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Ontogenetic Change in a Novel Function:

Waterfall Climbing in Adult Hawaiian Gobiid Fishes

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Abstract

Juveniles from three species of Hawaiian gobiid fishes climb waterfalls as part of an amphidromous life cycle, allowing them to re-penetrate adult upstream habitats after being swept out to the ocean upon hatching. The importance of climbing for juvenile stream gobies is well established, but adult fishes in upstream island habitats also face potential downstream displacement by catastrophic disturbances. Thus, retention of climbing ability could be advantageous for adult stream gobies. Climbing performance might be expected to decline among adults, however, due to the tendency for mass specific muscular power production to decrease with body size, and a lack of positively allometric growth among structures like the pelvic sucker that support body weight against gravity. To evaluate changes in waterfall climbing ability with body size in Hawaiian stream gobies, we compared climbing performance and kinematics between adults and juveniles from three species (*Awaous guamensis*, *Sicyopterus stimpsoni*, and *Lentipes concolor*). For species in which juveniles climbed using "powerbursts" of axial undulation, adult performance and kinematics showed marked changes: adult *A. guamensis* failed to climb, and adult *L. concolor* used multiple pectoral fin adductions to crutch up surfaces at slow speeds, rather than rapid powerbursts. Adult *S. stimpsoni*, like juveniles, still alternated attachment of oral and pelvic suckers to "inch" up surfaces and climbed at speeds comparable to those of juveniles. However, unlike juveniles, adult *S. stimpsoni* also add pectoral fin crutching to every climbing cycle. Thus, although powerburst species appear particularly susceptible to size-related declines in waterfall climbing performance, the addition of compensatory mechanisms prevents complete loss of this novel function in at least some species.

Introduction

Many of the functional demands that animals face result from the physical forces placed on them by the external environment. As animals grow, these forces may change as a function of an animal's increase in size. To accommodate growth-related changes in external forces, animals might exhibit compensatory mechanisms such as allometric growth of support or propulsive structures (Carrier, 1983, 1996; Toro et al., 2003; Blob, 2006), or changes in behavior (Higham et al., 2005), that help to maintain functional performance at a comparable level throughout growth. Alternatively, without such mechanisms, the performance of some functions could decrease as juveniles mature into adults, even if those functions might continue to be advantageous among larger individuals (Maie, Schoenfuss & Blob, in review).

The freshwater fauna of small volcanic islands are faced with particularly challenging environmental conditions. Streams on these islands are commonly subject to flash floods, high velocity flows, and waterfalls that obstruct access to upstream reaches (Fitzsimons & Nishimoto, 1995). Although the physical forces exerted by these hazards can be quite rigorous for stream-dwelling animals, many species have evolved novel life history strategies and functional capacities that allow them to accommodate such demands. For example, in the Hawaiian Islands, the five native species of stream fishes (four gobies and one eleotrid: Fitzsimons, Nishimoto & Yuen, 1993) hatch in freshwater but are swept by currents to the ocean, where they share an oceanic larval phase for several months before returning to freshwater habitats as juveniles (Ego,

1956; Fitzsimons & Nishimoto, 1995, 1996; Julius, Blob & Schoenfuss, 2005). Two of

these species (*Eleotris sandwicensis* and *Stenogobius hawaiiensis*) stay in lower

stream reaches for the rest of their lives as they grow into adults. However, the three

remaining species face further challenges because the adult stream habitats to which

they return are in upstream reaches that are frequently obstructed by waterfalls ranging

from hundreds to thousands of body lengths in height (Fitzsimons & Nishimoto, 1995;

Schoenfuss & Blob, 2003). Juveniles of these species have been observed using two

different mechanisms to climb these waterfalls (Nishimoto & Kuamo'o, 1992;

Schoenfuss & Blob 2003; Blob et al., 2006). Juveniles of *Awaous guamensis* and

Lentipes concolor propel themselves against falling water by using rapid "powerbursts"

of axial undulation initiated by pectoral fin adduction, which are then followed by periods

of rest during which fish adhere to the substrate using a ventral sucking disc formed by

fusion of the pelvic fins. In contrast, the third species, *Sicyopterus stimpsoni*, slowly

inches up vertical surfaces by alternately attaching the pelvic disc and a second, oral

disc to the substrate with little or no fin motion or axial undulation. The ability of juvenile

gobies to scale waterfalls using these mechanisms allows the repopulation of upstream

habitats by oceanic larvae even after catastrophic disturbances such as floods or

hurricanes (Fitzsimons & Nishimoto, 1995, 1996).

The importance of climbing to the juvenile life stages of *A. guamensis*, *L.*

concolor, and *S. stimpsoni* has been well established by previous studies. However,

adult fishes in upstream Hawaiian habitats face the same demanding environmental

conditions as juveniles, such as rapid stream flows and displacement by catastrophic

disturbances (Fitzsimons & Nishimoto, 1995). As a result, the retention of climbing

ability could be a considerable advantage to adult fishes. However, as body size

increases among adult fishes, their ability to climb waterfalls might decline for several

reasons. First, given the potential for mass specific power production to decrease with

body size (Wilson, Franklin & James, 2000; Irschick et al., 2003; Toro et al., 2003), size-

related limits to power production might restrict the maximum body size at which

"powerbursting" in particular is a viable style of climbing (Blob et al., 2006). This factor

might limit the ability of *A. guamensis* and *L. concolor* to climb as adults, or force these

species to adopt different climbing mechanics. Power demands might not substantially

limit climbing by *S. stimpsoni* because of the slow cycle frequencies of inching and

because this species keeps one sucker attached to the substrate throughout climbing

bouts (Schoenfuss & Blob, 2003). However, studies of ontogenetic scaling of body

proportions in gobies (including *S. stimpsoni*) have shown that the area of the pelvic

sucking disc grows isometrically with body length (i.e., scales $\propto BL^2$) and, thus, does not

keep pace with increases in the mass of the body ($\propto BL^3$) that it must keep attached to

the substrate (Maie et al., in review). As a result, even inching climbers like *S.*

stimpsoni might experience a decline in waterfall climbing performance as they grow

larger in size.

In this study we test for size-related changes in climbing performance and

mechanisms by evaluating the climbing of adult Hawaiian stream gobies (*A. guamensis*,

L. concolor, and *S. stimpsoni*) and comparing their performance and kinematics to

previous data (Schoenfuss & Blob, 2003; Blob et al., 2006) from juveniles of the same

species. Because of possible limits to muscular power production and isometric scaling

of the adhesive sucker, we expect the climbing performance of adults to decrease

relative to the performance of conspecific juveniles. We expect these changes to be especially dramatic for powerburst climbing species because the high frequencies of their locomotor movements (Schoenfuss & Blob, 2003) are particularly disadvantageous for the high power production that would be required for climbing by larger fishes. Thus, species that use powerburst climbing as juveniles also might require major changes in locomotor behavior if they are to climb successfully as adults.

Materials & Methods

During three field seasons between 2003-2005, adult individuals of the waterfall climbing species of Hawaiian stream gobies were collected with 'opae nets (fine mesh nets with a narrow opening at the top of a bowl shaped basket) from their native habitats above waterfalls in the mid-stream (*A. guamensis* and *S. stimpsoni*) or upstream (*L. concolor*) reaches of Hakalau Stream on the Island of Hawai'i. Within two hours of capture, animals were transferred in stream water to a research facility provided by the Hawai'i Department of Land and Natural Resources, Division of Aquatic Resources (DAR). Animals were separated by species into small groups of three to five individuals and then acclimated together for several hours at ambient stream temperatures (19 °C). Trials were conducted on individuals that overlapped in body size (Table 1).

Our experimental arena consisted of a climbing chute that extended up from a catch basin (adapted from Blob et al., 2006). The basin was 60 cm long x 45 cm wide x

15 cm deep, half filled with acclimated Hakalau stream water and contained a rock to provide cover for the fish. Climbing chutes were constructed from 1.5 m long sections of 4-inch diameter PVC pipe (cut in half longitudinally) and were extended from the basin at 57° from horizontal. To generate flow over the climbing surface, a siphon was used to direct acclimated Hakalau stream water from a 20 l bucket down the chute at a flow rate of 200 ml min^{-1} (within the range of flow rates these fish encounter in nature: HLS, pers. obs.). We began each climbing trial by introducing groups of three to five fish (from a single species) into the catch basin with water flowing down the chute. If climbing did not commence within 20 min of the introduction of the fish, all animals were removed and a new group of fish was placed into the basin.

Climbing performance and kinematics were tested on chutes with fine-grained sand (grain size mean $\pm$ S. D. = 392 $\pm$ 99 μm; range 250 – 675 μm; n = 50) attached to the pipe with spray glue. For several days prior to the climbing trials, stream water was run over these chutes to remove any chemical residue. A distance scale was marked on the cut surface of each PVC chute, out of sight of the climbing fish.

Two types of video records were collected from these trials. First, to measure climbing performance over a distance of several body lengths, a Sony DTV 1020 digital camcorder was used to film animals in dorsal view at a framing rate of 30 Hz as they climbed up the chute. The camera's field of view was adjusted to cover a 20 cm segment of pipe approximately 10 cm above the water level in the catch basin (water level in the catch basin varied slightly as water drained from the chute into the basin). Each animal was used in only a single trial and then returned to the stream. To follow Hawaiian tradition, we also returned the rock providing cover for the fish to the stream at

the end of the filming sessions. Trials were only analyzed if video records indicated that

the fish had climbed the entire 20 cm segment. Footage from mini DV tapes was

transferred to an Apple Macintosh G4 computer using iMovie software. One of us (MB)

tracked individual animals frame by frame in the videos. The beginning and end of each

climbing bout was recorded, as were rest periods between sequential climbing bouts. In

addition, instances of pectoral fin and tail use during climbing bouts were also recorded.

Second, to evaluate details of climbing kinematics for adult gobies, high-speed

digital video recordings were collected for a subset of trials using either a Phantom V4.1

(200 Hz) or Redlake Motionscope (250 Hz) high-speed camera. Fish were filmed in

dorsal view as they climbed up the chute (as in 30 Hz videos). High-speed video

sequences of climbing were saved as .AVI files, and the positions of landmarks on the

bodies of the fishes were digitized for each frame of analyzed climbing cycles using a

modification of the public domain NIH Image program for Apple Macintosh, developed

at the U.S. National Institutes of Health and available on the internet at

http://rsb.info.nih.gov//nih-image/ (the modification, QuickImage, was developed by J.

Walker and is available at http://www.usm.maine.edu/~walker/software.html). For the

inching climber *S. stimpsoni*, points at the base and tip of both pectoral fins were

digitized along with four points on the head representing the maximal anterior, left, and

right margins of the oral sucker, as well as the midpoint between the eyes. At least nine

additional points along the midline body axis were also digitized, with the most posterior

point representing the base of the caudal peduncle. For powerburst climbing species

without an oral sucker, the same points were digitized except for those on head, for

which the three sucker landmarks for *S. stimpsoni* were replaced with points at the

anterior midline of the face and the left and right eyes.

Custom programs written in Matlab 5.0 (Mathworks, Inc.; Natick, MA, USA) were

used to calculate kinematic variables from digitized coordinate data. For all trials, fin

movements and the displacement of the front lip through the course of each cycle were

evaluated. To quantify fin movements, the angle of each fin relative to the direction of

travel (determined by the two midline head points) was calculated for each frame,

allowing us to calculate a profile of fin movements throughout locomotor cycles and

determine fin excursion angles. In addition, for *S. stimpsoni*, an index of the

instantaneous area of the oral sucker also was calculated as:

$$\text{mouth area} = (\pi/4)\ (D_{LR})\ (D_{AP}) \tag{1}$$

where D_{LR} is the instantaneous distance between the left and right edges of the mouth,

and D_{AP} is the instantaneous distance from the point between the eyes to the point

marking the anterior edge of the mouth. This index simplifies the shape of the mouth

and, therefore, does not measure the actual area of the oral disc; however, it allows us

to evaluate changes in anteroposterior and lateral mouth dimensions that effectively

track whether the oral disc is increasing or decreasing in area (Schoenfuss & Blob,

2003). After evaluating these parameters, we used QuickSAND software (Walker,

1998; program available at http://www.usm.maine.edu/~walker/software.html) to fit a

quintic spline to the kinematic calculations for each trial, smooth the data, and normalize

all trials to the same duration in order to calculate mean kinematic profiles for each variable.

Statistical analyses were performed using GraphPad Prism version 4.0c for Macintosh (San Diego, California, USA) or Statview 5.0 for Macintosh (SAS Institute, Inc., Cary, NC, USA). Mean durations of single locomotor cycles were calculated from high-speed video trials, whereas mean durations of climbing and rest periods for entire bouts of climbing (over the 20 cm distance) were calculated from standard video trials. Net climbing speed (accounting for rest periods) was calculated by dividing the 20 cm distance climbed by the sum of the climbing and rest times. In addition, the percentage of time that each individual spent in motion (described as "duty factor" by Weinstein, 2001) was calculated as the time spent in motion divided by the sum of the motion and rest time periods. The significance of differences in kinematic and performance variables between species and between juveniles and adults of the same species were evaluated using non-parametric Mann-Whitney U tests.

Results

Climbing by Adult Gobies: Intraspecific Comparisons to Juveniles

Awaous guamensis

In stark contrast to the behavior of powerbursting juveniles (Schoenfuss & Blob, 2003; Blob et al., 2006), none of 10 adult *A. guamensis* successfully climbed (or even

attempted to climb) the experimental apparatus. Since adults of the other two focus

species were able to climb (see following sections), and field and lab experience have

not indicated adult *A. guamensis* to be especially skittish in their behavior (RWB, TM &

HLS, pers. obs.), the failure of *A. guamensis* to climb seems unlikely to be an artifact of

the climbing apparatus itself or our experimental design. On the contrary, the lack of

climbing by *A. guamensis* corresponds to observations from Kaua'i populations, in

which streams devastated by Hurricaine Iniki showed delays of several months before

the reappearance of reproductive adult *A. guamensis* (Fitzsimons & Nishimoto, 1995).

[HEIKO – see note in Discussion about checking accuracy of this statement]

Sicyopterus stimpsoni

Much like juvenile *S. stimpsoni*, adults use inching movements to climb waterfalls (Figs.

2a-d, 3a-e). As in juveniles, adult climbing cycles begin with a decrease in mouth area

(Fig. 3b, d), reflecting detachment of the oral sucker from the substrate (Schoenfuss &

Blob, 2003). During this time there is little forward advancement of the body in adults

until the minimum mouth area (i.e., sucker detachment) is achieved (Fig. 3c-d).

However, unlike climbing by juveniles, in adult *S. stimpsoni* each climbing cycle is

accompanied by moderate angular excursions of the pectoral fins (Figs 2a-d, 3e). From

an initial position with the pectoral fin tips pointed posteriorly, by mid-cycle the fins

abduct anteriorly to an angle nearly 40° from a vector pointing posteriorly along the

body midline (Table 1; Figs. 2a-c, 3e). This position is held through the middle third of

the climbing cycle (Fig. 3e), during which time the body begins to advance (Fig. 3c).

Thus, initial pectoral fin movements may put the fins into a position that helps to hold adult *S. stimpsoni* in place on the substrate, but initial forward movement of adults in this species appears to be achieved strictly through advancement of the head. Advancement of adults through the final third of the climbing cycle, however, is synchronous with adduction of the pectoral fins back to their initial position, suggesting that the pectoral fins might make contributions to climbing propulsion in adult *S. stimpsoni* that are not observed in juveniles. Some individuals made occasional moderate bending movements of the distal tail during some climbing cycles, but this behavior occurred irregularly. Because climbing proceeded for many cycles without these movements, they do not appear necessary for climbing to succeed.

With regard to climbing performance, adults show a small (0.016 s), statistically significant increase in climbing cycle duration compared to juveniles, but a much larger (and significant) decrease (~37%) in the duration of climbing bouts (i.e., sequences of consecutive climbing cycles: Table 1). In absolute terms, adult *S. stimpsoni* climbed the 20 cm distance significantly (>3 times) faster than juveniles; however, the adults evaluated were almost 2.5 times larger than juveniles, and a difference in climbing speed was no longer evident between juveniles and adults when their speeds were normalized by body length (Table 1). In fact, when climbing speed was calculated based only on the duration of time that fish were actually moving (i.e., speed during motion without consideration of time spent resting between bouts of consecutive climbing cycles), juveniles are slightly (but significantly) faster than adults (Table 1). Adults spent a greater portion of time than juveniles moving, rather than resting (67±16% versus 54±21%), but high variance in these measurements decreased our

power to reject the null hypothesis of no difference between adults and juveniles in this

parameter.

Lentipes concolor

L. concolor adults showed dramatic changes in climbing kinematics compared to

juveniles, changes that are so substantial that it is inappropriate to describe adult *L.*

concolor as "powerburst" climbers. First, in contrast to juveniles, in adult *L. concolor*

rapid undulation of the body axis is absent during climbing (Fig. 2e-h). In addition, adult

L. concolor use a sequence of pectoral fin abduction and adduction with every

locomotor cycle, not just a single phase of fin adduction to initiate a climbing bout.

Unlike juveniles, in which the pectoral fins are only adducted once to initiate a bout of

climbing (Schoenfuss & Blob, 2003), in adults a cycle of pectoral fin abduction and

adduction accompanies every climbing cycle. Abduction begins early in the cycle,

advancing to an average angle 107.1±29.2° from a vector pointing posteriorly along the

body midline, a value that does not differ significantly from that exhibited by powerburst

climbing juveniles (Figs. 2f, 3g; Table 1). Pectoral fin adduction begins soon after the

peak abduction angle is achieved (Fig. 3g) and is closely followed by upward

advancement of the body at approximately 60% through the climbing cycle (Figs. 2g-h,

3f). As in *S. stimpsoni*, some *L. concolor* made moderate bending movements of the

distal tail in some climbing cycles, but this behavior was irregular and climbing

frequently took place without any lateral tail movements.

Climbing performance of adult *L. concolor* also shows some substantial

differences from that of juveniles. Although the duration of adult climbing cycles is

significantly (over ten times) longer than that of juveniles, the duration of climbing bouts does not increase significantly among large individuals (Table 1). Moreover, the absolute climbing speed of adults (not normalized for body size) is also not significantly different from that of juveniles (Table 1). As a result, climbing speeds normalized for body size are more than eight times lower in adult *L. concolor* than in juveniles (Table 1). To achieve even these lower levels of performance, adults must spend a significantly greater portion of time moving, rather than resting (37±19%), compared to juveniles (22±11%: Table 1).

Interspecific Comparisons of Adult Climbing in Hawaiian Stream Gobies

Although the two species of Hawaiian stream gobies that retain the ability to climb as adults (*S. stimpsoni* and *L. concolor*) used different styles of climbing as juveniles (inching versus powerbursts), as adults the kinematics and performance of these species become much more similar. Pectoral fin excursion angles differ significantly between adults of these species (with greater angles achieved by *L. concolor*: Table 1), but similarity is demonstrated between the species because, unlike juveniles, *S. stimpsoni* and *L. concolor* adults both incorporate pectoral fin abduction/adduction sequences with every locomotor cycle (although fin adduction in *L. concolor* is more closely synchronized with body advancement: Fig. 3). Both species also show very similar locomotor cycle durations as adults (Table 1). Although mean cycle durations are still statistically different between adults of these species, the difference between adult *S. stimpsoni* and *L. concolor* (0.251 s versus 0.229 s) is much less than that

between juveniles of these species (0.235 s versus 0.02 s: Table 1). Climbing bout

durations show a similar pattern, with mean values for adults still significantly different

between the species, but converging much more closely (4.7 s versus 2.7 s for *S.*

stimpsoni and *L. concolor*, respectively) than is typical in juveniles (7.6 s versus 2.0 s).

Convergence in locomotor performance between adult *S. stimpsoni* and *L.*

concolor is also evident through comparisons of their climbing speed. Although juvenile

L. concolor are dramatically faster climbers than juvenile *S. stimpsoni* (Schoenfuss &

Bllob, 2003; Blob et al., 2006), as adults the climbing performance of these species is

much more similar (Table 1; Fig. 4a-b). Specifically, speeds of *L. concolor* have

converged on those of *S. stimpsoni*: speeds of adult *S. stimpsoni* differ little from those

of juveniles when normalized for body length, but speeds of adult *L. concolor* have

decreased substantially from those of juveniles, so much so that adult *L. concolor* are

significantly slower than adult *S. stimpsoni* (Table 1; Fig. 4b). One comparison that

remains similar in adults to patterns observed in juveniles is that adult *S. stimpsoni* still

spend a significantly longer portion of time in motion than adult *L. concolor* (Table 1;

Fig. 4c). However, as noted previously, adults of both species show an increase in the

portion of time spent moving over that observed in juveniles (Table 1).

Discussion

Body size has pervasive effects on animal function, many of which relate to changes in

the forces that animals experience as they grow (McMahon, 1973; Schmidt-Nielsen,

1984; LaBarbera, 1989; McHenry & Lauder, 2005). Unless growing animals adopt

mechanisms to accommodate these changing forces, the performance of a variety of

functions might be impaired or precluded among larger individuals (Maie et al., in

review). The three species of Hawaiian stream gobies capable of climbing waterfalls as

juveniles show differing functional responses as they increase in size. In *A. guamensis*,

the ability to climb waterfalls appears to have been lost among adult individuals.

However, in the other two species of Hawaiian stream gobies, moderate to dramatic

changes in locomotor kinematics appear to preserve at least some degree of climbing

ability among adults, allowing continued opportunity for the use of this novel behavior to

meet the functional requirements of a habitat with extreme physical demands.

Ontogenetic Changes in Waterfall Climbing: Mechanisms for Accommodating

Larger Body Size

Two general mechanisms might enable animals to accommodate size-related increases

in the locomotor forces that they experience (or must produce) as a result of growth:

allometric increases in morphological features (e.g., fin length or breadth) or

physiological parameters (e.g., muscle force generation); or, changes in locomotor

behavior. Although morphological and physiological scaling have not been completely

surveyed in climbing gobies, at least one morphological feature integral to climbing, the

area of the pelvic sucker, fails to exhibit allometric growth that could help to

accommodate the greater gravitational forces that must be resisted by larger individuals

as they climb vertical surfaces (Maie et al., in review). This led us to predict that if adult

gobies were able to climb successfully, they would be expected to show behavioral adjustments relative to juveniles that would facilitate the maintenance of climbing performance. Such adjustments appeared in both goby species (*S. stimpsoni* and *L. concolor*) that continued to climb as adults. In adults of both species, movements of the pectoral fins were added to every climbing cycle, contrasting with climbing patterns in juveniles in which the pectoral fins were used once (*L. concolor*) or not at all (*S. stimpsoni*) during climbing bouts. In *S. stimpsoni* pectoral fin movements are less extensive than in *L. concolor* and fin adduction is delayed until well after forward advancement has already commenced (Fig. 3c, e). Thus, extension of the fins may initially help stabilize the body of *S. stimpsoni* against the substrate during extension of the head, only aiding forward propulsion toward the end of each climbing cycle. In contrast, the broadly sweeping adductions of the pectoral fins in *L. concolor* are closely synchronized with upward advancement of the body, and appear to provide the primary upward thrust in this species in which axial motions are severely reduced compared to powerburst climbing juveniles.

Size-related changes in climbing performance and kinematics among the species that used powerburst climbing as juveniles were more drastic than those exhibited by the inching climber *S. stimpsoni*: one powerburst species (*A. guamensis*) did not climb as adults, and the other (*L. concolor*) shifted from using the body axis as a main propulsive structure to primary reliance on the pectoral fins. This divergence in climbing performance and mechanics between juveniles and adults suggests that powerbursts, as used by juveniles, may no longer be a viable climbing mechanism for larger individuals. *A. guamensis* and *L. concolor* do not appear incapable of performing the

body movements associated with powerburst climbing as adults: the body axis movements seen among juvenile climbers (Schoenfuss & Blob, 2003) can still be observed in swimming adults (RWB and HLS, pers. obs.), and the pectoral fin movements used by climbing adult *L. concolor* simply represent more frequent use of a behavior (performed over the same range of angles) exhibited by juveniles (Table 1). Instead, the use of powerburst climbing might be precluded because of its high demand for muscular power production (Blob et al., 2006). Ascension of vertical surfaces typically has high power requirements (Dial, 2003; Irschick et al., 2003), but muscles contracting at the high frequencies used during powerburst climbing tend to have reduced power producing capacities (Johnson et al., 1994; Rome et al., 1996; Rome, 1998). Because mass-specific power production also tends to decrease with body size (Wilson et al., 2000; Irschick et al., 2003; Toro et al., 2003), it might not be possible to climb using rapid movements at larger body sizes, leaving slower movements like pectoral fin crutching and the inching behavior of *S. stimpsoni* as the only viable mechanisms for adult gobies to use to scale waterfalls.

Physiological Implications of the Climbing Performance of Adult Hawaiian Stream Gobies

Like juveniles, both species that successfully climb waterfalls as adults still use intermittent bouts of locomotion during climbing. For other species moving over terrestrial substrates, the use of intermittent locomotion has the potential to significantly increase the distance that animals are able to travel before fatigue (Full & Weinstein,

1992; Weinstein & Full 1992, 1998, 1999, 2000). This benefit results if pauses are of

appropriate duration (absolute and relative to the time of motion) to allow the removal of

fatigue-inducing products (such as inorganic phosphate) and the restoration of

metabolic fuels (Weinstein & Full, 2000; Allen & Westerblad, 2001; Kramer &

McLoughlin, 2001). Intermittent bouts of inching by *S. stimpsoni* juveniles did not

appear to meet these criteria (Blob et al., 2006), as their duration of activity ranged up to

twice as long as their rest periods, a ratio that did not result in performance

improvements in other studies (Weinstein & Full, 1998, 1999). Adult *S. stimpsoni* did

not differ significantly from juveniles in the ratio of time that they spent climbing versus

resting (Table 1); thus, it appears that adults of this species are also unlikely to increase

the distance that they travel before fatigue through the use of intermittent bouts of

climbing. In contrast, juvenile powerburst climbers rest for time periods from three to

five times longer than they spend moving (Blob et al., 2006). With the short durations of

their periods of activity (< 2 s), the distribution of motion and rest used by juvenile

powerburst climbers is appropriate to increase their pre-fatigue travel distance

(Weinstein & Full, 1998, 1999) at the cold temperatures typical of their stream habitats

(18-19° C: Schoenfuss, Julius, & Blob, 2004). Although adult *L. concolor* rested for a

significantly shorter portion of their climbing trials than juvenile *L. concolor*, they still

rested for over 1.7 times the duration of their activity periods (i.e., the ratio of motion to

rest time was 0.59); in addition, adult locomotor bouts did not last significantly longer

than those of juveniles (Table 1). This ratio of activity to rest periods is close to the ratio

of 0.5 observed to increase distance capacity in species ranging from crabs to geckos

(Weinstein & Full, 1998, 1999). Thus, although adult *L. concolor* use climbing

mechanisms substantially different from those of juveniles, they may still achieve some of the same performance benefits.

The slow speed of climbing by adult gobies may also help to prevent fatigue during this behavior. Climbing cycle frequencies for adult *S. stimpsoni* and *L. concolor* (calculated from climbing cycle durations listed in Table 1) average 4.0 and 4.4 Hz, respectively, and climbing speeds for both species (considering periods of motion only) are less than 0.25 body lengths s^{-1} (Table 1). Motion at such low speeds and frequencies in fishes is typically powered by slow oxidative (red) muscle without accompanying activity in other fiber types (Jayne & Lauder, 1993, 1994; Gillis, 1998; Altringham & Ellerby, 1999). If adult gobies used only red muscle during climbing, their potential for fatigue could be reduced substantially because the activity of red muscle depends on aerobic metabolism that is well suited for endurance behaviors (Driedzic & Hochachka, 1978; Sänger & Stoiber, 2001). Extensive development of fast glycolytic (white) fiber would still be expected in adults of these species, however, as they would be of great importance for the swimming in rapid currents that likely constitutes the majority of locomotion in these animals.

Ecological Implications of the Climbing Performance of Adult Hawaiian Stream Gobies

The climbing performance exhibited by adult stream gobies can be related to several aspects of the ecology of these species. First, because *A. guamensis* juveniles have

been shown to be the poorest climbers among juveniles of the three climbing stream

goby species (Blob et al., 2006), it is not surprising that this species appears to have

lost the ability to climb as adults that was retained by the other species we examined.

Moreover, if climbing ability has been lost among larger *A. guamensis*, it would help to

explain why this species showed the greatest delay in the upstream appearance of

reproductive adults in streams on the island of Kaua'i that were devastated by

Hurricaine Iniki in 1992, as repopulation may have been achieved solely by small

individuals and juveniles migrating in from the ocean (Fitzsimons & Nishimoto, 1995).

[HEIKO – please check if you feel this statement in red is accurate – I only have the

Env. Biol. Fishes paper to go on, not the one in the Devick volume].

One of the most striking features of the climbing behaviors exhibited by juvenile

Hawaiian stream gobies is the diversity of climbing mechanisms and levels of

performance that they employ. Not only do juveniles exhibit two dramatically different

styles of climbing (inching versus powerbursts), but the performance exhibited by

different species of powerburst climbers also differs dramatically. This diversity

resembles the "many-to-one" mapping of morphology to function seen in studies of jaw

function in labrid fishes (Alfaro, Bolnick & Wainwright, 2005; Wainwright et al., 2005),

and indicates that a wide range of performance capabilities can be maintained even

under the strong selective pressures of extreme environmental conditions (Blob et al.,

2006). However, among adult climbers this functional diversity appears to be

diminished, suggesting a "fewer-to-one" mapping of performance to functional demand.

[second half of previous sentence too over the top?] Although there are still statistically

significant differences in the values of several performance variables between adult *S.*

stimpsoni and *L. concolor*, the magnitude of those differences is considerably smaller between adults than it was between juveniles (Table 1). Moreover, adults of both species incorporate use of the same additional propulsive structure (adduction by the pectoral fins) beyond those seen in juveniles (Table 1). Thus, adult *S. stimpsoni* and *L. concolor* appear to have converged considerably in their strategies and capacity for climbing waterfalls. Although functional diversity might be maintained among juveniles of these species despite strong selective pressure for successful waterfall climbing, the additional pressures of increasing body size may finally limit the range of mechanics and performance that can be used to execute this novel behavior.

Acknowledgements

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FIGURE LEGENDS

Figure 1 (a) Falls of Nanue Stream on the Hamakua Coast of the island of Hawai'i, a

waterfall climbed by juveniles of all three species of climbing Hawaiian stream gobies:

powerburst climbing *Awaous guamensis* and *Lentipes concolor*, and the inching climber

Sicyopterus stimpsoni. (b) Photo of adult *A. guamensis*. (c) Photo of adult male *L.

concolor*. (d) Photo of adult male *S. stimpsoni*.

Figure 2 Still images from high-speed video of adult Hawaiian stream gobies climbing

up the chute of the artificial waterfall arena. (a-d) *Sicyopterus stimpsoni*. (e-h) *Lentipes

concolor*. In each panel, left pectoral fin of the fish is outlined with a dashed line, and

time (in milliseconds, ms) through a single climbing cycle for each species is indicated

in the lower left corner of the panel.

Figure 3 Mean profiles of kinematic variables for *Sicyopterus stimpsoni* juveniles (a-b, *n*

= 26: data replotted for comparison with adults from Schoenfuss & Blob, 2003),

Sicyopterus stimpsoni adults (c-e, *n* = 23), and *Lentipes concolor* adults (f-g, *n* = 32).

Plots for the same variable are organized in columns to facilitate comparison among

these groups. Variables include cumulative displacement of the front lip since the start

of the cycle, measured in body lengths (a, c, f); instantaneous area of the mouth (i.e.,

oral sucker: b, d); and excursion angle of the pectoral fin (e, g). Each climbing cycle

was normalized to the same duration and values of kinematic variables were

interpolated for 25 equally spaced increments through the cycle using QuickSAND

software (Walker, 1998). Plots for each variable illustrate means ± 1 SE for each 4%

increment of time through the cycle. Fin excursion angle was not plotted for juvenile *S. stimpsonsi* and mouth area was not plotted for adult *L. concolor* because these groups did not produce the movements represented by these variables. No plots are shown for adult *Awaous guamensis* because climbing was not elicited from adults of this species.

Figure 4. Box plots comparing (a) absolute climbing speed (in cm s-1), (b) relative climbing speed normalized for body length (in BL s-1), and (c) portion of time spent moving (% time in motion) for adults of the Hawaiian stream gobies *Sicyopterus stimpsoni* (n = 25) and *Lentipes concolor* (n = 43). Speeds are calculated over the entire 20 cm climbing distance (see text) and account for periods of rest between bouts. For each plot, the box extends from 25th to 75th percentile, with a line indicating the median. Bars demarcate the range of values. *** Significant difference at P < 0.001 (Mann-Whitney U-test).