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REPRODUCTION, DEVELOPMENT AND GROWTH OF THE MILK-SPOTTED PUFFERFISH *CHELONODON KAPPA* (TETRAODONTIFORMES, TETRAODONTIDAE) IN CAPTIVITY

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ABSTRACT

Five specimens of the milk-spotted pufferfish, *Chelonodon kappa,* collected from the Hanechi Inland Sea, Okinawa, Japan in January 2021, were reared in a closed circulation system and maintained with fish meat and processed fish food. They spawned spontaneously in the tank in August 2022, the fertilized eggs [diameter 0.96 ± 0.02 (mean ± standard deviation) mm] being spherical, transparent, demersal and adhesive. Of the 2,220 eggs collected, 1,157 (52.1%) had been fertilized, and 205 hatched after 4 days (17.7% of fertilized eggs). Hatched larvae [2.26 mm in notochord length (NL) and 2.39 mm in total length (TL)] reached the juvenile stage [8.07 mm in body length (BL) and 10.3 mm TL] 30 days after hatching. The adult body color pattern of the species was attained by 60 days after hatching. By 120 days, specimens were 33.1 mm BL and 39.9 mm TL, growing to 70.4 mm BL and 84.6 mm TL by 360 days after hatching. Von Bertalanffy growth equations were fitted to the data.

Keywords: *Chelonodon kappa*, reproduction, aquarium, tetrodotoxin

INTRODUCTION

Pufferfish (family Tetraodontidae) include excellent food species, although many poisoning incidents have been reported in East and Southeast Asia (e.g., YAMAGUCHI PREFECTURAL FOOD HYGIENE ASSOCIATION, 2011; NAGASHIMA *ET AL.*, 2015). Due to the lack of knowledge about pufferfish (family Tetraodontidae) and their toxicity, they are still found in Southeast Asian fish markets, even though local and national governments in countries around the South China Sea have banned the sale of pufferfish for food (MATSUURA, 2015).

Toxicity in pufferfish is known to be exogenous, resulting from ingested food organisms (e.g., NOGUCHI *ET AL.*, 2006). Whereas cultured tiger pufferfish (*Takifugu rubripes*) fed with non-toxic food remained non-toxic (e.g., NOGUCHI *ET AL.*, 2012), cultured juvenile grass pufferfish (*T. alboplumbeus*) (mean 6.1 mm total length [TL], 18 days old) fed with toxic flatworm larvae had already become toxic (ITOI *ET AL.*, 2018). However, when and how wild pufferfishes develop toxicity is poorly known, except in the case of juvenile milk-spotted pufferfish (*Chelonodon kappa*) (15–55 mm TL), which accumulated tetrodotoxin (TTX) (e.g., NOGUCHI & HASHIMOTO, 1973; MAHMUD *ET AL.*, 2001; GHOSH *ET AL.*, 2004; TERUYA *ET AL.*, 2006) by feeding on flatworm larvae and the goby *Yongeichthys criniger* (ITOI *ET AL.*,

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2020; ITO *ET AL.*, 2022). Cases of food poisoning by ingesting milk-spotted pufferfish have also been reported in many Asian countries such as Japan, China, Philippines, Thailand, and Bangladesh (e.g., CHULANETRA *ET AL.*, 2011; MATSUURA, 2017; BARMAN *ET AL.*, 2018; SOUMYA & SAMANTA, 2018).

Chelonodon kappa or milk-spotted pufferfish is characterized by: mouth below a horizontal line through dorsal end of gill opening; nasal organ in the form of a depression with slightly raised margin expanded before and behind into a pair of elongate flaps; 2 lateral lines on body, upper lateral line joining lower behind anal fin, and lower extending forward above anal fin; brownish gray body with many round white spots; 5 or 6 dark relatively wide bars on body, first across eye and last on caudal peduncle; a yellow longitudinal streak running along ventrolateral corner of body from below mouth to caudal peduncle; 9–11 dorsal-fin rays; and, 8–10 anal-fin rays (MATSUURA & MOTOMURA, 2022 [as *Chelonodontops patoca*]; BRITZ *ET AL.*, 2024). The species had been known as *Chelonodon patoca* (e.g., KOTTELAT *ET AL.* 1993: MATSUURA 2001; YAMADA & YANAGISITA, 2013) or *Chelonodontops patoca* (e.g., KOTTELAT, 2013; MATSUURA, 2015, 2017; TAKI *ET AL*, 2021; MATSUURA & MOTOMURA, 2022; OKAMURA & ENDO, 2022) for a long time until a recent taxonomic re-assessment by BRITZ *ET AL.* (2024) that showed that the correct generic name for this group of pufferfishes is *Chelonodon,* and the correct scientific name of the species is *Chelonodon kappa*. The species inhabits mangrove, sea grass and coral reef areas, and is widely distributed from the coast of Pakistan, India, Myanmar, eastwards to Indonesia, Australia, and Japan (OKAMURA & ENDO, 2022; BRITZ *ET AL.*, 2024). The species is thought to settle as juveniles of ca. 10–20 mm in total length (TL), growing in mangrove or brackish water areas; juveniles from 7.5 mm TL (mostly from ca. 10 mm) have been recorded from Australia (ROBERTSON & DUKE, 1990), from 20 mm TL from Sri Lanka (SILVA & PUNCHIHEWA, 2023), and from 15 mm TL from the Ryukyu Islands, Japan (NANJO *ET AL.*, 2008; ITOI *ET AL.*, 2020; ITO *ET AL.*, 2022). Determining the life history (ontogeny) of *C. kappa* is important for understanding the development of toxicity. However, the early life history of milk-spotted pufferfish is not well understood.

Many wild pufferfish species, particularly those inhabiting fresh and brackish water, are traded worldwide as ornamental (aquarium) fishes (EBERT, 2001), thereby raising concerns about the negative impact of such trade on wild stocks (PENNING *ET AL.*, 2009). From a biodiversity conservation perspective, it has become increasingly important to develop breeding techniques for such fishes in captivity (BARONGI *ET AL.*, 2015; DOI *ET AL.*, 2015, 2022a, 2022b; MOMOTA *ET AL.*, 2022, 2023).

Milk-spotted pufferfish are comparatively common and are not listed as Endangered (LARSON *ET AL.*, 2021). However, as SDGs (Sustainable Development Goals) (UNITED NATIONS, 2015) are presently being discussed worldwide, it is necessary for aquarium displays to also develop in a sustainable way (JANSE *ET AL.*, 2017; POWELL, 2018; WAZA, 2020). For this reason, it will become increasingly important to develop breeding techniques for any ornamental fish species, including milk-spotted pufferfish, thereby promoting study of the development and growth of aquarium-maintained *C. kappa* in order to improve breeding techniques and to elucidate its early life history.

MATERIALS AND METHODS

Parental Fish and Spawning

On 11 January 2021, five juvenile milk-spotted pufferfish, *Chelonodon kappa* (8.9 ± 0.4 cm mean total length [TL], 7.2 ± 0.4 cm mean body length [BL], and 15.5 ± 0.9 g mean weight [BW]), were collected from the Hanechi Inland Sea (26°39′00″N 127°59′18″E) off Wakukawa, Nakijin Village, Kunigami-gun, Okinawa Prefecture, Japan. The fish were subsequently reared in an acrylic tank (120 × 50 × 40 cm, 240 L) with a closed circulating filtration system installed at NIFREL, Osaka, from 27 January, 2021. Water temperature was maintained at 23–26°C, and salinity at 30–33‰, under a 12L12D lighting regime (23.5 W, Clear LED Power III 900, Gex Co., Ltd.). About one-tenth of the tank water was exchanged with new seawater daily, and leftover food and excrement were removed by a syphon. The daily diet consisted of horse mackerel *Trachurus japonicus*, capelin *Mallotus villosus*, river prawns *Macrobrachium* sp., and processed fish food (Hikari Cat, Kyorin Co., Ltd., and Σ Grow F, Dojo Laboratory).

At 0700 h on 2 August 2022, spawned eggs were found scattered on the tank floor. They were quickly collected by siphoning for subsequent rearing and observations.

Incubation of Eggs

Eggs were housed in a cylindrical polycarbonate tank (30 L). The water was aerated, with temperature maintained at 26°C, with similar salinity and light conditions as for the parental fish. One-third of the tank water was changed daily.

Rearing of Larvae and Juveniles

Hatched larvae were reared in two cylindrical polycarbonate tanks (30 L). The rearing water was slightly aerated, and maintained at a temperature of 23–28°C and salinity of 30–33‰. Lighting was provided by a 32 w white-fluorescent lamp (FL32SW, Toshiba Lighting & Technology Corp.) constantly for the first 11 days in order to enhance encounters with initial food items, and then shortened to 12L12D thereafter.

From 30 days after hatching, pufferfish were reared in two polycarbonate tanks (30 L) and 3 glass tanks (60 × 30 × 30 cm, 54 L), and then from 100 days, in one polycarbonate tank (30 L), two glass tanks (54 L) and two acrylic tanks (90 × 45 × 30 cm, 121.5 L).

Newly hatched larvae were fed with freshwater chlorella-enriched S type rotifers (*Brachionus* sp.) at a density of 30 individuals/mL for 21 days, and additionally with *Artemia* spp. Nauplius from 22 to 24 days after hatching. Subsequent feeding regimes included: from 25 to 56 days, commercially prepared Otohime B2 (Marubeni Nisshin Feed Co., Ltd.), in addition to *Artemia* spp. nauplius; from 57 to 149 days, frozen *Artemia* Clean brine shrimp (Kyorin Co., Ltd.), commercially prepared Otohime B2, Hikari Megabite red S (Kyorin Co., Ltd.), and biological control food for goldfish (Japan Pet Design Co., Ltd); from 150 days, chironomid larvae, Hikari Megabite red S and biological control food for goldfish.

Observations of Eggs, Larvae, Juveniles and Young Fish

Eggs, larvae and juveniles were collected from the rearing tanks with a pipette or small net, and observed under a stereomicroscope (OLYMPUS SZ61 binocular stereomicroscope [equipped with measuring software Anyty Microscope with 3R-WDKMCO2, 3R System, Olympus Co., Tokyo)] without anesthesia. Eggs and larvae were measured to the nearest 0.01 mm, and juveniles photographed with a digital camera. Standard length (BL, notochord length in early larval stages) and TL were measured.

Designation of developmental intervals followed OKIYAMA (2014): larval period, before complete development of countable characters (with three steps as follows: preflexion step, before notochord flexion; flexion step, incorporating notochord flexion; postflexion step, following completion of notochord flexion); and juvenile period, following the larval period, characterized by adult complements of countable characters (e.g., fin rays). Measurement and counting methods followed OKIYAMA (2014).

Growth

The von Bertalanffy growth formulae of *C. kappa* were estimated following the standard form of Richards' model (RICHARDS, 1959) by comparing the residual sum of squares (AKAMINE, 1988, 2004), calculated using MS Excel (Microsoft Office 365).

RESULTS

Parental Fish and Spawning

The five parental individuals had grown to 12.4 ± 1.9 cm BL, 15.3 ± 2.2 cm TL, and 93.7 ± 39.4 g BW by 13 May 2022, and 13.9 ± 1.8 cm BL, 17.0 ± 2.3 cm TL, and 106.5 ± 29.9 g BW by July 11, 2022. At this time, sperm were observed by stripping the smallest male individual (10.7 cm BL, 13.0 cm TL, and 56.1 g BW). The other four individuals (sex not determined) ranged from 14.1 to 15.0 cm BL (mean 14.7 cm), 17.4 to 19.0 cm TL (mean 18.0 cm), and 103.5 to 123.5 g BW (mean 119.1 g).

On 2 August 2022 at 0700 h (moon age ca. 3.4–4.4, mid-tide), spawned eggs were found scattered on the tank floor. Spawning behavior had not been observed, but no bite marks were apparent on any of the fish. The fish pecked at the spawned eggs, which were immediately collected from the tank.

On 4 August 2022, sperm could still be stripped from the smallest male, which was determined as the spawner (Fig. 1a). A comparison of the weights of the remaining four individuals with those recorded on July 11, indicated one individual (14.1 cm BL, 17.4 cm TL, and 96.5 g BW) as having decreased in weight, being therefore the likely spawner (Fig. 1b). Sex of the other specimens remained undetermined.

Eggs

Eggs collected from the parental tank numbered 2,220, and 1,157 (52.1 %) were fertilized. The fertilized eggs were demersal and adherent, separated eggs having a circular yolk [diameter 0.96 ± 0.02 mm (n = 10) (Fig. 2a)]. Embryoid bodies formed two days after

Figure 1. Parental milk-spotted pufferfish, *Chelonodon kappa*; a, male (10.7 cm BL); b, female (14.1 cm BL). Photographs by Yasuyuki Tai.

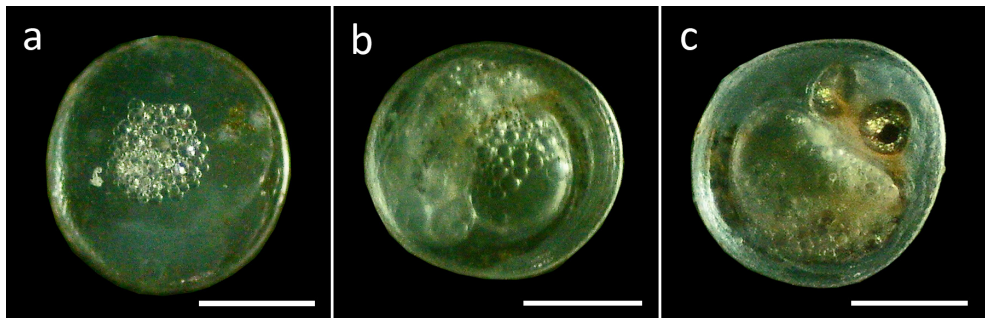

Figure 2. Egg development in the milk-spotted pufferfish *Chelonodon kappa*; a, fertilized egg; b, 2 days after fertilization; c, 3 days after fertilization. Scale bars indicate 0.5 mm. Photographs by Hiroyuki Doi.

spawning (Fig. 2b). After three days, the eyes developed, and the embryonic body moved occasionally (Fig. 2c). Stellate melanophores were scattered on the yolk, and melanophores, erythrophores and xanthophores appeared on the embryonic body. Four days after spawning, 205 larvae (17.7 % of fertilized eggs) hatched.

Larvae and Juveniles

Hatched larvae (Fig. 3a) were 2.26 ± 0.05 mm NL and 2.39 ± 0.04 mm TL (n = 10), with 7 + 13 = 20 myomeres, the mouth already opened anteriorly, pectoral fins formed, and yolk sac remaining. Stellate melanophores and xanthophores were present on the trunk to tail and sub-ocular regions. Melanophores occurred on the yolk surface.

One day after hatching, preflexion larvae were 2.27 ± 0.06 mm NL and 2.40 ± 0.07 mm TL (n = 10). Two days after hatching, preflexion larvae were 2.29 ± 0.05 mm NL and 2.46 ± 0.02 mm TL (n = 10), the yolk having been absorbed. Melanophores were present from the upper region of the eye to the dorsal, lateral and abdominal surfaces of the body. Xanthophores were present behind the eye and along the lateral body surface to the anus. Larvae characteristically moved to the water surface and engaged in food-chasing behavior.

Ten days after hatching (Fig. 3b), preflexion larvae were 3.09 ± 0.03 mm NL and 3.35 ± 0.06 mm TL (n = 10). Dorsal and anal fin soft rays, and hypurals had begun to form.

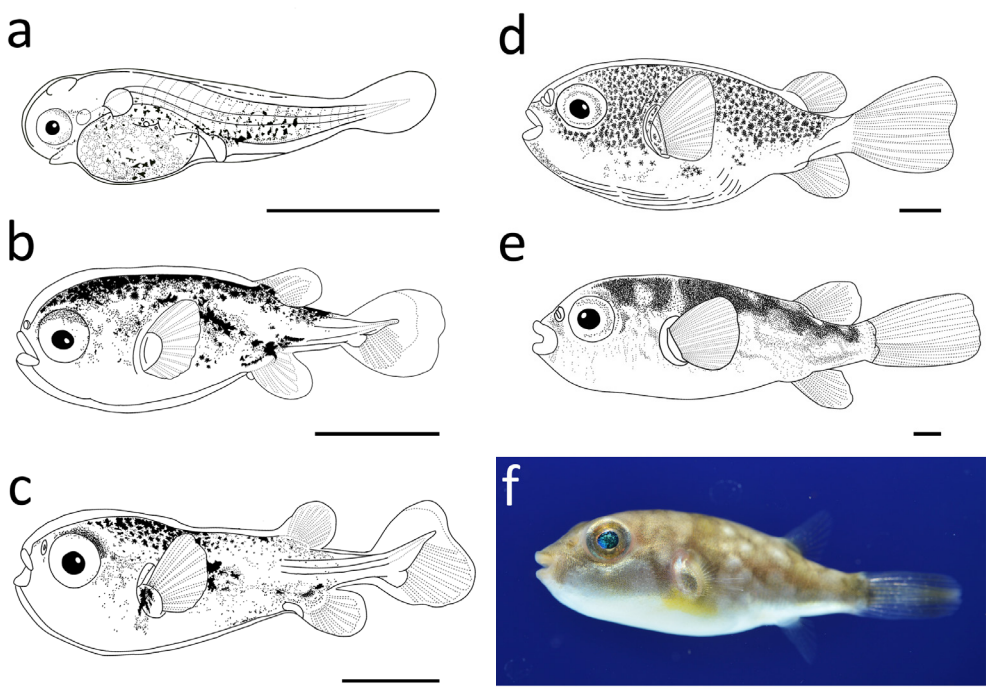

Figure 3. Larvae and juveniles of the milk-spotted pufferfish *Chelonodon kappa*; a, newly hatched larva, 2.26 mm NL, 2.39 mm TL; b, preflexion larva, 10 days after hatching, 3.07 mm NL, 3.44 mm TL; c, flexion larva, 18 days after hatching, 4.34 mm NL, 4.69 mm TL; d, juvenile, 30 days after hatching, 7.95 mm BL, 10.18 mm TL; e, juvenile, 40 days after hatching, 12.73 mm BL, 15.83 mm TL; f, juvenile, 60 days after hatching, ca. 21 mm BL, 28 mm TL; Scale bars indicate 1 mm. Drawings by Koyuki Matsuda and photograph by Yasuyuki Tai.

Melanophores were densely distributed from the rostral and parietal regions to the dorsal body surface and around the anus. Orange to yellow xanthophores were apparent on the lateral body surface, abdomen, tail and fins. Individuals sometimes appeared swollen.

Eighteen days after hatching (Fig. 3c), flexion larvae of 4.33 ± 0.04 mm NL and 4.70 ± 0.10 mm TL (n = 10) initiated notochord flexion. Melanophores were scattered over the entire body, except for fins, being especially dense around the base of the pectoral fins and the anus. Xanthophores were widely distributed over the entire body, being especially orange or yellow from the eyes to the abdomen. Teeth were formed.

Thirty days after hatching (Fig. 3d), individuals had reached the juvenile period (8.07 ± 0.35 mm BL and 10.3 ± 0.5 mm TL [n = 10]), having completed notochord flexion, and attained full fin ray complements (10 dorsal, 8 anal, and 14 pectoral fin soft rays). Melanophores occurred densely on the dorsal surface, and dense xanthophores on the body and abdomen. Individuals began to respond to light and noise, and gather at the water surface when feeding.

Forty days after hatching (Fig. 3e), juveniles were 11.9 ± 0.6 mm BL and 15.4 ± 0.5 mm TL (n = 10), with white spots forming on the dorsal surface, and an indistinct black transverse band apparent dorsally between the pectoral fin bases. Xanthophores on the body and abdomen had disappeared, the abdomen becoming whitish.

Sixty days after hatching (Fig. 3f), juveniles were 20.7 ± 1.7 mm BL and 26.7 ± 2.1 mm TL (n = 10), and had attained the overall adult body color pattern, including white spots on the darker lateral and dorsal body surfaces. The black transverse band on the dorsal surface between the pectoral fin bases had become clearer, with two additional dark transverse bands on the dorsal surface. A single yellow longitudinal line was present below the pectoral fin base.

Individuals (33.1 ± 1.7 mm BL and 39.9 ± 1.6 mm TL [n = 10]) actively pecked at the bottom of the tank 120 days after hatching, and grew to 43.6 ± 5.9 mm BL and 54.4 ± 6.5 mm TL (n = 10), and 70.4 ± 4.5 mm BL and 84.6 ± 4.4 mm TL (n = 10), respectively, 180 days and 360 days after hatching.

Throughout development, no mutual biting behavior was observed between individuals.

Growth

Approximated von Bertalanffy growth formulae for BL and TL (n = 200, including both sexes) are summarized below, the correlation coefficient being 0.981 (BL) and 0.973 (TL), where Lt is estimated length, and t is days after hatching (Fig. 4):

BL: $Lt = 94.0502 \times (1\text{-}e^{-0.0036(t+2.3587)})$. $R^2 = 0.984$

TL: $Lt = 106.2923 \times (1\text{-}e^{-0.0041(t+1.4051)})$. $R^2 = 0.986$

DISCUSSION

Milk-spotted pufferfish, maintained in a small recirculating tank (240 L), spawned spontaneously 15 months after capture on a diet of fish meat and processed fish food. The larvae were relatively easily reared in small tanks (30.0–121.5 L), being fed freshwater chlorella-enriched S-type rotifers (*Brachionus* sp.) as initial food items, and eventually growing to about 70 mm BL after one year. These findings contributed to the propagation program for sustainable aquarium displays of pufferfish.

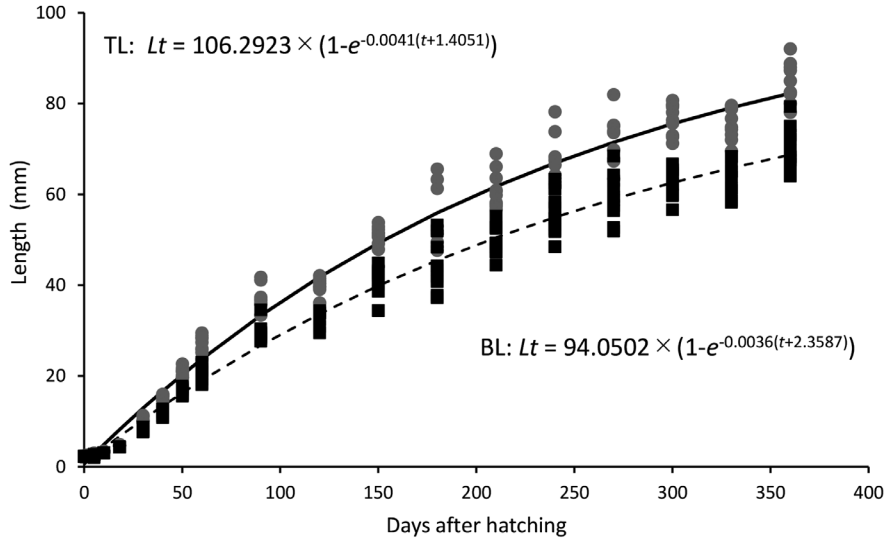

Figure 4. Growth of *Chelonodon kappa*. Black squares and dotted line indicate BL, and grey circles and solid line, TL. von Bertalanffy growth formulas for BL and TL also shown.

ROBERTSON & DUKE (1990) reported that milk-spotted pufferfish occurred in mangrove systems as juveniles of ca. 10 mm TL. The present study showed that the larvae reached the juvenile stage at 8.07 ± 0.35 mm BL and 10.3 ± 0.50 mm TL 30 days after hatching, a size at which they would probably have settled in mangrove or brackish water areas.

Milk-spotted pufferfish are already toxic as juveniles of 15–55 mm TL (ITO *ET AL.*, 2022) by feeding on toxic diets (ITOI *ET AL.*, 2020; ITO *ET AL.*, 2022). According to NANJO *ET AL.* (2008), wild *C. kappa* (as *C. patoca*) individuals (33–79 mm BL) in Okinawa feed on detritus and crabs, and larger individuals (84–161 mm BL) on larger benthic invertebrates, such as crabs, snails, and bivalves, with many of these prey species containing TTX (e.g., NOGUCHI *ET AL.*, 1982a, 1982b, 1983; MIYAZAWA *ET AL.*, 1988). Wild milk-spotted pufferfish would become more toxic during the growth. Further studies on the development of toxicity with growth, both in juvenile puffers and in their prey, are needed to clarify the ontogenetic process of TTX accumulation.

It is known that cultured tiger puffers (*Takifugu rubripes*) become non-toxic when fed a TTX-free diet (e.g., NOGUCHI *ET AL.*, 2012). On the other hand, it has also been established that the survival rate of toxic juveniles fed toxic diets is greater than that of non-toxic juveniles, presumably because the former are less likely to be taken by predators (SAKAKURA *ET AL.*, 2017) than the latter. It is possible that aquarium-bred pufferfish may become non-toxic. In an aquarium, where a variety of fish species including predatory ones are often kept in the same tank, it may be important in the future to induce toxicity in bred pufferfish using an appropriate toxification mechanism, so as to increase the survival rates of the latter.

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REFERENCES

Akamine, T. 1988. Estimation of parameters for Richards' model. *Bull. Jap. Sea Reg. Fish. Res. Lab.* 38: 187–200.

Akamine, T. 2004. Statistical test and model selection of fish growth formula. *Bull. Jap. Soc. Fish. Oceanogr.* 68: 44–51. (in Japanese)

Barman, A. A., S. K. Mustafi, M. Hasan, N. C. Roy, M. M. Hossain, P. P. Barman, and M. G. Rasul. 2018. Puffer fish poisoning: An assessment of consumer awareness and availability in Sylhet region, Bangladesh. *Int. J. Fish. Aquat. Stud.* 6: 110–116.

Barongi, R., F. A. Fisken, M. Parker, and M. Gusset (eds.). 2015. *Committing to conservation, The World Zoo and Aquarium conservation strategy*. WAZA executive office, Gland. 69 pp.

Britz, R., H. H. Tan, and L. Rüber. 2024. *Chelonodontops bengalensis* Habib *et al*. 2018, a junior synonym of *Tetrodon patoca* Hamilton 1822, with a type species designation for *Chelonodon* Müller 1841. *Zootaxa* 5519: 561–570.

Chulanetra, M., N. Sookrung, P. Srimanote, N. Indrawattana, J. Thanongsaksrikul, Y. Sakolvaree, M. Chongsa-Nguan, H. Kurazono, and W. Chaicumpa. 2011. Toxic marine puffer fish in Thailand seas and tetrodotoxin they contained. *Toxins* 3: 1249–1262.

Doi, H., T. Akita, and H. Sakai. 2022a. Reproduction and development in captivity of the Southeast Asian freshwater pufferfish *Pao palembangensis*. *Aquacult. Sci.* 70: 197–199.

Doi, H., K. Momota, H. Obata, and H. Sakai. 2022b. Reproduction in captivity of three southeast Asian freshwater pufferfish species of the genus *Pao*. *Aquacult. Sci.* 70: 221–229.

Doi, H., H. Sakai, Y. Yamanoue, T. Sonoyama, and T. Ishibashi. 2015. Spawning of eight Southeast Asian brackish and freshwater puffers of the genera *Tetraodon* and *Carinotetraodon* in captivity. *Fish. Sci.* 81: 291–299.

Ebert, K. 2001. *The puffers of fresh and brackish waters*. Aqualog Verlag GmbH, Mörfelden-Walldorf. 96 pp.

Ghosh, S., K. H. Alok, S. Banerjee, and B. Mukherjee. 2004. The seasonal toxicological profile of four puffer fish species collected along Bengal coast, India. *Ind. J. Mar. Sci.* 33: 276–280.

Ito, M., R. Furukawa, S. Yasukawa, M. Sato, H. Oyama, T. Okabe, R. Suo, H. Sugita, T. Takatani, O. Arakawa, M. Adachi, T. Nishikawa, and S. Itoi, 2022. Local differences in the toxin amount and composition of tetrodotoxin and related compounds in pufferfish (*Chelonodon patoca*) and toxic goby (*Yongeichthys criniger*) juveniles. *Toxins* 14: 150.

Itoi, S., T. Sato, M. Takei, R. Yamada, R. Ogata, H. Oyama, S. Teranishi, A. Kishiki, T. Wada, K. Noguchi, M. Abe, T. Okabe, H. Akagi, M. Kashitani, R. Suo, T. Koito, T. Takatani, O. Arakawa, and H. Sugita. 2020. The planocerid flatworm is a main supplier of toxin to tetrodotoxin-bearing fish juveniles. *Chemosphere* 249: 126217.

Itoi, S., H. Ueda, R. Yamada, M. Takei, T. Sato, S. Oshikiri, Y. Wajima, R. Ogata, H. Oyama, S. Teranishi, T. Shitto, K. Okuhara, T. Tsunashima, E. Sawayama, and H. Sugita. 2018. Including planocerid flatworms in the diet effectively toxifies the pufferfish, *Takifugu niphobles*. *Sci. Rep.* 8: 12302.

JANSE, M., B. ZIMMERMAN, L. GEERLINGS, C. BROWN, AND L. A. J. NAGELKERKE. 2017. Sustainable species management of the elasmobranch populations within European aquariums: a conservation challenge. *J. Zoo Aquarium Res*. 5: 172–181.

KOTTELAT, M. 2013. The fishes of the inland waters of southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raff. Bull. Zool. Suppl*. 27: 1–663.

KOTTELAT, M., A. J. WHITTEN, S. N. KARTIKASARI, AND S. WIRJOATMODJO. 1993. *Freshwater fishes of Western Indonesia and Sulawesi*. Periplus Editions, Hong Kong. xxxviii+259 pp. 84 pls.

LARSON, H., K.T. SHAO, K. MATSUURA, J. LIU, G. HARDY, J. L. LEIS, AND M. LIU. 2021. *Chelonodon patoca*. The IUCN Red list of threatened species 2021: e.T172416A147760777. https://dx.doi.org/10.2305/IUCN.UK.2021-1.RLTS.T172416A147760777.en, accessed on 12 Dec. 2023.

MAHMUD, Y., M. B. TANU, T. TAKATANI, E. ASAYAMA, O. ARAKAWA, AND T. NOGUCHI. 2001. *Chelonodon patoca*, a highly toxic marine puffer in Japan. *J. Nat. Toxins* 10: 69–74.

MATSUURA, K. 2001. Tetraodontidae. Pages 3954–3957 *in* K. E. Carpenter, and V. H. Niem (eds.), *FAO species identification guide for fishery purposes. The living marine resources of the western central Pacific*. Vol. 6. FAO, Rome.

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

MATSUURA, K. 2017. *Pufferfishes and their allies of Japan*. Tokai university Press, Hadano, p. 127. (in Japanese)

MATSUURA, K., AND H. MOTOMURA. 2022. *Identification guide to pufferfishes (Tetraodontidae, Tetraodontiformes) of the South China Sea*. The Kagoshima University Museum, Kagoshima, 40 pp.

MIYAZAWA, K., M. HIGASHIYAMA, K. ITO, T. NOGUCHI, O. ARAKAWA, Y. SHIDA, AND K. HASHIMOTO. 1988. Tetrodotoxin in two species of ribbon worm (Nemertini), *Lineus juscoviridis* and *Tubulanus punctatus*. *Toxicon* 26: 867–874.

MOMOTA, K., H. DOI, H. OBATA, AND H. SAKAI. 2022. Spawning and development of ocellated pufferfish *Leiodon cutcutia* in captivity. *Aquacult. Sci*. 70: 9–15.

MOMOTA, K., H. DOI, S. MURAKAMI, H. OBATA, AND H. SAKAI. 2023. Reproduction and early development of the green pufferfish *Dichotomyctere fluviatilis* in captivity. *Aquacult. Sci*. 71: 49–55.

NAGASHIMA, Y., O. ARAKAWA, AND S. SATO. 2015. Tetrodotoxin. Pages 33–103 *in* K. Matsuura, and Y. Nagashima (eds.), *Natural history of poisonous fishes*. Hokkaido Univ. Press, Sapporo. (in Japanese)

NANJO, K., H. KOHNO, AND M. SANO. 2008. Food habits of fishes in the mangrove estuary of Urauchi river, Iriomote Island, southern Japan. *Fish. Sci*. 74: 1024–1033.

NOGUCHI, T., O. ARAKAWA, AND T. TAKATANI. 2006. TTX accumulation in pufferfish. *Compar. Biochem. Physiol. Pt. D: Genom. Proteom*. 1: 145–152.

NOGUCHI, T., AND Y. HASHIMOTO. 1973. Isolation of tetrodotoxin from a goby *Gobius criniger*. *Toxicon* 11: 305–307.

NOGUCHI, T., J. MARUYAMA, Y. UEDA, K. HASHIMOTO, AND T. HARADA. 1982a. Occurrence of tetrodotoxin in the Japanese ivory shell *Babylonia japonica*, *Bull. Jap. Soc. Sci. Fish*. 47: 909–913.

NOGUCHI, T., H. NARITA, J. MARUYAMA, AND K. HASHIMOTO. 1982b. Tetrodotoxin in the starfish *Astropecten polyacanthus*, in association with toxification of a trumpet shell "Boushubora" *Charonia sauliae*. *Bull. Jap. Soc. Sci. Fish*. 48: 1173–1177.

NOGUCHI, T., K. ONUKI, AND O. ARAKAWA. 2012. Production of non-toxic tiger puffer by culture. Pages 84–97 *in* Y. Nagashima, O. Murata and S. Watabe (eds.), *Forefront studies on puffer fish and technological development for tiger puffer production*. Kouseisha Kouseikaku, Tokyo. (in Japanese)

NOGUCHI, T., A. UZU, K. KOYAMA, AND K. HASHIMOTO. 1983. Occurrence of tetrodotoxin as the major toxin in xanthid crab *Atergatis floridus*. *Bull. Jap. Soc. Sci. Fish*. 49: 1887–1892.

OKAMURA, K., AND H. ENDO. 2022. First record of *Chelonodontops patoca* from Shikoku Island, Japan. *Ichthy, Nat. Hist. Fish. Jap*. 34: 15–18 (in Japanese).

OKIYAMA, M. (ed.) 2014. *An atlas of early stage fishes in Japan, second edition*. Tokai University Press, Hadano, p. xxvii. (in Japanese)

PENNING, M., G. McG. REID, H. KOLDEWEY, G. DICK, B. ANDREWS, K. ARAI, P. GARRATT, S. GENDRON, J. LANGE, K. TANNER, S. TONGE, P. VAN DEN SANDE, D. WARMOLTS, AND C. GIBSON. (eds.) 2009. *Turning the tide: A global aquarium strategy for conservation and sustainability*. World Association of Zoos and Aquariums, Bern. 80 pp.

POWELL, D. M. 2018. Collection planning for the next 100 years: What will we commit to save in zoos and aquariums? *Zoo Biol*. 38: 139–148.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. *J. Exp. Bot*. 10: 290–300.

ROBERTSON, A. I., AND N. DUKE. 1990. Recruitment, growth and residence time of fishes in a tropical Australia mangrove system. *Estuar. Coast. Shelf. Sci*. 31: 723–743.

SAKAKURA, Y., T. TAKATANI, J. NAKAYASU, H. YAMAZAKI, AND K. SAKIYAMA. 2017. Administration of tetrodotoxin protects artificially raised juvenile tiger puffer *Takifugu rubripes* from predators. *Fish. Sci*. 83: 191–197.

SILVA, M. P. C., AND N. N. PUNCHIHEWA. 2023. Evaluation of utilization of mangroves as a food source of fish species inhabiting the Chilaw lagoon using gut content analysis. *Glob. Res. Environ. Sustain*. 1: 22–32.

SOUMYA, J., AND K. SAMANTA S., 2018. Seasonal variation in toxicity of puffer fish, *Arothron immaculatus*, *Chelonodon patoca* and *Lagocephalus scleratus* along Tamil Nadu coast. *IJMS* 47: 231–239.

TAKI, Y., R. OHTSUKA, M. KOMODA, Y. NATORI, K. UTSUGI, K. SHIBUKAWA, T. OIZUMI, S. OTTOMANSKI, B. PRAXAYSOMBATH, K. PHONGSA, W. MAGTOON, P. MUSIKASINTHORN, C. GRUDPAN, J. GRUDPAN, A. SUVARNARAKSHA, N. SO, P. THACH, P. T. NGUYEN, D. D. TRAN, AND L. X. TRAN (eds.). 2021. *Fishes of the Indochinese Mekong*. Nagao Natural Environment Foundation (NEF), Tokyo. xii+546 pp.

TERUYA, N., N. OSHIRO, AND K. TAMANAHA. 2006. Toxicity of pufferfishes from Okinawa. *Rep. Okinawa. Pref. Health. Environ*. 40: 93–97 (in Japanese).

UNITED NATIONS. 2015. Transforming our world: the 2030 agenda for sustainable development. https://sdgs.un.org/publications/transforming-our-world-2030-agenda-sustainable-development-17981, accessed on 12 Dec. 2023.

YAMADA, U., AND N. YANAGISITA, 2013. Tetraodontidae. Pages 1728–1742 *in* T. Nakabo (ed.), *Fishes of Japan with pictorial key to the species third edition* II. Tokai University Press, Hadano. (in Japanese)

YAMAGUCHI PREFECTURAL FOOD HYGIENE ASSOCIATION. 2011. *Pufferfish – public awareness of correct knowledge for pufferfish poisoning prevention*. Yamaguchi Prefectural Food Hygiene Association, Yamaguchi, pp. 43–60. (in Japanese)

WAZA. 2020. *Protecting our planet sustainability strategy 2020–2030*. WAZA executive office, Gland. 33 pp.