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Metamorphosis in the Cranium of Postlarval *Sicyopterus stimpsoni*, an Endemic Hawaiian Stream Goby[1]

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Abstract—The endemic goby *Sicyopterus stimpsoni* is unique among the five species of Hawaiian freshwater fishes because it undergoes a rapid metamorphosis of its cranial structures during postlarval development. These fish are amphidromous and return after a prolonged stay in the ocean to Hawaiian streams where they are confined to estuaries until completion of cranial restructuring. Within 48 hours after entering fresh water, snout length, as well as head height and width increase greatly while total length and head length remain unchanged. The upper lip enlarges greatly, and the mouth position shifts from terminal to nearly ventral. After completion of metamorphosis *S. stimpsoni* is able to climb waterfalls by alternating use of the pelvic sucking-disk and the mouth, with which it also is able to scrape diatoms from the surface of rocks by rapid rostrocaudal movement of the upper-jaw complex. Macroscopic and microscopic anatomical evidence, as well as observations of live fish, confirm the dynamics and extent of the metamorphosis. Non-linear, highly dynamic cranial development with extensive reallocation of cranial function has not been described previously for fishes, and its study provides a vehicle for a better understanding of ontogenetic development in vertebrates.

Introduction

The Hawaiian freshwater ecosystem is host to five species of native freshwater fishes, which occupy different stream reaches according to their ability to hold position against high-velocity currents (see Fitzsimons et al. 1997). Three species have adapted to climbing, but only *Sicyopterus stimpsoni* undergoes a metamorphosis of the cranial anatomy during its early ontogeny before it is able to climb waterfalls. Metamorphosis occurs rapidly and results in profound differences in

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[1] ASIH symposium on freshwater gobies

diet and spatial distribution between larval and post-metamorphosed fish. The metamorphosis of *S. stimpsoni* is one of the most dynamic anatomical reorganizations in vertebrate ontogeny. While non-linear ontogenetic development in fishes has been described before (e.g., Mullaney & Gale 1996, Corti et al. 1996), it seldom occurs as rapidly or to the degree as in *S. stimpsoni.*

Hawai'i provides an ideal setting for the study of metamorphosis in light of evolutionary processes because of its geographic isolation and low species diversity. Hawai'i is situated approximately 3500 km from the nearest continental shelf and is a relatively young group of islands. The Hawaiian Chain stretches from a northwestern to southeastern direction with the largest and youngest island, Hawai'i, its southernmost extension (Fig. 1). The oldest and most eroded island in the present day Hawaiian Archipelago (excluding submerged islands) is Kaua'i with an age of about 5.1 million years. The "Big Island of Hawai'i" is an estimated 0.4 million years old and is still growing due to its two active volcanoes, Mauna Loa and Mauna Kea. The prevailing northeastern tradewinds are loaded with moisture causing frequent orographic rainstorms on islands high enough to block the passage of the clouds. Most Hawaiian streams are fed ultimately by the orographic rain which mainly accumulates on the northeastern side of the islands, thus the separation into "wet" and "dry" sides of the islands (Armstrong 1983).

Two species, *Stenogobius hawaiiensis* and *Eleotris sandwicensis,* are only found in the lower reaches of Hawaiian streams. Recruiting fishes and invertebrates are preyed upon by *E. sandwicensis,* which attacks from hiding spots in the gravel. *Eleotris sandwicensis* is unable to withstand strong currents or climb waterfalls. *Stenogobius hawaiiensis* is a small omnivore of the estuarine community that lives in areas with mud and debris substrate. It also has no ability to withstand strong currents or climb waterfalls.

The remaining three species are more capable of withstanding the strong currents of Hawaiian streams and can climb. *Awaous guamensis,* the only species of freshwater fish not endemic to Hawai'i, feeds on algae and invertebrates (Kido 1996). It can be found in habitats from the stream mouth to the mid stream reaches. The adults reach a total length of up to 35 cm. The large size and plump body shape of the adult *A. guamensis* limit their ability to colonize stream reaches with faster currents (see Fitzsimons et al. 1997). *Lentipes concolor* feeds on small animal and plant matter and matures in the upper stream reaches, often above the highest waterfalls, where it may be the only vertebrate in the stream.

Awaous guamensis and *L. concolor* both climb waterfalls by accelerating against the current with a rapid movement of the caudal fin, followed by a resting period during which fish are attached to the surface of the waterfall by their pelvic sucking disk. This sequence is repeated until the upper edge of the waterfall is reached. This climbing behavior can best be described as "powerburst climbing" and involves a phase during which the fish has no contact with the substrate and moves up waterfalls by free swimming. The pelvic sucking disk, formed from fused pelvic fins, is a feature common to gobioid fishes and is used by many to attach to the substrate as a means to withstand strong currents (e.g., Alexander 1965, Annandale & Hora in Wake 1993, Benjamin 1986).

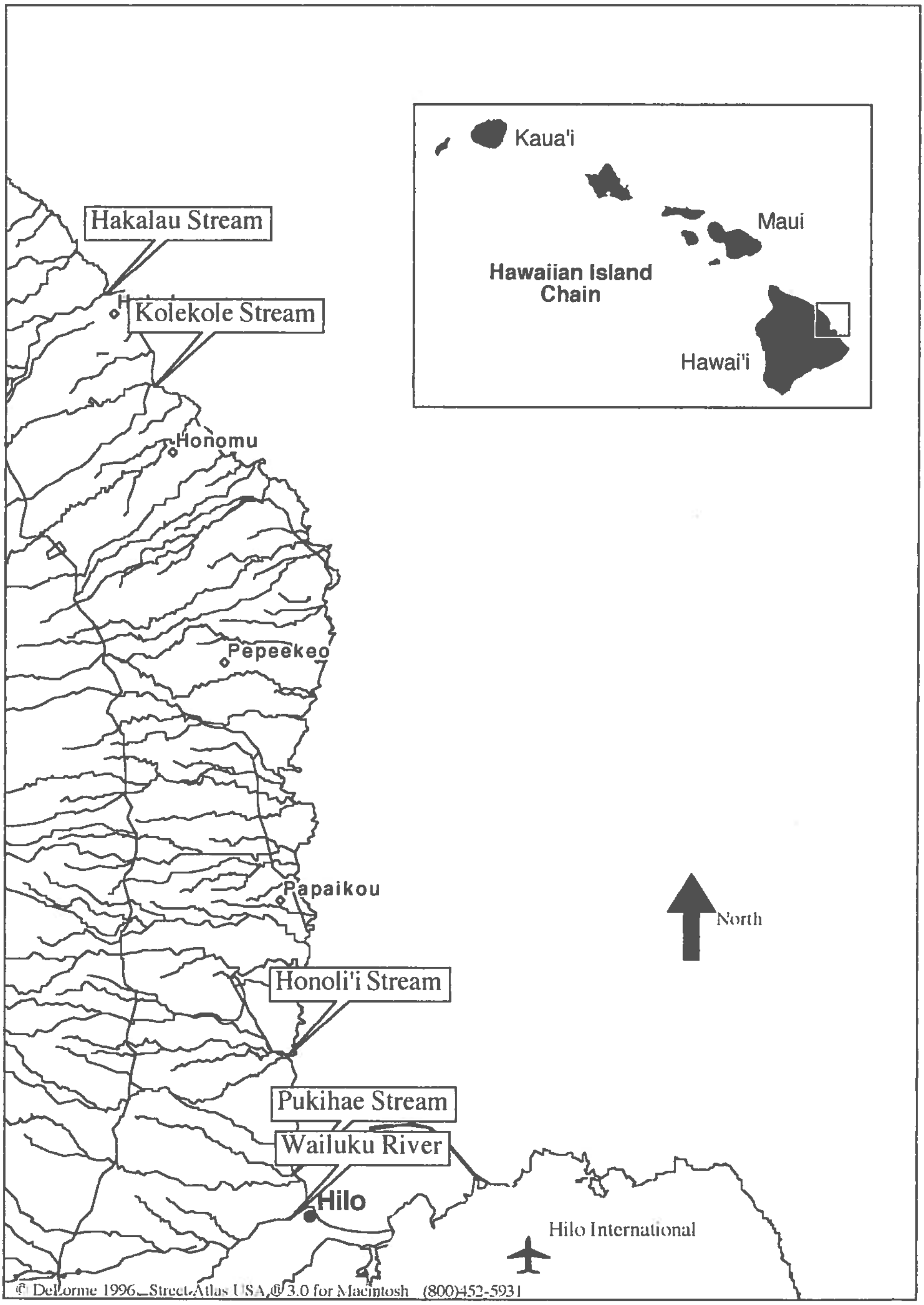

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Figure 1. Collection sites on the Hawaiian Archipelago.

Sicyopterus stimpsoni has an amphidromous life cycle in common with all native freshwater fishes of Hawai'i. Courtship behavior and mating occurs in the mid to upper stream reaches (Fitzsimons et al. 1993) where eggs are deposited. After hatching, larvae are washed into the ocean where they remain for approximately six months before returning to fresh water. Anecdotal evidence suggests that major migrations of postlarval *S. stimpsoni* occur after flash floods. When entering estuaries, recruiting fish are subjected to intense predatory pressure by *E. sandwicensis* and some marine fishes, like *Kuhlia sandvicensis,* which prey on the incoming juveniles (Kahiapo & Fitzsimons pers. comm.). Non-metamorphosed *S. stimpsoni* are unable to reach the upper parts of the streams, because of terminal waterfalls and riffle areas that commonly block their migration. In contrast to *L. concolor* and *A. guamensis, S. stimpsoni* does not climb by "powerbursts" and remains in the estuaries until its mouth develops into a second sucking disk. Only after the mouth has changed in the newly recruited *S. stimpsoni* from a terminal to a ventral position is the fish able to climb. The change in mouth position is vital to the survival of the fish once it has entered the stream and allows the fish to feed on rocks in swift current. Correlation between cranial restructuring and diet has previously been observed in other fishes (Mullaney & Gale 1996), but never in association with changes in locomotory ability. Mouth-assisted climbing has been described for gobioid fishes of the genus *Sicyopterus* (Fukui 1979), but this is the first account of the ontogenetic events creating the necessary anatomical mechanisms for mouth-climbing. Sucker-aided climbing is little understood and has seldom been analyzed functionally (Wake 1993). The climbing ability of *S. stimpsoni* is even more astonishing in light of the metamorphosis that precedes it. The purpose of this study is to describe the anatomical changes occurring during metamorphosis, and to analyze their functional implications for feeding and locomotion in *S. stimpsoni.*

Materials and Methods

Fish for this study were collected at several localities along the Hamakua Coast on the Island of Hawai'i and at some locations on Kaua'i and Maui (Fig. 1). Fish were observed and collected during three field seasons. During a two-week sampling period in February 1995, fish were observed in the streams, and adult specimens were collected. Collection was hampered by an extended dry period, which prevented migration. A second field season from February 15 to March 10, 1996 provided a better opportunity to observe newly recruiting *S. stimpsoni* but was interrupted three times by strong flash floods. A final sampling period on Hawai'i from July 28 to August 11, 1996 allowed continuation of experiments with live *S. stimpsoni* begun during the previous spring. Additional specimens were collected by Darrell Kuamo'o (LSUMZ 11841/11842) and Jan Smith (LSUMZ 11862) on the Hamakua Coast, by Skippy Hau (LSUMZ 11857/11858) on Maui, and David Tate on Kaua'i (LSUMZ 11860).

Fish were preserved in 6% borax-buffered neutral formalin. Fish used in this study are cataloged in the collection of fishes at the Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana. Specimens examined: LSUMZ 11573 (3 specimens), 11574 (2), 11575 (30), 11576 (10), 11841 (11), 11842 (13), 11843 (2), 11844 (2), 11845 (1), 11846 (5), 11847 (7), 11848 (3), 11849 (2), 11850 (2), 11851 (2), 11852 (15), 11853 (9), 11854 (2), 11855 (4), 11856 (1), 11857 (7), 11858 (8), 11859 (7), 11860 (4), 11861 (10), 11862 (35), 11863 (40). A map of the collections sites was prepared with DeLorme software for the Power Macintosh (Street Atlas USA 3.0, 1996).

All *S. stimpsoni* collected in this study were measured for total length, standard length, head length, snout length, prefrontal length, height and width at eye level, and weight. Morphological measurements were performed with an Olympus dissection microscope and digital calipers (Sylac Fowler Ultri-Cal II). Fish were weighed to 0.1 g with a digital laboratory scale (Sargent-Welch SWE 500).

Recruiting postlarvae were trapped as they entered the mouth of the stream with a device resembling a modified breeder trap adapted by Kuamo'o to fit our particular needs. The trap was positioned as close as possible to the stream mouth, usually within 5 m of the surf, but was placed further inland under severe weather conditions. The trap was emptied hourly, and after collection fish were maintained in fresh water for varying lengths of time (2–48 hours) so that morphological changes over a 48 hour period could be examined (LSUMZ 11841, 11 specimens, 0–18 hours; LSUMZ 11842, 13 specimens, 20–48 hours). This method made it possible to establish a sequence of changes at two hour intervals that ultimately led to the cranial reorganization in metamorphosed *S. stimpsoni.* Some recruiting *S. stimpsoni* were caught while climbing the waterfall of a stream by scraping the surface of the waterfall with a dip-net. Adult fish were either speared or caught with an 'opae net, consisting of two bamboo rods with a net-pouch suspended between them.

All *S. stimpsoni* were subjected to radiographic imaging with a soft x-ray apparatus located in the Department of Radiology, LSU School of Veterinary Medicine using the radiological methods outlined by Miller & Tucker (1979).

A number of newly recruited *S. stimpsoni* were prepared for sectioning with standard histological procedures for Tricolor, Maisson, and H&E staining (LSUMZ 11843/11850 sagittal sections; LSUMZ 11843 frontal section). Tissues for the sections were either embedded in paraffin or plastic, depending on the required resolution of the final slide. Sections were taken in all three planes of the body. All histological slides were prepared in the Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, Texas. Slides were examined and photographed with a Zeiss compound microscope with camera attachment for a Nikon camera body.

Some specimens (LSUMZ 11574/11575) in different developmental stages (pre-metamorphosed, during metamorphosis, post-metamorphosed, and adult) were subjected to clearing and staining methods as outlined by Song & Parenti (1995). The specimens were stained for demonstration of bone, cartilage, and nerves. After the procedure was completed, the specimens were stored in 100% Glycerin to avoid destaining or degeneration of the specimens. Photos of these specimens were taken on a Polaroid M4 copy stand with an Olympus camera body and lenses.

Some fish caught in the traps at the mouth of Hakalau Stream were kept alive for behavioral experiments and for the study of cranial movements during feeding and climbing. An artificial, Plexiglas waterfall with a video-camera facing the surface of the waterfall was constructed by Kuamo'o to record climbing behavior by *S. stimpsoni*. Images were recorded onto Sony Video Hi8 Metal tape, which allows single-frame viewing without distortion. In a second experimental setup, postlarval and adult *S. stimpsoni* were placed in either of two containers, each with water cascading down one surface. The water for both containers originated from the same source and was provided through a 1.25 cm diameter hose. One container was partitioned by a screen, with a large predator (*E. sandwicensis*) on one side of the screen and a newly recruited *S. stimpsoni* on the other.

Results

No significant changes over time were recorded in total length, standard length, prefrontal length, head length, or weight of the fish. Changes in snout length, height of head and width of the head were significant (Fig. 2). The upper lip enlarged greatly during the first 48 hours the new recruits spent in fresh water. The three notches in the upper lip, which are characteristic for *S. stimpsoni* (Tate et al. 1992), became better developed as the mouth position shifted from terminal to ventral. Bristle-like structures in the upper and lower lips appeared during the same period of time.

Radiographic images demonstrated the osteological differences between fishes just entering fresh water (0 time) and those that were preserved after the metamorphosis (Fig. 3 a,b). The 0-time fish had a terminal mouth position reflecting the cranial anatomy of the new recruits, with premaxilla and maxilla only slightly rostral to the dentary. The dentary and maxilla were equally well developed and ossified. After metamorphosis, the dentary had greatly retracted and become situated below the neurocranium substantially farther caudal than previously. Premaxilla and maxilla were protracted and greatly increased in size and degree of ossification. The mouth position shifted to a subterminal position during the metamorphosis.

Histological results concur with the radiographic evidence (Fig. 3 c,d). The premaxillary-maxilla complex was greatly enlarged not only in the dense connective tissues but also in epithelial and muscular composition. A large gland had formed deep to the epithelium of the upper lip and occupied almost exclusively the space between maxilla and epithelium. The mucosal membrane of the upper lip was highly differentiated and exhibited numerous folds. The *Musculus adductor mandibulae* was visible on the lateral aspect of the cheek in the histological sections. The lower lip was inconspicuous and lined by a smooth and undifferentiated mucosal membrane. The cleared and stained specimens of *S. stimpsoni* (Fig. 4 a,b) at the end of metamorphosis exhibited a similar developmental pattern by having a greatly enlarged upper lip and a receded lower lip.

Videographic and photographic evidence, as well as personal observations, confirmed the use of the mouth as a secondary sucking disk in *S. stimpsoni*. While

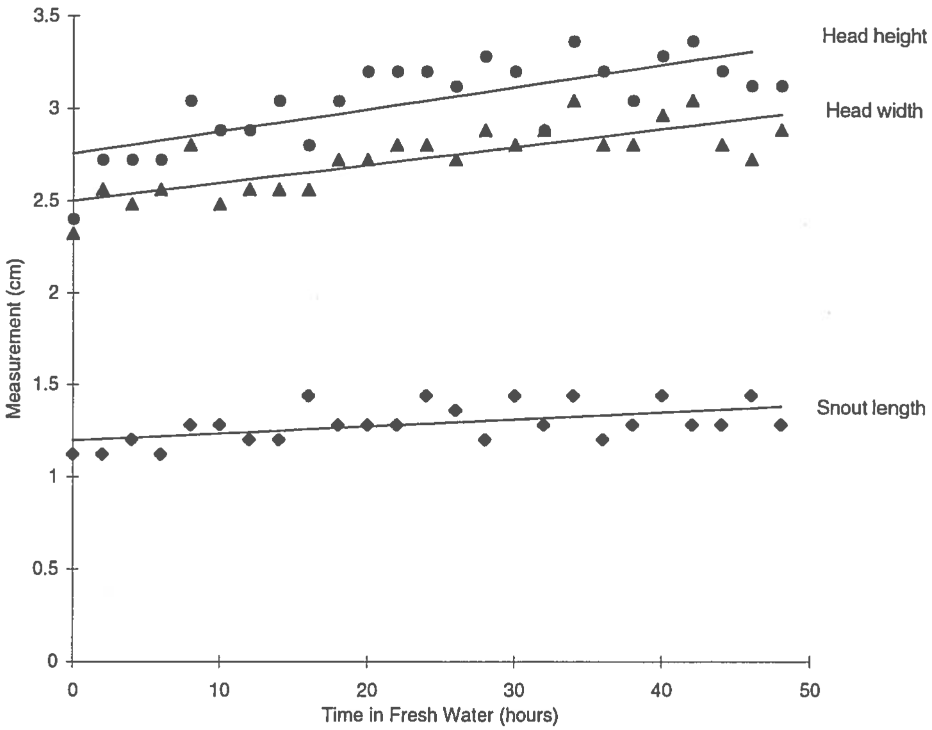

Figure 2. Linear regression demonstrating significant changes of head height and width, as well as snout length. (head height: r-square = 0.618, p = 3.15 × E-6, f = 37.27; head width: r-square = 0.55, p = 2.13 × E-5, f = 28.28; snout length: r-square = 0.28, p = 0.006, f = 9.06).

climbing, *S. stimpsoni* alternated in creating a sucking force with its pelvic sucking disk and its mouth. By doing so, the fish worked its way up the surface of the waterfall without loosing contact with the surface. The fish advanced along the periphery of the waterfall where water pressure was lowest. Larval fish frequently followed each other while climbing as if following a path established by the first fish. *Sicyopterus stimpsoni* had great difficulties advancing over sharp ridges in their climb.

Under laboratory conditions, we were able to induce larger *S. stimpsoni* to climb by adding a large predator (*E. sandwicensis*) to the pool below the artificial waterfall, where the two fish were separated only by a screen. Under these circumstances, juvenile *S. stimpsoni* to 3 cm total length were observed climbing. In Hawaiian streams, larger juveniles or adults were never observed climbing.

Once in their upstream habitat new recruits and adults established territories which often centered around "feeding rocks" freed of most vegetation. *Sicyopterus stimpsoni* were usually found on top of such rocks where they scraped and engulfed diatoms by suction from the surface. While feeding, the fish maintained position with the pelvic sucking disk, while the premaxilla and maxilla were pro-

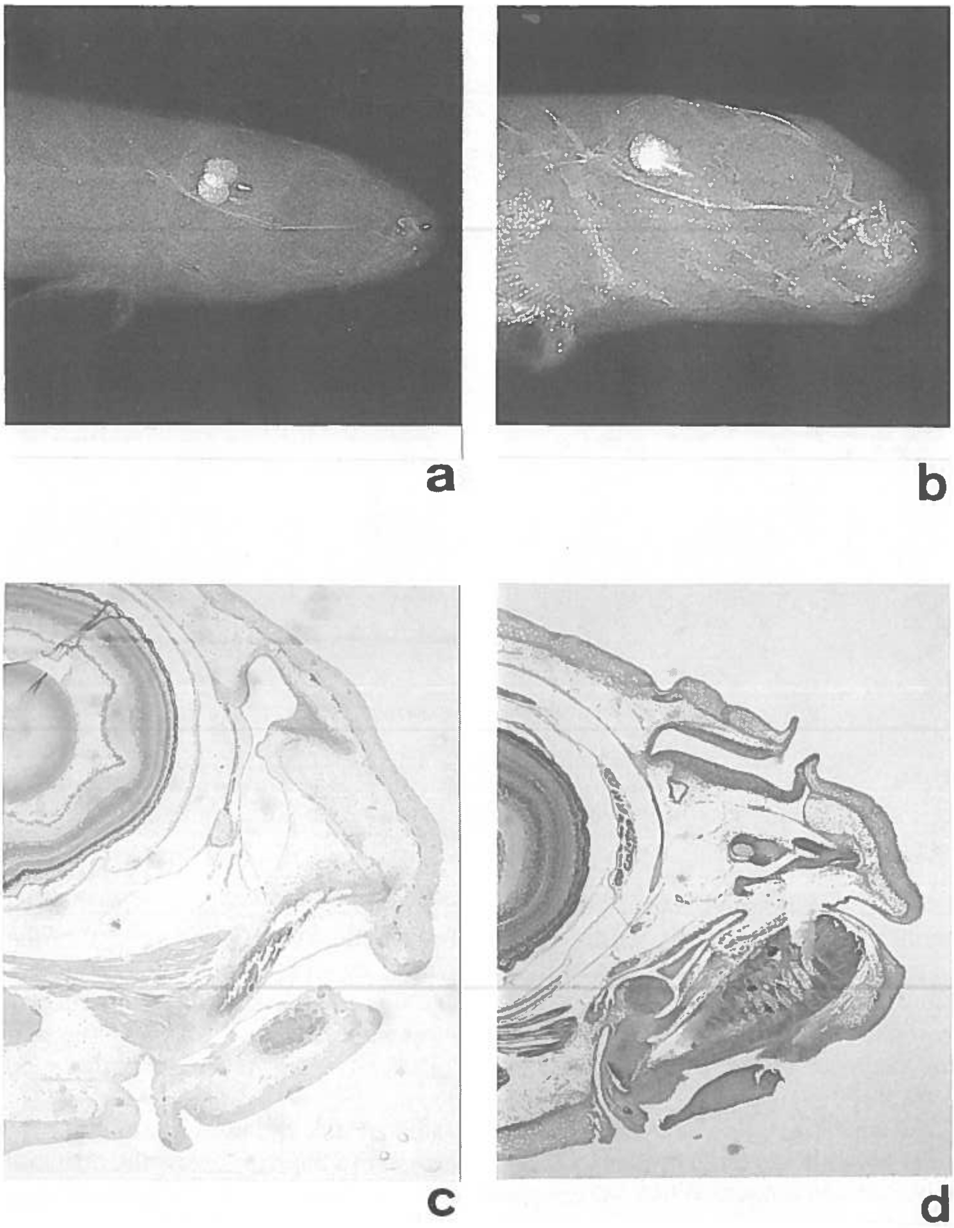

Figure 3. Radiographic image (top, a and b) and sagittal section through cranial structures (bottom, c and d) of postlarval *Sicyopterus stimpsoni*: a and c are fish before metamorphosis; b and d are fish after metamorphosis.

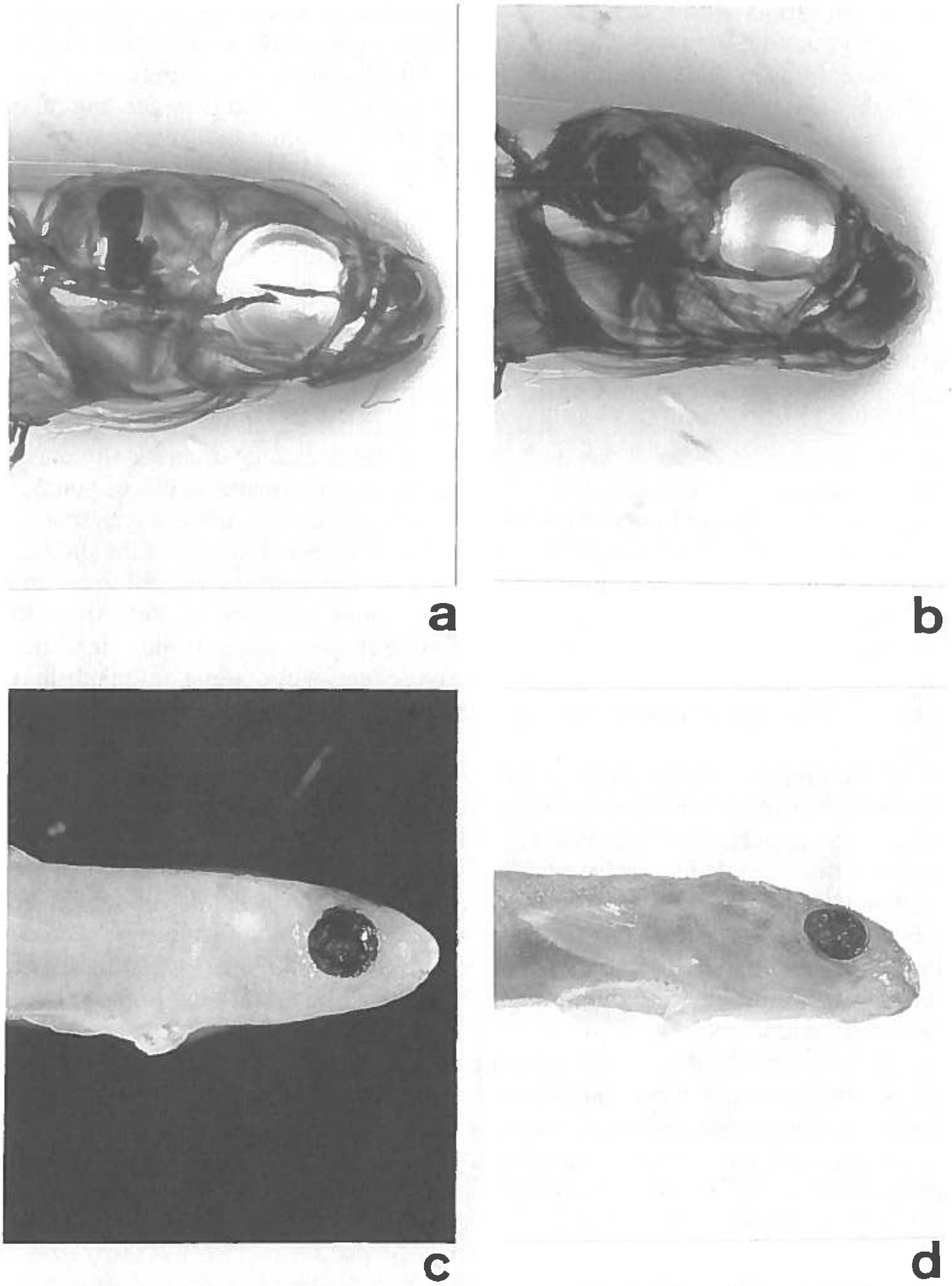

Figure 4. Cleared and stained (top, a and b) and intact (bottom, c and d) specimens of *Sicyopterus stimpsoni*: a and c are fish before metamorphosis; b and d are fish after metamorphosis.

truded and retracted in a rapid sequence. The head was moved from side to side while the mouth parts were engaged in continuous scraping of substrate. The tail was not employed while feeding and was often shifted by the changing currents while the fish appeared to be undisturbed in its feeding action. The feeding mode of the metamorphosed *S. stimpsoni* led to the conclusion that post-metamorphosed *S. stimpsoni* are obligate herbivores feeding on diatoms and algae (Kido 1996, Kinzie 1988).

Discussion

Morphological measurements, observations of live fish, and anatomical evidence document the remarkable metamorphosis in recruiting *S. stimpsoni.* Metamorphosis begins immediately when the fish returns into fresh water and continues rapidly. No indications of the onset of metamorphosis before entering freshwater have been found. Several external features change dramatically during metamorphosis and can be used to determine the developmental status of young *S. stimpsoni.* The enlarged upper lip of metamorphosed *S. stimpsoni* is the most obvious feature and is the result of the protraction and repositioning of the premaxilla and maxilla. Retraction of the dentary, which comes to rest caudal to the cranial edge of the neurocranium, causes the mouth position to shift from the terminal, pre-metamorphic condition to a ventral, post-metamorphic condition. The shift of the mouth position and the broadening of the upper lip result in an overall robust appearance of the head in comparison to the rest of the body (Fig. 4 c,d).

Climbing by *S. stimpsoni* is divisible into a sequence of six functional events, which are repeated continuously during climbing: (1) The fish attaches to the substrate with the pelvic sucking disk; (2) The buccal structures release from the substrate surface; (3) A forward stretching along the long axis of the body occurs; (4) A suction force firmly attaches the buccal structures to the substrate; (5) The pelvic sucking disk releases; (6) Shortening of the body along its long axis causes the pelvic sucking disk to be pulled forward toward the cranium. Preceding the initial sequence is accelerated swimming, often propelling the fish out of the water to the first attachment site.

A shift in feeding mode occurs simultaneously with the metamorphosis. Larval *S. stimpsoni* are most likely intermittent suction feeders, which approach planktonic food items and engulf them through the negative pressure created in the buccal and oral cavities. A terminal mouth position with a fairly small mouth is ideal for this feeding mode. The post-metamorphic *S. stimpsoni* are obligate herbivores that scrape diatoms and algae from rocks by rapid rostrocaudal movement of the upper jaw.

Climbing behavior is functionally related to feeding behavior in post-metamorphic *S. stimpsoni.* During both feeding and climbing, the upper lip is greatly extended forward and retracted. During climbing this negative pressure is used to create a suction on the surface of the rock. During feeding, the same suction is used to transport food items into the pharynx. The inability of climbing *S. stimp-*

soni to pass over sharp ridges indicates the need for a tight seal to establish negative pressure in the buccal and oral cavities. The three conspicuous notches in the upper lip of *S. stimpsoni* allow the seal to be broken at the end of the climbing sequence in a manner similar to the small lip on a suction cup that must be released to facilitate its removal.

Observations that post-metamorphic *S. stimpsoni* follow one another while climbing suggest the presence of a scent gland. Other researchers were unsuccessful in locating a glandular structure in the pelvic sucking disk (R. J. F. Smith, pers. comm.). The gland in the upper lip of *S. stimpsoni* (described above) may secrete a scent that marks the path of a climbing fish.

It is unlikely that climbing ability in *S. stimpsoni* evolved independently, because of the close functional proximity that it shares with *Sicyopterus japonicus,* which climbs in a similar manner. The prolonged offshore larval phase could provide for a transport mechanism of a common ancestor to the Hawaiian islands. Regardless of origin, the ontogeny of climbing ability is linked to the diet of the different developmental stages. As such, colonizing *S. stimpsoni,* or an ancestral form, may have been able to exploit abundant herbivore food resources without competition. To do so efficiently requires the fish to be able to anchor itself with its pelvic sucking disk while scraping and ingesting food from the rock surface without being washed away. Metamorphosis of feeding structures from the offshore larval phase is vital for the survival of the fish once they have entered fresh water. The rapid metamorphosis is essential to allow the new recruits to begin feeding on benthic algae and to escape predation. Continuing studies of anatomical and evolutionary processes underlying the rapid metamorphosis of *S. stimpsoni* will provide new insights into the development and function of cranial structures in vertebrates.

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