

Larval Development, Growth and Age Determination in the Sharpnose Pufferfish *Canthigaster valentini* (Teleostei: Tetraodontidae)

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Abstract The eggs, early larvae and juveniles of the sharpnose pufferfish *Canthigaster valentini* are described, based on material collected in Great Barrier Reef waters. Eggs were obtained in the field by divers and reared in the laboratory. The eggs are spherical, strongly adhesive, 0.68–0.72 mm in diameter, possess a dense cluster of small oil droplets, and hatch around sunset 3 to 5 days after fertilization. Newly hatched larvae have a small yolk sac, pectoral fin folds, 17 myomeres (6 pre-anal, 11 post-anal) and measure 1.30–1.40 mm in notochord (standard) length. The eggs of *C. valentini* differ from those of other tetraodontids in being much smaller and having a longer incubation time. The larvae can be distinguished from other tetraodontid larvae by pigmentation, myomere count and size at hatching. Growth is most rapid during the first day of larval life. Age determinations (based on otolith microstructure) of field collected juveniles, both pelagic and newly settled, indicate a pelagic phase of between 64 and 113 days for this species. This estimate appears consistent with the extended pelagic juvenile stages observed in other tetraodontiform fishes and could indicate that *C. valentini* can delay settlement for some time after becoming competent to settle at a minimum age of 64 days.

The tetraodontid subfamily Canthigasterinae (Tyler, 1980) is composed of a single genus *Canthigaster* containing 24 species (Allen and Randall, 1977; Lubbock and Allen, 1979). Despite the fact that members of the Canthigasterinae are common over a wide geographical area, the egg and larval development has only been recently described in detail for one species: *Canthigaster rivulata* (Arai and Fujita, 1988). In the present report we describe the eggs, early larvae and juveniles of *C. valentini* (Bleeker) based on laboratory reared and field collected specimens. Data on growth and ageing of larvae and juveniles, obtained by examining daily increments on otoliths (Brothers et al., 1976; Struhsaker and Uchiyama, 1976) are also presented. *C. valentini* is one of the more common and widespread members of the genus and is found on coral reefs of the tropical Indo-Pacific from shallow water to depths of at least 25 metres. A full description of the adult is given by Allen and Randall (1977).

Materials and methods

Field collections. Field work and rearing were

conducted at the Lizard Island Research Station in northern Great Barrier Reef of Australia. Courting individuals of *C. valentini* were followed underwater by divers using SCUBA and the time and location of spawning noted. Eggs were collected attached to the algae in which they were laid (see below) a few minutes after spawning and transferred to the surface in plastic bags. They were then placed into buckets of fresh seawater and aerated with a portable air pump until transported to the aquaria.

Pelagic juveniles were collected under night lights with a dip net and, except where noted otherwise, were immediately preserved in 80% ethanol. Settled juveniles were collected from a series of small artificial reefs using ichthyocides. These reefs were cleared of individuals weekly, therefore juveniles collected this way were never more than seven days settled, assuming no inter-reef movement of settled individuals.

Eggs were reared in 100 litre opaque, plastic, circular tanks following the method of Houde and Ramsay (1971) and Houde (1973). Temperature during six rearing experiments ranged from 23.5 to 27.1°C. Three days after hatching, larvae were

fed on a diet of rotifer *Brachionus plicatilis* and wild size-sorted zooplankton. Natural illumination (under 70% shade cloth) was used at all times. A total of 93 larvae were sequentially removed and preserved in 5% buffered seawater formalin and used for descriptive material. A further 16 were taken along with the above series and preserved on 80% ethanol for otolith age-verification studies. All specimens are housed in the larval fish collection at the Australian Museum, Sydney.

Terminology. Terminology of the developmental stages follows that of Leis and Rennis (1983) who consider the transition between larva and juvenile to coincide with the attainment of full meristic characters and the loss of temporary specializations to pelagic life.

Meristics and morphometrics. All counts and measurements were made with a binocular dissecting microscope and ocular micrometer. Abbreviations used in fin ray formulae follow those of Leis and Rennis (1983). Illustrations were drawn with the aid of a camera lucida.

Definitions of morphometric measurements are as follows: standard length (SL) is the distance from the tip of the snout to the posterior tip of the notochord (before hypural formation) or posterior margin of the hypurals; pre-anal length is measured from the tip of the snout along the midline to a vertical line through the posterior edge of the anus; head length is the horizontal distance from the tip of the snout to the upper edge of the gill opening; snout length is the horizontal distance from the tip of the snout to the anterior margin of the fleshy orbit; eye diameter is the maximum horizontal distance measured from anterior to posterior margin of the fleshy orbit; pre-dorsal length is measured from the tip of the snout along the midline to a vertical line through the origin of the dorsal fin; body depth is the vertical distance between body margins (exclusive of fins) through the anterior margin of the pectoral fin base; body width is the transverse distance between body margins at the pectoral fin base.

Myomeres (pre-anal plus post-anal) were counted on each larva when they could be distinguished. Several larvae were stained with the vital protein "Rose Bengal" to aid in counting myomeres (Marcy, 1975).

Osteology. Three juveniles (13.9–16.4 mm SL) were cleared with trypsin and stained with alizarin

(Taylor, 1967) in order to study ossified structures.

Otolith Studies. Lapilli and sagittae were removed from each larva by teasing them from fresh or ethanol-preserved specimens with the aid of fine stainless steel needles. The otoliths were transferred to clean microscope slides wetted with immersion oil. Generally, no further preparation was necessary—the otoliths were immediately examined in immersion oil or, after drying, permanently mounted with the neutral mounting medium "Pro-texx". Otoliths removed from juveniles were treated as above, but sometimes required etching for 1–2 minutes in a 1% solution of HCl before being mounted (Struhsaker and Uchiyama, 1976).

The smallest growth increments of the mounted otoliths were counted with a compound microscope at magnification of 400–1,500 \times after contrast was maximized by a polarizing filter. Increments were counted from the nucleus to the longest axis since they were most clearly discernible in this region. Three consecutive counts were made of the increments on each otolith: if the difference between these counts was <2 they were considered acceptable counts. In cases where this difference was >2 the otolith was re-counted and either a series of counts accepted, or the data discarded. Consistent (5% error) accepted counts were obtained between the four otoliths of each individual examined. Accepted counts were averaged over all four otoliths to give an estimate of the age of an individual in days. This procedure was unnecessary for laboratory reared larvae due to the ease and consistency in reading their otoliths.

Verification of the daily nature of otolith growth increments was obtained by comparing the number of growth increments observed in the otoliths of laboratory reared larvae with the known chronological ages of these larvae.

Results

Spawning. At Lizard Island *Canthigaster valentini* spawns year round. This species has a male-dominated social structure and undergoes pair spawning between 0800 and 1530 hours. Spawning generally takes place in coral rubble areas where the eggs are laid on the bottom attached to filamentous algae (Fig. 1A). Each of five females followed laid a cluster of eggs numbering between 15 and 876 (mean = 314).

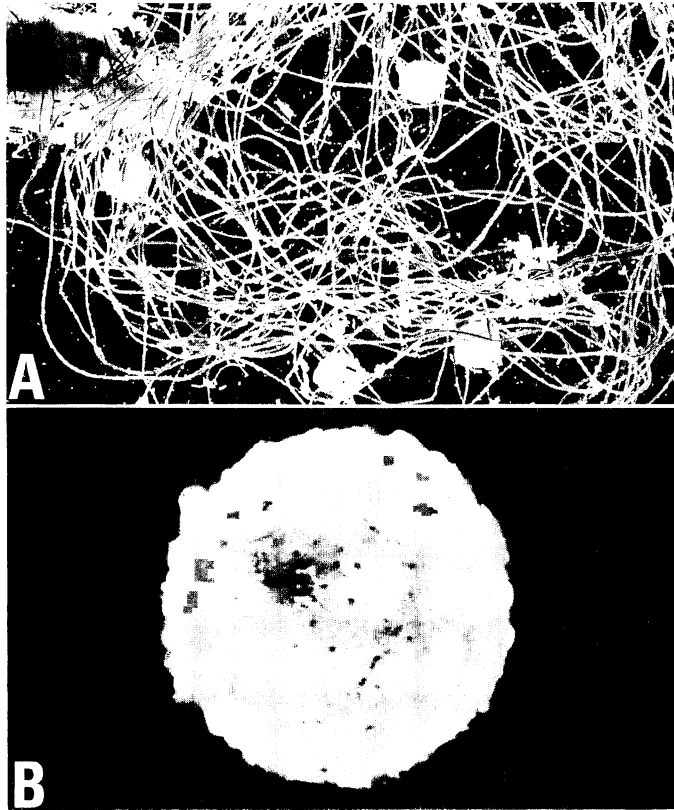


Fig. 1. Photomicrograph of *Canthigaster valentini* eggs. A: Eggs attached to filamentous algae. Each egg is approximately 0.70 mm diameter. B: A single egg measuring 0.71 mm diameter.

Description and incubation of eggs. Egg size ranged from 0.68 to 0.72 mm diameter (mean = 0.70, $n=49$). The eggs were transparent, spherical and possessed a dense cluster of small oil globules ranging from 0.01 to 0.05 mm in diameter. The egg membrane consisted of nine radial layers and has a strongly adhesive outer coating. Fine sediment sticking to this adhesive coating tended to give the egg an opaque, almost whitish appearance (Fig. 1B).

Incubation time of aquarium reared eggs, which were kept attached to the algae on which they were laid, varied inversely with temperature and ranged from 126.5 hours from spawning at 23.5°C to 81 hours at 27.1°C. However, this time was further constrained by the apparent requirement for hatching shortly after sunset. Hatching of all six batches reared occurred between 1844 hours (winter) and 2045 hours (summer) and was always within one hour of nautical twilight (Nautical Almanac, British Admiralty): range 14 to

50 minutes.

Description of larvae and juveniles. At hatching, the larvae of *C. valentini* possessed an ovoid head, a relatively deep body and measured 1.30–1.40 mm in standard length. The small yolk sac contained numerous oil globules ranging from 0.01 to 0.05 mm diameter along its dorsal and posterior margins (Fig. 2A). The mouth was not yet formed, the gut was straight and the anus unopen. Each larva was enclosed in a dermal sac which, except for small sections of the dorsal and ventral fin folds, was completely covered in numerous minute tubercles. The pectoral fin folds were small, but present in newly hatched larvae.

By 24 hours posthatching at 23.5°C the yolk sac was considerably reduced and the mouth was beginning to form. The anus was still unopen (Fig. 2B). There was no significant change in morphology by 48 hours. By 72 hours posthatching the yolk sac was almost completely absorbed,

the mouth was formed, the gut was coiled and extended to about mid-body, and the anus was open (Fig. 2C). Most of the smaller oil globules had coalesced by this time and the larvae had commenced feeding. Neither yolk nor oil globules were visible in larvae 6 days posthatching (Fig. 2D, E). The gill opening had, by this time, closed into a small pore. By 9 days posthatching the nasal capsules were visible and the head and body had become slightly more robust (Fig. 2F). Notochord flexure had still not occurred.

Laboratory reared larvae grew from an average 1.35 mm SL at hatching to an average of 1.82 mm SL after nine days when the last of the survivors was taken for age studies. From the graph (Fig. 3) it can be seen that growth was most rapid during the first day of larval life.

Morphometric growth and changes in relative character size with growth is shown in Table 1 for several laboratory reared larvae and field collected juveniles. It can be seen that there are marked differences in relative morphometric proportions with the change from larval to juvenile status. This change is least with the size of the eye which gradually increases in relative size with larval growth and reaches a maximum apparently about the time of settlement, before regressing. Head length (and snout length), body depth, preanal length and body width have all increased markedly in juveniles as compared with the larvae, reflecting the change towards robust body shape finally attained by these fishes. For the laboratory reared larvae, body width increases to Day 3 then decreases. This possibly indicates a loss of condition of the larvae due to inadequate diet leading to death.

Myomeres were often obscured by the dermal sac and/or heavy pigment, but in those larvae in which they could be distinguished (1.30–1.64 mm SL, $n=16$) the total number of myomeres was always 17 (6 pre-anal plus 11 post-anal). The vertebral formula was given by Allen and Randall (1977) and Tyler (1980) for *C. valentini* as 8+9=17. Total myomeres thus agree 1:1 with vertebral count (cf Miller et al., 1979), although the numbers of pre-anal myomeres and pre-caudal vertebrae differ. No fin rays were present in the larvae up to age 9 days which had only fin folds. Fin rays most probably develop during late pre-flexion and flexion stages as occurs in other tetraodontids (Leis and Rennis, 1983). Fin ray counts

of 12 juveniles were D. 9, A. 9, P₁. 16–17, C. 5+6. These are within the range of adult values (Allen and Randall, 1977).

Newly hatched larvae had unpigmented eyes although there was a band of pigment running from behind the posterior section of the eye to the otic capsule (Fig. 2A). By 1.52 mm SL (24 hours posthatching) this internal pigment had extended further antero-dorsally (Fig. 2B). The eyes began to develop pigment by the third day when the larvae were about 1.68 mm SL and yolk sac absorption was virtually complete (Fig. 2C). A large subdermal melanophore, situated approximately midway between the posterior margin of the eye and upper end of the operculum, had developed by the time larvae had reached 1.70 mm SL (6 days posthatching). A large stellate external melanophore, just posterior to the base of the pectoral fin fold, was also present at this stage of development (Fig. 2D).

Brain pigmentation appeared around 1.52 mm SL (24 hours posthatching) in the form of a small stellate subdermal melanophore situated posterolaterally on one, or both sides of the hindbrain (Fig. 2B). By 1.70 mm SL (6 days posthatching) these small melanophores had been replaced by a single large stellate subdermal melanophore located on the posterior region of the hindbrain (Fig. 2D, E).

The yolk sac of a newly hatched larva exhibited numerous small, scattered, stellate melanophores particularly along its dorsal margin where they formed a line of heavy pigment (Fig. 2A). A similar line, almost continuous with one of the yolk sac, ran along the dorsal edge of the gut. One to five small, stellate melanophores were present on the myomeres situated above the gut region (Fig. 2A) however, by 1.52 mm SL (24 hours posthatching) these melanophores had disappeared (Fig. 2B). As development continued the gut region became heavily pigmented anteriorly, dorsally and dorsolaterally, but remained unpigmented ventrally (Fig. 2C, D, F).

Tail pigmentation in newly hatched larvae consisted of a series of melanophores which extended from around the level of the anus to five or six myomeres caudad as well as from the dorsal to ventral body margins (Fig. 2A). By 1.52 mm SL (24 hours posthatching) the melanophores comprising this series had become more numerous and formed a distinct pigment band on the tail (Fig.

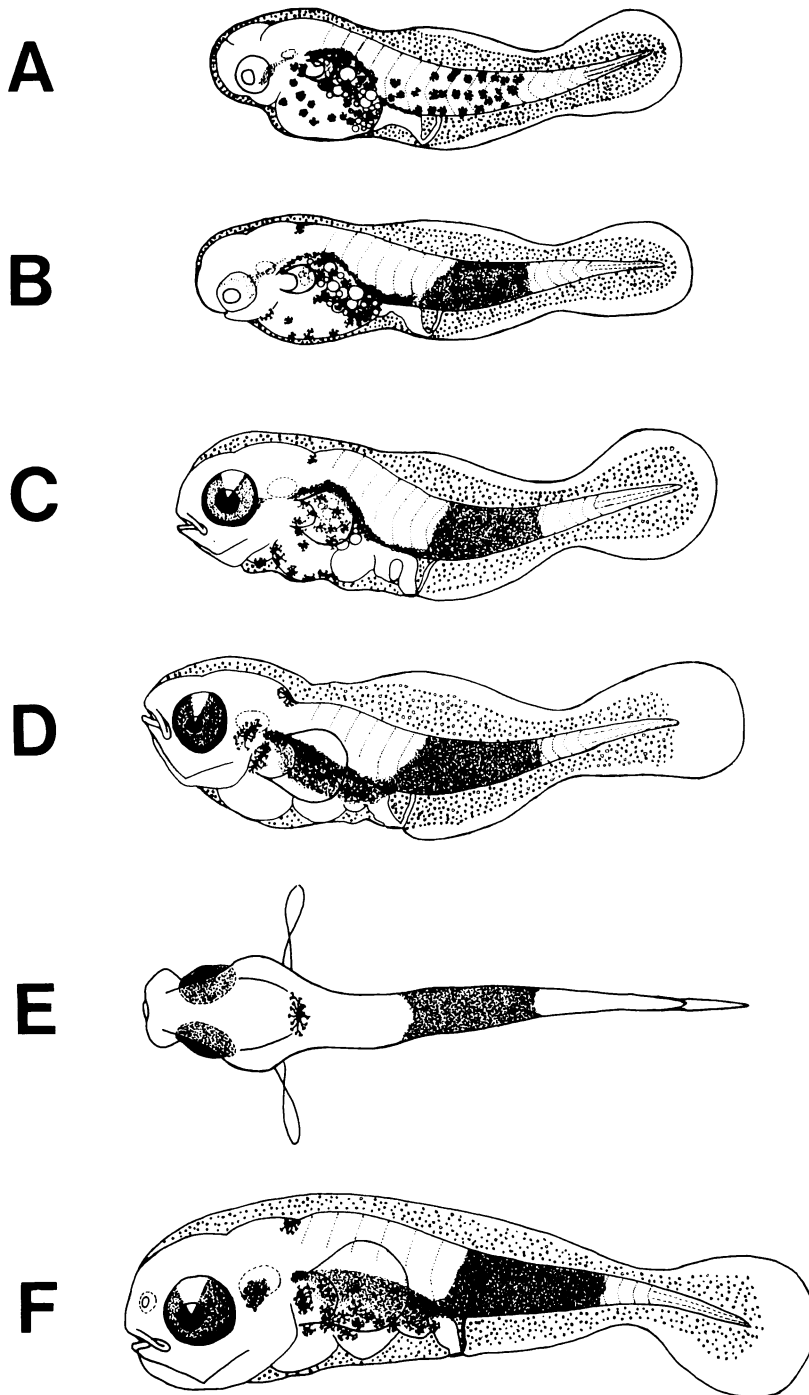


Fig. 2. Larval development of *Canthigaster valentini*. A, lateral view of newly hatched larva (1.32 mm SL); B, 24 hours after hatching (1.52 mm SL); C, 72 hours after hatching (1.68 mm SL); D, 6 days after hatching (1.70 mm SL); E, 6 days after hatching (1.70 mm SL), dorsal view; F, 9 days after hatching (1.82 mm SL).

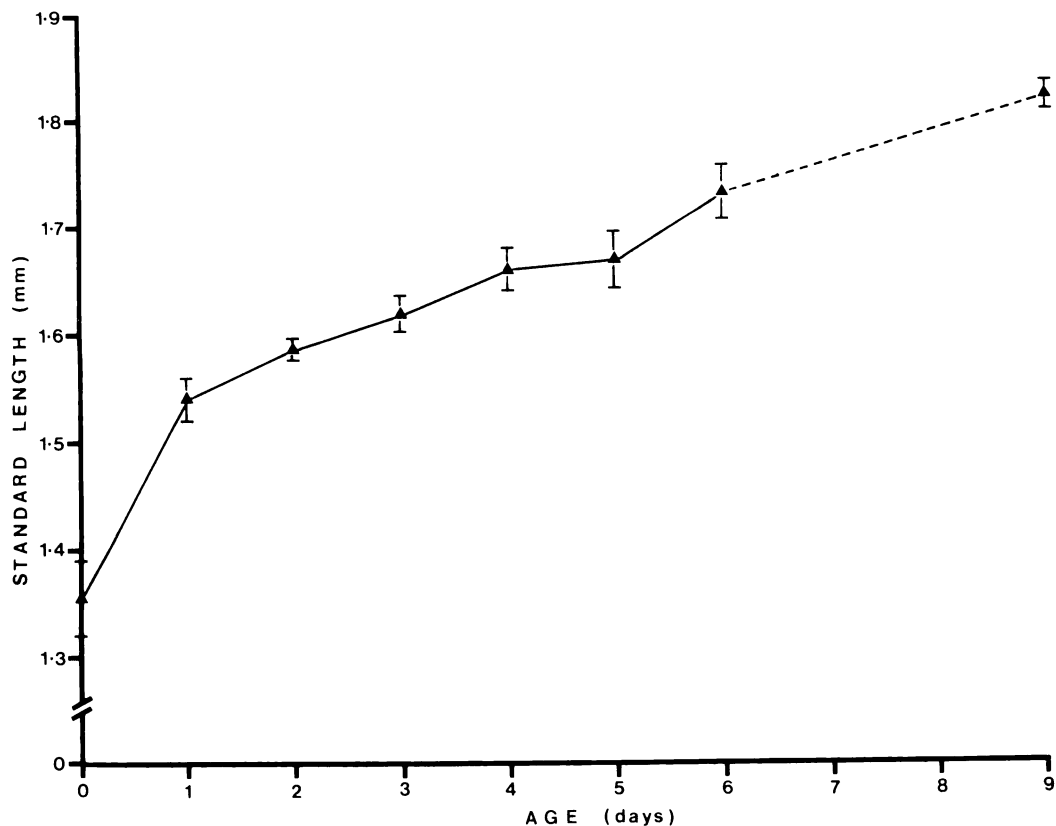


Fig. 3. Comparison of standard length (mm) with age (days) for laboratory reared larvae at 23.0°C; mean \pm SE, $n=31$.

2B). This pigment band was present in all other stages of larvae which were examined (Fig. 2C-F). The pigmentation of juveniles was typical of that of the adults albeit with fewer brown spots on the head and body laterally.

Ossification is apparently completed before settlement as all adult structures were ossified in three newly settled juveniles (93–113 days old; 13.88–16.37 mm SL) that had been cleared and stained. The vertebral formula in each individual was 8+9=17. The nine dorsal fin rays were supported by seven basal pterygiophores situated between the neural spines of the seventh abdominal vertebra and the third caudal vertebra (i.e. between vertebra 7 and 11). The nine anal fin rays were supported through fibrous tissue by five basal pterygiophores. The dorsal edges of these pterygiophores articulate by fibrous tissue with the ventral edges of the haemal spines of the caudal vertebrae: Pterygiophore 1 was attached to the first caudal

vertebra, pterygiophores 2, 3, 4 and 5 were attached to caudal vertebrae 2, 3, 4 and 4 respectively. The number and/or arrangement of dorsal and anal pterygiophores thus differs somewhat from that of *Canthigaster rostrata* described by Tyler (1980).

Age determination. Larvae were reared for up to 7 days after hatching. Of the several counts of each of the four otoliths removed from each individual at days one, three, six and nine respectively, the number of rings counted agreed exactly with the age in days except with one sagitta from one of the nine day larvae which had only eight rings. There are thus no rings laid down during the egg stage.

Field collected newly settled juveniles ranged in age between 64 and 113 days based on otolith counts (Table 1). Actual age at settlement could, however, be up to seven days less than this as settlement reefs were only visited once per week.

The one pelagic juvenile ageable gave an otolith age of 105 days which, subtracting the five days this individual was in an aquarium before extraction of its otoliths, is consistent with the estimates from settled individuals. No obvious "settlement" or transition marks of the type described by Victor (1982) and Brothers et al. (1983) were observed in the individuals examined.

Discussion

The literature on tetraodontid eggs and larvae deals primarily with temperate species of the tetraodontine genera *Fugu*, *Sphoeroides*, *Tetraodon*

(including subgenera *Chelonodon*, *Monotreta* and *Tetraodon*) and *Torquigener*. The eggs of these species are typically spherical, 0.85–1.41 mm in diameter, contain a dense cluster of small oil globules and are covered with an adhesive coating (Welsh and Breder, 1922; Schreitmuller, 1930; Munro, 1945; Uno, 1955; Fujita, 1962; Leis, 1984). These eggs are generally demersal and cemented to stones and rocks (Welsh and Breder, 1922; Schreitmuller, 1929, 1930; Randow, 1934; Sterba, 1962; Honma et al., 1980; Richter, 1982) or partly buried in the sand (Breder and

Table 1. Morphometric measurements (in percentage of the standard length) for larvae and juveniles of *Canthigaster valentini*. * Pelagic juveniles, the remainder being collected as settled individuals. ** Damaged specimens. ? Otoliths unreadable.

Age (days)	SL (mm)	Eye diameter	Predorsal length	Body depth	Body width	Pre-anal length	Head length	Snout length
Laboratory reared larvae:								
0	1.30	9.2	—	26.2	18.5	52.3	18.5	4.6
0	1.32	9.0	—	27.2	19.7	48.5	18.2	3.0
0	1.36	8.8	—	27.9	17.6	51.5	19.1	4.4
0	1.38	8.7	—	26.1	17.4	50.0	18.8	4.3
0	1.40	8.6	—	24.3	17.1	49.3	18.6	4.3
1	1.52	11.8	—	28.9	19.7	50.0	22.4	5.3
1	1.54	10.4	—	28.6	22.1	48.1	23.4	5.2
1	5.56	11.5	—	26.9	21.8	52.6	22.4	3.8
2	1.58	11.4	—	27.8	22.8	46.8	22.8	5.1
2	1.58	12.7	—	29.1	21.5	48.1	24.1	5.1
2	1.59	12.6	—	30.8	22.6	46.5	23.9	6.3
2	1.60	12.5	—	28.8	21.3	47.5	25.0	6.3
3	1.60	12.5	—	28.8	22.5	47.5	23.8	5.6
3	1.62	13.0	—	28.4	22.2	46.9	24.7	6.2
3	1.62	12.3	—	27.8	21.6	48.1	23.5	6.2
3	1.63	12.3	—	27.0	22.1	46.6	24.5	6.1
4	1.64	12.2	—	26.8	22.0	47.6	23.2	4.9
5	1.64	12.2	—	25.6	19.4	47.6	23.2	4.9
4	1.68	11.9	—	28.6	19.0	47.6	22.6	4.8
6	1.70	11.8	—	25.9	15.3	48.2	21.8	5.9
Field collected juveniles:								
64	11.81	17.0	78.8	48.2	37.6	83.5	42.3	16.4
?	12.22*	18.2	81.8	51.1	43.2	85.3	43.2	15.9
87	12.29	18.1	80.2	49.7	39.5	83.6	41.8	16.9
76	12.36	18.0	82.0	48.3	38.2	85.4	45.0	19.1
105	12.64*	16.5	79.1	50.6	39.6	85.7	41.8	15.3
89	13.19	15.8	80.0	45.3	35.8	85.3	43.1	20.0
104	13.60	16.3	82.7	49.0	36.8	82.7	48.0	20.4
93	13.88**	15.0	—	—	—	84.1	46.0	—
85	14.45	16.3	81.7	46.2	35.6	83.6	44.2	21.2
106	15.69**	15.9	—	—	—	85.0	48.7	—
?	16.15	17.2	80.0	46.4	35.2	86.0	43.0	23.2
113	16.39	15.3	77.1	44.9	39.0	86.5	47.5	26.3

Clark, 1947; Uno, 1955). The eggs of *Canthigaster valentini* are also spherical, demersal, adhesive and possess a dense cluster of oil globules, but differ in being much smaller in diameter (0.68–0.72 mm) and in being laid attached to clumps of filamentous algae (Gladstone, 1987a) rather than on rocks or in sand depressions. Richter (1982) reported that the freshwater puffer *Carinotetraodon lorteti* (named *C. somphongsi* in his article) spawned in algal clumps, however his photographs show the eggs to be scattered primarily over rocks. In Japan however, the marine pufferfish *Fugu pardalis* is known to spawn in seagrass beds (Fujita, 1962). Arai and Fujita (1988) recently described spawning in an aquarium by *Canthigaster rivulata*; unlike the former demersal spawning species a pair of *C. rivulata* released their gametes together above the substratum.

There is some additional information on the eggs of *Canthigaster* spp. Gladstone (1987b) recently reported that the eggs, and larvae, of *C. valentini* are unpalatable to several species of reef fish. A consequence of this is that parents do not defend nests of fertilized eggs. Fujita (1962) described the unfertilized eggs of *C. rivulata* as transparent, spherical, 0.67–0.71 mm in diameter with a mass of small oil droplets each measuring 0.017–0.035 mm in diameter. Fujita (1962) also noted that the egg membrane consisted of several radial layers. In a more recent paper Arai and Fujita (1988) described the fertilized eggs of this species and counted 14 radial layers in the egg membrane. The eggs of *C. rivulata* thus appear to be very similar to those of *C. valentini* however, *C. valentini* has a slightly larger egg diameter (0.68–0.72 mm), a slightly greater range of oil droplet size (0.01–0.05 mm diameter), and fewer layers in the egg membrane (nine), than the former species.

In the literature, the only other marine pufferfish which produces eggs of similar size to *C. valentini* is the tropical inshore species *Lagocephalus lunaris spadiceus* from Japan. Fujita (1966) described their eggs as colourless, transparent, spherical, 0.61–0.70 mm in diameter and possessing a cluster of oil globules each measuring 0.009–0.07 mm in diameter. He also mentioned that their eggs are demersal, but only faintly adhesive in nature. They had an incubation time of 76 hours at 21.0–24.5°C. This contrasts with the eggs of *C. valentini* which are strongly adhesive, slightly larger and

with a noticeably longer incubation time (126 hours at 23.5°C).

In several species of tetraodontids the male guards the eggs until hatching occurs (Schreitmuller, 1930; Sterba, 1962; Richter, 1982). It has been reported that the males of *Monotreta cutcutia* also shelter their hatched young in a sand depression and guard them for a further period in a manner not unlike that of certain cichlids (Schreitmuller, loc. cit.). In contrast, no nest guarding or other form of parental care has been observed in *C. valentini* (Gladstone, 1987a).

Larvae of *C. valentini* can be distinguished by their smaller size at hatching (1.30–1.40 versus 1.60–3.20 mm notochord length) and lower myomere count (17 versus 17–27) of the preflexion larvae of other tetraodontid fishes that have been described (cf. Welsh and Breder, 1922; Munro, 1945; Fujita, 1956a, b, 1962, 1966; Fujita and Ueno, 1956; Shojima, 1957; Miller et al., 1977; Leis and Rennis, 1982). The pigmentation, particularly the band of pigment extending from the dorsal to ventral margins of the tail, is also very helpful in separating this species from others. Few tetraodontid larvae have been collected in plankton tows around Lizard Island (J. M. Leis, pers. comm.; Talbot, Goldman and Stroud, unpubl.) and of these only one species resembles *C. valentini*. The preflexion larvae of these two species differ in length of the pigment band on the tail. In *C. valentini* the pigment band extends 5–8 myomeres compared with only 3–5 myomeres in the other species which remains unidentified at the present time. Juvenile *C. valentini* are easily separable from juveniles of other *Canthigaster* species by colour pattern and meristics (Allen and Randall, 1977), which also distinguishes them from other tetraodontid genera (Leis and Rennis, 1983).

The otolith age determinations of field collected juveniles indicate a pelagic phase of between 64 and 113 days for this species. The wide range in values is probably not due to non-daily ring deposition, given the consistent results for the reared larvae. This range in age at settlement could indicate that *C. valentini* can delay settlement for some time (as much as 49 days based on our results) after becoming competent to settle at a minimum age of 64 days. Members of the tetraodontiform families Diodontidae, Monacanthidae and Balistidae also have extended pelagic juvenile stages and are often of large size by the

time they settle out from the plankton (Berry and Vogele, 1961; Berry and Baldwin, 1966; Randall, 1971; Randall and Klauswitz, 1973; Leis, 1978). There is also some evidence to suggest that at least one ostracodontid species may be able to prolong the length of its pelagic juvenile stage (Moyer, pers. comm.; Thresher, 1985). Ambivalence in settling and consequent repeated re-entry into the plankton (Fluchter, 1965; Marliave, 1977) may have also contributed to the wide range observed in newly settled individuals of *C. valentini*.

Other reef fishes may have pelagic periods less variable and/or of shorter duration. Larvae of *Haemulon flavolineatum* (Pomadasysidae) for instance settle in 11–15 days (Brothers and McFarland, 1981) and *Thalassoma bifasciatum* (Labridae) settles in 40–72 days (Victor, 1982). Brothers et al. (1983) examined the otoliths of 43 species of newly settled reef fishes from One Tree Island, Great Barrier Reef and found the otolith ages of individuals within a given species to vary from one day in some species to 22 days in others. Unfortunately, only small numbers of individuals were examined for the majority of species. Due to the limited available information on age at settlement in reef fishes at the present time we cannot say how typical *Canthigaster valentini* is of reef fishes in its widely variable length of pelagic stage.

Acknowledgments

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Literature cited

- Allen, G. R. and J. E. Randall. 1977. Review of the sharpnose pufferfishes (Subfamily Canthigasterinae) of the Indo-Pacific. *Rec. Austr. Mus.*, 30: 475–517.
- Arai, H. and S. Fujita. 1988. Spawning behavior and early life history of the sharpnose puffer, *Canthigaster rivulata*, in the aquarium. *Japan. J. Ichthyol.*, 35(2): 194–202.
- Berry, F. H. and W. J. Baldwin. 1966. Triggerfishes (Balistidae) of the Eastern Pacific. *Proc. Calif. Acad. Sci.*, 34: 429–474.
- Berry, F. H. and L. E. Vogele. 1961. Filefishes (Monacanthidae) of Western North Atlantic. *U. S. Fish Wildl. Serv., Fish Bull.*, 61: 61–109.
- Breder, C. M., Jr. and E. Clark. 1947. A contribution to the visceral anatomy, development, and relationships of the Plectognathi. *Bull. Amer. Mus. Nat. Hist.*, 88: 291–319.
- Brothers, E. B. and W. McFarland. 1981. Correlations between otolith microstructure, growth and life history transitions in newly recruited French grunts (*Haemulon flavolineatum*). *Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer.*, 1978: 396–374.
- Brothers, E. B., C. P. Mathews and R. Lasker. 1976. Daily growth increments in otoliths from larval and adult fishes. *U. S. Fish Wildl. Serv., Fish. Bull.*, 74: 1–8.
- Brothers, E. B., D. McB. Williams and P. F. Sale. 1983. Length of larval life in twelve families of fishes at "One Tree Lagoon", Great Barrier Reef, Australia. *Mar. Biol.*, 76: 319–324.
- Fluchter, J. 1965. Versuche zur Brutaufzucht der Seezunge *Solea solea* in kleinen Aquarien. *Helgolander Wiss. Meeresunters.*, 12: 395–403. (In German with English summary.)
- Fujita, S. 1956a. On the development of the egg and prelarval stages of the puffer *Fugu (Shosaifugu) stictonotus* (Temminck et Schlegel). *Sci. Bull. Fac. Agr., Kyushu Univ.*, 15: 525–530. (In Japanese with English summary.)
- Fujita, S. 1956b. On the development of the egg and prelarval stages of the puffer *Fugu (Shosaifugu) poecilnotus* (Temminck et Schlegel). *Sci. Bull. Fac. Agr., Kyushu Univ.*, 15: 531–536. (In Japanese with English summary.)
- Fujita, S. 1962. Studies on the life history and culture of common pufferfishes in Japan. *Nagasaki Pref. Fish. Res. Stn. Rep.*, (2), 121 pp., 40 pls. (In Japanese.)
- Fujita, S. 1966. Egg development, larval stages and rearing of the puffer *Lagocephalus lunaris spadiceus* (Richardson). *Japan J. Ichthyol.*, 13: 162–168. (In Japanese with English summary.)
- Fujita, S. and M. Ueno. 1956. On the egg development and prelarval stages of *Fugu (Torafugu) rubripes*

- (Temminck et Schlegel). Sci. Bull. Fac. Agr., Kyushu Univ., 15: 519–524. (In Japanese with English summary.)
- Gladstone, W. 1987a. The courtship and spawning behaviors of *Canthigaster valentini* (Tetraodontidae). Env. Biol. Fish., 20: 255–261.
- Gladstone, W. 1987b. The eggs and larvae of the sharpnose pufferfish *Canthigaster valentini* (Pisces: Tetraodontidae) are unpalatable to other reef fishes. Copeia, 1987: 227–230.
- Honma, Y., T. Ozawa and A. Chiba. 1980. Maturation and spawning behaviour of the puffer *Fugu niphobles*, occurring on the coast of Sado Island in the Sea of Japan (a preliminary report). Japan J. Ichthyol., 27: 129–13.
- Houde, E. D. 1973. Some recent advances and unsolved problems in the culture of marine fish larvae. Proc. World Maric. Soc., 3: 83–112.
- Houde, E. D. and A. J. Ramsay. 1971. A culture system for marine fish larvae. Prog. Fish Cul., 33: 156–158.
- Leis, J. M. 1978. Systematics and zoogeography of the porcupine fishes (*Diodon*, Diodontidae, Tetraodontiformes), with comments on egg and larval development. U.S. Fish Wildl. Serv., Fish. Bull., 76: 535–567.
- Leis, J. M. 1984. Tetraodontioidei: Development. Pages 447–450 in H. G. Moser and W. J. Richards, eds. Ontogeny and systematics of fishes. Special Publication No. 1, American Society of Ichthyologists and Herpetologists. Allen Press, Kansas, ix + 760 pp.
- Leis, J. M. and D. S. Rennis. 1983. The larvae of Indo-Pacific coral reef fishes. Univ. N.S.W. Press, Sydney, 269 pp.
- Lubbock, R. and G. R. Allen. 1979. *Canthigaster leoparda* a new sharpnose pufferfish (Teleostei: Tetraodontidae) from the central Indo-Pacific. Rev. Fr. Aquariol., 6: 87–90.
- Marcy, B. 1975. Mechanics of handling ichthyoplankton. Pages 11–12 in Ichthyoplankton. Report of the CICAR Ichthyoplankton Workshop. UNESCO Technical Papers in Marine Science No. 20, Div. of Marine Sciences, UNESCO, Paris, 46 pp.
- Marliave, J. B. 1977. Substratum preferences of settling larvae of marine fishes reared in the laboratory. J. Exp. Mar. Biol. Ecol., 27: 47–60.
- Miller, J. M., W. Watson and J. M. Leis. 1979. An atlas of common nearshore marine fish larvae of the Hawaiian Islands. Sea Grant Misc. Rep. UNIH-SeaGrant-MR-80-02, 127 pp.
- Munro, I. S. R. 1945. Postlarval stages of Australian fishes. No. 1. Mem. Qld. Mus., 12: 136–153.
- Randall, J. E. 1971. The nominal triggerfishes (Balistidae) *Pachynathus nycteris* and *Oncobalistes erythropterus*, junior synonyms of *Melichthys vidua*. Copeia, 1971: 462–469.
- Randall, J. E. and W. Klausewitz. 1973. A review of the triggerfish genus *Melichthys*, with description of a new species from the Indian Ocean. Senckenb. Biol., 54: 57–69.
- Randow, H. 1934. *Tetraodon cutcutia* and *Tetraodon fluviatilis* Hamilton-Buchanan. Wochenschr. Aquar. Terrarienk., 31: 561–563. (In German.)
- Richter, H. J. 1982. Spawning Somphongs' puffer *Carinotetraodon somphongsii*. Trop. Fish Hobbyist, 31: 8–25.
- Schreitmuller, W. 1929. *Tetraodon fluviatilis* (Ham-Buch) green ballfish. Aqu. Life, Baltimore, 13: 23–26.
- Schreitmuller, W. 1930. Kugelfische. Das Aquarium, Berlin, Jan.: 12–16, Feb.: 20–26. (In German.)
- Shojima, Y. 1957. On the development of eggs and rearing of larvae of a puffer, *Fugu (Higanfugu) pardalis* (Temminck et Schlegel). Sci. Bull. Fac. Agr., Kyushu Univ., 16: 125–126. (In Japanese with English summary.)
- Sterba, G. 1962. Freshwater fishes of the world. Longacre Press, London, 878 pp.
- Struhsaker, P. and J. H. Uchiyama. 1976. Age and growth of the nehu, *Stolephorus purpureus* (Pisces: Engraulidae), from Hawaiian Islands as indicated by daily growth increments of sagittae. U.S. Fish Wildl. Serv., Fish. Bull., 74: 9–17.
- Taylor, W. R. 1967. An enzyme method of clearing and staining small vertebrates. Proc. U.S. Natn. Mus., 122: 1–17.
- Thresher, R. E. 1985. Reproduction of reef fishes. T. F. H. Publ., Neptune City, New Jersey, 399 pp.
- Tyler, J. C. 1980. Osteology, phylogeny and higher classification of fishes of the Order Plectognathi (Tetraodontiformes). NOAA Tech. Rep., Circ. 434, 420 pp.
- Uno, Y. 1955. Spawning habit and early development of a puffer *Fugu (Torafugu) niphobles* (Jordan and Schneider). J. Tokyo Univ. Fish., 41: 169–183.
- Victor, B. C. 1982. Daily otolith increments and recruitment in two coral reef wrasses, *Thalassoma bifasciatum* and *Halichoeres bivittatus*. Mar. Biol., 71: 303–308.
- Welsh, W. W. and C. M. Breder Jr. 1922. A contribution to the life history of the puffer *Spheroides maculatus* (Schneider). Zoologica (N.Y.), 2: 261–276.
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シマキンチャクフグの仔魚の発生、成長と日令決定

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Great Barrier Reef で採集した材料に基づいて、シマキンチャクフグ *Canthigaster valentini* の卵、初期仔魚及び稚魚について述べる。卵は野外で潜水夫によって採集され、実験室で飼育された。本種の卵は球形、強粘着性で卵径 0.68–0.72 mm、小油球の濃密な塊をもち、

受精後 3–5 日の日没時にふ化する。ふ化仔魚は小さな卵黄嚢、胸鰭をそなえ、筋肉節数 17 (6+11) で脊索長 (SL) 1.30–1.40 mm である。シマキンチャクフグの卵は卵径がずっと小さいこととふ化所要時間が長いことで他のフグ類の卵と異なる。本種の仔魚は色素細胞の分布、筋肉節数及びふ化時の大きさで他のフグ類仔魚と識別できる。成長は仔魚第 1 日目が最も急速である。浮游生活期ならびに新たに定着した野外採集稚魚の日令決定（耳石の微細構造にもとづく）結果は、本種の浮游生活期が 64 日ないし 113 日であることを示している。この概算は他のフグ目魚類で認められている延長浮游稚魚期とも矛盾しないようであり、シマキンチャクフグが 64 日の最小日令で定着能力を得た後は、ある期間定着を後らすことができることを示しているものであろう。