Finding paradise: cues directing the migration of the waterfall climbing Hawaiian gobioid \textit{Sicyopterus stimpsoni}


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A series of waterfall-climbing trials were conducted to identify cues that direct the climbing of juvenile \textit{Sicyopterus stimpsoni}. In the first experiment, whether climbing juveniles preferentially ascend water sources with conspecifics or whether the presence of just stream water is sufficient to attract fish to ascend a climbing path were assessed. In the second experiment, whether climbing juveniles create a trail of mucus that facilitates the ability of conspecifics to follow their lead was determined. The results indicate that juvenile \textit{S. stimpsoni} are less likely to climb in waters devoid of organic cues but are strongly attracted to stream water with or without the odour of conspecifics. Once climbing, performance did not differ for juveniles climbing in differing water choices, suggesting an all-or-nothing commitment once climbing commences. Climbing \textit{S. stimpsoni} did produce a mucous trail while climbing that was associated with a mucous gland that dramatically increases in size just prior to juveniles gaining the ability to climb. The trail was not followed closely by subsequent juveniles traversing the same channel, however, suggesting only weak trail-following in waterfall climbing \textit{S. stimpsoni}. Previous genetic studies suggest that juvenile \textit{S. stimpsoni} do not home to natal streams in the face of strong near-shore oceanic currents. Instead, these fish appear primarily to rely on cues that suggest the presence of organic growth in streams, a factor that may indicate suitable habitat in an ever-changing stream environment but which may also be vulnerable to interference through human activity.

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INTRODUCTION

Among the many challenges to migratory fishes the detrimental effect of in-stream barriers such as dams and diversion structures are well established (Baxter 1977; Jungwirth \textit{et al.}, 1998; Schiemer, 2000; Katano \textit{et al.}, 2006). For many diadromous fishes residing in freshwater streams on oceanic islands, however, these barriers may provide opportunities if they produce safe reproductive habitats, or exclude piscivorous predators and dietary competitors from segments of streams (Schoenfuss & Blob, 2007). For example, the amphidromous life cycle of stream gobioids with a larval, passive downstream migration into the ocean and an active juvenile migration
upstream, leads these species to encounter man-made and natural (i.e. waterfalls) in-stream obstacles; nonetheless, they have repeatedly evolved the ability to scale such barriers, including waterfalls up to 330 m high in some species (Schoenfuss & Blob, 2007; McDowall, 2010).

Despite the ability of many stream gobies to climb, their in-stream migrations are fraught with natural and man-made dangers. As *Sicyopterus stimpsoni* (Gill 1860) return to fresh water from the ocean, they do not home to natal streams (Zink *et al*., 1996; Chubb *et al*., 1998; Schoenfuss *et al*., 2004; Moody *et al*., 2012) but instead have to rely on environmental cues to find appropriate climbing routes to reach adult habitats beyond in-stream obstacles. In contrast to sea lampreys *Petromyzon marinus* L. 1758, which also lack homing behaviour into natal streams (Bergstedt & Seelye, 1995) and whose migration is largely driven by well-characterized conspecific pheromones (Li *et al*., 2003), little is known about the environmental cues that provide guidance to migrating amphidromous goboids on tropical islands. The increasing pressure on freshwater resources, especially in tropical islands with limited water supplies (Brasher, 2003), however, raises the spectre that anthropogenic changes such as aquatic pollution and climate change may alter or obstruct cues (Schlaepfer *et al*., 2002; Tierney *et al*., 2010) needed by juvenile gobies to reach their upstream habitats. In this study, cues that may guide juvenile gobies during their in-stream migration were examined, with a particular focus on whether cues provided by conspecifics may help to determine the paths used by climbing gobies in the ascent of waterfalls obstructing access to their adult upstream habitats.

Gobioids of the Sicydiinae sub-family represent the most specious vertebrate taxon with an estimated 2000 species (Thacker, 2003) inhabiting mainly oceanic near-shore environments. In addition to strictly marine habitats, however, many streams on tropical volcanic islands in the Caribbean Sea, Atlantic, Indian, and Pacific Oceans also have been populated by goboids (McDowall, 2010). One factor potentially contributing to the penetration of such habitats is that in Sicydiinae the pelvic fins are highly modified into a rounded suction disc (diagnostic for the taxon) which allows fishes to adhere to the substratum in stream currents and while moving upstream. Examples of goboids gaining climbing ability have been reported from nearly all tropical islands including Japan (Fukui, 1979; Iida *et al*., 2008, 2010a, b) and the Hawaiian Islands (Schoenfuss, 1997; R. T. Nishimoto & D. G. K. Kuamo'o, unpubl. data) in the Pacific Ocean, Reunion in the Indian Ocean (Keith, 2003; Keith *et al*., 2008; Valade *et al*., 2009), Dominica in the Caribbean (Bell, 1994; Keith, 2003; Maie *et al*., 2007), Vanuatu and New Caledonia (Lord *et al*., 2010), Fiji (Jenkins *et al*., 2009), Solomon Islands (Boseto *et al*., 2007) and Australia (Thuesen *et al*., 2011). Perhaps best studied in the context of climbing behaviour and performance are several species on the Hawaiian Islands, including *Lentipes concolor* (Gill 1860), *Awaous guamensis* (Valenciennes 1837) and *S. stimpsoni*. The last, an endemic goby to the Hawaiian Islands, shares with many other goboids an amphidromous life cycle (Yamamoto & Tagawa, 2000). Following a six-month oceanic larval phase (Radtke *et al*., 1988), post-larvae (Keith *et al*., 2008; Valade *et al*., 2009) enter stream estuaries and undergo a rapid metamorphosis that is characterized by a shift of the mouth from anterior to ventral and a dramatic enlargement of the upper lip to form an oral ‘sucker’ (Schoenfuss, 1997). Approximately 36 h after entering estuaries, this metamorphosis is completed and fishes that are now considered juveniles have gained the ability to climb, which they will maintain throughout life.
climb using an inching-up style in which alternate attachment of the pelvic suction disc and the oral sucker allows the fish to climb in-stream obstacles while remaining in constant contact with the substratum (Schoenfuss & Blob, 2003).

The Hawaiian Islands and their native fish fauna provide an excellent system to examine the environmental cues used by gobioids to guide their upstream migration, which may clarify the extent to which this process is vulnerable to anthropogenic interference (Schlaepfer et al., 2002; Kristan, 2003; Tierney et al., 2010). Over the past century, the ecosystem of the Hawaiian Islands has been altered dramatically, first through agricultural practices that required the diversion of rivers for irrigation and later through urban development (Brasher, 2003). As a consequence, many Hawaiian streams contain diversion structures, dams for hydroelectric power generation, and receive agricultural or urban runoff. Understanding the environmental cues used by migrating gobioids will provide much needed information to guide conservation efforts and may help to ward off future detrimental alterations to stream environments. If human activity alters environmental cues that direct in-stream migration, *S. stimpsoni* may be lead into ecological traps through maladaptive habitat choice (Kristan, 2003). In this study, two separate but related questions were investigated to better understand the in-stream migration of this species: (1) Do *S. stimpsoni* use odour cues in choosing the water sources into which they climb? (2) Do climbing *S. stimpsoni* deposit a mucous trail that subsequent climbers use to guide their climbing path?

Investigating whether odours influence the choice of water source into which *S. stimpsoni* climb is important, as the high rainfall volumes on the windward side of the islands generate many small, ephemeral water flows that could divert migrating juveniles into unsuitable habitats that are prone to dry out within a few days of dry weather conditions. In this study, choice trials were used to assess whether conspecific odours guide migrating juveniles to follow a specific climbing path and whether climbing juveniles deposit a mucous trail that guides subsequent climbing juveniles was examined. Previous studies have shown that waterfall climbing exerts a strong selective force on migrating juvenile *S. stimpsoni* (Blob et al., 2008, 2010), so that choosing an incorrect climbing route might preclude a migrating juvenile from successfully scaling a high waterfall. Together, these studies will provide evidence for the extent to which environmental cues influence the climbing behaviour of amphidromous gobies. Given the close phylogenetic relationship among tropical climbing gobiods, this information may be able to guide resource managers across oceanic islands.

**MATERIALS AND METHODS**

**SPECIMENS**

Juvenile *S. stimpsoni*, c. 6 months old (27–31 mm total length, $L_T$) and recently migrated into the estuary of Hakalau and Honolii streams (Hamakua Coast, Island of Hawai‘i, HI, U.S.A.), were captured with aquarium dip-nets (Blob et al., 2010). Fish were transferred in buckets containing aerated stream water to the research field station of the Department of Land and Natural Resources (DLNR), State of Hawai‘i in Hilo, HI, within 2 h of capture. Fish were maintained at the field station in aerated stream water at ambient stream temperature (c. 20°C) for no longer than 48 h prior to being used once for any of the trials outlined.
below. All water used for the choice and path-finding trials were acclimated to the same temperature as that of these holding enclosures in order to exclude temperature as a confounding factor during experiments. Stream rocks containing native diatom flora, the exclusive food source of the obligate herbivore *S. stimpsoni* (Kido, 1996; Julius *et al*., 2005), were provided throughout the holding period and were grazed upon by the captured juveniles. Juveniles not used for histological examinations were returned to their native streams upon completion of the respective trials. Collections were approved by the DLNR and animal use and care in all experiments was approved by the St Cloud State University Animal Care and Use Committee (IACUC).

**WATER SOURCE CHOICE EXPERIMENTS**

To assess whether migrating juvenile *S. stimpsoni* rely on odour cues to guide upstream migration, juveniles were allowed to climb, one fish at a time, artificial waterfalls with paired water sources (Fig. 1 and Table 1). In the first experiment, waterfalls were supplied with (1) activated carbon-filtered well water and (2) activated carbon-filtered well water in which conspecific *S. stimpsoni* had been maintained for the previous 12 h. The rationale for this experiment was to determine whether conspecific odour, in a matrix largely devoid of any other environmental cue (hence the use of carbon-filtered well water) would direct the upstream migration of the juveniles. In the second experiment, (1) water collected from the lower stream reaches of Hakalau stream was paired with (2) water collected further upstream and beyond the reach of *S. stimpsoni*. The rationale for this set of trials was to assess whether the complex environmental matrix of upstream water (without the odour of conspecifics) would be as attractive as downstream water containing odour of conspecific fish living in Hakalau stream. The results of these two experiments prompted a third pairing consisting of (1) carbon-filtered well water and (2) upstream Hakalau water, to assess whether any biological odours influenced the migratory choice of the juvenile *S. stimpsoni*.

All experiments followed the same standard climbing procedure, modifying methodology developed and published for previous studies (Blob *et al*., 2006, 2010). First, the right and left pairing of water was determined by coin-flip for each trial, and 20 l of each water source was positioned above the artificial waterfall. Water was siphoned onto the climbing ramp (58° angle to horizontal) at a rate of c. 180 ml min\(^{-1}\) (Fig. 1). The upper two-thirds of the climbing ramp was split vertically to allow the two water sources to establish thin sheets of water that would then mix in the bottom third of the climbing surface. In the preparatory phase, dyes were added to the water source buckets to ensure that mixing of the two water sources would occur before the water entered the pool containing the fish. A single juvenile was netted at random from the holding tank and added to the pool at the base of the waterfall, which consisted of a 20 l tub with a stone that provided shelter for the fish. Three trials were run simultaneously on three adjacent artificial waterfalls, with an observer noting the time at which an individual fish began its climb, which side of the main water flow the fish exited the tub at the base of the waterfall, and the choice of water to which the fish migrated. A fish was scored for a water source after it climbed at least 8 cm (c. \(5 \times L_T\)) of the split climbing surface. Fish that did not climb the waterfall within 20 min of the start of the trial were considered non-climbers (Blob *et al*., 2006).

**TRAIL FOLLOWING EXPERIMENT**

To assess whether climbing juveniles deposit a mucous trail that could serve as a guide for subsequent climbing juveniles, an artificial waterfall was constructed out of opaque plexiglass (Fig. 2). As climbing gobies are strongly positively rheotactic (*i.e.* swim into flowing water; Fitzsimons *et al*., 1997), it was important to distinguish between fish following the same trail due to advantageous water flow conditions *v.* the presence of a mucous trail. Consequently, the waterfall was constructed so that the main climbing surface [area outlined by hashed rectangle in Fig 2(a)] could be moved laterally between individual climbing fish. For these experiments, the water source was downstream Hakalau water fed from a 20 l bucket by siphon onto the climbing surface at a rate of 180 ml min\(^{-1}\) (as in the first set of water source choice experiments). Great care was taken to create an even sheet of water across much
Fig. 1. Water-source choice trial climbing setup. (a) Two buckets containing the different water sources (Table I) gravity-feed water to the climbing surface in even sheets at a rate of c. 180 ml min$^{-1}$. One juvenile at a time is placed into the pool (grey container) at the bottom of the climbing ramp and allowed to climb. Oblique barriers force water from both sources to mix just above the pool [(b)–(d), insets with magnified mixing zone). (b) A juvenile fish is climbing the mixing zone and eventually follows (d) the left water source. Exit location from pool (left, centre, right) did not affect the water source followed. Adobe Photoshop CS5 for Macintosh was used to enhance image quality. All manipulations (cropping, converting to greyscale, auto-adjust tone and contrast) were applied to entire images.
Table I. Results of three sets of water source choice experiments with climbing juvenile *Sicyopterus stimpsoni*

<table>
<thead>
<tr>
<th>Carbon-filtered well water without or with conspecific odour</th>
<th>Water only</th>
<th>Water with conspecifics</th>
<th>Non-climbers</th>
<th>Sign-test between choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choice (n = 66)</td>
<td>28</td>
<td>12</td>
<td>26</td>
<td><em>P</em> &lt; 0.05</td>
</tr>
<tr>
<td>Downstream Hakalau v. upstream Hakalau water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choice (n = 65)</td>
<td>30</td>
<td>30</td>
<td>5</td>
<td><em>P</em> &gt; 0.05</td>
</tr>
<tr>
<td>Upstream Hakalau v. carbon-filtered well water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choice (n = 65)</td>
<td>44</td>
<td>13</td>
<td>8</td>
<td><em>P</em> &lt; 0.001</td>
</tr>
</tbody>
</table>

of the climbing surface to minimize any factors associated with specific flow conditions. Similar to the first experiment, single fish were introduced into the tub containing a rock and were allowed to climb the waterfall. After three fish had climbed the waterfall (all fish were introduced to the apparatus and tested individually to avoid any visual cues), the climbing surface was laterally transposed by a mean distance of 4.2 cm (equivalent to 10 body-widths), and an additional three juveniles were allowed to climb one at a time. The entire experiment was captured by a stationary 30 Hz digital video camera to allow for later tracing of the climbing routes of each fish. The rationale behind the lateral transposition of the climbing surface was as follows: if the second three fish followed the same path as the first three fish, their movement was directed by some cue provided by their conspecifics. However, if the paths of the second set of three fish were offset from the paths of the first three fish by the distance the climbing surface had been moved, their climbing path was largely determined by localized water flow conditions. Between trials, the entire climbing apparatus was thoroughly washed with a soapy solution, extensively rinsed in carbon filtered well water and dried before use in subsequent trials.

To assess whether a mucous trail was a likely cue for subsequent climbing fish, climbing surfaces over which fish had climbed were stained using a HP Yellow (Anatech Ltd; www.anatech ltdusa.com) stain. For staining, the surface was wetted with deionized water, periodic acid applied for 10 min and then the surface misted with HP Yellow for 5–7 min. After washing the surface with deionized water, mucous trails were photographed under UV light. The oral suckers of several *S. stimpsoni* were also excised, and these tissues were stained for mucous-producing goblet cells. Adult specimens were captured and transported to the Hilo field station for dissections and processing. The upper lip was extracted, labelled and fixed in 10% buffered formalin. Tissues were then packaged and shipped to Saint Cloud State University for histological analysis. Upon arrival, the tissues were dehydrated through a graded series of ethanol and xylene before being embedded in paraffin wax for microtome sectioning at 4 μm thickness. The staining procedure was modified from a commercial mucin stain kit (Mayer Mucicarmine, Newcomersupply; www.newcomersupply.com). Sections were deparaffinized and hydrated through graded ethanol to distilled water prior to staining with diluted mucicarmine stock stain solution (one part stock to three parts of chlorine free tap water) for 180 min at room temperature. After placing cover slips, slides were evaluated by an experienced histologist using a Leica DM750 microscope and Leica EC3 digital camera system (www.leica.com) attached to a 2.26 GHz Quad-Core Intel Xeon Macintosh Computer (www.apple.com).
CUES DIRECTING MIGRATION OF *SICYOPTERUS STIMPSONI* 7

Fig. 2. (a) Trail following setup with water-filled basin at the bottom (darker area) and water flow from the top (>). Note the two plus (+) signs in upper left corner and plus sign shift in (b) to reflect the shift in the main climbing surface (outlined by hashed line). The first three fish individually climb the surface prior to the shift [climbing surface position in (a)]; the second three fish individually climb the surface after it has been shifted to the right [position in (b)]. Adobe Photoshop CS5 for Macintosh was used to enhance image quality for publication. All manipulations (cropping, converting to greyscale, auto-adjust tone and contrast) were applied to entire images.

STATISTICAL ANALYSIS

All statistical analyses were performed in Graphpad Prism 5.0d for Macintosh (www.graphpad.com). To test whether *S. stimpsoni* discriminated between water sources in the water source choice experiments, results were evaluated using a two-tailed sign test with equal probability of the two outcomes (water choice). Non-climbers were excluded from this analysis. A Fisher’s exact test was used to assess whether the number of climbers was influenced by the water source.

To assess whether fish followed mucous trails deposited by conspecifics, all climbing trails were traced from video recordings and the distance from the edge of the climbing sheet was measured for each 1 cm of vertical climbing progress. Linear regression analysis (ANCOVA) of each trial allowed for the calculation of slope and intercept for trails produced by the groups of three fish before and after the shift of the climbing surface. The magnitude of positional shift in distance to the reference line observed between pre and post-shift trails was used to assess the influence of the mucous trail and any other cue that might influence the climbing direction of climbing juvenile *S. stimpsoni*. A probability of *P* < 0.05 was set as level of significance for all comparisons.

RESULTS

Juvenile *S. stimpsoni* readily climbed the climbing surface with >75% of juveniles climbing the ramp past the split between water sources within the allotted 20 min. The willingness to climb is noteworthy as the fish, in contrast to most choice trials, have the option not to climb at all and remain in the pool below the ramp (an option they exercise if disturbances occur in the vicinity of the climbing ramp). Consequently, a reasonable assumption can be made that the climbing behaviour observed was voluntary and entailed the same decision making behaviours as in natural settings.
WATER SOURCE CHOICE EXPERIMENTS

When given the choice between carbon-filtered well water containing conspecific odours vs. carbon-filtered water alone, significantly more *S. stimpsoni* climbed into the water free of conspecific odours ($P < 0.05$; Table I). This aversion to conspecific odours, however, did not persist when downstream Hakalau water (a stream with a dense population of *S. stimpsoni*) was compared with upstream Hakalau water collected beyond the upstream reach of this species. In this comparison, juvenile *S. stimpsoni* were as likely to choose the climbing path sourced by downstream water as the one sourced by upstream water ($P > 0.05$; Table I). Finally, climbing juveniles overwhelmingly preferred upstream Hakalau water over carbon-filtered well water ($P < 0.001$; Table I), suggesting that the overall odour of stream water is a greater attractant than water largely devoid of organic material. A Fisher’s exact test also revealed that the number of non-climbers was significantly greater in the first choice experiment (26 non-climbers in 66 trials; carbon-filtered well water with or without conspecifics) than in the second or third experiments, which both included at least one choice of stream water (second experiment: five non-climbers in 65 trials, $P < 0.001$, Fisher’s exact test; third experiment: eight non-climbers in 65 trials, $P < 0.001$, Fisher’s Exact test).

TRAIL-FOLLOWING EXPERIMENT

Fish readily climbed the artificial waterfall in this experiment (63 of 66 fish) in 11 trials conducted over several days (Table II). The trails generated by the three fish that were allowed to climb the ramp after the ramp surface had been moved laterally by $10 \times L_T$ fell into three distinct patterns (Table II and Fig. 3): (1) the post-shift fish (linear regression of all three climbing trails) overlapped the pre-shift trails (trials 1, 6 and 10; Table II). The trails were considered to be overlapping if the mean distance between pre and post-shift trails was $<1$ cm, $c$, the total width of the fish with fins in an abducted position, (2) trails of the post-shift fish shifted in the opposite direction (i.e. the ramp was shifted to the right, the trail of fish climbing after the ramp shift was further left than the pre-shift trails) as the shift of the ramp surface (defined as a positive shift, trials 2 and 8) and (3) post-shift fish shifted in the direction of the water flow, compensating for the shift of the climbing surface to the right (defined as a negative shift, trials 3, 4, 5, 9 and 11). In no instance did the compensation of the post-shift fish in their climbing location match the total shift of the climbing surface (mean shift $= 4.2$ cm; Table II).

Since post-shift fish did not fully compensate their trail location for the shift in climbing surface, other possible cues that may influence the climbing path of juvenile *S. stimpsoni* were explored. Specifically, whether juveniles created a trail that may be followed by subsequent climbing fish was assessed. A probable source of any conspecific cue, which persisted beyond shifting the climbing surface and removing the fish, hence excluding water-born fish odour as a source, is the glandular upper lip of *S. stimpsoni* [Fig. 4 (a), (b)]. This structure increases dramatically in size and prominence during the metamorphosis of the juveniles migrating back into fresh water [Fig. 4(b)] and consists largely of tissue that appears glandular in nature (Schoenfuss, 1997). Histological preparations of this tissue excised from climbing *S. stimpsoni* confirmed the presence of a glandular sheet on the ventral surface of the upper lip [Fig. 4(c)]. The glandular sheet is dominated by goblet cells [arrows...
Table II. Summary of 11 trail following trials. Slope (\(b\)) and intercept (\(a\)) values were calculated to identify the difference (in cm) between the pre-shift and post-shift regressions for each trial.

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>Mean ± s.e. (number of trails)</th>
<th>Linear regression</th>
<th>Magnitude of shift (cm)</th>
<th>Interpretation of shift in climbing trails</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-shift (cm)</td>
<td>Post-shift (cm)</td>
<td>(b)</td>
<td>(a)</td>
</tr>
<tr>
<td>1(^a)</td>
<td>8.5 ± 0.1 (3)</td>
<td>7.8 ± 0.1 (2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>2(^a)</td>
<td>7.8 ± 0.1 (3)</td>
<td>11.1 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>3</td>
<td>8.8 ± 0.2 (3)</td>
<td>7.6 ± 0.1 (1)</td>
<td>NS</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>4</td>
<td>8.7 ± 0.1 (2)</td>
<td>6.9 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>5</td>
<td>8.2 ± 0.1 (3)</td>
<td>7.1 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>6</td>
<td>10.4 ± 0.1 (3)</td>
<td>11.1 ± 0.2 (3)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>12.3 ± 0.1 (3)</td>
<td>8.9 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>8</td>
<td>10.4 ± 0.2 (3)</td>
<td>12.4 ± 0.1 (3)</td>
<td>(P &lt; 0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>9(^a)</td>
<td>11 ± 0.1 (3)</td>
<td>9.4 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>10</td>
<td>11.4 ± 0.1 (3)</td>
<td>10.4 ± 0.1 (3)</td>
<td>NS</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>11</td>
<td>12.8 ± 0.1 (3)</td>
<td>9.7 ± 0.1 (3)</td>
<td>(P &lt; 0.01)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, non-significant (\(P > 0.05\)).

\(^a\)Regression analysis for these trials detailed in Fig. 3.

\(^b\)Post-shift values are adjusted for the shift of the climbing surface so that similar values between pre and post-shift trails indicate 100% overlap.
Fig. 3. Figure legend on next page.
in Fig. 4(c)] actively secreting mucus onto the ventral surface of the upper lip, which is in direct and almost constant contact with the surface during waterfall climbing (Schoenfuss & Blob, 2003). When applying the HP Yellow stain to a climbing surface over which adult *S. stimpsoni* (chosen for their much larger size) had moved, a stained path indicating the presence of mucus became visible [Fig. 4(d)]. Similarly, after allowing c. 20 juvenile *S. stimpsoni* to climb a surface (to assure that enough mucus would be deposited to be visible using the staining procedure), staining also revealed a mucous layer deposited onto the surface of the climbing ramp [Fig. 4(e)]. Finally, when traces of these juveniles, captured with a 30 Hz digital video camera, were overlaid on the image of the mucous-stained climbing ramp, the most intense mucous staining overlapped with the traces of the juvenile *S. stimpsoni* [Fig. 4(f)].

**DISCUSSION**

This study was designed to elucidate cues used by climbing juvenile *S. stimpsoni* to direct their climbing path during upstream migration into adult habitats. Two types of cues for this migration that have not been evaluated previously were assessed in these experiments. First, it was determined that water sources influence the climbing direction of juvenile *S. stimpsoni*. Second, that *S. stimpsoni* deposit a mucous trail while climbing, and that the trail may serve as one of several cues in determining the climbing route of subsequently climbing con-specics.

Juvenile *S. stimpsoni*, migrating from the ocean into Hawaiian freshwater streams, face many challenges before reaching adult habitats with few native predators and abundance of algal aufwuchs (Julius *et al.*, 2005). In addition to obstacles common to many migrating fishes, including physico-chemical alterations in the environment and predation, the need to climb barriers presents additional challenges that are poorly understood. The water source choice experiments suggest that odour cues influence the direction of upstream migration by juveniles. Interestingly, while water devoid of any biological matter still elicited climbing behaviour by almost half of the tested fish in the first choice experiment, an almost similar number did not climb at all. Furthermore, the same water source, but populated by conspecifics for 12 h prior to its use, elicited an even lower climbing response, potentially indicating a repelling property to this source water. Although beyond the scope of the study, previous research into the presence of Schreckstoffe (alarm pheromones) indicates that other fishes in stressful conditions (*e.g.* fathead minnows *Pimephales promelas* Rafinesque 1820) may release (actively or passively) alarm pheromones that deter conspecifics from following (Smith, 1992). Since the odour-donating fish were maintained under highly sanitized conditions to avoid the introduction of biological odours other than

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**Fig. 3.** Analysis of trail following in climbing juveniles of *Sicyopterus stimpsoni*. (a) Trial 1, overlap between pre and post-shift trails, suggesting that juveniles climbing after the shift followed the path of the mucous trail, rather than strictly water flow. (b) Trial 2, trail shifts opposite to shift in climbing surface and previous mucous trails, suggesting juveniles climbing after the shift of the surface were not following previously deposited mucous trail or location specific to water-flow characteristics. (c) Trial 9, trail shifts towards the water flow preferred by pre-shift fish, but does not completely overlap with the shift of the mucous trail.
Fig. 4. Evidence for the use of mucus as a clue in climbing juveniles of *Sicyopterus stimpsoni*. (a) Juvenile *S. stimpsoni* (c. 6 months old) shortly after entering the estuary of an Hawaiian stream. (b) Head of a juvenile *S. stimpsoni* with prominent upper lip (→) where mucous sheet is located. (c) Mucous sheet in upper lip of *S. stimpsoni*, →, mucous-secreting goblet cells highlighted by mucicarmine stain. (d) Three mucous trails of *S. stimpsoni* highlighted on the climbing surface using the HP Yellow stain and UV light source. (e) Climbing surface after multiple juvenile *S. stimpsoni* climbed surface; bright area indicates mucus deposited by climbing fish. (f) Overlay of individual climbing trails on the climbing surface depicted in (e). Adobe Photoshop CS5 for Macintosh was used to enhance image quality. All manipulations (cropping, auto-adjust tone and contrast) were applied to entire images.

those emitted by the fish, it is possible that pheromones made this water source least desirable for juveniles at the bottom of the climbing ramp.

In contrast, when fish were given the choice between stream water collected from just below a large population of conspecifics and water collected upstream beyond the reach of *S. stimpsoni*, no preference was evident among climbing juveniles. Results of this experiment corroborate the hypothesis that odour-donating fish in the first experiment were producing some deterrent that repelled juveniles from climbing, as no such effect was seen in the second experiment where odours were collected with
stream water from wild fish not subjected to artificial confinement. In addition, both stream-water sources were clearly more desirable than the carbon-filtered well water used in the first water source choice experiment, as >90% of juveniles climbed in the comparisons using stream water sources. This suggests that the general physico-chemical cues associated with stream water provide a strong attraction to migrating juveniles. To evaluate this possibility, a third water-source choice experiment was initiated in which upstream water and carbon-filtered well water were compared (neither containing conspecific fish odour). While the fraction of non-climbers was comparable with the second experiment, this time more than three quarters of climbing juveniles were attracted to the stream water source, supporting the hypothesis that stream water, regardless of the inclusion of wild conspecific odours, strongly attracts migrating juveniles. Furthermore, the results of the third experiment also suggest that in the second experiment, the total mass of carbon present in upstream and downstream water either did not differ enough to affect fish choice, or if different, did not influence the fish’s choice.

These results fit well into the larger natural history context of climbing Hawaiian gobioids (McDowall, 2010). Previous studies by Fitzsimons & Nishimoto (1996) have documented that the larval oceanic phase of Hawaiian gobioids serves as a recruitment pool to re-populate streams after natural disasters common to small, volcanic, tropical, oceanic islands with small watersheds. For example, Hurricane Iniki completely stripped aquatic vegetation and fish populations from numerous streams on the Hawaiian Island of Kaua‘i in 1993. These streams, however, were quickly re-populated by juveniles returning from the relative safety of the ocean at the end of their larval oceanic phase. Similarly, Schoenfuss et al. (2004) documented the colonization of a newly formed Hawaiian stream (after volcanic eruptions covered the landscape in lava) by juvenile *S. stimpsoni*. In both these instances, conspecific cues would have been mostly or completely absent in the streams into which juveniles chose to migrate. The presence of adequate algal aufwuchs, however, would have been broadcasted to arriving larvae through the overall physico-chemical conditions and biological odour of the stream. The latter information is of particular significance to *S. stimpsoni* among the five Hawaiian amphidromous stream gobies, as it is the only obligate herbivore and furthermore has a highly restricted diet consisting mostly of diatoms (Kido, 1996; Julius et al. 2005). This may explain why *S. stimpsoni* was the first (and most abundant) species to repopulate newly opened habitat after the fracture of the covering lava sheet exposed a small freshwater habitat (Schoenfuss et al., 2004).

Beyond re-population of habitat after catastrophic events, directing migration into water flows containing odorants that indicate adequate food supply is significant for these obligate herbivores, as juveniles tend to migrate *en masse* into estuaries following flash flood events (Nishimoto & Kuamo‘o, 1997). These flooding events not only flush out silt from the main stem of the stream (Julius et al., 2005) but also produce many ephemeral small streamlets that could guide migrating juveniles away from the primary stream channel and into temporary swamps that will quickly dry out between rain events (H. L. Schoenfuss, pers. obs.). Important in this context is the tendency of *S. stimpsoni* to climb at the very edge of water flows (Schoenfuss & Blob, 2003) suggesting that current itself may be of lesser significance to choices made by these fish during migration than in other migrating fish species. Maladaptive habitat choice creates ecological traps that could remove recruiting juveniles from
repopulating adult habitats (Kristan, 2003). Stream odorants, therefore, may keep juveniles on track in their upstream migration.

While climbing, route selection may also affect whether a juvenile *S. stimpsoni* will be successful in scaling the obstacle. Previous studies by Blob et al. (2008, 2010) have indicated that even on a small waterfall (2 m high), failure rates for *S. stimpsoni* juveniles average 30–40%, suggesting that under natural conditions with waterfalls tens to hundreds of metres high, failure rates may be much higher. This possibility is supported by 20 years of observational data indicating that annual recruitment into upstream reaches is small despite tens of thousands of juveniles returning into streams annually (Nishimoto & Kuamo’o, 1997; H. L. Schoenfuss pers. obs.). By following a climbing route successfully completed by a previous juvenile, subsequent juveniles may improve their chances for a successful ascent.

The histological analysis identified a glandular sheet in the upper lip of juveniles that produces mucus. Furthermore, staining of the climbing surface indicated that mucus is deposited during upstream migration, and that subsequent juveniles tend to follow similar pathways even if the previously climbing juvenile has already been removed from the climbing structure (thus ruling out visual cues) (Fig. 4). As juvenile *S. stimpsoni* are strongly rheotactic, however, the experimental design also attempted to distinguish between any flow conditions, which may direct climbing juveniles and mucous cues deposited by juveniles that climbed the surface previously. By shifting the climbing surface after three individuals had climbed, water flow conditions were effectively separated from cues emanating from the mucous trail of juveniles that climbed the surface previously.

Interestingly, in six of the 11 trials, fish compensated at least partially for the shift in the climbing surface by climbing near the same location (in relation to water flow) that the pre-shift juveniles had climbed. While this compensatory behaviour moved the fish away from the previously secreted mucous trails, the compensatory shift was never as large as the actual shift of the climbing surface, suggesting that the mucous trail might present some attraction. This hypothesis was corroborated by the behaviour of post-shift fish in three trials where the juveniles did not compensate for the shift in the climbing surface, but instead followed closely the trails of the three fish that climbed prior to the shift in climbing surface, indicating that the mucous trail served as a guide for the upstream climbing. In two trials, however, post-shift fish did not appear to follow any of the cues that had been hypothesized (water flow and mucous trail) and instead moved even further away from the pre-shift climbing routes and created completely new trails. It is possible, however, that these locations had equally suitable flow conditions as the pre-shift climbing trails. The use of a combination of hydrological and odorant cues has also been suggested for the in-stream migration of Chinook salmon *Oncorhynchus tshawytscha* (Keefer et al., 2006) (Walbaum 1792), which preferentially used ladder passages on the side of the dam that was in closer proximity to upstream natal tributaries.

The presence of the glandular sheet along the medial edge of the upper lip in juvenile *S. stimpsoni*, and the reliance of this species on the oral sucker as a secondary locomotor structure during climbing in this species, strongly suggests a role for this structure in upstream migration. As the mouth serves as a secondary locomotor organ in *S. stimpsoni* (Schoenfuss & Blob, 2003), allowing for the inching-up climbing style of this species, it is conceivable that the mucus deposited by the fish onto the climbing surface (Fig. 4) principally serves a locomotor function, potentially
by providing a better seal to the rough climbing surface. As such, production of a mucous trail could be an incidental by-product of the adhesive mechanism of this species. The studies also demonstrate that, independent from the ultimate purpose of mucous production, juveniles climbing after some individuals have already climbed may extract cues from the mucous trail that could enhance their climbing success. Juveniles also extract migratory cues from the stream of water into which they climb. While these cues do not appear to be generated exclusively by conspecifics, they nevertheless suggest that alterations to upstream habitats may influence migratory behaviour (Schlaepfer et al., 2002; Kristan, 2003) and success in climbing juvenile \textit{S. stimpsoni}.

As Hawaiian streams, and stream habitats in many oceanic islands, are subjected to diverse anthropogenic pressures (Brasher, 2003), challenges arise for water resource managers (Katano et al., 2006). For example, stream diversion structures, common in Hawaiian streams and across oceanic islands, can alter flow patterns and change the flora of upstream reaches (Fitzsimons et al., 1997; Blob & Rivera, 2008). If \textit{S. stimpsoni} relies on upstream cues that indicate adequate food supplies, fish may be less inclined to migrate into these streams. Furthermore, if agricultural or urban runoff is allowed to enter upstream reaches (a common problem in small, steep watersheds), it may generate stress for conspecific fish populations (Scott & Sloiman, 2004), which may respond with the production of alarm pheromones (Smith, 1992) as were implicated in the first water-source choice experiment. Finally, odours entering the stream from non-point sources may mask attractants and further diminish the likelihood of juveniles to migrate upstream (Tierney et al., 2010). Studies of other amphidromous gobiotics suggest considerable migratory plasticity (Tsunagawa & Arai, 2008), thus raising the spectre that even with sustained fish populations, stream reaches may remain devoid of migrating juveniles if environmental cues are masked by anthropogenic alterations to the landscape. Even though climbing gobiotics may be less affected by in-stream obstacles than most migrating fish species, this study presents evidence that stream alterations may interfere with environmental cues and in-stream migration of oceanic island fishes.

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References


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