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Pelvic-Fan Flaring and Inflation in the Three-Tooth Puffer, *Triodon macropterus* (Tetraodontiformes: Triodontidae), with Additional Observations on Their Behavior in Captivity

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Triodon macropterus, the Three-tooth Puffer, is the sole extant representative of Triodontidae. It is characterized by a large pelvic fan that it flares when disturbed. Unlike Tetraodontidae (pufferfishes) and Diodontidae (porcupinefishes), *T. macropterus* has not previously been documented to inflate its abdomen although some nineteenth-century reports implied that it can. Those reports were rejected by a mid-twentieth-century anatomical study, and no new information about inflation in *T. macropterus* has been reported in the intervening 70 years, in part because the species was rarely collected. In this study, we used a combination of imaging techniques to investigate if *T. macropterus* can inflate. We examined 13 photographs of *T. macropterus* in which the pelvic fan was prominently flared; in seven of these, the abdomen was also inflated. We also studied captive *T. macropterus* at the Okinawa Churaumi Aquarium, which allowed us to make videographic and ultrasound studies of live individuals to confirm inflation behavior in this species. Videography shows that pelvic-fan flaring always preceded inflation. Ultrasound data from four trials of one captive individual show that water is buccal pumped for inflation into the stomach to produce an increase in volume of 30%. Powerful adduction of the suspensorium correlates with stomach inflation. We prepared x-rays and dissected three specimens to evaluate mobility of the long pelvic bone and its role in expanding the ventral abdominal recess during pelvic-fan flaring. We also studied the digestive tract and discovered a thin-walled pyloric region of the stomach that allows inflation to occur. Differences between the inflation mechanism of *T. macropterus* and those of tetraodontids and diodontids include: (1) inflation is slower and less extreme in *T. macropterus*; (2) *T. macropterus* has a pelvic bone that expands the ventral abdominal recess; (3) *T. macropterus* has smaller folds in the peritoneum and these are ventral to, rather than dorsal to, the digestive tract; (4) the ribs and long postcleithra of *T. macropterus* limit abdominal inflation; (5) the first branchiostegal ray of *T. macropterus* is much smaller than in tetraodontids and diodontids, which use the expanded bone to rapidly pump water for inflation; and (6) the pectoral girdle of *T. macropterus* is larger and much less mobile than that of tetraodontids and diodontids. Although we interpret that inflation serves similar roles in defense, the differences in the inflation mechanism of *T. macropterus* suggest that it evolved independently, and optimization of inflation behavior on three phylogenetic hypotheses for Tetraodontiformes supports this interpretation.

AN inflation mechanism of the stomach and abdomen, usually interpreted as a synapomorphy of Diodontidae + Tetraodontidae, allows the body to expand several times its ordinary size by taking in large amounts of water or air if the fish is removed from water (e.g., Brainerd, 1994). Inflation is a defensive mechanism to appear larger because it occurs when, for example, other fishes or wading birds attack puffers (e.g., Recher and Recher, 1968; Shepherd et al., 2019; there is no support for other speculations, such as inflation with air allowing pufferfishes to be blown along the water's surface by wind or that inflation serves in respiration; see Rosén, 1912: 12). In diodontids and tetraodontids, the broad and flat first branchiostegal ray and large *hyohyoideus abductores* are used to rapidly pump water into the buccal cavity and subsequently into the stomach, where it is contained by sphincters associated with a ventral, expansible, sac-like diverticulum of the stomach (Gabriel, 1940; Breder and Clark, 1947; Brainerd, 1994; Wainwright and Turingan, 1996, 1997; McGee and Clark, 2014). In some species, such as *Diodon holocanthus*, inflation results in a 300% increase in body

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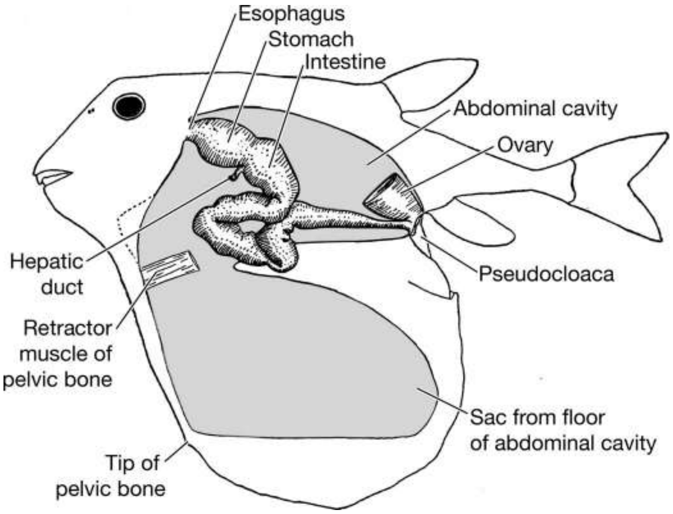

Fig. 1. Illustration from Breder and Clark (1947: fig. 3) showing their terminology for the viscera of *Triodon macropterus* (SU 13747, 391 mm SL). Gray shading indicates their interpretation of the extent of the abdominal cavity into the pelvic fan. In this paper, we revise their interpretations of anatomical structures and show that, contrary to their interpretation, *T. macropterus* can inflate.

volume and causes the large body spines to erect, further contributing to defense (Brainerd, 1994).

Although the common name "Three-tooth Puffer" suggests that the single extant species of Triodontidae, *Triodon macropterus*, can inflate, literature reports are unclear. Until the early 2000s, *T. macropterus* has remained rarely collected and poorly known (Matsuura, 2015; see review of historical collections in Tyler, 1967; for osteology, Tyler, 1962, 1980; and for myology, Winterbottom, 1974). The descriptions of *Triodon macropterus* Lesson 1829 and *Triodon bursarius* Cuvier 1829 (a synonym of *T. macropterus*; see Fricke et al., 2022) mention the large pelvic fan below the abdomen in the midline plane (= dewlap of Lesson, 1829, ventral flap of Matsuura et al., 2017). The specific epithet "bursarius" implies a bursa or pouch; however, Cuvier did not describe one. Forty years later, Günther (1870: 270) implied that *T. macropterus* inflates, stating that the abdomen is "dilatable into a very large, compressed, pendant sac, the lower part of which is merely a flap of skin, into which the air does not penetrate. The sac is kept expanded by the very long pelvic bone, the bottom of which is just a flap of skin." Jordan and Snyder (1901: 230) and Herre (1925: 167) closely paraphrased Günther's description but did not add any new information. Rosén (1912: 5, 6) was skeptical of Günther's assessment because the specimens that Günther examined were dry and stuffed, preventing examination of visceral anatomy. In a comparative study of the visceral anatomy of tetraodontiforms, Breder and Clark (1947: 294, fig. 3) described the digestive tract of *T. macropterus* as uniform in width and thickness and illustrated the abdominal cavity extending into the pelvic fan in an hourglass shape (Fig. 1). They explicitly refuted the ability of *T. macropterus* to inflate, stating: "…from the dissections described in this paper it can be concluded that inflation is not possible in this fish" (Breder and Clark, 1947: 294).

Almost all preserved specimens of *Triodon macropterus* have a prominently flared pelvic fan with a large ocellus (eyespot). In contrast, Matsuura et al. (2017) discovered that live individuals in the Okinawa Churaumi Aquarium typically hold the pelvic fan in a retracted position against the ventral body wall (Fig. 2A). When disturbed, they rotate the long pelvic bone (see "Skeleton and muscles," below), which flares the pelvic fan, increases the apparent size of the body, and displays the prominent ocellus (Fig. 2B; Supplemental Video 1; see Data Accessibility). Matsuura et al. (2017) described the morphology of the pelvic fan, including elongate scales that allow the fan to seamlessly retract. When the pelvic fan is fully retracted, the body has a torpedo-like shape (Fig. 2A), like that of some uninflated pufferfishes, such as species of *Lagocephalus*. Individuals of *T. macropterus* at the Okinawa Churaumi Aquarium occasionally flare their pelvic fan, for example, in response to other fishes, to new surroundings, or during buccal cavity expansion (= "yawning" of Matsuura et al., 2017).

There is broad agreement about the monophyly of each of the ten families of Tetraodontiformes, but relationships among families are resolved differently, and the placement of Triodontidae is particularly uncertain. Based on morphology, Triodontidae is the sister group of Molidae + Diodontidae + Tetraodontidae (muscle characters, Winterbottom, 1974; skeletal characters, Tyler, 1980: figs. 1, 2, and Santini and Tyler, 2003, 2004). Some molecular analyses (e.g., Arcila et al., 2015; 1 mitochondrial and 15 nuclear loci) and combined analyses of molecular and morphological datasets (Arcila and Tyler, 2017) also support this topology. More recent studies recover *T. macropterus* in more basal positions within Tetraodontiformes. Betancur-R. et al. (2017) placed *T. macropterus* as sister to ((Triacanthidae + Triacanthodidae) + (Aracanidae + Ostraciidae)); however, there is low bootstrap support for these higher relationships. Ghezelayagh et al. (2022) recovered *T. macropterus* as sister to (Aracanidae + Ostraciidae) with strong bootstrap support.

Given the conflicting literature on the ability of *Triodon macropterus* to inflate and to better understand the evolution of inflation in tetraodontiforms, we examined photographs of freshly captured *T. macropterus* taken by fishers in the field, studied live individuals with videography and ultrasound at the Okinawa Churaumi Aquarium, and used radiographs and dissections to study preserved specimens. We describe pelvic-fan flaring, and, for the first time, document and describe inflation in *T. macropterus* and relate it to the anatomy of the head, pectoral girdle, vertebral column, body cavity, and digestive tract. We also include new information about other behaviors of this species in captivity.

MATERIALS AND METHODS

Abbreviations.—Institutional abbreviations follow Sabaj (2020). We report lengths as standard length (SL), fork length (FL), or total length (TL).

Field photographs.—We studied 13 field photographs with validated locality information taken by colleagues or found by searching "*Triodon macropterus*" in Google Images (Fig. 2; see also Supplemental Fig. 1 for photographs and Supplemental Table 1 for data on the photographs; see Data Accessibility). Most fish in these photos had been caught using hook and line.

Collection of live specimens.—The Okinawa Churaumi Aquarium makes as many as 20 trips per year to waters off the Ryukyu Islands, Japan. The crew uses hand lines to collect specimens of deep-water fishes, such as *Triodon macropterus*. The lines are typically fished at 150–350 m; most *T. macropterus* are caught at 200–300 m and are brought up slowly (< 1 meter per second) to minimize barotrauma. If there are signs of barotrauma, a needle syringe is inserted into the upper abdomen to release the pressure. Fish are transported in water-filled coolers back to the aquarium.

Behavioral scoring and ultrasound examination of live specimens.—One of us (AK) has cared for and observed 45 individuals of *Triodon macropterus* at the Okinawa Churaumi Aquarium since 2003 (Supplemental Table 2; see Data Accessibility), where they are maintained on a diet of fishes, squids, cuttlefish, and shrimps. We observed and made video recordings of one individual (Fish 3) free swimming in its exhibit tank and of two others (Fish 1, Fish 2) in trial tanks for videography and ultrasound studies (Table 1). We conducted and recorded six inflation trials (two trials for Fish 1 and four trials for Fish 2) using a Sony FDR-AX45 (Sony Group Corporation, Tokyo, Japan) and a GoPro Hero5 (GoPro Inc., San Mateo, California, USA) from 19 June 2020 to 1 October 2020. For five of the six trials (two trials for Fish 1 and three trials for Fish 2), we used an ARIETTA Prologue (Hitachi-Aloka Medical Ltd., Tokyo, Japan) to observe inflation of the stomach. Trials ranged in length from 2.5 to 19 minutes. Some trials included a period of acclimation to the trial tank, whereas others did not (trial tanks were too small to maintain fish for periods longer than the trial). We analyzed ultrasound data using OsiriX (vers. 3.9.2–3.9.4, Pixmeo SARL, Geneva, Switzerland).

In trial tanks, we observed and made video recordings of *Triodon macropterus* under two handling conditions:

1. Free swimming: no contact with the fish.
2. Fish held in hand: fish was restrained for examination (indicated by shaded gray boxes in Fig. 3).

We scored two behaviors from the video recordings of the six trials based on external appearance of the fish and, where applicable, ultrasound data:

1. Pelvic-fan flaring. None of our video recordings during our six trials show the pelvic fan fully retracted because individuals had already partially flared the pelvic fan in response to being moved to the trial tank. We scored the fan as partially flared when the pelvic bone was rotated anteroventrally from 0° to 45° from the body axis. When the pelvic bone was rotated anteroventrally > 45°, we scored the fan as fully flared.
2. Inflation. We recognize three inflation states. The first is no externally visible sign of abdominal inflation. Our ultrasound recordings confirmed that water is buccal pumped for inflation into the stomach before any external evidence of inflation. Because we were not always recording ultrasound data to establish the start of inflation, we scored this state as beginning at the first external evidence of inflation. Similarly, deflation was assessed based on external evidence of decreasing abdominal size. We could not confirm deflation with ultrasound because the fish would not deflate when in contact with the ultrasound probe.

We estimated the volume of water buccal pumped during inflation in two ways. First, we subtracted the fish's weight

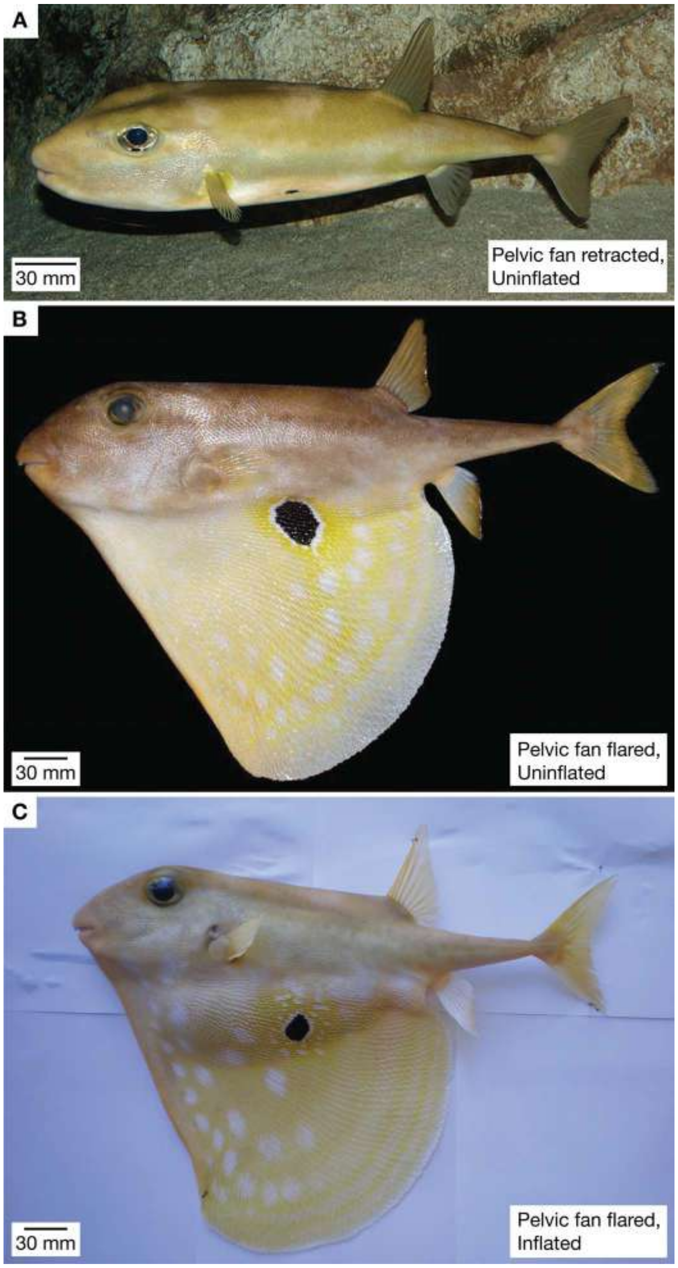

Fig. 2. Photographs of *Triodon macropterus* showing pelvic-fan flaring and inflation. (A) Individual (collected 8 February 2007), 300 mm TL, in display tank at the Okinawa Churaumi Aquarium showing pelvic fan fully retracted and no inflation. Photograph by Atsushi Kaneko; modified from Matsuura et al. (2017). Photograph reprinted with permission from the Ichthyological Society of Japan. (B) Specimen with pelvic fan flared, abdomen uninflated, LBRC-F 00805, 324.5 mm SL, sex unknown. Caught by hook and line in the Maluku Sea, Indonesia, and purchased at a fish market, 11 July 2009. Photograph by Teguh Peristiwady; modified from Wibowo et al. (2020: fig. 2a). Photograph reprinted with permission from the Jurnal Iktiologi Indonesia (see Supplemental Figure 1D, Supplemental Table 1; see Data Accessibility). (C) Individual photographed shortly after capture with pelvic fan flared and abdomen inflated. The fish was destructively sampled for parasites (see Beveridge et al., 2014; Bray and Justine, 2014), 361 mm FL, female. Caught by hook and line off Récif Toombo, Nouméa, New Caledonia (22°34.565′S, 166°27.636′E), 2 July 2009. Collected and photographed by Jean-Lou Justine (see Supplemental Fig. 1H, Supplemental Table 1; see Data Accessibility).

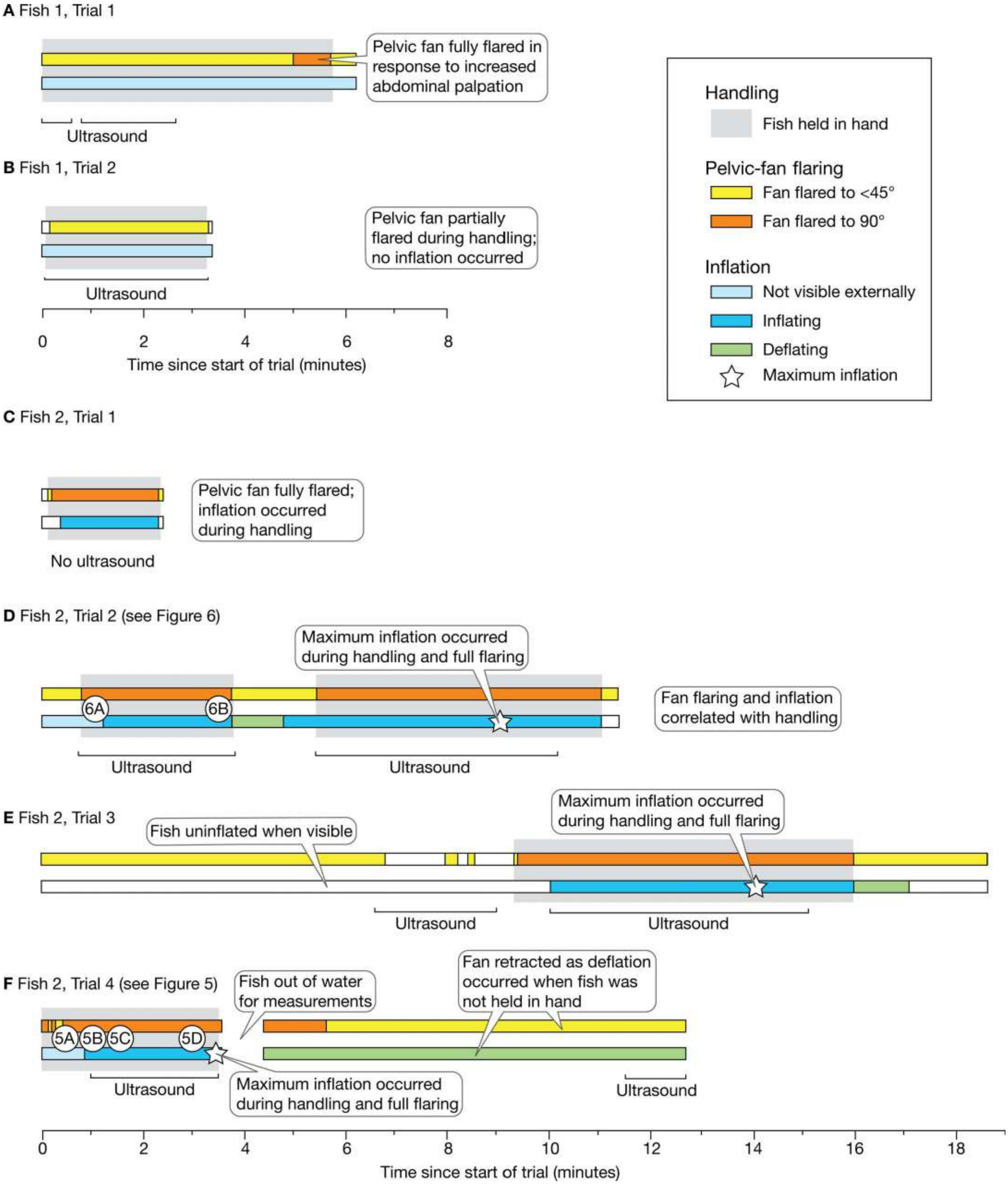

Fig. 3. Kinematics of pelvic-fan flaring and inflation in two individuals of *Triodon macropterus* at the Okinawa Churaumi Aquarium during six handling trials. Shaded gray boxes behind the bars indicate when the fish was held in hand; at other times during the trials the fish was free swimming. Brackets show periods of ultrasound examination. Numbers and letters in circles for parts D and F link figure numbers to the kinematic analysis. (A, B) Trials 1 and 2 of Fish 1 (308 mm SL). Fish 1 flared its pelvic fan in response to being handled but did not inflate in either trial. Fish 1 had been handled during routine care during 16 months in the aquarium. (C–F) Trials 1–4 of Fish 2 (376 mm SL). Fish 2 flared its pelvic fan and inflated in all four trials in response to handling; it had been handled at the aquarium but was a relatively new resident (3 months) at the time of the trials.

Table 1. Examined specimens of preserved and living individuals of *Triodon macropterus* at the Okinawa Churaumi Aquarium.

Fish or museum number, status or preparation, sex	Size (mm SL)	Collection information	Notes
Fish 1, live, sex unknown	308	Collected 15 February 2019 by Okinawa Churaumi Aquarium from 26°49′N, 127°41′E; 200 m depth	Studied with ultrasound; flared pelvic fan but never inflated (Fig. 3A, B)
Fish 2, live, sex unknown	376	Collected 21 March 2020 by Okinawa Churaumi Aquarium from 26°31′N, 127°51′E; 200 m depth	Studied with ultrasound; flared pelvic fan and inflated when handled (Figs. 3C–F, 5, 6; Supplemental Videos 4, 6)
Fish 3, live, sex unknown	320	Collected 27 February 2019 by Okinawa Churaumi Aquarium from 26°38′N, 127°45′E; collection depth unknown	Videorecorded and observed during nighttime (Fig. 4; Supplemental Fig. 3; Supplemental Videos 2, 5)
USNM 451516, whole EtOH specimen, male	358	Collected 12 March 2006 by S. Nakamura off Sesoko Island, Okinawa Prefecture, Japan; 100–200 m depth	Studied with x-ray (Fig. 8A); dissected to examine digestive tract
NSMT P93783, whole EtOH specimen stained with alizarin red, male	343	Collected 25 January 1999 by M. Katoh off Yaeyama, Ryukyo Islands, Okinawa Prefecture, Japan; depth unknown	Studied with x-ray; dissected to examine branchiostegals and digestive tract (Figs. 7, 10)
NSMT P0110597, whole EtOH specimen, sex unknown	360	Collected 22 August 2012 by S. Chiba and E. Katayama from fish market of Chahana Fishing Port, Yoron Town, Ryukyo Islands, Kagoshima Prefecture, Japan; depth unknown	Studied with x-ray (Fig. 8B); dissected to examine pelvic fan and abdominal musculature (Fig. 9)

before inflation from its weight when fully inflated (Fish 2, Trial 4). Second, we measured changes in the diameter of the stomach during ultrasound to estimate volume changes during inflation (Fish 2, Trial 2).

We recorded nighttime behavior of a single individual (Fish 3, 320 mm SL) during one overnight period (16 November 2020) using a high-sensitivity nighttime video camera (Canon ME20F-SH) installed in front of the exhibit tank (4.4 m x 2 m x 2.7 m) that also contained several other species (Fig. 4; Supplemental Video 2; see Data Accessibility). The images are bright because of the camera used, but the fishes were in darkness throughout the recordings.

Approval for animal handling from the Institutional Animal Care and Use Committee of the Okinawa Churashima Foundation is waived for fishes; nevertheless, animal handling was done following guidelines for animal care and welfare for reptiles, birds, and mammals.

We also studied two video recordings made before our study: (1) a video of a wild individual observed by a remotely operated vehicle (ROV) and reported by Matsuura et al. (2017: fig. 5; Supplemental Video 1; see Data Accessibility); and (2) a video of a fourth captive *Triodon macropterus* at the Okinawa Churaumi Aquarium showing an encounter with a Deepwater Squirrelfish (*Ostichthys kaianus*; Supplemental Video 3; see Data Accessibility).

Anatomical study and terminology.—To study anatomical specializations for pelvic-fan flaring and inflation, we prepared x-rays and dissections of three specimens of *Triodon macropterus* (Table 1). Terminology for the skeleton follows Tyler (1980). Myological terminology follows Winterbottom (1973, 1974). We introduce a new term, ventral abdominal recess, to describe the portion of the abdominal cavity formed by rotation of the pelvic bone during pelvic-fan flaring and inflation. Terminology for the abdominal cavity and digestive tract is modified from Breder and Clark (1947) and Brainerd (1994). "Stomach loss" has been reported in some tetraodontiforms based on the absence of gastric glands in the walls of the region posterior to the esophagus (e.g., Fagundes et al., 2016, for *Sphoeroides testudineus*; Suyehiro, 1942, for *Mola mola*). We did not study the histology of the region that we refer to herein as the stomach but instead base our interpretation of it on the presence of esophageal and pyloric sphincters. We distinguish the body and pyloric regions of the stomach following general terminology for fish stomachs discussed by Barrington (1957: 115). We use the term bile duct instead of hepatic duct (used by Breder and Clark, 1947) because the duct drains the gall bladder. Johnson and Britz (2005) were the first to describe Tyler's pouch, which is a thin-walled expansion of the intestine of unknown function. Following Johnson and Britz (2005: fig. 3c–f), the region of the intestine posterior to Tyler's pouch is the rectum. Breder and Clark (1947) described an invagination of the pelvic fan as the "pseudocloaca," into which the anus and genital organs open. A broad comparative anatomical and histological survey of the digestive tract of tetraodontiforms is needed to establish consistent terminology.

Specimen imaging.—Images were taken using a Canon 7D with a 24-70 mm lens. Specimens were illuminated either by daylight LED lights or, when anatomical features were difficult to view due to being lightly stained and surrounded by soft tissue, high-energy royal-blue lights (440–460 nm)

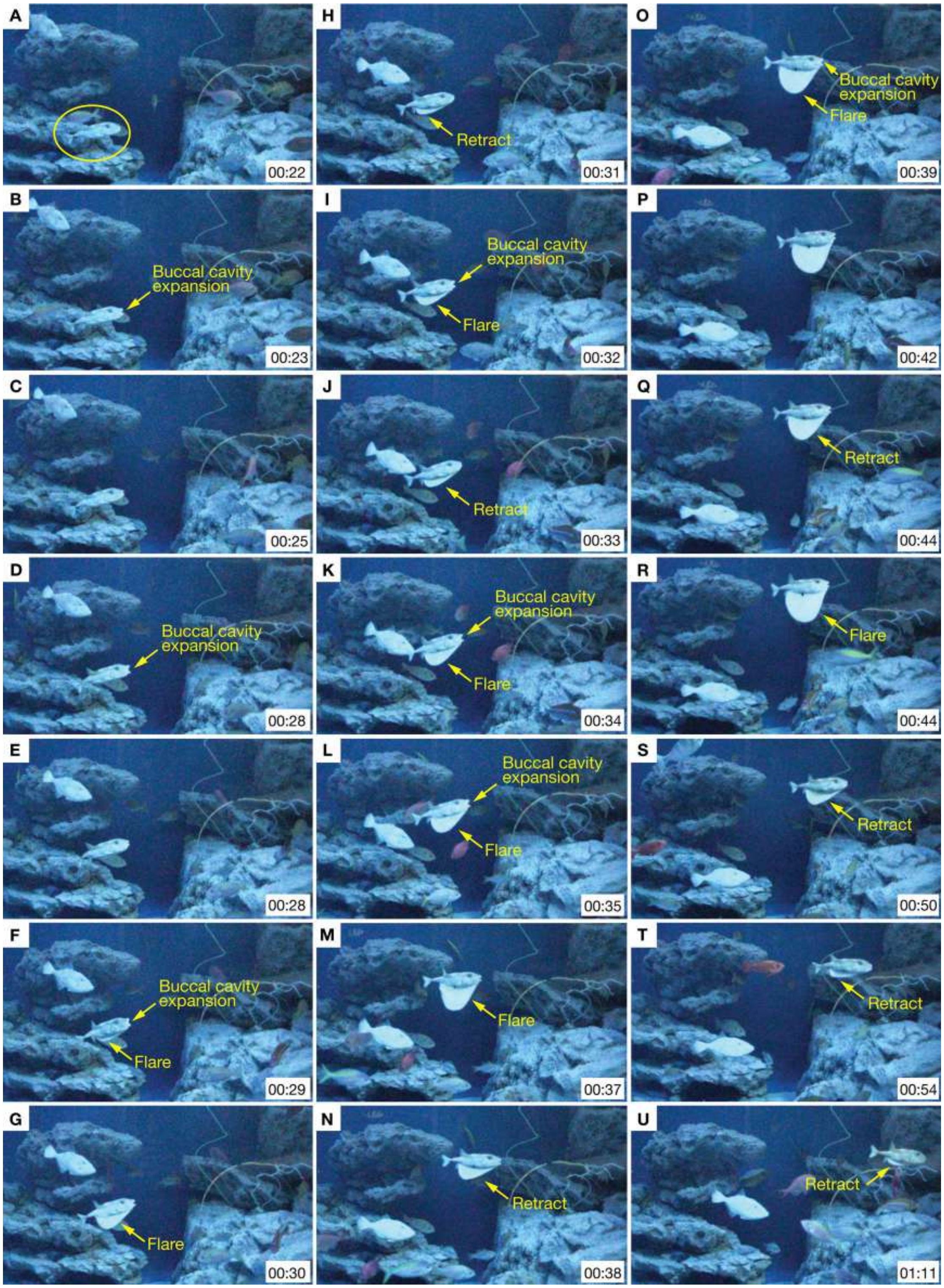

Fig. 4. Frames from a video of *Triodon macropterus*, Fish 3 (320 mm SL), recorded during the night (1900–0400 hours) of 16 to 17 November 2020 at the Okinawa Churaumi Aquarium. Frames A–U show behaviors that occurred during a 1:11 minute interval that included 13 buccal cavity expansions and 11 pump-like expansions and retractions of the pelvic fan. We term this behavior pump flaring. No inflation occurred. Supplemental Video 2 (see Data Accessibility) was the basis for this figure.

following Smith et al. (2018), with filter and LED-light modifications of Girard et al. (2020, 2022).

Character analyses.—We evaluated two behavioral characters, pelvic-fan flaring and inflation, in the ten families of Tetraodontiformes. We scored the presence or absence of pelvic-fan flaring, defined as moving the pelvic bone anteroventrally to expand the skin in the ventral region of the abdomen. If fan flaring occurs in any member of a family, then we scored pelvic-fan flaring as present. We scored inflation behavior as present if any member of a family buccal pumps water into the stomach to cause inflation. We also scored the condition of pelvic-fan flaring and inflation in an outgroup, Lophiiformes, the extant sister group of Tetraodontiformes. We scored pelvic-fan flaring and inflation as absent in Lophiiformes (Table 2).

We optimized pelvic-fan flaring and inflation onto topologies of Arcila and Tyler (2017), Betancur-R. et al. (2017), and Ghezelayagh et al. (2022) using parsimony analysis in TNT v.1.5 for Windows (Goloboff and Catalano, 2016).

RESULTS

Lines of evidence documenting pelvic-fan flaring and inflation behaviors come from: (1) field photographs of wild-caught individuals; (2) a video of a wild specimen recorded using an ROV; (3) observations and video recordings of three individuals in the Okinawa Churaumi Aquarium (two of which we studied concurrently with ultrasound); (4) a video of a captive fish recorded prior to this study; and (5) x-rays and dissections of three specimens.

Field photographic documentation of pelvic-fan flaring and inflation.—In the 13 field photographs of freshly caught *Triodon macropterus* that we studied, all individuals had partially to fully flared pelvic fans (Supplemental Fig. 1; see Data Accessibility). Six individuals (e.g., Fig. 2B; Supplemental Fig. 1A–F; see Data Accessibility) showed no externally visible inflation, but seven did (e.g., Fig. 2C; Supplemental Fig. 1G–M; see Data Accessibility).

Pelvic-fan flaring and inflation in living specimens.—Both pelvic-fan flaring and inflation increase body size (Fig. 2). Body depth increases two to three times when the pelvic fan is fully flared. During inflation, the ventral abdominal region expands and the ocellus size increases.

We observed three types of pelvic-fan flaring. The first was seen in a video of a wild individual observed by an ROV and reported by Matsuura et al. (2017: fig. 5; Supplemental Video 1; see Data Accessibility). In that sequence, the fish gradually flared its pelvic fan during a period of six seconds, and, from the time it began flaring, no retraction occurred. When the pelvic fan had flared about 80°, the fish rapidly swam away and retracted its fan. We refer to this as gradual pelvic-fan flaring.

We observed a second type of pelvic-fan flaring in a nighttime video of a captive individual (Fish 3) at the Okinawa Churaumi Aquarium (Fig. 4; Supplemental Video 2; see Data Accessibility). The pelvic fan became fully flared within 15–20 seconds after a series of 13 buccal cavity expansions and 11 pump-like extensions and partial retractions of the fan, each one resulting in greater expansion of the fan (Fig. 4 and Supplemental Video 2 [see Data Accessibility] show one flaring event; we observed two other similar flaring events by Fish 3 during the one night of recording). We refer to this as pump flaring and only observed it in an individual seemingly undisturbed in its surroundings.

A third type of flaring, which we call rapid flaring, occurred when we netted and handled individuals during moves to the trial tank. In rapid flaring, the pelvic fan is fully flared in < 1 second. We also observed rapid flaring in a video of an individual swimming up to, and aggressively flaring at, a Deepwater Squirrelfish (*Ostichthys kaianus*; Supplemental Video 3; see Data Accessibility).

Table 2. Matrix of pelvic-fan flaring and inflation behaviors in Tetraodontiformes. Absent = 0, present = 1. Behaviors were scored as present in a family if at least one member demonstrates them.

Family	Number of valid extant species	Pelvic-fan flaring	Inflation
Lophiiformes (outgroup)	~358	0	0
Triacanthodidae	24	0	0
Triacanthidae	7	0	0
Balistidae	45	1	0
Monacanthidae	112	1	1
Aracanidae	13	0	0
Ostraciidae	26	0	0
Triodontidae	1	1	1
Molidae	5	0	0
Diodontidae	18	0	1
Tetraodontidae	196	0	1

The pelvic fan can be flared while the fish is stationary or swimming. The three types of pelvic-fan flaring demonstrate fine muscular control of the pelvic fan.

Figure 3 shows kinematic analyses of pelvic-fan flaring and inflation behaviors in Fish 1 and Fish 2. When moved to the trial tank, both individuals rapidly flared their pelvic fans. The degree of flaring ranged from partial (Fig. 3, yellow bars) to full (Fig. 3, orange bars) in relation to handling. For example, the pelvic fan was fully flared when the fish was handled (Fig. 3D, E) but only partially flared when it was free swimming in the trial tank (Fig. 3D, E). At the time of our investigations, Fish 1 had been in captivity for 16 months and had been handled for routine care at the aquarium. Fish 2 had only been in captivity for three months. Fish 1 appeared to be acclimated to handling because it only flared its pelvic fan fully when, in addition to being held, its abdomen was palpated (Fig. 3A); it did not inflate in either trial (Fig. 3A, B). In contrast, Fish 2 flared the pelvic fan fully whenever it was handled, and flaring was followed by inflation in all four trials (Fig. 3C–F). Inflation was always associated with pelvic-fan flaring.

Figure 5 shows frames from a video recording of Fish 2, Trial 4 (kinematics of this trial are presented in Fig. 3F). In Figure 5A, the pelvic fan is flared, but there is no external sign of inflation and the ocellus (white arrow) is not displaced by inflation. Abdominal inflation was visible 49 seconds into the trial (Fig. 5B) and later extended into an area ventral to the gills and cleithra (Fig. 5D; we term this space the ventral abdominal recess of the abdominal cavity; described below). As inflation progressed, the ocellus was displaced ventrolaterally (Fig. 5B–D, white arrows). By weighing Fish 2 (376 mm SL) before (924.5 grams) and after (1204.5 grams) inflation, we estimated that it buccal pumped 280 ml of water in three minutes 50 seconds, inflating the stomach and abdomen and representing a volume increase of 30%.

In Trial 2 for Fish 2, we measured changes in circumference and cross-sectional area of the stomach using ultrasound to estimate volume change and to determine where in the digestive tract inflation occurs. We found that most expansion occurred in a thin-walled pyloric extension of the stomach. Thirty seconds into Trial 2, the stomach was 13.0

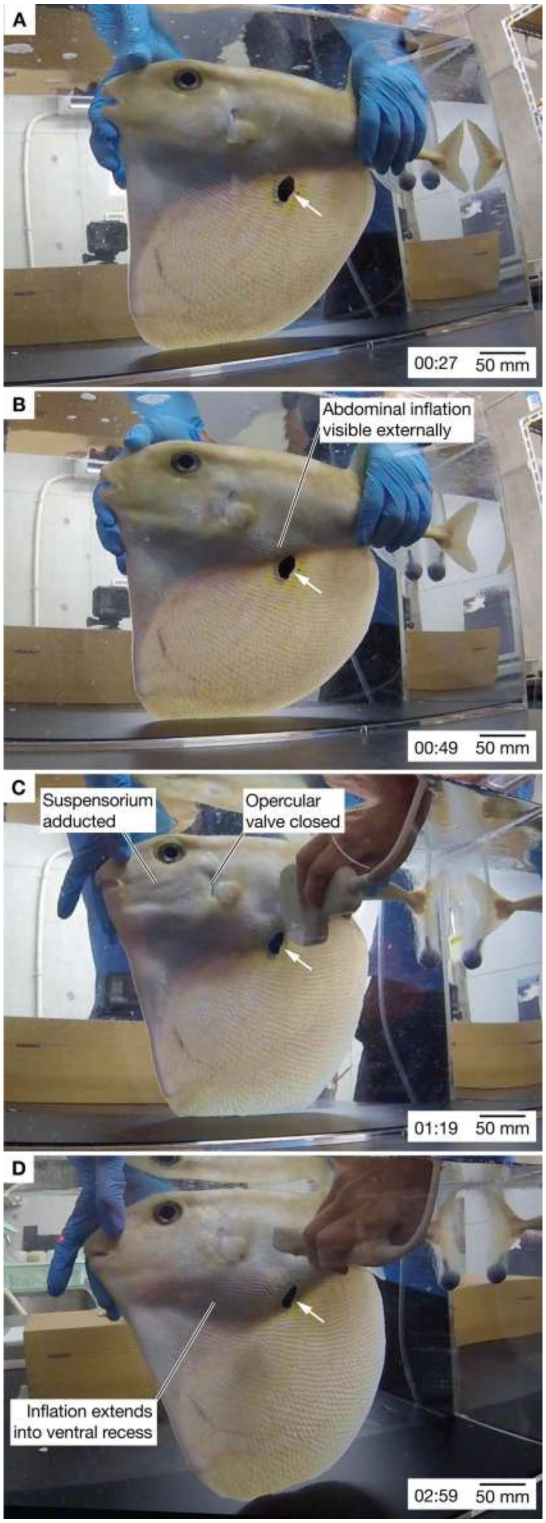

Fig. 5. Frames from a video of *Triodon macropterus*, Fish 2 (376 mm SL), inflating underwater during handling for Trial 4 at the Okinawa Churaumi Aquarium. The kinematic record of this trial is shown in Figure 3F. During this 2:59 minute sequence, the fish buccal pumped water into the stomach for inflation. As the abdomen expanded, the position of the ocellus changed (white arrows; changes in the position of the ocellus are primarily the result of inflation and not further pelvic-fan flaring). (A) At 27 seconds into the trial, Fish 2 showed no external signs of inflation. (B) At 49 seconds, external signs of inflation became visible. (C) At 01:19 minutes into being held, the fish was moderately inflated because water had been buccal pumped for inflation into the stomach; note that the suspensorium is adducted and the opercular valve is closed, indicating that water is not flowing out of the opercular opening. (D) Maximum inflation in this trial was reached at 2:59 minutes, although the fish probably had not achieved its full inflation potential.

cm in circumference with a slice area of 10.8 cm^2 (Fig. 6A). Two and a half minutes later, the stomach had expanded to 15.5 cm in circumference with a slice area of 17.3 cm^2 (Fig. 6B), a 62% increase in the area of this slice near the thin-walled pyloric region of the stomach.

Paired video and ultrasound recordings show that Fish 2 buccal pumped water into the stomach to inflate it (Supplemental Video 4; see Data Accessibility). In videos of Fish 2 Trial 2 (Fig. 3), we observed alternating periods of buccal cavity expansion and powerful buccal pumping movements lasting < 1 second that resulted in stomach inflation. For example, in Figure 5C, adduction of the suspensorium is visible externally. Buccal pumping for inflation differs from normal gill ventilation, which we also observed throughout the videos, in that the opercular valve closes so that water does not flow out of the gill opening as it does during gill ventilation. Buccal pumping for body inflation also includes elevation of the lower jaw and deployment of the lower oral valve. We were unable to visualize movements of branchiostegal 1, but the hyoid apparatus as a whole moves anterodorsally during buccal pumping for inflation. The pectoral girdle and fins do not abduct laterally during inflation.

After ultrasound examination, both individuals seamlessly retracted the pelvic fan against the abdominal region, reducing the size of the ocellus. In Fish 2, inflation diminished simultaneously with pelvic-fan retraction (Fig. 3D–F). Water from inflation was released from the mouth, although we could not rule out that it also exits from the restricted gill openings.

Anatomical features related to pelvic-fan flaring and inflation

Skeleton and muscles.—*Triodon macropterus* has six branchiostegal rays (Fig. 7). The first branchiostegal ray is broader than the others and has a small medial flange, but it lacks a well-developed hinge joint with the ceratohyal (e.g., as found in *Diodon*; Tyler, 1980: fig. 285; Wainwright et al., 1995: 618, 619). The small *hyohyoidei abductores* of *T. macropterus* attaches to the proximal portion of branchiostegal 1 and more extensively between branchiostegals 2 and 3 (Fig. 7).

Figure 8 compares radiographs of two specimens preserved with partial pelvic-fan flaring: ~25° (Fig. 8A) and ~45° (Fig. 8B) from the body axis. The anterior tip of the long cleithrum lies just below the lower-jaw articulation with the quadrate and the posteroventral portion of the articular (lower-jaw joint; Fig. 8). The cleithrum articulates with posterior dermal bones of the pectoral girdle, which are the supracleithrum, dorsal postcleithrum, and ventral postcleithrum (the dorsal and ventral postcleithra cannot be distinguished from each other in Fig. 8; but see Tyler, 1980: 250, fig. 187). Together, the dorsal and ventral postcleithra form a large, posteroventrally directed strut that sweeps across the anterior portion of the abdomen (Fig. 8A).

The long right and left pelvic bones are interdigitated and move as one unit; herein, we refer to them together as the

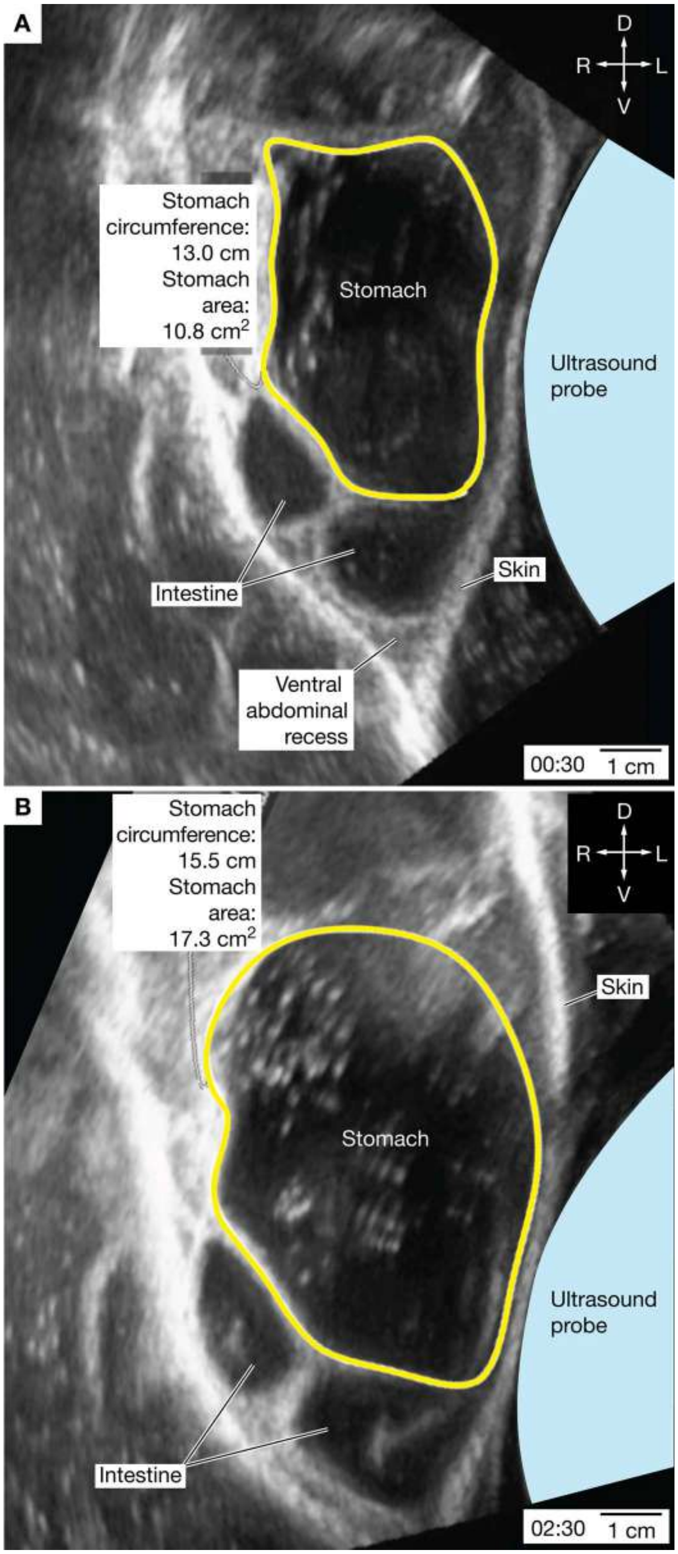

Fig. 6. Ultrasound recording of *Triodon macropterus*, Fish 2 (376 mm SL), at the Okinawa Churaumi Aquarium. The kinematic record of this trial is shown in Figure 3D. The circumference of the stomach is outlined with a yellow line. Black areas within the stomach are water; white spots are air bubbles. (A) Ultrasound image showing the circumference (13.0 cm) of the stomach and the area of one slice of the stomach (10.8 cm^2) 30 seconds into the trial. (B) Ultrasound image showing increase in the circumference (15.5 cm) and the slice area of the stomach (17.3 cm^2) 2:30 minutes after the image shown in Figure 6A.

pelvic bone. There is no evidence of a pelvic-fin spine or fin rays along the length of the pelvic bone (Tyler, 1980: 251); however, at its posterior end are small cartilages that might be remnants of pelvic-fin-ray elements (see Tyler and Matsuura, 1981: 64, 65, fig. 5). Other than the elongate scales, the ventral margin of the pelvic fan has no skeletal support. The sturdy pelvic bone extends from a region ventral to the quadrate and passes between the left and right cleithra to the anteroventral tip of the pelvic fan, to which it connects by tough ligaments. White stars (Fig. 8) indicate the center of rotation of the pelvic bone at the connection with the cleithra. In Figure 8A (specimen with ~25° of fan flaring), the anterior tip of the pelvic bone is beneath the oral cavity; in Figure 8B (specimen with ~45°of fan flaring), the tip of the pelvic bone is rotated to a position above the lower-jaw joint.

The pelvic fan is flared by contraction of the *infracarinalis anterior*, which originates on the anteroventral tip of the cleithrum and inserts in a deep groove on the anterior face of the pelvic bone (Figs. 7–9). The long *infracarinalis medius*, which originates on the anterior surface of the first anal pterygiophore, retracts the pelvic fan (Fig. 9A; see Winterbottom, 1974: 49 for more on this muscle). It extends lateral to the abdominal wall and the ventral abdominal recess to insert as a sheet of fibers on the lateral side of the pelvic bone about one-third the way along its length (Figs. 7, 8). Lines of actions of the *infracarinalis* muscles are indicated in Figure 8B. When the *infracarinalis anterior* depresses the pelvic bone, the abdominal cavity expands to form the ventral abdominal recess below the cleithrum and gills (Fig. 8B). The ventral abdominal recess ends anteroventrally just beyond the ventralmost edge of the *infracarinalis medius* but continues posteriorly (see white dashed line in Fig. 8B for ventral boundary).

As the stomach fills with water, it pushes the intestines into the ventral abdominal recess (Fig. 6A). Ventral peritoneal folds (Fig. 9B) allow the expansion to occur. Photographs of more inflated specimens show that inflation can extend deeply into the ventral abdominal recess (e.g., Supplemental Fig. 1H–M; see Data Accessibility).

Triodon macropterus has seven abdominal ribs (Tyler, 1980: fig. 182). The first rib is short, but ribs 2–7 are comparatively long and extend ventrally to the lower edge of the abdominal region when the pelvic fan is retracted (Fig. 8A).

Viscera.—The stomach was everted into the buccal cavity of all three specimens that we dissected (Fig. 8B), presumably caused by barotrauma; in one specimen, the swim bladder had ruptured. The esophagus is short and broad, and a sphincter marks its junction with the stomach (Fig. 10A, B). The stomach is 8 cm long and about 1.5 cm in diameter (Fig. 10A, B; specimen length 343 mm SL). It is divisible into a thick-walled body and a thinner-walled, bag-like pyloric region (Fig. 10A, B). As in other tetraodontiforms, the bile duct enters the body of the stomach (e.g., *Mola mola*; Bemis, 2019). The lining of the body of the stomach has longitudinal rugae of varying width (Fig. 10B). In contrast, the thin wall of the pyloric region has a smooth lining (Fig. 10B).

The intestine joins the dorsal side of the stomach, about 6 cm from the esophageal sphincter (Fig. 10B). A well-developed pyloric sphincter separates the stomach and intestine. We distinguish three intestinal regions. The first region is 8 cm long. Its walls have strong rugae of similar width along its length. There is no formal name for this

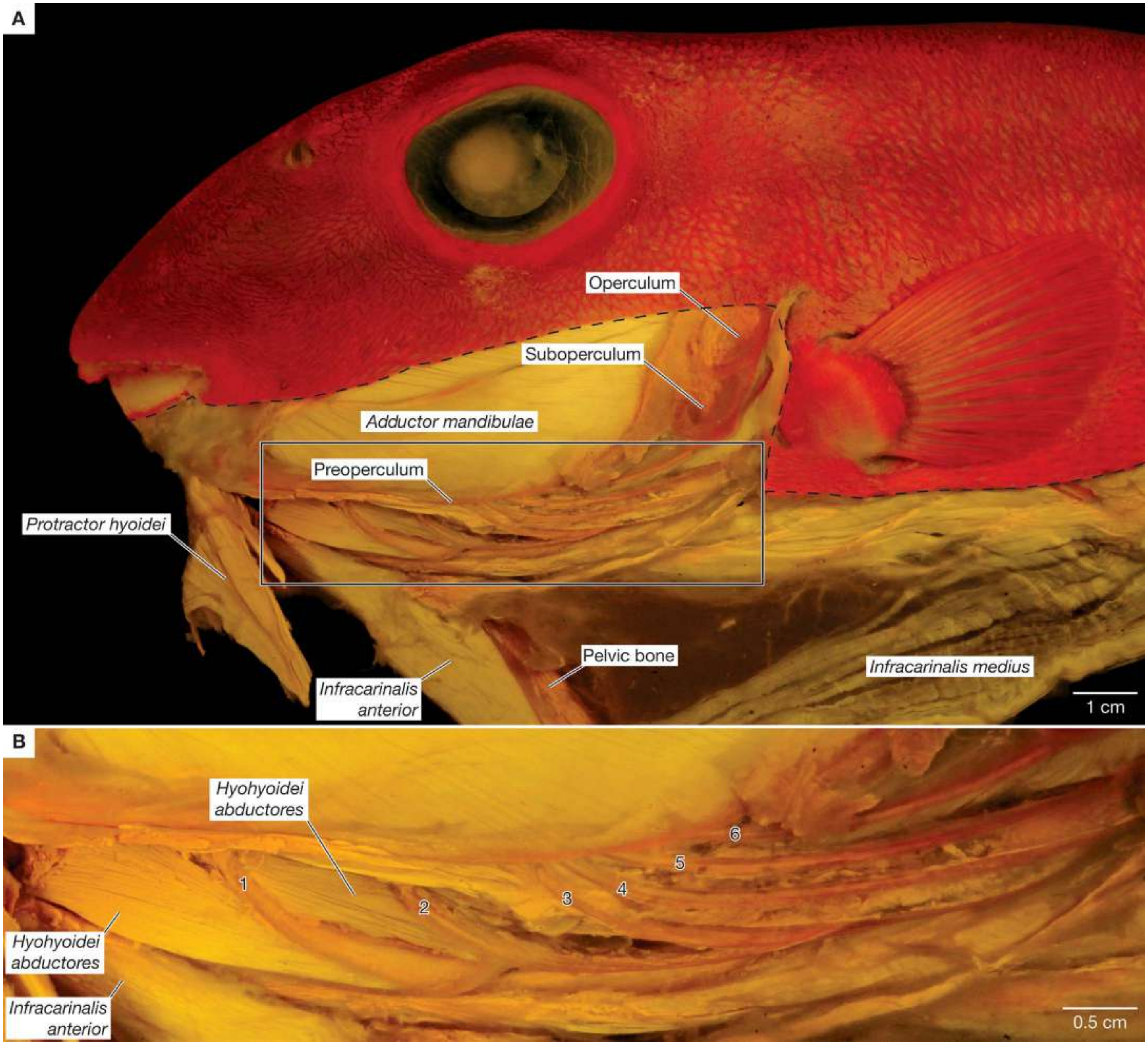

Fig. 7. Dissection of branchiostegal rays and muscles of *Triodon macropterus* (NSMT P93783, 343 mm SL). The specimen was photographed using high-energy royal-blue light to differentiate autofluorescing alizarin-stained bones and scales from muscles (see Smith et al., 2018). (A) Overview to show position of branchiostegal rays. Rectangular box indicates area shown in part B. (B) Higher magnification view to show relative size of branchiostegal 1 in comparison with branchiostegals 2–6 and the position and size of the *hyohyoidei abductores* (numbers on photograph indicate branchiostegal rays).

region, which corresponds approximately to the duodenum of other vertebrates; however, unlike the duodenum of other vertebrates, the bile duct enters the stomach of *Triodon macropterus*. The second region is thin-walled and wider; it lacks rugae and has sphincters at each end. Johnson and Britz (2005: 177) termed this region Tyler's pouch. We found unidentifiable hard parts of prey in Tyler's pouch in the specimens we dissected. The third region of the intestine, posterior to Tyler's pouch, is 8 cm long and is termed the rectum; it is about the same diameter as the first region and has similar strong rugae along its length (Fig. 10C).

The digestive tract opens into the pseudocloaca, an invagination of skin in the posterodorsal region of the pelvic fan that also receives the urinary and genital ducts (Fig. 10A, C; Breder and Clark, 1947). We did not study pseudocloacal anatomy, but this unusual structure deserves further investigation.

Character optimization.—Character optimizations for pelvic-fan flaring and inflation on three topologies are shown in Figures 11 and 12 and discussed below.

***Additional behavioral observations on captive* Triodon macropterus.**—Rapid fluttering of the caudal fin, caudal peduncle, and posterior part of the body resulted in quivering of the pelvic fan during flaring (quivering cannot be seen in the

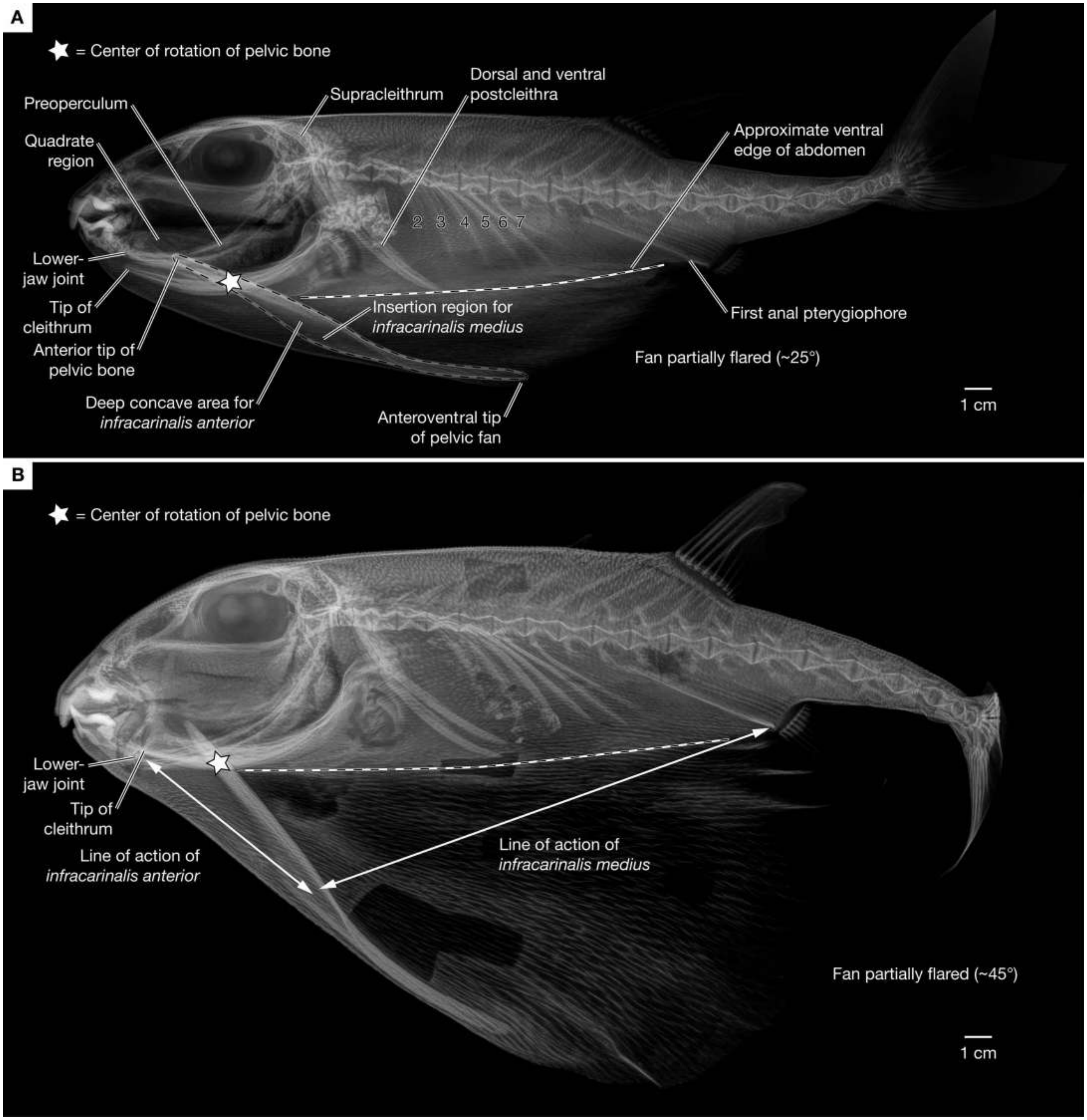

Fig. 8. X-rays of two specimens of *Triodon macropterus* showing differences in pelvic bone position related to extent of pelvic-fan flaring. The anterior tip of the long cleithrum extends to the lower-jaw joint. White stars indicate center of rotation of the pelvic bone at its connection with the cleithrum. (A) USNM 451516, 358 mm SL. Fan partially flared to ~25°. Long ribs are numbered 2–7. (B) NSMT P0110597, 360 mm SL. Fan partially flared to ~45°. White arrows indicate lines of actions of the muscles that flare and retract the pelvic fan. Patches of skin and scales were removed from this specimen for another study (see Matsuura et al., 2017).

single frames shown in Figure 4, but it can be seen in Supplemental Video 5; see Data Accessibility).

In the aquarium, individuals of *Triodon macropterus* search for food by forcefully blowing a stream of water from the mouth, displacing the sand (Supplemental Video 6; see Data Accessibility). We did not observe individuals coughing out unwanted items ingested during feeding (a "cough," *sensu* Wainwright and Turingan, 1997). When we fed Fish 2 hard-shelled crabs, which are harder than the typical diet provided in captivity, nothing was coughed up nor were any crab shell remains found on the sandy aquarium bottom the next morning.

Captive *Triodon macropterus* occasionally made grunting sounds, for example, when handled and lifted out of the

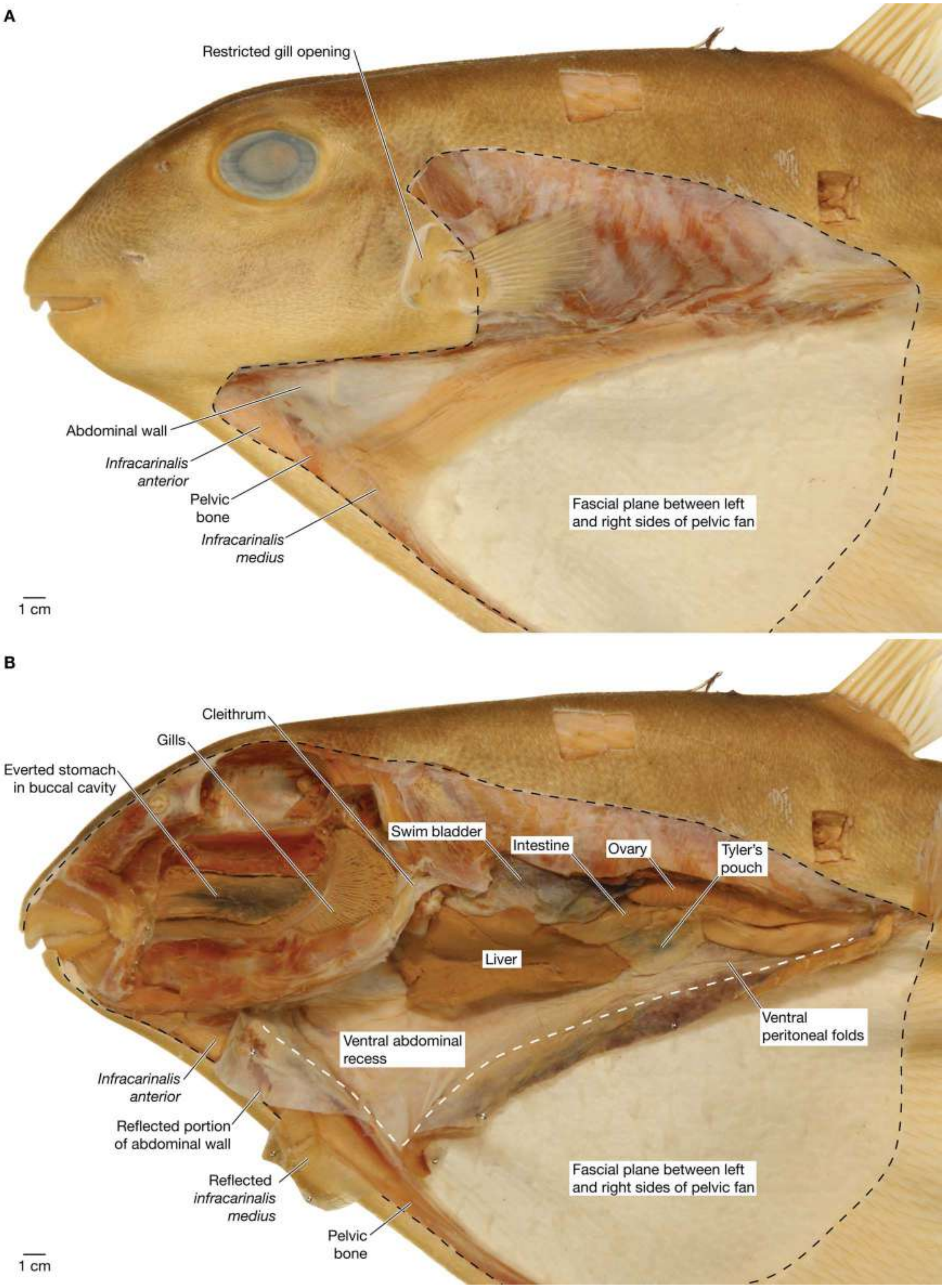

Fig. 9. Dissection of *Triodon macropterus*, NSMT P0110597, 360 mm SL. Black dashed lines indicate cut edges. (A) Skin of the pelvic fan and abdomen removed to show muscles of the pelvic fan. (B) Dissection to show the viscera and extent of the ventral abdominal recess (white dashed line). Postcleithral bones removed to view abdominal cavity. The abdominal cavity has small ventral peritoneal folds. The stomach in this specimen everted into the buccal cavity because of barotrauma. It tore away from its connection with the intestine at the pyloric region; the rest of the intestinal tract, including Tyler's pouch, is in place as in life.

water for transfer between tanks. We did not investigate the mechanism of this sound production, but we suppose that it involves the grinding together of the jaws and triturating teeth as in other tetraodontiforms (e.g., *Sphoeroides maculatus* and *Chilomycterus schoepfi*; Fish et al., 1952).

Individuals of *Triodon macropterus* in the same exhibit tank usually do not interact, although sometimes they are close together (Supplemental Fig. 2; see Data Accessibility). Occasionally, one of us (AK) has seen individuals lightly bite the distal portion of the caudal fin of other individuals. This

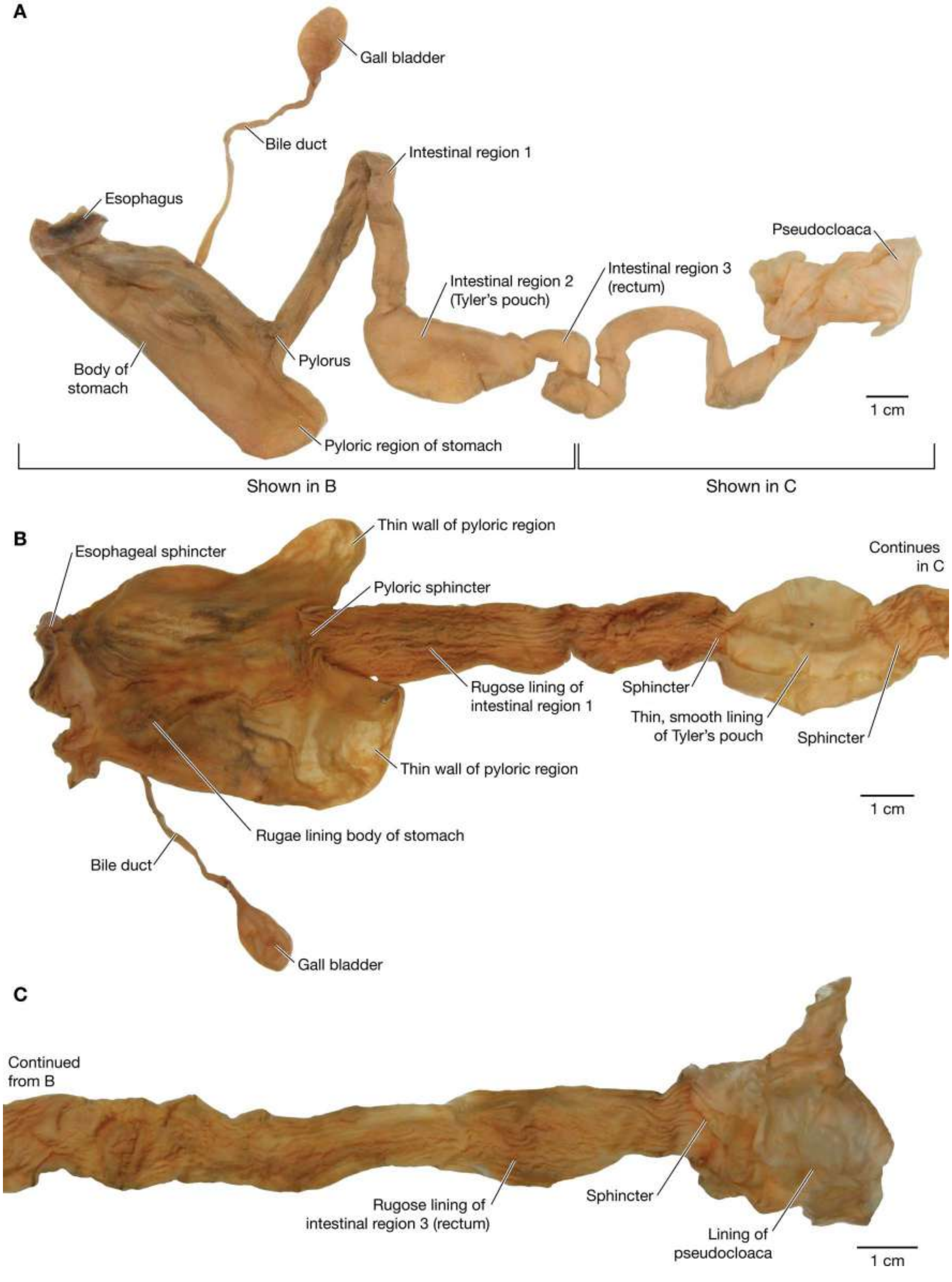

Fig. 10. Photographs of digestive tract removed from *Triodon macropterus*, NMST P93783, 343 mm SL, anterior to left. (A) Digestive tract from esophagus to pseudocloaca showing the rectangular-shaped stomach with the bile duct entering the body of the stomach. The thin-walled pyloric region of the stomach is where most of the inflation occurs. (B) Lining of anterior region of digestive tract. (C) Lining of posterior half of the digestive tract.

behavior does not result in excision of large pieces of fin; rather, the fin membranes are damaged. Caudal-fin biting is not correlated with size, as smaller fish are just as likely to nibble the fin of a larger tank mate as the reverse.

During 18 years of displaying *Triodon macropterus* at the aquarium, AK has never observed courtship or breeding behavior, even during a nine-month period when three to five individuals (undetermined sex) were kept in the same tank.

Videos of Fish 3 at night show that this individual swam more slowly and was less active at night, mostly hovering in place using its pectoral, dorsal, and anal fins to maintain its position. Occasionally it moved vertically (both up and down; ≤ 1 m) and horizontally the width of the tank (Supplemental Fig. 3; see Data Accessibility). As described above and shown in Figure 4, we observed nocturnal pelvic-fan flaring, buccal cavity expansion, fluttering of the caudal fin, and quivering of the rear of the body; all of these behaviors were also observed during the day.

DISCUSSION

We synthesized information from (1) field photographs of *Triodon macropterus* by fishers, (2) direct observations, video recordings, and ultrasound studies of three individuals in captivity, and (3) anatomical study of three preserved specimens to document pelvic-fan flaring and inflation behavior. Our results show that *T. macropterus* can inflate, disproving the interpretation that *T. macropterus* cannot inflate (Breder and Clark, 1947: 294).

Behaviors.—*Triodon macropterus* uses two behaviors that increase its apparent body size: pelvic-fan flaring and inflation. When handled, individuals flare the pelvic fan and buccal pump water to inflate the stomach and abdomen. Because handling induces pelvic-fan flaring and inflation, we interpret them as defensive behaviors. Our online search for photographs of *T. macropterus* found two images of individuals with damaged pelvic fans (e.g., Supplemental Fig. 1L; see Data Accessibility). These photographs support our hypothesis that the pelvic fan is flared during encounters with predators because flaring is the only way that the fan could be exposed to a predator given that it is typically retracted against the ventral body wall. One video recording of a captive *T. macropterus* at the Okinawa Churaumi Aquarium showed it swimming up to a Deepwater Squirrelfish (*Ostichthys kaianus*) and aggressively flaring its pelvic fan (Supplemental Video 3; see Data Accessibility). Pelvic-fan flaring and inflation might also be used for intraspecific communication; however, when two or more individuals lived in the same tank, they were never observed to flare their pelvic fans nor to inflate when interacting with one another.

Superficially, pelvic-fan flaring resembles dewlap extension in anole lizards, and the pelvic fan of *Triodon macropterus* is sometimes referred to as a dewlap (e.g., Tyler, 1980: 250). The dewlap of anoles is associated with the hyoid apparatus and functions in territorial defense and courtship (Nicholson et al., 2007). In contrast, the pelvic fan of *T. macropterus* is associated with a highly modified pelvic girdle and has a presumed defensive role. Kawase (2005: 195) reported that during courtship and aggressive displays, male *Brachaluteres jacksonianus* (Monacanthidae) flare their pelvic fan and synchronously quiver the fan and caudal fin. We observed similar pelvic-fan quivering in nocturnal video recordings of *Triodon macropterus* (Supplemental Video 5 [see Data Accessibility]; Fish 3). When we observed this behavior, no conspecifics were in the tank.

Photographs of freshly caught wild individuals of *Triodon macropterus* showed levels of inflation ranging from none (e.g., Supplemental Fig. 1A; see Data Accessibility) to obvious (e.g., Supplemental Fig. 1I; see Data Accessibility). Some increase in abdominal sizes in these photographs may have resulted from swim bladder expansion caused by barotrauma. In extreme cases, the stomach everts into the buccal cavity and the swim bladder bursts; in less severe cases, the swim bladder likely expands beyond its normal size and contributes to apparent inflation. We could not determine how greatly barotrauma contributed to abdominal swelling because we did not have access to the specimens shown in Supplemental Figure 1 (see Data Accessibility).

In six trials of two individuals, Fish 1 never inflated, but Fish 2 inflated four times (Fig. 3). It is possible that the captive fish we studied had habituated to handling and thus showed a reduced inflation response as described for *Diodon holocanthus* (Brainerd, 1994). We likely did not observe the maximum potential inflation in any of the six trials because, compared with the maximum inflation seen in field photographs (Supplemental Fig. 1; see Data Accessibility), we only observed partial inflation in the ventral abdominal recess (Fig. 5D).

Inflation in tetraodontids and diodontids results in a balloon-shaped body, whereas inflation in *Triodon macropterus* is restricted to the ventral abdominal region. Brainerd (1994) studied inflation in *Diodon holocanthus* (Diodontidae), one of the most dramatic inflators. She induced individuals to inflate by holding them and applying pressure to the head. The average volume change in *D. holocanthus* was 3.1±0.2 times the resting non-inflated volume, and it occurred very rapidly, within 14.5±4.1 seconds; deflation took about twice as long (Brainerd, 1994: 247). While individuals of *D. holocanthus* were held tightly, they remained inflated; when the grip was loosened, they started to deflate and tried to swim away. We found that *T. macropterus* inflated much more slowly (49 seconds for inflation to be visible externally and three minutes 50 seconds for maximum inflation observed; Figs. 3F, 5) and to a lesser extent (0.3 times resting non-inflated volume in the single estimate made by weighing the fish before and after inflation); we were not able to estimate deflation time. Our times potentially underestimate how long inflation takes because we had disturbed the fish by moving them to the trial tanks.

In many animals, ocelli (eyespots) function in intraspecific communication, predator deflection, or intimidation (see review in Hemingson et al., 2020). The eyespots of *Triodon macropterus* (one on each side of the pelvic fan) are typical ocelli consisting of a black spot bounded by a white border; the shape and size of the black spot vary between individuals (and are occasionally absent; Mochizuki et al., 2021) and change with the degree of fan flaring and inflation. How other fishes perceive these eyespots, including potential mates or predators, is unknown, but the eyespots increase in size when the pelvic fan is flared (Figs. 2, 5).

Anatomical observations relevant to inflation.—Breder and Clark (1947) compared visceral anatomy among tetraodontiforms, including *Triodon macropterus* (Fig. 1; preserved female specimen; SU 13747, 391 mm SL; the viscera of this specimen were unavailable for reexamination, Dave Catania, pers. comm., 5 August 2020). We found three discrepancies in Breder and Clark (1947). First, the digestive tract is not uniform in diameter, nor are its walls uniform in structure, as stated by Breder and Clark (1947: 298). Instead, each region of the gut can be characterized by distinctive features (Fig. 10). Second, we found a much smaller ventral abdominal recess (Fig. 9B) than illustrated by Breder and Clark (1947: fig. 3, which they called "Sac from floor of abdominal cavity," see Fig. 1). It is not constricted into an hourglass shape and does not extend as far into the pelvic fan as Breder and Clark

(1947) reported. Third, Breder and Clark (1947) concluded that *T. macropterus* could not inflate; however, we show that it can.

The 20 mm SL juvenile specimen of *Triodon macropterus* studied by Johnson and Britz (2005) has a developing, elongated pelvic bone but lacks a pelvic fan and ocellus. Johnson and Britz (2005: 177) described the viscera of that specimen as follows: "The oesophagus opens into the anterodorsal side of the very large, translucent, and elastic stomach, the most prominent organ in the abdominal cavity." They noted it had a "very large distended stomach" that was "lacking in the adult" (Johnson and Britz, 2005: 179). They described Tyler's pouch as an S-shaped loop of the intestine located between the first region of the intestine and the third region (rectum). The function of Tyler's pouch remains unknown. An illustration in Johnson and Britz (2005: fig. 2a, c) shows the specimen with a rounded abdomen; however, the shape of the abdomen was estimated. Johnson and Britz (2005) did not comment on inflation, and it remains unknown if juvenile *T. macropterus* inflate.

Comparisons with other fishes that inflate.—In addition to the Swell Shark (*Cephaloscyllium ventriosum*; Clark, 1947), inflation of the stomach occurs in several teleosts. Examples include the Pelagic Goby (*Sufflogobius bibarbatus*; Smith, 1956) and Sea Raven (*Hemitripterus americanus*; John Galbraith, pers. comm., 18 December 2021). Long and Farina (2019) reported gill chamber (i.e., not the stomach) inflation in chaunacids during ventilation and suggested that such inflation may play a defensive role because it increases body size. Inflation may occur in frogfishes (Antennariidae; see Pietsch and Arnold, 2020: 452–454; also Ferreira et al., 2022), but the evidence and mechanism are unclear.

Among tetraodontiforms, some monacanthids can inflate, including *Brachaluteres* (four species) and *Paraluteres* (two species; Clark and Gohar, 1953; Matsuura, 1979; Hutchins and Swainston, 1985; Hutchins, 1988). Inflation among monacanthids is best exemplified by *B. jacksonianus*, in which the entire stomach expands (Hutchins and Swainston, 1985). Inflation has not been reported in Triacanthodidae, Triacanthidae, Balistidae, Aracanidae, Ostraciidae, or Molidae.

Many anatomical features have been interpreted as correlates of inflation in tetraodontiforms (Rosén, 1912; Brainerd, 1994; Wainwright et al., 1995; Wainwright and Turingan, 1997). These include derived features of the skin, digestive tract, muscles, and skeleton, but they have yet to be comprehensively surveyed within tetraodontiforms and combined with detailed behavioral observations. Such a survey is beyond the scope of this study, but we note and briefly compare seven intriguing anatomical differences between the inflation mechanism of diodontids (which is the best understood and most extreme example of inflation) and that of *Triodon macropterus*.

1. Diodontids lack a pelvic bone, whereas *Triodon macropterus* has an elongate, highly mobile pelvic bone that plays an essential role in pelvic-fan flaring and inflation by expanding the ventral abdominal recess (Fig. 9B).
2. Brainerd (1994: fig. 3) described large dorsal peritoneal folds in *Diodon holocanthus* that allow the abdomen to expand. We did not observe dorsal peritoneal folds in *Triodon macropterus*, but there are some small ventral peritoneal folds below the digestive tract (Fig. 9B).
3. Unlike other tetraodontiforms, which lack ribs, *Triodon macropterus* has seven relatively long ribs with associated epipleurals (Fig. 8; Tyler, 1980: figs. 182, 189). The ribs extend ventrolaterally, thus constraining inflation to ventral regions of the abdomen. Brainerd (1994: 249) considered that the absence of the ventral processes on most abdominal vertebrae and the absence of ribs on all abdominal vertebrae contributed to the ability of *Diodon holocanthus* to inflate.
4. The single postcleithrum of diodontids is very short (e.g., Tyler, 1980: fig. 281, 286 for *Diodon holocanthus*), allowing balloon-like abdominal expansion. In contrast, the two postcleithra of *Triodon macropterus* form a long strut that sweeps ventrally across the abdomen. Like the ribs, this strut constrains inflation to the ventral abdominal region.
5. Brainerd (1994) reported that the vertebral column of *Diodon holocanthus* has a kyphotic curve (concave side directed ventrally) when the fish inflates. We did not observe vertebral flexion or extension during inflation in the ultrasound videos of *Triodon macropterus*.
6. The expanded first branchiostegal ray of diodontids has a well-developed hinge joint with the ceratohyal (e.g., Tyler, 1980: fig. 285 for *Diodon holocanthus*). The massive *hyohyoideus abductores* inserts on the first branchiostegal and rapidly pumps water into the digestive tract (Wainwright et al., 1995: figs. 1, 2). The first branchiostegal ray of tetraodontids is similarly expanded (e.g., Tyler, 1980: fig. 207 for *Canthigaster rostrata*). In contrast, the first branchiostegal of *Triodon macropterus* is much narrower and lacks the specialized articulation with the ceratohyal found in diodontids and tetraodontids (Fig. 7). It has a small medial flange for attachment of a slip of the *hyohyoideus abductores* (Fig. 7; Tyler, 1980: fig. 186; Winterbottom, 1974: 46). Although we could not discern movements of the first branchiostegal ray in our videos or ultrasound recordings for *T. macropterus*, its small size suggests that it plays a negligible role in buccal pumping for inflation compared with that in diodontids and tetraodontids.
7. The pectoral girdle of diodontids is shorter and more mobile than that of *Triodon macropterus*. Diodontids have a mobile joint between the cleithrum and supracleithrum that allows girdle abduction and inflation to achieve a balloon shape (Wainwright et al., 1995). Their cleithra only extend partway toward the lower jaw and are rotated posteriorly during inflation (Wainwright et al., 1995: fig. 8). In contrast, the anterior tips of the cleithra of *T. macropterus* extend nearly to the lower-jaw joint and cannot be rotated during inflation because their anterior location is linked to rotation of the pelvic bone during pelvic-fan flaring (Fig. 8).

Based on these seven differences, and others not discussed herein, we conclude that the anatomical substrates for inflation behavior evolved independently in the diodontid–tetraodontid clade and *Triodon macropterus*. Thus, defensive inflation is achieved through different anatomical specializations.

Phylogenetic interpretations of pelvic-fan flaring and inflation.—Phylogenetic analyses using characters of muscles (Winterbottom, 1974) and bones (e.g., Santini and Tyler,

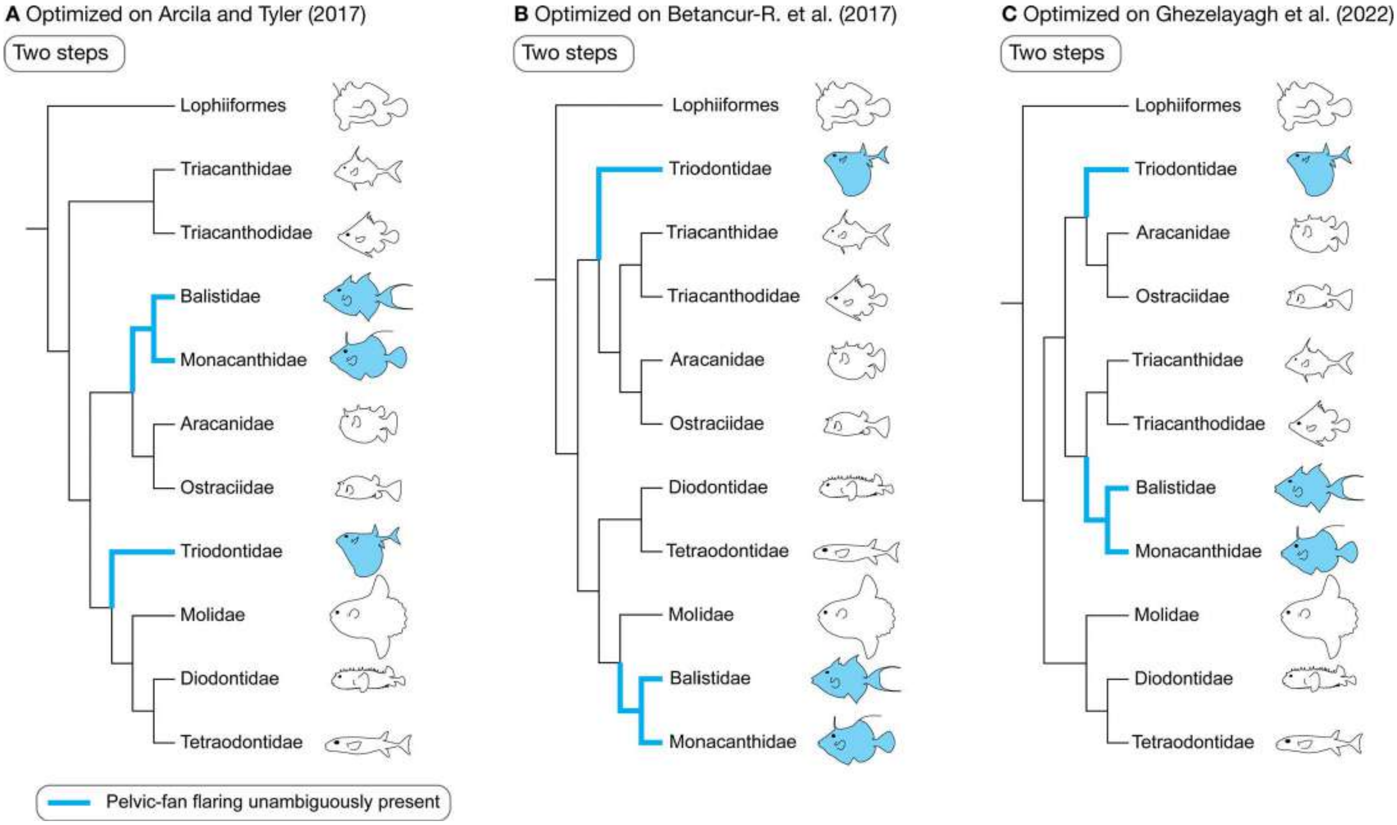

Fig. 11. Pelvic-fan flaring in Tetraodontiformes. Blue indicates presence of pelvic-fan flaring behavior. (A) Pelvic-fan flaring has two steps when optimized on the topology of Arcila and Tyler (2017; 16 loci and morphology). (B) Pelvic-fan flaring has two steps when optimized on the topology of Betancur-R. et al. (2017; nuclear and mitochondrial loci). (C) Pelvic-fan flaring has two steps when optimized on the topology of Ghezelayagh et al. (2022; ultraconserved elements). Data on the occurrence of pelvic-fan flaring are summarized in Table 2.

2003) recovered *Triodon macropterus* as the sister group of Molidae + (Diodontidae + Tetraodontidae). Early studies based on larvae (e.g., Leis, 1984) and molecular characters (e.g., Holcroft, 2005) did not include *T. macropterus* because of its rarity. Arcila and Tyler (2017) combined morphology and molecules and recovered the same topology as Santini and Tyler (2003). In contrast, Betancur-R. et al. (2017) and Ghezelayagh et al. (2022) placed *T. macropterus* in two different and more basal positions within Tetraodontiformes based on molecular data. To explore these different topologies, we optimized pelvic-fan flaring and inflation on all three trees (Figs. 11, 12) to consider different interpretations about the evolution of these behaviors. In all three trees, pelvic-fan flaring evolved independently in (Monacanthidae + Balistidae) and Triodontidae (Fig. 11). In Figure 12A, it is ambiguous whether inflation is a synapomorphy of Triodontidae + (Molidae + (Diodontidae + Tetraodontidae)) or whether it evolved independently in Triodontidae and (Diodontidae + Tetraodontidae). Figure 12B, C shows that inflation evolved independently in Triodontidae, Diodontidae + Tetraodontidae, and Monacanthidae. Our anatomical data favor independent evolution of inflation in Triodontidae, but it will be important to examine other anatomical characters associated with inflation in future research. For example, monacanthids, diodontids, and tetraodontids lack acid secretion in the stomach, a loss that Ferreira et al. (2022) linked to inflation ability. The presence of acid secretory cells in the stomach of *Triodon macropterus* is unknown.

Conclusion.—Contrary to the prevailing interpretation from Breder and Clark (1947: 294), *Triodon macropterus* can inflate. Inflation is always preceded by pelvic-fan flaring, and in combination, these two behaviors increase body size. We interpret these behaviors as defensive, but they might also play roles in intraspecific communication. Based upon the anatomical differences between *T. macropterus* and diodontids, we consider that inflation evolved independently in Triodontidae and in the Diodontidae + Tetraodontidae clade. Combinations of live observations and anatomical studies may further elucidate the behavioral and phylogenetic diversity of pelvic-fan flaring and inflation in tetraodontiforms.

DATA ACCESSIBILITY

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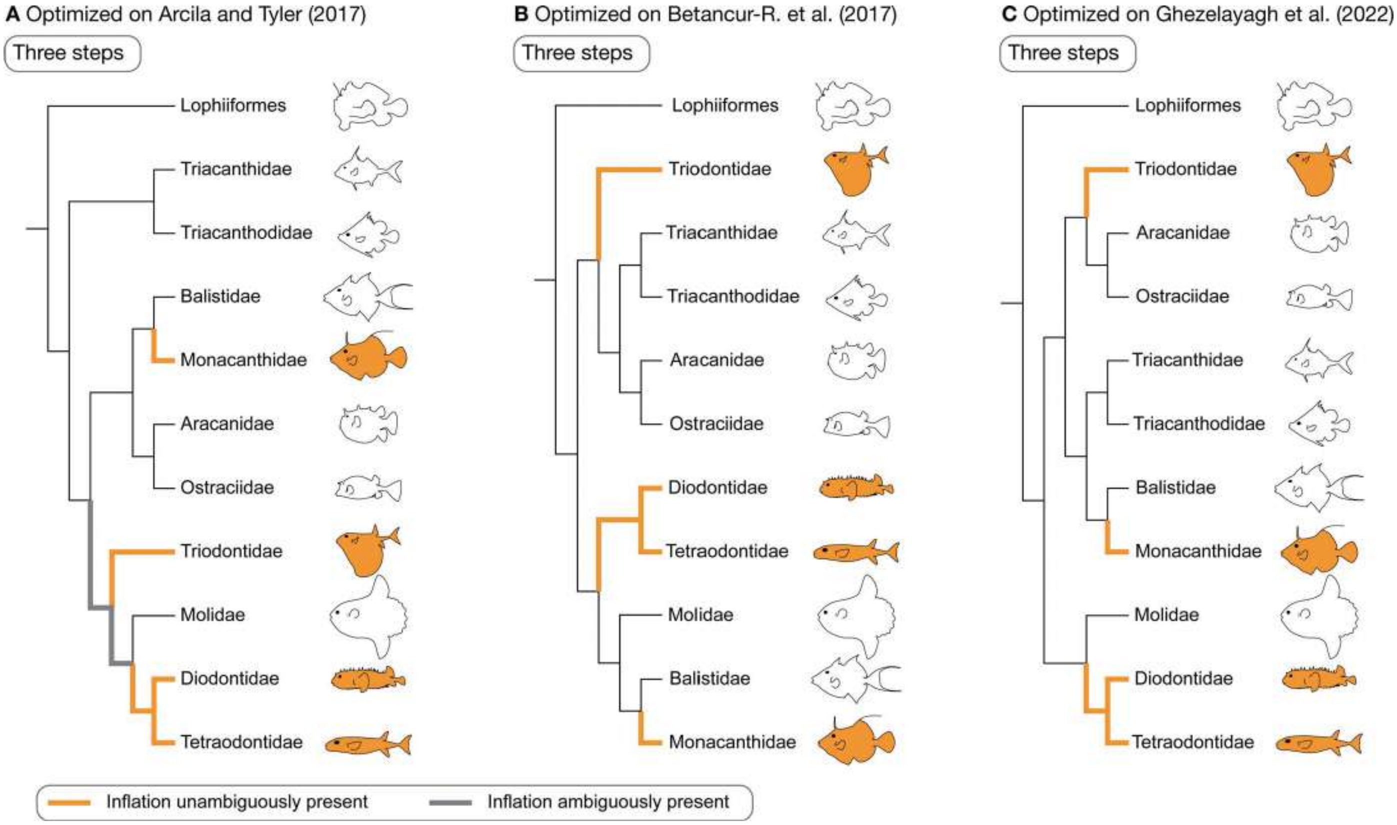

Fig. 12. Inflation in Tetraodontiformes. Orange indicates presence of inflation behavior. (A) Inflation has three steps when optimized on the topology of Arcila and Tyler (2017; 16 loci and morphology). (B) Inflation has three steps when optimized on the topology of Betancur-R. et al. (2017; nuclear and mitochondrial loci). (C) Inflation has three steps when optimized on the topology of Ghezelayagh et al. (2022; ultraconserved elements). Data on the occurrence of inflation are summarized in Table 2.

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LITERATURE CITED

Arcila, D., R. A. Pyron, J. C. Tyler, G. Orti, and R. Betancur-R. 2015. An evaluation of fossil tip-dating versus node-dating calibrations in tetraodontiform fishes (Teleostei: Percomorphaceae). Molecular Phylogenetics and Evolution 82:131–145.

Arcila, D., and J. C. Tyler. 2017. Mass extinction in tetraodontiform fishes linked to the Palaeocene–Eocene thermal maximum. Proceedings of the Royal Society B 284: 20171771.

Barrington, E. J. W. 1957. The alimentary canal and digestion, p. 109–161. *In*: The Physiology of Fishes. M. E. Brown (ed.). Academic Press, New York.

Bemis, K. E. 2019. Studies on the anatomy of teleosts. Unpubl. Ph.D. diss., College of William & Mary, Williamsburg, Virginia.

Betancur-R., R., E. O. Wiley, G. Arratia, A. Acero, N. Bailly, M. Miya, G. Lecointre, and G. Ortí. 2017. Phylogenetic classification of bony fishes. BMC Evolutionary Biology 17: 162.

Beveridge, I., R. A. Bray, T. H. Cribb, and J.-L. Justine. 2014. Diversity of trypanorhynch metacestodes in teleost fishes from coral reefs off eastern Australia and New Caledonia. Parasite 21:60.

Brainerd, E. L. 1994. Pufferfish inflation: functional morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). Journal of Morphology 220:243–261.

Bray, R. A., and J.-L. Justine. 2014. A review of the Zoogonidae (Digenea: Microphalloidea) from fishes of the waters around New Caledonia, with the description of *Overstreetia cribbi* n. sp. PeerJ 2:e292.

Breder, C. M., and E. Clark. 1947. A contribution to the visceral anatomy, development and relationships of the Plectognathi. Bulletin of the American Museum of Natural History 88:287–319.

Clark, E. 1947. Notes on the inflating power of the Swell Shark, *Cephaloscyllium uter.* Copeia 1947:278–280.

Clark, E., and H. A. F. Gohar. 1953. The Fishes of the Red Sea: Order Plectognathi. Publications of the Marine Biological Station, Al Ghardaqa (Red Sea) No. 8:4–80. Fouad I University Press, Cairo, Egypt.

Cuvier, G. 1829. Le Règne Animal, distribué d'après son organisation, pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Edition 2. Vol. 2:i–xv + 1–406. Paris.

Fagundes, K. R. C., M. M. Rotundo, and R. B. Mari. 2016. Morphological and histochemical characterization of the digestive tract of the puffer fish *Sphoeroides testudineus* (Linnaeus 1758) (Tetraodontiformes: Tetraodontidae). Anais da Academia Brasileira de Ciências 88:1615–1624.

Ferreira, P., G. T. Kwan, S. Haldorson, J. L. Rummer, F. Tashiro, L. F. C. Castro, M. Tresguerres, and J. M. Wilson. 2022. A multitasking stomach: functional coexistence of acid–peptic digestion and defensive body inflation in three distantly related vertebrate lineages. Biology Letters 18: 20210583.

Fish, M. P., A. S. Kelsey Jr., and W. H. Mowbray. 1952. Studies on the production of underwater sound by North Atlantic coastal fishes. Journal of Marine Research 11:180–193.

Fricke, R., W. N. Eschmeyer, and R. van der Laan (Eds.). 2022. Eschmeyer's Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 8 January 2022).

Gabriel, M. L. 1940. The inflation mechanism of *Spheroides* [sic] *maculatus*. Biological Bulletin 79:372. [Abstract]

Ghezelayagh, A., R. C. Harrington, E. D. Burress, M. Campbell, J. Buckner, P. Chakrabarty, J. R. Glass, W. T. McCraney, P. Unmack, C. Thacker, M. E. Alfaro, S. T. Friedman, W. B. Ludt, P. F. Cowman . . . T. J. Near. 2022. Prolonged morphological expansion of spiny-rayed fishes following the end-Cretaceous. Nature Ecology & Evolution 6:1211–1220.

Girard, M. G., M. P. Davis, and W. L. Smith. 2020. The phylogeny of carangiform fishes: morphological and genomic investigations of a new fish clade. Copeia 108: 265–298.

Girard, M. G., M. P. Davis, H. H. Tan, D. J. Wedd, P. Chakrabarty, W. B. Ludt, A. P. Summers, and W. L. Smith. 2022. Phylogenetics of archerfishes (Toxotidae) and evolution of the toxoid shooting apparatus. Integrative Organismal Biology 4:obac013.

Goloboff, P. A., and S. A. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics 32:221–238.

Günther, A. 1870. Catalogue of the Fishes in the British Museum. Volume 8. London.

Hemingson, C. R., P. F. Cowman, and D. R. Bellwood. 2020. Body size determines eyespot size and presence in coral reef fishes. Ecology and Evolution 10:8144–8152.

Herre, A. W. C. T. 1925. A supplement to poisonous and worthless fishes. Philippine Journal of Science 25:167–168.

Holcroft, N. I. 2005. A molecular analysis of the interrelationships of tetraodontiform fishes (Acanthomorpha: Tetraodontiformes). Molecular Phylogenetics and Evolution 34:525–544.

Hutchins, J. B. 1988. The comparative morphology and phylogeny of the monacanthid fishes. Unpubl. Ph.D. diss., Murdoch University, Perth, Australia.

Hutchins, J. B., and R. Swainston. 1985. Revision of the monacanthid fish genus *Brachaluteres*. Records of the Western Australian Museum 12:57–78.

Johnson, G. D., and R. Britz. 2005. A description of the smallest *Triodon* on record (Teleostei: Tetraodontiformes: Triodontidae). Ichthyological Research 52:176–181.

Jordan, D. S., and J. O. Snyder. 1901. A review of the gymnodont fishes of Japan. Proceedings of the U.S. National Museum 24:229–264.

Kawase, H. 2005. Spawning behavior of the Pygmy Leatherjacket *Brachaluteres jacksonianus* (Monacanthidae) in southeastern Australia. Ichthyological Research 52:194–197.

Leis, J. M. 1984. Tetraodontiformes: relationships, p. 459–463. *In*: Ontogeny and Systematics of Fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, and S. L. Richardson (eds.). Allen Press, Lawrence, Kansas.

Lesson, R. P. 1829. Poissons, livraison 9, pl. 4. *In*: Voyage autour du monde, exécuté par ordre du Roi sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 1825. L. I. Duperrey, Paris.

Long, N. P., and S. C. Farina. 2019. Enormous gill chambers of deep-sea coffinfishes (Lophiiformes: Chaunacidae) support unique ventilatory specialisations such as breath holding and extreme inflation. Journal of Fish Biology 95:502–509.

Matsuura, K. 1979. Phylogeny of the superfamily Balistoidea (Pisces: Tetraodontiformes). Memoirs of the Faculty of Fisheries, Hokkaido University 26(1, 2):49–169.

Matsuura, K. 2015. Taxonomy and systematics of tetraodontiform fishes: a review focusing primarily on progress in the period from 1980 to 2014. Ichthyological Research 62:72–113.

Matsuura, K., A. Kaneko, and E. Katayama. 2017. Underwater observations of the rare deep-sea fish *Triodon macropterus* (Actinopterygii, Tetraodontiformes, Triodontidae), with comments on the fine structure of the scales. Ichthyological Research 64:190–196.

McGee, G. E., and T. D. Clark. 2014. All puffed out: Do pufferfish hold their breath while inflated? Biology Letters 10:20140823.

Mochizuki, K., J. Ohtomi, K. Matsuura, and H. Motomura. 2021. Record of an abnormal coloration specimen of *Triodon macropterus* from the Kusagaki Islands, and the first specimen-based record of the species from the Tokara Islands, Kagoshima Prefecture, Japan. Ichthy, Natural History of Fishes of Japan 15:27–32.

Nicholson, K. E., L. J. Harmon, and J. B. Losos. 2007. Evolution of *Anolis* lizard dewlap diversity. PLoS ONE 2: e274.

Pietsch, T. W., and R. J. Arnold. 2020. Frogfishes: Biodiversity, Zoogeography, and Behavioral Ecology. Johns Hopkins University Press, Baltimore, Maryland.

Recher, H. F., and J. A. Recher. 1968. Comments on the escape of prey from avian predators. Ecology 49:560–562.

Rosén, N. 1912. Studies on the plectognaths. 2: The air-sac, with notes on other parts of the intestines. Arkiv för Zoologi 7:1–23.

Sabaj, M. H. 2020. Codes for natural history collections in ichthyology and herpetology. Copeia 108:593–669.

Santini, F., and J. C. Tyler. 2003. A phylogeny of the families of fossil and extant tetraodontiform fishes (Acanthomorpha, Tetraodontiformes), Upper Cretaceous to Recent. Zoological Journal of the Linnean Society 139:565–617.

Santini, F., and J. C. Tyler. 2004. The importance of even highly incomplete fossil taxa in reconstructing the phylogenetic relationships of the Tetraodontiformes (Acanthomorpha: Pisces). Integrative and Comparative Biology 44: 349–357.

Shepherd, B., H. T. Pinheiro, and L. A. Rocha. 2019. Sometimes hard to swallow: attempted feeding on a porcupinefish results in death of both predator and prey. Western Indian Ocean Journal of Marine Science 18:87–89.

Smith, J. L. B. 1956. Self-inflation in a gobioid fish. Nature 177:714.

Smith, W. L., C. A. Buck, G. S. Ornay, M. P. Davis, R. P. Martin, S. Z. Gibson, and M. G. Girard. 2018. Improving vertebrate skeleton images: fluorescence and the non-permanent mounting of cleared-and-stained specimens. Copeia 106:427–435.

Suyehiro, Y. 1942. A study on the digestive system and feeding habits of fish. Japanese Journal of Zoology 10:1–303.

Tyler, J. C. 1962. *Triodon bursarius*, a plectognath fish connecting the Sclerodermi and Gymnodontes. Copeia 1962:793–801.

Tyler, J. C. 1967. A redescription of *Triodon macropterus* Lesson, a phyletically important plectognath fish. Koninklijke Nederlandse Akademie van Wetenschappen 70:84–96.

Tyler, J. C. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). NOAA Technical Report, NMFS Circular 434:1–422.

Tyler, J. C., and K. Matsuura. 1981. Comments on the osteology of balistoid fishes (Tetraodontiformes), with notes on the triodontid pelvis. Proceedings of the Biological Society of Washington 94:52–66.

Wainwright, P. C., and R. G. Turingan. 1996. Muscular basis of buccal pressure: inflation behavior in the Striped Burrfish *Chilomycterus schoepfi*. Journal of Experimental Biology 199:1209–1218.

Wainwright, P. C., and R. G. Turingan. 1997. Evolution of pufferfish inflation behavior. Evolution 51:506–518.

Wainwright, P. C., R. G. Turingan, and E. B. Brainerd. 1995. Functional morphology of pufferfish inflation: mechanism of the buccal pump. Copeia 1995:614–625.

Wibowo, K., M. Huda, R. S. Wicaksono, M. Bataona, and T. Peristiwady. 2020. Catatan distribusi ikan buntal laut dalam langka *Triodon macropterus* (Tetraodontiformes: Triodontidae) dari Laut Maluku dan Laut Sawu, Indonesia. Jurnal Iktiologi Indonesia 20(1):73–79. [In Bahasa Indonesian with English abstract]

Winterbottom, R. 1973. A descriptive synonymy of the striated muscles of the Teleostei. Proceedings of the Academy of Natural Sciences of Philadelphia 125:225–317.

Winterbottom, R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. Smithsonian Contributions to Zoology 155:1–201.