Functional Morphology of Pufferfish Inflation: Mechanism of the Buccal Pump

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The mechanism of the buccal pump used to fill the stomach with water during inflation behavior was studied in the striped burrfish, *Chilomycterus schoepfi*. The movement patterns of the pectoral girdle and the anterior region of the hyoid apparatus were inferred from simultaneous impedance and buccal pressure recordings. Impedance was measured between two electrodes; a stationary electrode implanted in the roof of the mouth and a second electrode implanted either in the anterior tip of the cleithrum or the musculature surrounding the anterior end of the hyoid apparatus. In addition, simultaneous buccal pressure and electromyographic (EMG) recordings were made during inflation sequences to determine the activity patterns of eight cranial muscles. Impedance recordings indicate that buccal expansion is caused by synchronous posteroventral rotation of the hyoid apparatus and the pectoral girdle, whereas buccal compression is accomplished by hyoid and pectoral girdle anterodorsal rotation. EMG data revealed that periods of reduced buccal pressure occurring at the onset of each inflation cycle were associated with activity of several muscles that open the mouth (levator operculi, dilatator operculi), depress the hyoid apparatus (hyohyoideus abductor, sternohyoideus), and retract the pectoral girdle (levator pectoralis). Periods of positive buccal pressure, during which water was forced into the stomach, were associated with muscular activity indicating mandibular adduction (adductor mandibulae), hyoid protraction (protractor hyoideus), and pectoral girdle protraction (protractor pectoralis). The key components of the buccal pump that, among tetraodontiform fishes, are derived for the Diodontidae plus the Tetraodontidae are the highly kinetic joint between the cleithrum and supracleithrum which permits extensive pectoral girdle protraction and retraction and the massively developed hyohyoideus abductor muscle that flexes a joint between the ceratohyal and the first branchiostegal ray, providing a novel mechanism of buccal floor depression.

FISHES in the teleost superfamily Tetraodontoidea (families Diodontidae and Tetraodontidae) are able to inflate their body by swallowing mouthfuls of water. The increased size of the inflated body is believed to make these slow-swimming fishes more difficult for predators to swallow (Randall, 1967; Myer, 1989). Although the ability to inflate the body has interested biologists for some time (Thilo, 1899; Parr, 1927; Brainerd, 1994) the musculoskeletal mechanism that underlies inflation behavior is poorly known.

Descriptions of tetraodontiform skeletal anatomy are provided by Tyler (1968, 1980), and the comparative musculature is reviewed by Rosen (1913) and in detail by Winterbottom (1974a). Brainerd (1994) describes features of postcranial morphology in *Diodon* that allow a dramatic change in the shape of the fish as the stomach fills with water. A few authors offer interpretations of how aspects of the buccal pump function. Thilo (1899) proposes that the enlarged postcleithral apparatus of tetraodontids functions prominently during inflation by spreading laterally and thus pulling water into the expanding body. This proposal is refuted by Parr (1927) who believes the postcleithrum and the remainder of the pectoral girdle to be "quite passive during the swelling" phase of the inflation process. Winterbottom (1971) describes the nature of the joint between the cleithrum and supracleithrum that we find to be a central feature of the buccal pump. Gabriel (1940), in a brief note, postulates that the enlarged first branchiostegal rays and their associated hyohyoideus abductor muscles "depress a pad covering the ceratohyals, thus expanding the mouth cavity and drawing in air or water."

The purpose of this study was to elucidate the mechanism of inflation in a representative tetraodontoid, the striped burrfish *Chilomycterus schoepfi* (Diodontidae). We describe patterns of pressure recorded in the buccal cavity, patterns of hyoid and pectoral girdle movement inferred from impedance data, and patterns of muscle activation from electromyographic recordings.

This study represents the first kinematic and electromyographic analysis of the buccal pumping mechanism of a pufferfish. Based on these observations, we present an interpretation of how water is drawn into the buccal cavity and repeatedly forced into the stomach during the extraordinary inflation behavior of pufferfishes.

MATERIALS AND METHODS

We collected specimens of *Chilomycterus schoepfi* from seagrass beds near the Florida State University Marine Laboratory in the northeastern Gulf of Mexico. Fish were housed separately in 100-liter laboratory aquaria at room temperature (22 ± 2 C) and fed a mixed diet of squid, bay shrimp, snails, and crab. A total of 21 live individuals ranging in size from 160–250 mm standard length (mean = 209 mm SL) were used in the experiments.

In addition to the 21 experimental specimens, we dissected three formalin preserved specimens (170–215 mm SL), one fresh-dead animal (153 mm), and one specimen that was cleared and stained for bone and cartilage (109 mm) following a modification of Dingerkus and Uhler (1977). Dissections and manipulation of dead animals helped elucidate the patterns of motion that are possible in the burrfish skull and the probable actions of all muscles included in the electromyographic analysis. Our anatomical terminology follows Winterbottom (1974a) for muscles and Tyler (1980) for skeletal elements. See Winterbottom (1974b) for discussions of muscle homology.

In vivo experiments.—Inflation behavior in *Chilomycterus*, as in other tetraodontoid species (Parr, 1927; Randall, 1983; Brainerd, 1994), involves repeated swallowing of mouthfuls of water. During each cycle of inflation, water is drawn into the buccal cavity and then forced into the stomach. Our major goal in this study was to determine the musculoskeletal mechanisms of oral expansion and compression that underlie this behavior. During all experiments, we recorded pressure from the buccal cavity with a Millar SPR-407 catheter tipped pressure transducer positioned at the opening of a plastic canula into the buccal cavity. We threaded the pressure transducer through the chronically implanted canula positioned on the dorsal midline of the neurocranium between the orbits. The plastic canula was implanted once the fish had been gradually anesthetized in a solution of tricaine methanesulfonate (MS-222) at a concentration of not more than 0.7 g/liter.

We simultaneously recorded pressure with impedance or electromyograms. The use of impedance measurements was motivated by initial observations made on a cineradiographic video (Siemens Cienradiograph with a Sirecon image intensifier and a 60 Hz video camera) of inflation in a 134-mm specimen of *Diodon holocanthus*. From this radiographic video, it appeared that considerable pectoral girdle motion (posteroventral and anteroventral rotation) accompanied inflation behavior. Impedance measurements made on *Chilomycterus* confirmed these initial observations and determined the timing of these movements relative to buccal pressure patterns. We implanted monopolar impedance recording electrodes into two structures (see below), and a UFI-2991 impedance converter determined changes in impedance to current passing between the electrodes as they moved close to and apart from each other with the motions of the implanted cranial structures. Impedance recordings do not permit exact measurements of distances between the two electrodes, but they indicate direction of movement (i.e., the electrode tips moved closer together or apart) and the time course of motion. We constructed unipolar impedance electrodes from 1.0-m pieces of 0.051-mm diameter insulated stainless steel wire. We scraped insulation off the ends of the electrodes under a dissecting microscope to expose 3.0-mm steel tips and threaded the electrodes into hypodermic needles with the exposed electrode tips bent back against the shaft of the needle, forming a hook that held the electrode in place when the needle was inserted into its recording position in the fish.

We used impedance to investigate movement patterns of two structures: (1) the anterior midline region of the hyoid apparatus relative to the roof of the buccal cavity (two fish), and (2) the anterior end of the pectoral girdle relative to the roof of the buccal cavity (two fish). In both cases, we implanted one monopolar electrode into the roof of the buccal cavity by inserting the hypodermic mounting needle anterior to the margin of the orbit obliquely until it emerged at the midline through the oral mucosa in the roof of the mouth. The electrode tip was exposed inside the buccal cavity and pulled back against the roof of the mouth as the needle was retracted. Hence, this electrode was mounted on the neurocranium and provided a fixed reference point for defining movements of the hyoid apparatus and pectoral girdle.

For recordings of hyoid movement, we implanted the second electrode into the anterodorsal surface of the hyohyoideus abductor
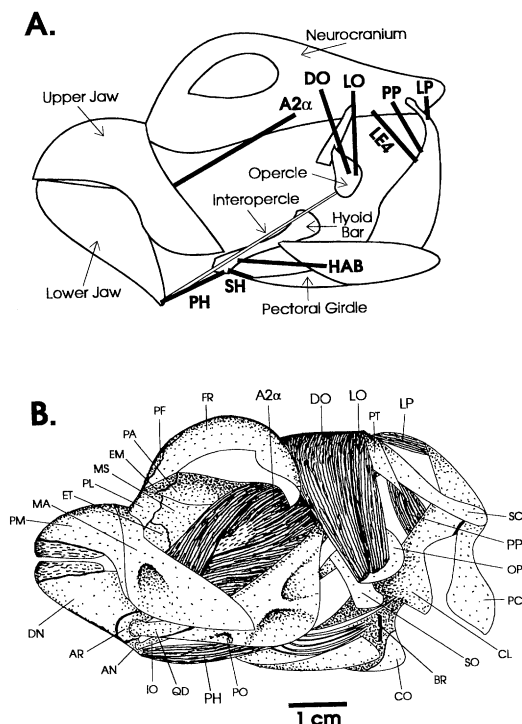
A.

B.

A.

B.

Fig. 1.   Lateral view diagram of the cranial musculoskeletal components of the buccal pumping mechanism in *Chilomycterus schoepfi*. (A) Relationships among the major skeletal elements of the inflation system are shown, and the positions of major muscles are indicated as thick black lines. Most of the suspensorium and the branchial apparatus are not shown. (B) Diagram emphasizing the configuration of the muscles indicated in part A. Abbreviations: A2α, adductor mandibulae; AN, angular; AR, articular; BR, branchiostegal ray; CL, cleithrum; CO, coracoid; CR, ceratohyal; DN, dentary; DO, dilatator operculi; EM, ethmoid; ET, ectopterygoid; FR, frontal; HAB, hyohyoideus abductor; IO, interopercle; LE4, fourth levator externus; LO, levator operculi; LP, levator pectoralis; MA, maxillary; MS, mesopterygoid; OP, opercle; PA, parasphenoid; PC, postcleithrum; PF, prefrontal; PH, protractor hyoideus; PL, palatine; PM, premaxillary; PO, preopercle; PP, protractor pectoralis; PT, pterotic; QD, quadrate; SC, supracleithrum; SH, sternohyoideus; SO, subopercle.
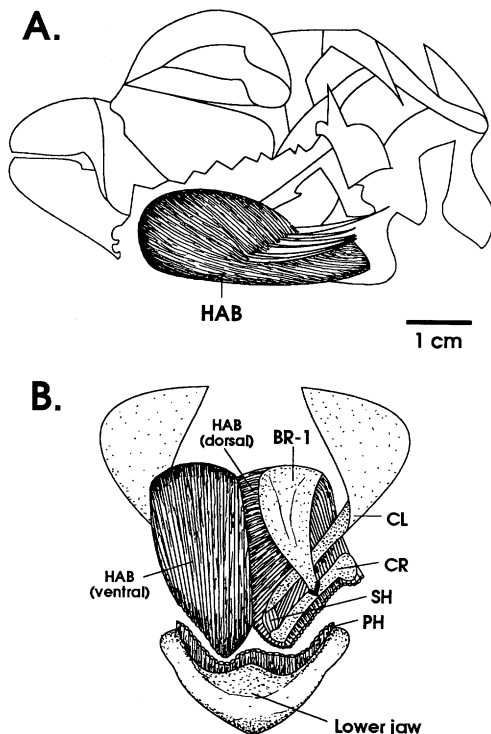
Fig. 2.   Diagrams of the complex arrangement of the hyohyoideus abductor muscle. (A) In lateral view, note that the hyohyoideus envelops the first branchiostegal ray (BR1) and the hyoid apparatus. (B) In ventral view, right side is shown with the ventral half of the hyohyoideus removed to reveal its dorsal fibers and the underlying skeletal elements. Abbreviations as in Figure 1.

muscle, where this muscle envelops the anterior portion of the paired hyoid bars (Figs. 1–2). We first implanted a short plastic canula through the roof of the skull immediately posterior to the pressure canula. Then we passed a hypodermic mounting needle through the canula and into the hyohyoideus abductor muscle in the floor of the buccal cavity. This arrangement permitted the electrode to move freely through the canula as the hyoid structures moved ventrally and dorsally during inflation sequences.

For records of pectoral girdle movement, we

implanted a second monopolar electrode into the anterior end of the left cleithrum. This electrode was positioned with the hypodermic needle by first making an incision in the ventral skin, exposing the cleithrum between and dorsal to the two enlarged first branchiostegal rays. During inflation, virtually all of the skin on the ventral side of the body of *Chilomycterus* expands away from the underlying musculoskeletal system. To permit this expansion and prevent it from dislodging the electrode positioned in the cleithrum, we loosely coiled about 80 mm of wire and lodged it beneath the skin, between the two first branchiostegal rays. The incision in the skin was then closed with a cyanoacrylate adhesive.

We made electromyographic recordings (EMGs) from 13 fish to document the sequence of muscle activation relative to the buccal pressure pattern. We recorded EMGs through bipolar electrodes constructed from 1.0-m pieces of 0.051-mm diameter insulated stainless steel wire. We glued together the two pieces of wire

along 15 cm at the recording ends and exposed 0.5-mm tips by scraping insulation off the wires under a dissecting microscope. The electrodes were mounted in 26 gauge, 12.7-mm long hypodermic needles, and the electrode tips were bent back against the shaft of the needle to form a hook. We inserted electrodes percutaneously into anesthetized fish and bundled all electrodes into a common cable that was sutured to the dorsum of the fish, just posterior to the bony ridge over the orbit. We amplified electrical signals 10,000 times with Grass P511 amplifiers, using a bandpass of 100–1000 Hz and always employing the 60 Hz notch filter. A TEAC XR-5000 14-channel FM tape recorder with a simultaneous voice track recorded all data (pressure, impedance, and electromyograms).

We recorded electromyograms from nine muscles that function during inflation behavior. These included the levator operculi (LO, data collected from three individuals), dilatator operculi (DO, four individuals), section 2α of the adductor mandibulae (AM2, three individuals), valvulus muscle (VAL, three individuals), levator pectoralis (LP, three individuals), protractor pectoralis (PP, six individuals), sternohyoideus (SH, two individuals), protractor hyoideus (PH, three individuals), and the hyohyoideus abductor (HAB). The HAB is massive and greatly modified in all tetraodontoids and has been implicated as a key muscle in the inflation mechanism (Gabriel, 1940). We recorded both from the region ventral to the enlarged first branchiostegal (eight individuals), the region dorsal to the branchiostegal (two individuals), and simultaneously from the left and right ventral regions (two individuals; Fig. 2).

We returned individuals to their aquaria after surgery to recover from the anesthesia and made recordings for both impedance and electromyograms several hours later on the same day or the following day. We induced fish to inflate by holding them by the caudal peduncle and applying gentle pressure. After recording several inflation sequences, we euthanized each fish by overanesthesia in a solution of MS-222 and confirmed electrode placements visually during a dissection of the fresh animal.

Data collection and analysis.—The objectives of the analyses of pressure, impedance, and electromyographic data were to quantify the timing of hyoid bar and pectoral girdle movements and activation of the eight muscles relative to features of the buccal pressure pattern. Following experiments, we played back the data on a Graphtec Mark-11 thermal array recorder at a speed that provided a resolution of 200 mm of chart paper per second of recorded data. From each
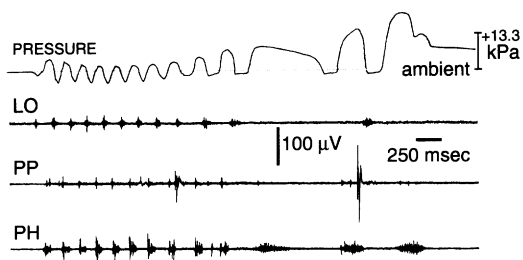

Fig. 3. Simultaneous recording of buccal pressure change and electromyographic activity from three cranial muscles showing an entire inflation sequence. Most of the sequence involves cycles with modest pressure levels until the final few cycles when pressures increase greatly and the time course of each cycle becomes longer. Muscle abbreviations: LO, levator operculi; PH, protractor hyoideus; PP, protractor pectoralis.

individual, we selected two or three inflation sequences for analysis. Each sequence was composed of from 5 to 20 cycles of buccal expansion and compression. We analyzed a total of 23 inflation sequences and 289 inflation cycles.

To quantify the shape of the buccal pressure traces, we measured five temporal and two amplitude variables from each inflation cycle using dial calipers (Figs. 3–7). For convenience, we defined ambient (= atmospheric) pressure as 0 kPa and measured buccal pressure changes above and below ambient. We used the beginning of subambient pressure as a reference time from which we measured times to the following four events in milliseconds: minimum pressure amplitude, ambient pressure as pressure was increasing, maximum pressure amplitude, and a second return to ambient pressure that marked the start of the subsequent cycle. A full cycle of inflation behavior was defined as the time between consecutive onsets of subambient pressure (see Fig. 4A).

We selected the onset of subambient pressure at the start of an inflation cycle as the reference time from which we measured the relative timing of impedance events and muscle activity. For impedance recordings, times were measured between the onset of subambient pressure and the following three events (Figs. 5–6): initial minimum impedance peak, maximum impedance peak, and second minimum impedance peak (= the start of the subsequent cycle).

During the electromyographic recordings, all eight muscles exhibited a single burst of activity per inflation cycle. For each muscle, we measured the duration of the activity burst and the onset of activity relative to the onset of subambient pressure (Figs. 4, 6). We made all temporal measurements to the nearest 0.1 mm with dial calipers at a resolution of 200 mm of chart pa-
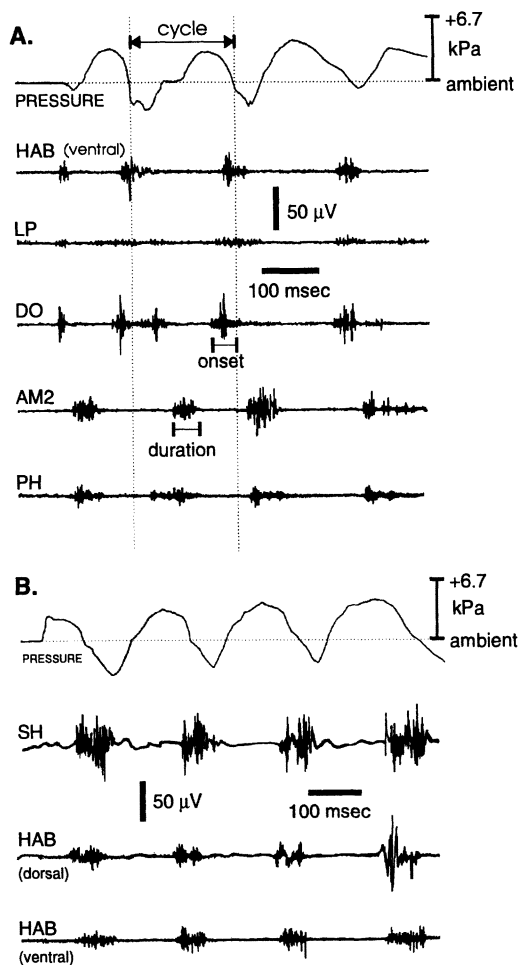
Fig. 4. Samples of simultaneously recorded buccal pressure change and electromyographic activity of head muscles during the early stages of inflation sequences. Note that muscles tend to be active in one of two periods: the HAB, LP, DO, and SH are all active as pressures initially drop below ambient. The remaining muscles, the AM2 and PH, are active as the pressure rises above ambient to its maximum peak. The dorsal and ventral regions of the HAB are active simultaneously. Muscle abbreviations: AM2, section 2 of the adductor mandibulae; DO, dilatator operculi; HAB, hyohyoideus operculi; LP, levator pectoralis; PH, protractor hyoideus; SH, sternohyoideus.
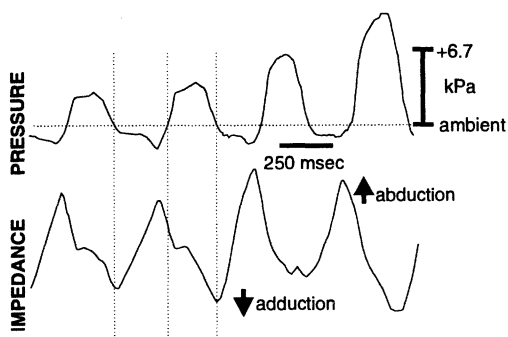

Fig. 5. Simultaneous recordings of buccal pressure change and impedance measured between an electrode in the roof of the mouth and an electrode located at the anterior tip of the cleithrum. Note that the onset of subambient pressure corresponds with the onset of increasing impedance, indicating abduction of the cleithrum from the roof of the mouth. The period of pressure increase is associated with adduction of the cleithrum, indicating compression of the buccal cavity.

per per second of recorded time, providing a measurement resolution of 0.5 msec per 0.1 mm of chart paper.

Results

Morphology.—A complex of skeletal and muscular features, characteristic of tetraodontoids (Diodontidae plus Tetraodontidae), functioned during inflation behavior of *Chilomycterus* (see

anatomical descriptions in Sarkar, 1960; Winterbottom, 1974a; Tyler, 1980). There are three principal skeletal characters: (1) The buccal cavity of *Chilomycterus* expanded ventrally and posterolaterally but not by lateral motion of the suspensoria. The suspensoria were firmly attached to the neurocranium, preventing lateral expansion of the buccal cavity. Also, the premaxillae were not protrusible, preventing anterior expansion.

(2) Two features of the pectoral girdle allowed it to play a key role in ventral expansion and compression of the buccal cavity. First, the girdle had a mobile joint with the neurocranium between the cleithrum and supracleithrum, as described by Parr (1927) and Winterbottom (1971) for *Sphoeroides.* This joint permitted the pectoral girdle (formed principally by the cleithrum) to rotate posteroventrally. During anterior rotation, the posterior regions of the pectoral girdle, the cleithra, also converged medially. We referred to these motions of the pectoral girdle as abduction and adduction, respectively. Second, the cleithra were elongate anteriorly, running as far as the midway point along the hyoid bars. The hyoid apparatus and the cleithra were intimately associated, being connected by the sternohyoideus muscle (see below) and connective tissue between the posterior rim of the first branchiostegal ray and the cleithrum (Figs. 1–2). As a result, these two structures could not move independently of each other.

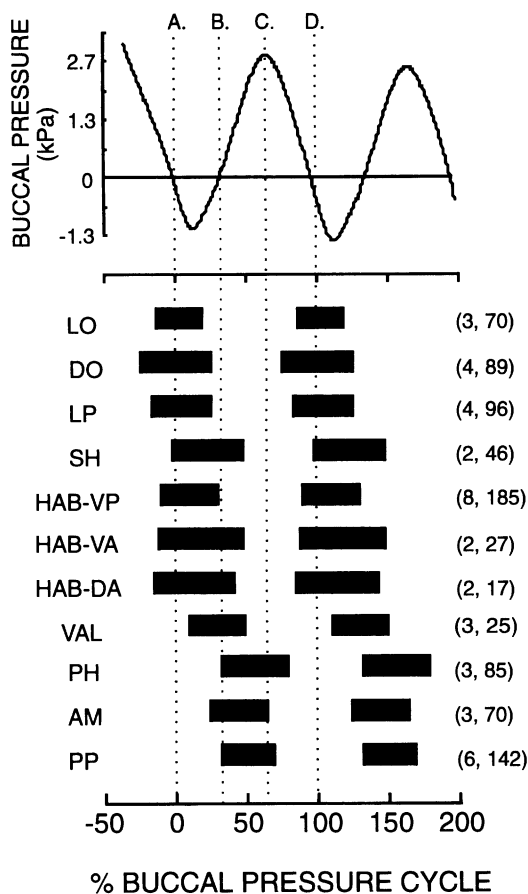(3) The first branchiostegal ray was greatly

Fig. 6. Summary diagrams of muscle activity patterns in eight muscles, relative to buccal pressure changes. Buccal pressure trace represents mean values of time to minimum pressure, time to ambient pressure, time to maximum pressure, and time to the onset of subambient pressure in the subsequent cycle (n = 289 cycles). Muscle activity bars indicate the average onset time of each muscle relative to the onset of subambient pressure (time A), and the duration of activity bursts as a percent of cycle duration. Note that this figure illustrates two cycles of a continuous behavior so that muscle activity at the far left and far right of the figure is incomplete. Numbers in parentheses indicate the number of individuals from which data were collected for that muscle and the total number of inflation cycles used to calculate the means. Dashed lines: A. onset of inflation cycle (ambient pressure), B. time to achieve ambient pressure following the initial pulse of negative pressure, C. time to maximum pressure, D. end of cycle (return to ambient pressure). Abbreviations as in Figure 1 and VAL = valvulus muscle.
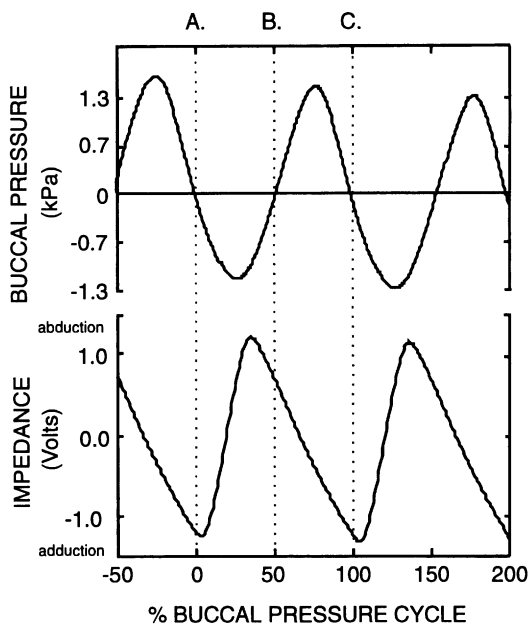

Fig. 7. Summary diagram of the relationship between buccal pressure and impedance measured between an electrode on the roof of the mouth and a second electrode either in the hyohyoideus abductor muscle or on the anterior tip of the cleithrum. The pressure trace represents mean values of time to minimum pressure, time to increase to ambient level, time to maximum pressure, and time to the onset of subambient pressure in the subsequent cycle. The impedance trace represents average values of time from the onset of subambient pressure to minimum impedance, maximum impedance, and the subsequent minimum impedance of the next cycle (n = 74 cycles). Data from the two impedance locations are combined. Standard errors for all temporal variables are less than 4%. Dashed lines: A. onset of inflation cycle (ambient pressure), B. time when pressure crosses ambient pressure following the initial negative pulse, C. end of cycle (return to ambient pressure).

enlarged and had a well-developed hinge joint with the ceratohyal. The branchiostegal was ligamentously connected, at its posterior border, to the cleithrum and to the suspensoria via the other branchiostegals. Because these connections prevented movements of the posterior region of the branchiostegal, if the angle between the anteroventral surface of the first branchiostegal and the ceratohyal was decreased, the anterior region of the hyoid apparatus rotated ventrally (Figs. 1, 8). This motion at the branchiostegal-hyoid joint was flexion and the subsequent motion of the hyoid apparatus was abduction.

In addition, there were four features of cranial musculature that functioned prominently in the inflation mechanism: (1) Several muscles were positioned near the articulation between the cleithra and the neurocranium and were positioned to either abduct or adduct the cleithra. These included the levator pectoralis, which
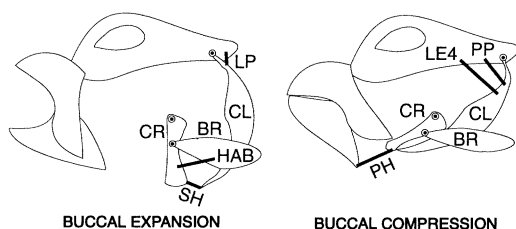
BUCCAL EXPANSION   BUCCAL COMPRESSION

Fig. 8. Schematic diagram of the mechanism of buccal expansion and compression. Expansion is caused by pectoral girdle retraction and depression of the hyoid apparatus. The cleithrum rotates about its joint with the supracleithrum; the ceratohyal rotates at its posterior connection with the suspensorium and the interopercular bone. These two structures are linked by the sternohyoideus muscle. The major depressor muscle of the hyoid apparatus is the hyohyoideus muscle which flexes the joint between the first branchiostegal ray and the ceratohyal. Pectoral girdle retraction is assisted by the levator pectoralis. Buccal compression involves protraction of the pectoral girdle by action of the protractor pectoralis and fourth levator externus muscles and protraction of the hyoid apparatus by the protractor hyoideus muscle. Abbreviations as in Figure 1.

passed from the pterotic and surrounding elements to the tip of the posterodorsal extension of the cleithrum, positioning the muscle to abduct the cleithrum on the supracleithrum (Figs. 1, 8). The protractor pectoralis connected the pterotic to the anterior region of the cleithrum, distal to the cleithrum-supracleithrum joint. Because of its position, the protractor pectoralis adducts the pectoral girdle during contraction (Figs. 1, 8). In addition, the fourth levator externus contributed a considerable portion of its mass to a section that connected the pterotic and prootic bones to the cleithrum distal to the attachment of the protractor pectoralis. This muscle formed a broad, but thin, sheet of muscle that was positioned to contribute to adduction of the pectoral girdle (Fig. 1).

(2) The hyohyoideus abductor muscle of *Chilomycterus* was massive and had complicated fiber arrangements. It completely covered the enlarged first branchiostegal ray, in both ventral and dorsal view (Figs. 1–2). Ventral fibers originated on the blade of the first branchiostegal and ran anteriorly around the anterior end of the hyoid apparatus before turning dorsally, enveloping the anterior end of the hyoid, and converging on the midline dorsal to the medial hyoid structures. Some fibers attached to the dorsal surface of the hyoid bars, but most attached to a midline raphe which separated the convergence of the left and right-side members of this muscle. Fibers that originated on the

dorsal surface of the first branchiostegal ray generally ran anteriorly and dorsally to converge at the midline and attach to the posterior region of the midline raphe. Flexion of the joint between the first branchiostegal ray and the ceratohyal in an anesthetized individual caused the anterior region of the hyoid to rotate ventrally, depressing the floor of the buccal cavity. Movement of the posterior region of the branchiostegal was prevented by its connection to the cleithrum.

The pectoral girdle and hyoid apparatus were connected anteriorly via a small sternohyoideus muscle between the anterior tips of the cleithra and the medial hyoid elements (Fig. 2B). As a result of this linkage between the pectoral girdle and hyoid apparatus, movement of one element caused a corresponding movement of the other element.

(3) The protractor hyoideus muscle was well developed and was positioned so as to assist the pectoral girdle muscles in adducting the hyoid apparatus during buccal compression. This muscle attached the posteroventral region of the mandible to the ceratohyal and the base of all branchiostegal rays (Fig. 1).

(4) The valvulus muscle and associated oral valve were well developed in *Chilomycterus*. The valve prevented water from escaping the mouth anteriorly during buccal compression.

Buccal pressure. —Inflation sequences were characterized by a cyclic pattern of decreasing and increasing pressure pulses (Fig. 3). A single sequence included from three to more than 20 cycles and took as little as 10 sec or as long as 34 sec to reach full inflation. Maximum pressure generally increased about twofold through the initial 85% of an inflation sequence (e.g., Fig. 3). However, as the fish approached complete fullness, peak positive pressure rose considerably during the final few cycles of an entire sequence. The highest pressure recorded was 81.6 kPa above atmospheric.

An average cycle (Fig. 6) began with a sharp reduction in pressure, which reached its minimum at $14.2 \pm 0.6\%$ of the total cycle, or 67.9 msec from the onset of pressure reduction (Figs. 5–6). Pressure dropped to an average value of 1.160 ± 0.05 kPa below atmospheric, before rising to atmospheric pressure at $31.9 \pm 0.98\%$ of the total cycle. Increased buccal pressure reached an average maximum value of 3.07 kPa ± 0.1 kPa above atmospheric at $65.6 \pm 0.9\%$, or an average of 313.5 msec following the onset of the cycle. Buccal pressure then dropped sharply until the start of the subsequent cycle (Fig. 5). Each pressure cycle lasted for an av-

erage of 477.83 msec, but cycle time varied greatly, from as short as 160 msec to as long as 2250 msec.

Impedance.—Impedance traces revealed similar patterns of movement for both the anterior hyoid region and the anterior section of the cleithra (Figs. 5, 7; Table 1). The pressure patterns reported here were based on sequences that were simultaneously recorded with impedance, and they differed slightly from average values reported from recordings made with electromyograms. The onset of subatmospheric buccal pressure (= the onset of the cycle) was nearly synchronous with minimum impedance, indicating that the electrodes had stopped moving toward one another and began to move apart as pressure dropped below ambient hydrostatic pressure (Table 1; Figs. 5, 7). Impedance rose sharply, indicating abduction of structures, until reaching its maximum at 33.1 ± 1.7% of the total cycle (Fig. 7). Maximum impedance occurred shortly before the time when buccal pressure increased to ambient at 48.9 ± 1.6% of the cycle (Fig. 7, time B). During rising impedance, pressure fell to its lowest level (at 30.7% of the cycle) and then rose to ambient. After crossing the ambient level, pressure rose to its maximum level and then dropped to begin the next cycle. This period of increasing and decreasing positive pressure (between times B and C on Fig. 7) was associated with a roughly constant decline in impedance, indicating adduction of the hyoid and pectoral girdle, with reference to the roof of the buccal cavity.

Muscle activity patterns.—Each of the eight muscles from which we recorded electromyograms fell into one of two general activity patterns. These patterns generally corresponded to two features of the buccal pressure pattern: (1) the period of declining buccal pressure, particularly once pressure dropped below ambient (the expansive phase); and (2) the period of increasing buccal pressure, above ambient (the compressive phase; Fig. 6). Five of the eight muscles showed activity during the period of declining and subambient buccal pressure: the levator operculi, dilatator operculi, levator pectoralis, sternohyoideus, and the hyohyoideus abductor (Figs. 3–4, 6). The hyohyoideus abductor muscle was bilaterally active during this period and on both the dorsal and ventral surface of the first branchiostegal ray (Fig. 6). Compressive phase muscles were all activated at about the time when the increasing buccal pressure reached ambient (Fig. 6, time A). The period of increasing buccal pressure was characterized

TABLE 1. STATISTICS FOR SIMULTANEOUSLY RECORDED BUCCAL PRESSURE AND IMPEDANCE FROM TWO LOCATIONS, THE ANTERIOR TIP OF THE CLEITHRUM AND THE ANTERIOR REGION OF THE HYOHYOIDEUS MUSCLE (TWO INDIVIDUALS PER LOCATION). Impedance measurements were made relative to a fixed point on the roof of the mouth.

Location of impedance electrode	Time to minimum pressure	Time to zero pressure	Time to maximum pressure	Minimum pressure	Maximum pressure	Time to minimum impedance	Time to maximum impedance
Hyohyoideus abductor m. (n = 25)	28.3 (1.8)	47.4 (3.4)	72.1 (2.5)	−1.33 (0.1)	0.85 (0.04)	5.7 (1.4)	35.8 (1.8)
Cleithrum (n = 49)	31.0 (1.6)	49.6 (1.7)	78.9 (1.4)	−0.97 (0.1)	2.08 (0.21)	2.1 (1.3)	31.7 (2.4)

Table entries are mean ± SE.
Time entries are in msec and pressures in kPa.

by activity in four muscles: section 2α of the adductor mandibulae, the protractor hyoideus, the valvulus, and the protractor pectoralis (Figs. 3–4, 6).

Activity durations varied from 37.4% of the cycle for the protractor pectoralis muscle to 68.3% of the cycle for the anterior ventral region of the hyohyoideus abductor muscle. There was a general trend for muscle burst durations to be strongly correlated with the duration of the pressure cycle (Fig. 3).

DISCUSSION

Inflation behavior in *Chilomycterus* consisted of repeated cycles of buccal expansion and compression as water was drawn into the mouth and pumped to the stomach. Our combined observations of the anatomy, buccal pressure patterns, hyoid and cleithrum movements, and muscle activity patterns of inflation behavior in *Chilomycterus* provided the basis for our interpretation of the underlying mechanisms.

Interpreting buccal pressure patterns.—In this section, we address two issues concerning this pattern of buccal pressure. First, what movements are associated with the pressure changes? And second, what are the likely patterns of water movement during the phases of the pressure cycle?

The impedance data for both the anterior tip of the cleithrum and the anterior region of the hyohyoideus abductor muscle clearly indicated that pectoral girdle and hyoid abduction, relative to the roof of the buccal cavity, occurred throughout the period of declining, subambient pressure. The hyoid apparatus and the pectoral girdle moved away from the roof of the mouth, expanding the buccal cavity, while pressure dropped to its lowest level (Fig. 7). The entire period of increasing and superambient buccal pressure was associated with hyoid and pectoral girdle adduction. These structures moved closer to the roof of the mouth, compressing the buccal cavity, while pressure increased to its maximum value and then dropped again to ambient (Figs. 5, 7). In summary, a cyclic pattern of abduction and adduction of the hyoid and pectoral girdle, indicating expansion and compression of the buccal cavity, corresponded closely to the periods of decreased and increased buccal pressure, respectively.

The period of subambient pressure was associated with relatively constant abduction of the hyoid and cleithra (see impedance traces in Figs. 5, 7). We suggest that abduction caused the volume of the buccal cavity to increase, cre-

ating the decline in buccal pressure. Buccal pressure then equilibrated as water entered the opened mouth, eliminating the pressure gradient that had been established against the area around the head. Hyoid and pectoral girdle abduction ceased at 33.1% of the cycle, prior to the time when the normalizing pressure reached ambient at 48.9% of the cycle (Figs. 5, 7). Mouth opening during this period was indicated by the pattern of EMG activity in the levator and dilatator operculi muscles during the early part of the expansion phase (Fig. 6).

Buccal compression was associated with a period of superambient pressure that included times of both increasing and decreasing pressure, much like a reverse image of the expansion phase (Fig. 7). We suggest that, as the buccal cavity was compressed, pressure increased to its maximum level and was then relieved as water was permitted to pass through the esophagus into the stomach. Our observation that pressures returned to ambient at the conclusion of all but the last few cycles of the inflation sequence suggests that during most of the inflation process the stomach was able to accommodate the change in volume without resistance.

This compliant change in shape and volume of the stomach and body of *Chilomycterus* has been noted in another diodontid, *Diodon*, and correlated with unusual mechanical properties of the skin (Brainerd, 1994). The skin of *Diodon* can undergo about a 50% increase in length before any resistance to stretch occurs (Brainerd, 1994). Although our observations suggest that *Chilomycterus* skin is not as deformable as that of *Diodon* [for example, *Chilomycterus* does not become spherical during inflation as *Diodon* does (Brainerd, 1994)], the repeated cycles of inflation during which buccal pressures return to ambient levels at the end of each cycle appear to correspond to increases in body volume that deform the skin within the range of unresisted stretching. Once resistance is encountered, only three or four more cycles of inflation are attempted by the fish. At the conclusion of each inflation sequence, the body of the fish became oblong but rounded, and the skin was stiff to the experimenter's touch.

Chilomycterus demonstrates an unusual capacity to generate extreme buccal pressures. Suction feeding sunfishes (*Lepomis*) routinely generate buccal pressure changes of 40 kPa below ambient during prey capture (Lauder, 1980). In comparison, the minimum values recorded in this study averaged 1.16 kPa subambient (compare to a range of 0.67–1.33 kPa subambient reported for *Diodon holocanthus* by Brai-

nerd, 1994) and occasionally were as low as 5.3 kPa below atmospheric. The key to strong inflation, however, is the increase in buccal pressure used to completely fill the stomach with water. Among our experimental individuals of *Chilomycterus*, maximum pressures reached an average of 48.1 kPa during the final cycles of an inflation sequence, and we measured pressures as high as 81.6 kPa superambient. In comparison, during the preparatory phase of suction feeding and during prey manipulations, *Lepomis* generates a positive pressure pulse of up to 18.7 kPa above atmospheric. This limited comparison suggests that *Chilomycterus* generates unusually high pressures during inflation sequences. Some caution is warranted in interpreting this comparison, however, because pressures will not be directly comparable across individuals with different sizes of buccal cavity nor across different behaviors. Nevertheless, unusually high buccal pressures do seem to be a feature of the inflation mechanism of *Chilomycterus*.

The valvulus muscle, found only in the Diodontidae and Tetraodontidae (Winterbottom, 1974a), is active during the superambient pressure phase of the inflation cycle suggesting that it functions to reinforce the oral valve, preventing the backflow of water out of the mouth.

Mechanisms of buccal expansion and compression.— Linked movements of the hyoid apparatus and pectoral girdle were responsible for ventral expansion of the buccal cavity and its subsequent compression. Of the components of the skull in *Chilomycterus*, only the ventral structures are sufficiently kinetic to contribute to buccal expansion and compression. The suspensoria are immovably fused at their articulation with the neurocranium, preventing the lateral abduction and adduction that characterizes most teleost suspensoria (Lauder, 1985). In addition, the upper jaw of *Chilomycterus*, as in most other tetraodontiforms, directly abuts the neurocranium and is not protrusible, so the buccal cavity cannot be expanded anteriorly. Therefore, our observations indicate that buccal expansion and compression were accomplished by movements of the ventral and posterior structures of the skull, the hyoid apparatus and pectoral girdle.

The key elements of the musculoskeletal mechanism underlying buccal expansion and compression are diagrammed in Figure 8. In the two-dimensional view (Fig. 8), the pectoral girdles move about their articulation with the neurocranium and the ceratohyals rotated about their connection to the medial surface of each suspensorium and interopercular bone. The

sternohyoideus connected the anterior end of the cleithra to the anteromedial hyoid elements. Any force that rotated either element also produced rotation of the other element, once slack is taken up in the sternohyoideus muscle. We give details of the mechanism for buccal compression first because it is simpler than that for expansion.

At least three muscles are in position to contribute to adduction of the pectoral girdle by flexion of the joint between the cleithrum and the supracleithrum. These are the protractor pectoralis and the fourth levator externus, which connect the neurocranium to the cleithrum, and the protractor hyoideus, which connects the posterior region of the mandible to the hyoid apparatus (Figs. 1, 8). Our EMG recordings from the protractor pectoralis and protractor hyoideus muscles indicated that they were active together with the adductor mandibulae during periods of buccal compression and, hence, adduction of the pectoral girdle (Figs. 3–4, 6).

Expansion of the buccal cavity involved abduction (posteroventral rotation) of the pectoral girdle and the hyoid apparatus. Our observations indicated that these motions were produced principally by the hyohyoideus abductor muscle, with assistance from the levator pectoralis and sternohyoideus muscles (Fig. 8). The massive hyohyoideus abductor muscle is positioned to flex the joint between the first branchiostegal and the ceratohyal (Figs. 2, 8). An important feature of this mechanism is the ligamentous connection between the posterior region of the first branchiostegal ray and the cleithrum, which prevents motion of this section of the branchiostegal ray, particularly in the dorsal-ventral plane. The ventral fibers of the hyohyoideus abductor muscle run parallel to the branchiostegal ray and the ceratohyal, envelop the anterior end of the ceratohyal, and attach either to the dorsal surface of the hyoid apparatus or to the midline raphe that is located dorsal to the hyoid apparatus. The dorsal fibers also run parallel to the branchiostegal ray and meet their antimeres at the posterior region of the midline raphe (Fig. 2). The arrangement of the ventral and dorsal regions of the hyohyoideus abductor muscle, together with the fixed position of the posterior region of the branchiostegal ray, result in flexion of the joint between the branchiostegal ray and the ceratohyal, this being the only motion permitted during contraction of the hyohyoideus abductor muscle (Fig. 8).

There has been some debate in the literature over the role of the postcleithral apparatus and the obliquus inferioris muscle in the pufferfish

inflation mechanism (Parr, 1927; Winterbottom, 1971). In *Chilomycterus*, the obliquus inferioris is reduced to a few slender slips of musculature that descend from the epaxialis and insert primarily on the postcleithrum and coracoid (section d of Winterbottom, 1971). Because of the very small size of the muscle in this species, we feel it is unlikely that it plays any significant role in movement of the pectoral girdle during inflation. The muscle contributes some fibers to the longitudinal cutaneous, a muscle thought to be significant in deflation of the inflated body (Brainerd, 1994). Other puffer species (e.g., *Sphoeroides nephalus*) have a well-developed obliquus inferioris muscle that may have a more pronounced role in pectoral girdle action.

Inflation mechanism as an evolutionary novelty.—
Within the Tetraodontiformes, inflation behavior is restricted to the pufferfish sister taxa Tetraodontidae and Diodontidae (Winterbottom, 1974a). Among the features of the buccal pumping mechanism that we describe in *Chilomycterus schoepfi*, many appear to be synapomorphies for the pufferfish clade. Key features of the inflation mechanism in *Chilomycterus* that are not known to occur in other tetraodontiform clades include the following: (1) a highly kinetic articulation between the supracleithrum and the cleithrum; (2) the enlarged first branchiostegal ray, including its well-developed hinge joint with the ceratohyal (Winterbottom, 1974a); and (3) the massively developed hyohyoideus abductor muscle associated with the enlarged first branchiostegal ray (Winterbottom, 1974a). An additional feature of the inflation apparatus that is unique within tetraodontiforms is the novel attachment of the fourth levator externus muscle on the cleithrum. We have confirmed the presence of the above-noted features of the inflation mechanism by dissection of at least one specimen of the following additional pufferfish species: *Arothron manillensis, Canthigaster rostrata, Chelonodon patoca, Diodon holocanthus, Lagocephalus scleratus,* and *Sphoeroides nephalus.* In conjunction with the anatomical observations on some of these, and other taxa by Winterbottom (1974a) and Tyler (1980), we conclude that these features are derived for pufferfishes.

In addition to these derived characteristics, a large suite of plesiomorphic anatomical and functional features are involved in the inflation mechanism. Most of the muscles that we studied retain their generalized function, although the roles of some muscles may be altered within the context of inflation behavior. For example, the

protractor pectoralis and levator pectoralis muscles retain their ability to protract and retract the cleithrum, respectively, but these actions occur within a new context during inflation. With the exception of the fourth levator externus, whose novel attachment to the cleithrum results in a novel action (i.e., being positioned to protract the cleithrum), all of the muscles we studied retain their plesiomorphic action. Even the hyohyoideus abductor muscle, that functions so prominently in the buccal expansion mechanism, exhibits its basic plesiomorphic function. In its generalized tetraodontiform condition, this muscle is positioned to flex the joint between the hyoid and the first branchiostegal ray (Winterbottom, 1974a). This function is modified in puffers, principally by the firm connection between the posterior margin of the branchiostegal ray and the cleithrum. The result is that, rather than abduct the branchiostegal ray, the muscle causes posteroventral rotation of the hyoid bar, causing buccal floor depression and posteroventral rotation of the pectoral girdle.

The existence of the hyohyoideus abductor mechanism for hyoid depression may be related to the presence of a highly mobile pectoral girdle and the trend toward reduction of the sternohyoideus in tetraodontiforms. The generalized teleost mechanism for depression of the hyoid bar involves contraction of the sternohyoideus muscle (Lauder, 1985). This mechanism relies upon the presence of a fixed cleithrum that serves as a solid framework against which the sternohyoideus could pull the hyoid elements posteroventrally upon contraction. The presence of the highly mobile pectoral girdle in puffers, and the absence of stabilizing hypaxial muscles posterior to the cleithrum, compromises the ability of the sternohyoideus muscle to act forcefully at its insertion on the hyoid elements. Pufferfishes lack the urohyal for insertion of the sternohyoideus, and the muscle is much smaller than seen even in more generalized tetraodontiforms (Winterbottom, 1974a; Turingan and Wainwright, 1993).

Comparative data are not yet available for the motor patterns and pressure data that we report here. Many tetraodontiforms are known to make extensive use of a buccal compression behavior in which they forcefully expel jets of water from their mouth to expose sand-covered prey (Fricke, 1975; Frazer et al., 1991). A key goal of our future research on the evolutionary origins of the pufferfish inflation mechanism will be to explore the existence of intermediate buccal compression behaviors in the outgroups to puffers.

LITERATURE CITED

BRAINERD, E. L. 1994. Pufferfish inflation: functional morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). J. Morphol. 220:243–262.

DINGERKUS, G., AND L. D. UHLER. 1977. Enzyme clearing of alcian blue stained whole vertebrates for demonstration of cartilage. Stain Tech. 52:229–232.

FRAZER, T. K., W. J. LINDBERG, AND G. R. STANTON. 1991. Predation of sand dollars by grey triggerfish, *Balsites capriscus,* in the northern Gulf of Mexico. Bull. Mar. Sci. 48:159–164.

FRICKE, H. W. 1975. Losen einfacher probleme bei einen fisch (freiswasser an *Balsites fuscus*). Z. Tierpsychol. 38:18–33.

GABRIEL, M. L. 1940. The inflation mechanism of *Sphoeroides maculatus.* Biol. Bull. 79:372.

LAUDER, G. V. 1980. The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. J. Exp. Biol. 88:49–72.

———. 1985. Aquatic feeding in lower vertebrates, p. 210–229. *In:* Functional vertebrate morphology. M. Hildebrand, D. Bramble, K. Liem, and D. Wake (eds.). Harvard Univ. Press, Cambridge, MA.

MYER, R. F. 1989. Micronesian reef fishes. Coral Graphics, Barrigada, Guam.

PARR, A. E. 1927. On the function and morphology of the postclavicular apparatus in *Sphoeroides* and *Chilomycterus.* Zoologica. 9:245–269.

RANDALL, J. E. 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanogr. 5:665–847.

———. 1983. Caribbean reef fishes. TFH Publications, Neptune City, NJ.

ROSEN, N. 1913. Studies of the Plectognathi, 4: the body muscles. Ark. Zool. (Stockholm). 8:1–14.

SARKAR, S. P. 1960. A study of the head of an Indian pufferfish, *Sphoeroides oblongus* (Bloch). Unpubl. Ph.D. diss., Univ. of Leiden, Leiden, The Netherlands.

THILO, O. 1899. Die Entsthung der Luftsacke bei den Kugelfishen. Anat. Anzeig. 16:73–87.

TURINGAN, R. G., AND P. C. WAINWRIGHT. 1993. Morphological and functional bases of durophagy in the queen triggerfish, *Balistes vetula* (Pisces, Tetraodontiformes). J. Morphol. 215:101–118.

TYLER, J. C. 1968. A monograph on the plectognath fishes of the Superfamily Triacanthoidea. Monogr. Acad. Nat. Sci. Philadelphia 16:1–364.

———. 1980. Osteology, phylogeny and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). NOAA Tech. Report, NMFS Circ. 434:1–422.

WINTERBOTTOM, R. 1971. A reinterpretation of the mechanism of extension of the pufferfish postclethral apparatus, with some notes on the oblique eye muscles of the Diodontidae (Plectognathi). Notulae Nature. 440:1–7.

———. 1974a. The familial phylogeny of the Tetraodontiformes (Acanthoperygii: Pisces) as evidenced by their comparative myology. Smith. Contrib. Zool. 155:1–201.

———. 1974b. A descriptive synonymy of the striated muscles of the teleostei. Proc. Acad. Nat. Sci. Philadelphia 125:225–317.

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