Note

Spawning and rearing of a porcupine puffer *Cyclichthys orbicularis* (Diodontidae, Tetraodontiformes) in captivity

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Abstract: *Cyclichthys orbicularis* (Diodontidae, Tetraodontiformes) were spawned in captivity, the larvae being reared for more than 60 days, in the Shimonoseki Marine Science Museum Aquarium. Isolated epipelagic eggs, 2.2 mm in mean diameter, hatched two days after spawning. Hatched larvae were 3.5 mm in total length, the head and trunk being covered by a “vesicular dermal sac”. Fin rays were completed in 17 days after hatching at 7.6 mm TL, some rudimentary spines appearing on the dorsum. Spines had become unmovable in 39 days after hatching at 20.8 mm TL. Growth rates from hatching to 8 days and thereafter from 9 days were different, growth regression equations between TL (\(y\)) and days after hatching (\(x\)) being \(y = 0.052x + 3.518\) and \(y = 0.295x + 2.046\), respectively.

Key words: Porcupine puffer; Spawning; Development; Growth

Porcupine puffers of the family Diodontidae include seven genera, 17 species and two subspecies, variously distributed throughout temperate to tropical seas worldwide (Froese and Pauly 2014). Four genera, including seven species, have been recorded from Japanese waters (Aizawa and Doiuchi 2013). A few reports on life history have indicated that isolated epipelagic eggs are spawned, juveniles and young fishes migrating to open pelagic waters (Nishimura 1960; Fujita 1962; Leis 1978; Sakamoto and Suzuki 1978; Fujita and Matsuura 2014), in contrast to tetraodontid puffers that spawn demersal adhesive eggs (Fujita 1962; Sakamoto and Suzuki 1978; Fujita and Matsuura 2014).

Two of the three known species of the genus *Cyclichthys* have been recorded from Japan, *Cy. orbicularis* (Bloch) and *Cy. spilostylus* (Leis and Randall) (Aizawa and Doiuchi 2013; Froese and Pauly 2014).

The present report details the first observations of spawning, eggs and larval development for more than 60 days of *Cy. orbicularis* held in the Shimonoseki Marine Science Museum Aquarium. Although the observations were not especially detailed, it was clear that this species spawns isolated epipelagic eggs just as other diodontids (Fujita and Matsuura 2014), the larval and juvenile development being similar to that of *Diodon holacanthus* Linnaeus, described by Sakamoto and Suzuki (1978).

Materials and Methods

Wild-caught adults of *Cy. orbicularis* from unknown locality, a female ca. 20 cm in standard length (SL) and a male ca. 15 cm SL,
were purchased from a fish dealer and maintained in a closed circulating-filtering exhibition aquarium of 1.1 m³ (0.75 × 1.00 × 1.50 m) with a large imitation coral rock on the coral sand bottom, in the Shimonoseki Marine Science Museum from August 2001. Water temperature was kept at 25°C and subject to cyclic 12 h light and dark periods (Fig. 1A). Two individuals of *Arothron nigropunctatus* (Bloch and Schneider) and each one of *A. manilensis* (Marion de Procé), *Tetrosomus concatenatus* (Bloch), *Lactoria diaphana* (Bloch and Schneider), *Lactophrys trigonus* (Linnaeus), *Chilomycterus antillarum* (Jordan and Rutter) and *Ch. antennatus* (Cuvier), all Tetraodontiformes, were maintained in the same aquarium, being fed three times weekly with processed giant tiger prawns and Manila clams.

Although sexing of adult *Cyclichthys orbicularis* is usually very difficult, the belly of the female became swollen several days before spawning. Both adults nestled close to each other on the bottom the day before spawning (Fig. 1B). Although the actual spawning behavior was not observed, the female belly shrank after spawning. Spawned eggs were found on five separate occasions in May 21 (18:00), June 22 (9:00), August 27 (16:00), September 26 (9:00 and 11:30) and November 6 (12:00), 2004. The eggs spawned in May and June were fertilized but not so on the remaining occasions. The rearing experiment was performed on the second set of eggs spawned on 22 June 2004. The rearing was continued more than 200 days, but measurement and morphological observation were performed only from eggs to 49 days after hatching.

When spawned eggs were found in the surface layer of the aquarium, water samples including the eggs were transferred to a 30 l plastic tank and incubated at 25°C with mild airation.

Hatched larvae were sucked up with a glass pipette and transferred to another 30 l plastic tank, and reared at 25°C without any special pH control. The water was partly exchanged for fresh water and excrement removed daily. The tank was illuminated continuously over 24 h daylight periods with two fluorescent lamps in order to promote continuous feeding (Yoseda et al. 2003).

Larvae, juveniles and young fish were fed several times a day with *Brachionus plicatilis* from the first 18 days, *Artemia* larvae from days seven to 27, frozen *Artemia* adults from days 18 to 48, and processed krill, giant tiger prawns and Manila clams from 48 days after hatching.
Results

Although the developmental stage was not determined when found, the isolated epipelagic eggs were spherical, 2.2 mm in mean diameter (1.9–2.4 mm, \( n = 181 \)), the embryo and primordial eyes forming within one day of spawning (Fig. 2A).

Hatching occurred two days after spawning. Hatched larvae (Fig. 2B, 2C) were 3.5 mm in total length (TL), with the mouth and anus closed. Membranous pectoral fins were obvious, and the head and trunk covered by a “vesicular dermal sac” (Leis 1978) or “pliable shell” (Sakamoto and Suzuki 1978). Melanophores were scattered on the body, tail and belly, with many xanthophores also on the body surface. The eyes were not pigmented.

Nineteen hours after hatching, the eyes had become pigmented and the mouth open (3.5 mm TL, Fig. 2D). Two days after hatching, membranous dorsal and anal fins were separated (3.5 mm TL, Fig. 2E). Five days after hatching, soft rays were apparent in the pectoral fins (3.7 mm TL, Fig. 2F), and seven days later, soft rays appeared in the dorsal and anal fins also (3.9 mm TL, no image).

Seventeen days after hatching, 12 dorsal, 10 anal and 21 pectoral fin rays were completed.
with some rudimentary spines (lumps) apparent on the dorsum (7.6 mm TL, Fig. 3A). By twenty-three days after hatching, the rudimentary spines had grown larger on the body (9.8 mm TL, Fig. 3B), becoming unmovable 39 days after hatching (20.8 mm TL, Fig. 3C). After spine development sequence was represented by conditions at 52 days (about 20 mm TL, no image) and 69 days (about 25 mm TL, Fig. 3D).

Growth of *Cy. orbicularis* from hatching to 49 days are shown in Table 1 and Fig. 4. Growth rates from hatching to 8 days and thereafter from 9 days indicated by regression equations between total length (\(y\)) and days after hatching (\(x\)).

![Fig. 3. Cyclichthys orbicularis young, 17 days after hatching (7.6 mm TL) (A); 23 days (9.8 mm TL) (B); 39 days (20.8 mm TL) (C); 69 days (about 25 mm TL) (D, live).](image)

![Fig. 4. Growth of Cyclichthys orbicularis from hatching to 49 days. Growth rates from hatching to 8 days and thereafter from 9 days indicated by regression equations between total length (\(y\)) and days after hatching (\(x\)).](image)

### Table 1. Growth of *Cyclichthys orbicularis* from hatching to 49 days

<table>
<thead>
<tr>
<th>After hatching</th>
<th>Total Length (mm)</th>
<th>Mean</th>
<th>SD*</th>
<th>Range</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Just</td>
<td></td>
<td>3.5</td>
<td>0.1</td>
<td>3.2–3.7</td>
<td>48</td>
</tr>
<tr>
<td>19 hours</td>
<td></td>
<td>3.5</td>
<td>0.1</td>
<td>3.4–3.7</td>
<td>11</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td>3.6</td>
<td>0.1</td>
<td>3.3–3.8</td>
<td>11</td>
</tr>
<tr>
<td>2 days</td>
<td></td>
<td>3.6</td>
<td>0.1</td>
<td>3.5–3.7</td>
<td>11</td>
</tr>
<tr>
<td>3 days</td>
<td></td>
<td>3.7</td>
<td>0.1</td>
<td>3.5–3.9</td>
<td>10</td>
</tr>
<tr>
<td>4 days</td>
<td></td>
<td>3.8</td>
<td>0.1</td>
<td>3.7–3.9</td>
<td>9</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>3.7</td>
<td>0.1</td>
<td>3.5–4.0</td>
<td>10</td>
</tr>
<tr>
<td>6 days</td>
<td></td>
<td>3.9</td>
<td>0.1</td>
<td>3.7–4.0</td>
<td>8</td>
</tr>
<tr>
<td>7 days</td>
<td></td>
<td>3.9</td>
<td>0.2</td>
<td>3.4–4.0</td>
<td>9</td>
</tr>
<tr>
<td>8 days</td>
<td></td>
<td>3.8</td>
<td>0.1</td>
<td>3.6–4.0</td>
<td>5</td>
</tr>
<tr>
<td>9 days</td>
<td></td>
<td>4.3</td>
<td>0.1</td>
<td>4.2–4.4</td>
<td>3</td>
</tr>
<tr>
<td>17 days</td>
<td></td>
<td>7.6</td>
<td>0.1</td>
<td>7.4–7.8</td>
<td>1</td>
</tr>
<tr>
<td>23 days</td>
<td></td>
<td>9.8</td>
<td>0.1</td>
<td>9.6–10.0</td>
<td>1</td>
</tr>
<tr>
<td>39 days</td>
<td></td>
<td>16.9</td>
<td>5.5</td>
<td>13.0, 20.8</td>
<td>2</td>
</tr>
<tr>
<td>43 days</td>
<td></td>
<td>14.6</td>
<td>2.3</td>
<td>11.9–20.0</td>
<td>10</td>
</tr>
<tr>
<td>44 days</td>
<td></td>
<td>13.4</td>
<td>2.0</td>
<td>11.1–16.6</td>
<td>7</td>
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<tr>
<td>45 days</td>
<td></td>
<td>15.6</td>
<td>2.4</td>
<td>12.7–19.3</td>
<td>7</td>
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<tr>
<td>49 days</td>
<td></td>
<td>20.0</td>
<td>0.5</td>
<td>17.0–23.0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Standard deviation.*
from 9 days were clearly different as visually shown in the growth lines in Fig. 4, regression equations between TL (y) and days after hatching (x) being $y = 0.052x + 3.518$ ($r = 0.663, P < 0.01, n = 132$) and $y = 0.295x + 2.046$ ($r = 0.809, P < 0.01, n = 32$), respectively.

**Discussion**

*Cyclichthys orbicularis* development was similar to but faster in early and slower in later stages than *D. holacanthus* (Sakamoto and Suzuki 1978), with slightly larger stage sizes (water temperature under 25°C and 24.2–27.9°C, respectively): eggs were 1.9–2.4 mm in *Cy. orbicularis* (1.7–1.9 mm in *D. holacanthus*), hatching occurred from 2 days after spawning, hatched larvae being 3.2–3.7 mm TL (102 h after spawning, 2.5–2.7 mm TL); eye pigmentation was 19 h after hatching at 3.4–3.7 mm TL (24 h, 2.6–2.7 mm TL); fin rays were completed and rudimentary spines appeared in 17 days at 7.6 mm TL (10 days, 4.9–5.9 mm TL); rudimentary spines grew larger in 23 days at 9.8 mm TL (13 days, 6.0–7.9 mm TL); spines were completed in 39 days at 20.8 mm TL (16 days, 11.0 mm TL).

*Cyclichthys orbicularis* spawned isolated epipelagic eggs, the larvae having a “vesicular dermal sac” or “pliable shell”, as in other diodontids (Fujita and Matsuura 2014). These characteristics are shared among three tetraodontiform families, viz. Diodontidae, Ostraciidae and Molidae (Sakamoto and Suzuki 1978; Fujita and Matsuura 2014), suggesting their close phylogenetic relationship (Sakamoto and Suzuki 1978). Although the analysis based on larval characteristics (Leis 1984) support such a relationship, other tetraodontiform phylogenetic analyses based on myology (Winterbottom 1974), spinal cord morphology (Uehara et al. 2014), osteology (Rosen 1984; Santini and Tyler 2003), and DNA sequence (Yamanoue et al. 2008; Santini et al. 2013) do not (Santini et al. 2013; Matsuura 2014), suggesting possible larval adaptation and convergence.

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**References**


Morphol., 276, 290-300.


メイタイシガキフグの水槽内での産卵と発育
土井啓行・石橋敏章・酒井治己

フグ目ハリセンポン科メイタイシガキフグ Cyclichthys orbicularis が下関市立しものせき水族館の水槽で産卵した。卵は分離浮遊性で平均直径は2.2 mm であり, 2 日でふ化した（25℃）。ふ化仔魚の全長は3.5 mm で, 頭部および躯幹部は厚い外膜“vesicular dermal sac”で覆われていた。ふ化後17日, 全長7.6 mm で各鱗条が完成し, 背面に棘原基が出現した。ふ化後39日, 全長20.8 mm で棘は不動となった。ふ化後 8 日までと 9 日以降では成長率が異なり, 全長（y）とふ化後日数（x）の関係式はそれぞれ \( y = 0.052x + 3.518 \) および \( y = 0.295x + 2.046 \)であった。