results of selection trials and the body shapes that would be expected to improve performance was good.

The calculation of selection differentials and gradients for both evasion of predators and for scaling waterfalls provided an opportunity to assess the extent of functional tradeoff between the demands posed by these pressures. It is striking that despite hydrodynamic predictions that optimal shapes for these functions should run counter to each other, very few variables (2 of 16 linear variables and 1 of 3 ratio variables) showed completely opposing patterns of selection (i.e., s' and/or β opposite in sign) between predation and climbing. More variables (5 of 16 linear) actually showed selection in the same direction across both predation and climbing trials, suggesting that the evolution of some traits (e.g., body mass) may be constrained by selection (Wilson et al. 2003). However, the most common pattern was for selection to be significant under one regime, but not the other (7 of 16 linear traits and 2 of 3 ratio variables). In the context of these results, negative values of off diagonals of the $\mathbf{F^T F}$ matrix (Ghalambor et al. 2003; Walker 2007) calculated from gradients for evading predators and for climbing waterfalls still supported the prediction of a functional tradeoff between these demands. However, the weak correlation between the gradients calculated for each of these selection pressures (Fig. 3) suggests that this tradeoff might not be as strong as initially predicted, potentially reflecting a complex, high-dimensional adaptive landscape for *S. stimpsoni* (Conrad 1990; Gavrilets 1999; Pigliucci 2008).

The significance of selection in the morphological divergence of subpopulations of Hawaiian stream gobies

Differences in stream characteristics between Kaua'i and Hawai'i (Blob et al. 2008) suggest that juvenile *S. stimpsoni* re-entering freshwater streams should experience geographic variation in the two primary functional pressures they face during migration upstream, with the demand to avoid predators preeminent on Kaua'i, and the demands of climbing waterfalls greater on Hawai'i. Differences in body shape between adult *S. stimpsoni* from these islands were well suited to meet the functional demands

believed to be primary on each island (Blob et al. 2008), raising the possibility that the differing selective regimes on each island could be promoting morphological divergence of these subpopulations. Comparisons of the morphology of juvenile *S. stimpsoni* recruiting to Kaua'i and Hawai'i support this possibility. We have not ruled out phenotypic plasticity (DeWitt et al. 1998; Domenici et al. 2008) as a factor contributing to divergence in body shape among adult *S. stimpsoni* subpopulations between islands; in fact, it may contribute to some of the disparity among islands in the difference in shape between juveniles and adults (e.g., heads are larger for fish from Hawai'i among juveniles, but larger for fish from Kaua'i among adults [Fig. 4, Table 3] [Blob et al. 2008]). However, the finding that juvenile *S. stimpsoni* differ significantly in shape upon reaching the island to which they recruit, before they are exposed to substantial selection in the stream, indicates that selection may have been contributing to the divergence of subpopulations over many generations of recruits, and that divergence in the body shapes of adults between islands is probably not a strictly plastic response. Such patterns of divergence between juveniles from different subpopulations could result if pelagic goby larvae are entrained near their source island (Taylor and Hellberg 1996) more than previous population genetic data have suggested (Fitzsimons et al. 1990; Zink et al. 1996; Chubb et al. 1998). In addition, differences in several aspects of body shape between *S. stimpsoni* from Kaua'i and Hawai'i match well between juveniles and adults and correspond to the different functional demands posed by each island (e.g., taller bodies in fish from Kaua'i, lower bodies in fish from Hawai'i) (Table 3; Blob et al. 2008). These patterns are consistent with the differing selective regimes on each island.

Further support for geographic variation in selection regimes that contributes to divergence in *S. stimpsoni* comes from consistency between the results of selection experiments and the patterns of divergence in body shape observed between juvenile fish from Kaua'i and those from Hawai'i. For example, height at mid-body and width of the body at the location of the anal pore both experience positive directional selection under predation, but negative directional selection under climbing (Table 1). Greater mid-body heights and wider bodies at the anal region are also the two highest scoring variables on PC1 and among the top variables on PC3 characterizing juvenile *S. stimpsoni* from Kaua'i (Table 3, Fig. 4), the island where selection by predators is likely of greatest prominence (Blob et al. 2008).

Head height also shows an interesting correspondence between the results of selection trials and the morphological differentiation of juveniles between islands. Contrary to adults (Blob et al. 2008), in juvenile *S. stimpsoni* head height (the third highest positive loading trait on PC1) is typically greater among fish from Hawai'i. Because production of thrust is enhanced by increased height of the posterior regions of the body relative to anterior regions (Walker 1997; Domenici 2003; Langerhans 2009b), it might be expected that the difference in head height between juvenile *S. stimpsoni* from Kaua'i and those from Hawai'i could have resulted from negative selection on head height during predation. However, our selection experiments indicate no significant selection on head height by predators; instead, we found significant "positive" selection on head height imposed by climbing (Table 1). Not only was this result somewhat surprising based on our hydrodynamic expectations (Blob et al. 2008, 2009), but it shows how complementary selection experiments can clarify the factors contributing to morphological differentiation in populations.

Future directions

One possible factor contributing to some differences we observed in patterns of selection versus the morphological differentiation seen across natural subpopulations of *S. stimpsoni* is the severity of selection we imposed. Although our failure rates in the predation and climbing experiments were ∼50% and ∼30%, respectively, the proportion of *S. stimpsoni* that fail to reproduce in nature is almost certainly much greater—for example, our own conservative estimate of rates of failure during the climbing of waterfalls in the wild was at least 90% (Blob et al. 2008). When subjected to the pressures of both climbing and predation, it is possible that only exceptional fish survive (Blob et al. 2006). Future experiments could evaluate whether similar patterns of selection emerge under more stringent failure rates, and what morphologies would emerge as successful if the pressures of predation and climbing were applied sequentially to the same animals, as they are in nature. Although we have focused on isolating the effects of selection imposed by predation or climbing, experiments subjecting survivors of predation to climbing trials could explore the interactions of these pressures in shaping the morphology of *S. stimpsoni*.

The group of roughly 30 juvenile *S. stimpsoni* from Kaua'i that plot outside the main cluster of fish from this island in our PCA of morphometric

variables (Fig. 4) suggest an intriguing possibility, potentially representing individuals that recruited to Kaua'i from other source islands, with morphology suited for different environmental conditions. Although these fish plot with the Kaua'i cluster along PC1, and with the Hawai'i cluster along PC3, they show the most extreme scores along both axes and, thus, do not exhibit clearly intermediate traits that might point toward an origin from one of the islands in the Hawaiian archipelago between Kaua'i and Hawai'i. Genetic comparisons of these individuals to fish from both of the islands in our sample, as well as the intervening islands, could resolve the likely source of these juveniles and help determine the extent to which morphological variation of fish within and between subpopulations is correlated with migration and gene flow.

An interesting result emerging from our analyses of functional tradeoffs was that the evolution of climbing performance appears to be more constrained than that of predator evasion. One factor potentially contributing to that result is that our selection trials were all conducted on fish from Hawai'i, the island where climbing performance is expected to be at a premium. If entrainment of oceanic larvae to their source island is more common than previously predicted (Zink et al. 1996, Chubb et al. 1998), then juvenile *S. stimpsoni* from Hawai'i may be derived from adults that have already been subjected to intense selection on climbing performance, leaving less opportunity for further change in response to selection, compared to potential responses to selection on other functions, such as evasion of predators. This possibility could be tested by conducting comparable selection trials on juvenile *S. stimpsoni* from other Hawaiian Islands, particularly Kaua'i, to evaluate whether the resistance of a specific functional attribute to evolutionary change varies depending on the primary pressure to which the population is exposed. Such population-level comparisons could be broadened to a wide range of other goby species, many of which are able to climb waterfalls on other volcanic island groups, ranging from the Caribbean to the Indian Ocean (Keith 2003; McDowall 2004; Maie et al. 2007). Such comparisons of the effects of similar selective pressures across a range of species are uncommon (Reznick and Ghalambor 2005; Irschick et al. 2007), but through the use of tools such as the selection experiments implemented in this study, they could provide substantial insight into the factors that link environmental variation with evolutionary divergence in the body form of fishes.

References

Arnold SJ. 1983. Morphology, performance, fitness. Am Zool 23:347–61.

Arnold GP, Weihs D. 1978. The hydrodynamics of rheotaxis in the plaice (*Pleuronectes platessa* L.). J Exp Biol 75:147–69.

Blake RW. 2004. Fish functional design and swimming performance. J Fish Biol 65:1193–222.

Blake RW. 2006. Biomechanics of rheotaxis in six teleost genera. Can J Zool 84:1173–86.

Blake RW, Li J, Chan KHS. 2009. Swimming in four goldfish *Carassius auratus* morphotypes: understanding functional design and performance employing artificially selected forms. J Fish Biol 75:591–617.

Blob RW, Bridges WC, Ptacek MB, Maie T, Cediel RA, Bertolas MM, Julius ML, Schoenfuss HL. 2008. Morphological selection in an extreme flow environment: body shape and waterfall-climbing success in the Hawaiian stream fish *Sicyopterus stimpsoni*. Int Comp Biol 48:734–49.

Blob RW, Bridges WC, Ptacek MB, Maie T, Cediel RA, Bertolas MM, Julius ML, Schoenfuss HL. 2009. Morphological selection in an extreme flow environment: body shape and waterfall-climbing success in the Hawaiian stream fish *Sicyopterus stimpsoni* (Erratum). Int Comp Biol 49:732–4.

Blob RW, Rai R, Julius ML, Schoenfuss HL. 2006. Functional diversity in extreme environments: effects of locomotor

style and substrate texture on the waterfall climbing performance of Hawaiian gobiid fishes. J Zool 268:315–24.

Blob RW, Wright KM, Becker M, Maie T, Iverson TJ, Julius ML, Schoenfuss HL. 2007. Ontogenetic change in novel functions: waterfall climbing in adult Hawaiian gobiid fishes. J Zool 273:200–9.

Carson HL, Clague DA. 1995. Geology and biogeography of the Hawaiian Islands. In: Wagner WL, Funk VA, editors. Hawaiian biogeography: evolution on a hotspot archipelago. Washington, DC: Smithsonian Institution Press. p. 14–29.

Carroll AM, Wainwright PC, Huskey SH, Collar DC, Turingan RG. 2004. Morphology predicts suction feeding performance in centrarchid fishes. J Exp Biol 207:3873–81.

Chubb AL, Zink RM, Fitzsimons JM. 1998. Patterns of mtDNA variation in Hawaiian freshwater fishes: the phylogeographic consequences of amphidromy. J Hered 89:8–16.

Conrad M. 1990. The geometry of evolution. BioSystems 24:61–81.

Coyne JA, Orr HA. 2004. Speciation. Sunderland, MA: Sinauer Associates Inc.

DeWitt TJ, Langerhans RB. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. J Sea Research 49:143–55.

DeWitt TJ, Sih A, Wilson DS. 1998. Costs and limits of phenotypic plasticity. TREE 13:77–81.

Domenici P. 2003. Habitat, body design, and the swimming performance of fish. In: Bels VL, Gasc J-P, Casinos A, editors. Vertebrate biomechanics and evolution. Oxford: BIOS Scientific Publishers. p. 137–60.

Domenici P, Turesson H, Brodersen J, Brönmark C. 2008. Predator-induced morphology enhances escape locomotion in crucian carp. Proc Roy Soc B 275:195–201.

Drucker EG, Jensen JS. 1997. Kinematic and electromyographic analysis of steady pectoral fin swimming in the surfperches. J Exp Biol 200:1709–23.

Efron B, Gong G. 1983. A leisurely look at the bootstrap, the jackknife, and cross-validation. Am Stat 37:36–48.

Ferry-Graham LA, Wainwright PC, Bellwood DR. 2001. Prey capture in long-jawed butterflyfishes (Chaetodontidae): the functional basis of novel feeding habits. J Exp Mar Biol Ecol 256:167–84.

Fitzsimons JM, Nishimoto RT. 1995. Use of fish behaviour in assessing the effects of Hurricane Iniki on the Hawaiian island of Kaua'i. Environ Biol Fish 43:39–50.

Fitzsimons JM, Schoenfuss HL, Schoenfuss TC. 1997. Significance of unimpeded flows in limiting the transmission of parasites from exotics to Hawaiian stream fishes. Micronesica 30:117–25.

Fitzsimons JM, Zink RM, Nishimoto RT. 1990. Genetic variation in the Hawaiian stream goby, *Lentipes concolor*. Biochem Syst Ecol 18:81–3.

Fuller RC, Baer CF, Travis J. 2005. How and when selection experiments might actually be useful. Int Comp Biol 45:391–404.

Garland T Jr. 2003. Selection experiments: an under-utilized tool in biomechanics and organismal biology. In: Bels VL, Gasc J-P, Casinos A, editors. Vertebrate biomechanics and evolution. Oxford: BIOS Scientific Publishers. p. 23–56.

Gavrilets S. 1999. A dynamical theory of speciation on holey adaptive landscapes. Am Nat 154:1–22.

Ghalambor CK, Reznick DN, Walker JA. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). Am Nat 164:38–50.

Ghalambor CK, Walker JA, Reznick DN. 2003. Multi-trait selection, adaptation, and constraints on the evolution of burst swimming performance. Int Comp Biol 43:431–8.

Higham TE, Malas B, Jayne BC, Lauder GV. 2005. Constraints on starting and stopping: behavior compensates for reduced pectoral fin area during breaking of the bluegill sunfish *Lepomis macrochirus*. J Exp Biol 208:4735–46.

Hoerner SF. 1958. Fluid-dynamic Drag. Midland Park, New Jersey: Published by the author.

Irschick D, Bailey JK, Schweitzer JA, Husak JF, Meyers JJ. 2007. New directions for studying selection in nature: studies of performance and communities. Physiol Biochem Zool 80:557–67.

Janzen FJ, Stern HS. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564–71.

Keith P. 2003. Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and Caribbean regions. J Fish Biol 63:831–47.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strength of phenotypic selection in natural populations. Am Nat 157:245–61.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. Evolution 37:1210–26.

Langerhans RB. 2008. Predictability of phenotypic differentiation across flow regimes in fish. Int Comp Biol 48:750–68.

Langerhans RB. 2009a. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. J Evol Biol 22:1057–75.

Langerhans RB. 2009b. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. Biol Lett 5:488–91.

Langerhans RB, Layman CA, Langerhans AK, DeWitt TJ. 2003. Habitat-associated morphological divergence in two neotropical fish species. Biol J Linn Soc 80:689–98.

Lauder GV, Drucker EG, Nauen JC, Wilga CD. 2003. Experimental hydrodynamics and evolution: caudal fin locomotion in fishes. In: Bels VL, Gasc J-P, Casinos A, editors. Vertebrate biomechanics and evolution. Oxford: BIOS Scientific Publishers. p. 117–35.

Liem KF. 1993. Ecomorphology of the teleostean skull. In: Hanken J, Hall BK, editors. The Skull, Vol. 3. Chicago: University of Chicago Press. p. 422–52.

Losos JB. 1990. The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. Evolution 44:1189–203.

Maie T, Schoenfuss HL, Blob RW. 2007. Ontogenetic scaling of body proportions in waterfall-climbing gobiid fishes from Hawai'i and Dominica: implications for locomotor function. Copeia 2007:755–64.

McDowall RM. 2003. Hawaiian biogeography and the islands freshwater fish fauna. J Biogeog 30:703–10.

McDowall RM. 2004. Ancestry and amphidromy in island freshwater fish faunas. Fish Fisheries 5:75–85.

McGuigan K, Franklin CE, Moritz C, Blows MW. 2003. Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104–18.

Mosimann JE, James FC. 1979. New statistical methods for allometry with application to Florida red-winged blackbirds. Evolution 33:444–59.

Neff NA, Marcus LF. 1980. A survey of multivariate methods for systematics. New York: Privately published.

Nishimoto RT, Fitzsimons JM. 1999. Behavioral determinants of the instream distribution of native Hawaiian stream fishes. In: Séret B, Sire J-Y, editors. Proceedings of the Fifth Indo-Pacific Fish Conference, Nouméa. Paris: Societe Francaise d'Ichtyologie. p. 813–8.

Nishimoto RT, Kuamo'o DGK. 1997. Recruitment of goby postlarvae into Hakalau Stream, Hawai'i Island. Micronesica 30:41–9.

Peres-Neto PR, Magnan P. 2004. The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species. Oecologia 140:36–45.

Pettersson LB, Hedenström A. 2000. Energetics, cost reduction, and functional consequences of fish morphology. Proc R Soc Lond B 267:759–64.

Pigliucci M. 2008. Is evolvability evolvable? Nat Rev Gen 9:75–82.

Reznick DN, Ghalambor CK. 2005. Selection in nature: experimental manipulations of natural populations. Int Comp Biol 45:456–62.

Rivera G. 2008. Ecomorphological variation in shell shape of the freshwater turtle Pseudemys concinna inhabiting different aquatic flow regimes. Int Comp Biol 48:769–87.

Rohlf FJ, Sokal RR. 1995. Statistical Tables. 3rd Edition. New York: WH Freeman.

Rosenberger LJ. 2001. Pectoral fin locomotion in batoid fishes: undulation versus oscillation. J Exp Biol 204:379–94.

Schluter D. 2001. Ecology and the origin of species. TREE 16:372–80.

Schoenfuss HL, Blanchard TA, Kuamo'o DGG. 1997. Metamorphosis in the cranium of postlarval Sicyopterus stimpsoni, an endemic Hawaiian stream goby. Micronesica 30:93–104.

Schoenfuss HL, Blob RW. 2003. Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface. J Zool 261:191–205.

Schoenfuss HL, Blob RW. 2007. The importance of functional morphology for fishery conservation and management: applications to Hawaiian amphidromous fishes. Bishop Mus Bull Cult Environ Stud 3:125–41.

Swain DP. 1992. Selective predation for vertebral phenotype in Gasterosteus aculeatus: reversal in the direction of selection at different larval sizes. Evolution 46:998–1013.

Taylor MS, Hellberg ME. 1996. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–9.

Vogel S. 1994. Life in Moving Fluids: The Physical Biology of Flow. 2nd Edition. Princeton, NJ: Princeton University Press.

Wainwright PC, Bellwood DR, Westneat MW. 2002. Ecomorphology of locomotion in labrid fishes. Env Biol Fishes 65:47–62.

Wainwright PC, Reilly SM. 1994. Ecological Morphology: Integrative Organismal Biology. Chicago: University of Chicago Press.

Walker JA. 1997. Ecological morphology of lacustrine threespine stickleback Gasterosteus aculeatus L. (Gasterosteidae) body shape. Biol J Linn Soc 61:3–50.

Walker JA. 2000. Does a rigid body limit maneuverability? J Exp Biol 203:3391–6.

Walker JA. 2007. A general model of functional constraints on phenotypic evolution. Am Nat 170:681–9.

Walker JA, Alfaro ME, Fulton CJ. 2010. Fluid dynamic drag, body shape, and endurance swimming performance among coral reef fishes. SICB 2010 Annual Meeting Abstracts. http://www.sicb.org/meetings/2010/SICB2010-oral.pdf.

Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN. 2005. Do faster starts increase the probability of evading predators? Funct Ecol 19:808–15.

Walker JA, Westneat MW. 2002. Performance limits of labriform propulsion and correlates with fin shape and motion. J Exp Biol 205:177–87.

Webb PW. 1975. Hydrodynamics and energetics of fish propulsion. Bull Fish Res Bd Can 190:1–159.

Webb PW. 1978. Fast-start performance and body form in seven species of teleost fish. J Exp Biol 74:211–26.

Webb PW. 1984. Body form, locomotion, and foraging in aquatic vertebrates. Am Zool 24:107–20.

Webb PW. 1986. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (Micropterus salmoides). Can J Fish Aquat Sci 43:763–71.

Webb PW. 1989. Station-holding by three species of benthic fishes. J Exp Biol 145:303–20.

Westneat MW. 1994. Transmission of force and velocity in the feeding mechanism of labrid fishes (Teleostei, Perciformes). Zoomorph 114:103–18.

Wilson AJ, Hutchings JA, Ferguson MM. 2003. Selective and genetic constraints on the evolution of body size in a stream-dwelling salmonid fish. J Evol Biol 16:584–94.

Zink RM, Fitzsimons JM, Dittmann DL, Reynolds DR, Nishimoto RT. 1996. Evolutionary genetics of Hawaiian freshwater fish. Copeia 1996:330–5.

Civil No. 19-1-0019-01 (JPC)
Defendant A&B/EMI's Exhibit AB-139
FOR IDENTIFICATION _____
RECEIVED IN EVIDENCE _____
CLERK _____