Parasites of Cultured Tiger Puffer (*Takifugu rubripes*) and their Seasonal Occurrences, with Descriptions of Two New Species of *Gyrodactylus*

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Parasites of cultured tiger puffer, *Takifugu rubripes*, produced in a hatchery, introduced to a culture site in Nagasaki Prefecture in summer and cultured for one year and a half in net cages, were examined bimonthly.

Thirteen species of parasites were recorded: ciliates, *Trichodina fugu* and *T. jadranica* on the gills, *Cryptocaryon irritans* in the fins and body surface; myxosporeans, *Kudoa shiomitsui* in the heart and Multivalvulida gen. sp. (syn.: *Septemcapsula yasunagai* Hsieh et Chen, 1984) in the brain; monogeneans, *Gyrodactylus rubripedis* sp. n. on the fins and body surface and *Heterobothrium okamotoi* on the gills and branchial cavity wall; trematodes, *Tetrochetus* sp. and *Maculifer pacificus* in the rectum; acanthocephalan, *Longicollum pagrosomi* in the rectum; copepods, *Caligus fugu* on the buccal cavity wall, *Pseudocaligus fugu* on the body surface and *Neobrachiella hugu* (syn: *Clavellopsis hugu* Yamaguti, 1939) on the buccal cavity wall.

Gyrodactylus rubripedis sp. n. is different from the most closely related *G. pardalidis* sp. n. collected from the fins of panther puffer, *Takifugu pardalis* and finespotted puffer, *T. poecilonotus* in smaller size of hamuli and shape of the marginal hook proper; the two new gyrodactylids are similar to ones from freshwater fish of the genus *Noemacheilus* in having inwardly bent root of hamuli, but clearly separated from them by the shape of the ventral bar.

In some selected species of parasites, seasonal occurrences are described and possible sources of infection are discussed.

Key words: *Takifugu rubripes*, tiger puffer, parasite fauna, new species, Monogenea

Culture of tiger puffer, *Takifugu rubripes*, started in western Japan in the 1950s (Okamoto, 1963), the annual production staying less than 100 metric tons during the 1950s–1970s. In those early years, feral puffers were caught in spring and summer, and cultured until winter, when most of puffers were consumed. Since the 1980s, however, the puffer culture industry has been rapidly growing. The annual culture production of tiger puffer exceeded 4,000 metric tons in 1992, about 10 times as much as that of 10 years before. This is mainly because of a plentiful supply of artificially produced juveniles, which has led to the recent expansion of tiger puffer culture. With the change of the source of seedlings, culture methods have substantially changed. Typically, hatchery-produced juveniles are available to fish farmers in summer, and cultured for about 1 year and a half until marketed in winter.

With regard to diseases associated with puffer culture, much remains to be studied, because of a rather short culture history. A total of 18 species of parasites have

so far been reported from tiger puffer, but information about their pathogenicity is quite limited. As for ciliate parasites, *Cryptocaryon irritans* was first reported from tiger puffer as an unidentified ciliate by Sikama (1937) and two species of *Trichodina* viz. *T. fugu* and *T. jadranica* were described by Imai *et al.* (1997). As for myxosporeans, *Kudoa shiomitsui* was described by Egusa and Shiomitsu (1983) and Multivalvulida gen. sp. by Egusa (1986). The latter myxosporean was named as *Septemcapsula yasunagai* as a new genus and a new species by Hsieh and Chen (1984), but there is some discussion about its validity of the new species (Egusa, 1986), and thus it is treated here as an unidentified myxosporean as above. There are 13 species of metazoan parasites, of which are 3 species of monogeneans: *Udonella caligorum* reported by Yamaguti (1958), *Neobenedenia girellae* by Ogawa *et al.* (1995) and *Heterobothrium okamotoi* first reported by Okamoto (1963) as *H. tetradonis* and later described by Ogawa (1991) as a new species, 3 species of trematodes:

Galactosomum sp. metacercaria by Yasunaga *et al.* (1981), *Pharyngora orientalis* by Yamaguti (1934) and *Psettarium japonicum* by Yamaguti (1938), 3 species of nematodes: *Anisakis* sp. larva by Yamaguti (1941), *Contracaecum* sp. larva by Fukuda *et al.* (1982) and Cuculanidae gen. sp. (originally ?*Spirruroides cuculanidae*?) by Okamoto and Ogasawara (1965), and 4 species of copepods: *Taeniocanthus yamagutii* first reported by Okamoto and Ogasawara (1965) as *Irodes yamagutii*, *Caligus fugu* described by Yamaguti and Yamasu (1959a), *Neobrachiella hugu*, originally described by Yamaguti (1939) as *Clavellopsis hugu*, *Argulus scutiformis* by Yamaguti and Yamasu (1959b).

In this paper, an attempt is made to identify parasites which appeared during a survey throughout the whole period of culture and to describe their patterns of occurrences, thus to provide basic information on parasite infections of cultured tiger puffer.

Materials and Methods

Hatchery-reared juveniles of tiger puffer cultured at a farm at Naru, Minami-Matsuura gun, Nagasaki Prefecture, Japan were used in this study. They were cultured in an off-shore net pen and fed daily with defrozen minced fish. Following the introduction of the puffer seedlings to the farm early in July 1986, 20 individuals were randomly collected bimonthly from a single fish stock and examined for parasites from July 1986, when they were 9.0 ± 0.8 cm in body length, 25.0 ± 7.4 g in body weight, until November 1987, soon before harvested (28.8 ± 1.2 cm long, 838 ± 145 g in weight) (Fig. 1). Water temperature ranged from 13.5 to 28.0°C during the survey period. Preliminary parasitological surveys of juvenile puffers were made on May 16, 1986, when the fish had still been reared in a hatchery in Nagasaki Pref., and on July 4, a few days after arrival of

Fig. 1. Fluctuation of water temperature and growth of tiger puffer during the study period (July 1986-Nov 1987) at Naru, Nagasaki Prefecture.

the seedlings at the farm, 25 days before the first regular sampling.

Parasitological surveys were carried out for ectoparasites on the gills, fins (pelvic, dorsal and anal) and body surface scrapings under a stereo-microscope, and the branchial and buccal cavity wall by the naked eye, and for endoparasites in the intestine, heart, brain under a stereo-microscope. Specifically, gill trichodinids were fixed in 5% formalin solution, and observed in wet mount under a compact microscope for identification. For practical reasons, trichodinids on the fins and body surface were not collected. Myxosporean spores in the brain, heart and gills were smeared on a slide glass and stained with May-Grunwald and Giemsa. Gyrodactylids on the fins and body surface were fixed in ammonium picrate-glycerin solution, and permanent mount preparations were made according to the method of Ergens (1969). Other helminths were fixed in AFA, stained with alum carmine or Heidenhein's iron hematoxylin, dehydrated in alcohol series and mounted in Canada balsam. Parasitic copepods were fixed in 70% alcohol and observed in lactic acid.

Gyrodactylus from tiger puffer were compared with those from other puffer species of the genus *Takifugu*, namely *T. pardalis* at Hagi, Yamaguchi Prefecture in April 1987 and *T. poecilonotus* at Nomo, Nagasaki Pref. in June 1986. Drawings of *Gyrodactylus* species were made with the aid of a camera lucida. Measurements are given in μm , unless otherwise stated.

Results

Description of parasites

Gyrodactylus rubripedis sp. n. (Fig. 2)

Habitats: fins and body surface of tiger puffer, *Takifugu rubripes* (Temminck et Schlegel).

Type locality and date: Naru, Nagasaki Prefecture, Japan (32° 52' N, 128° 56' E); May 21, 1987.

Additional record: Naru, Nagasaki Prefecture, Nov. 27, 1986, Mar. 16, 1987, July 23, 1987.

Specimens deposited: Meguro Parasitological Museum, Tokyo, M. P. M. Coll. No. 19696.

Description (based on 14 specimens): Body 0.39–0.56 mm long. Hamuli 47–53 long excluding folded part of root; base 40–46 long, point 27–29 long, root folded inwardly in middle. Ventral bar 20–25 long by 5–6 wide; well-developed ear-shaped processes, 9–15 long; membrane 11–15 long, with rounded margin. Dorsal bar 16–25 long by 1.5–2 wide, slightly swollen

in middle. Marginal hooks 19–21 in total length; handle 15–17 long; domus 8–10 long; hook proper 4 in length by 3.5–4 in distal width and 3–3.5 in proximal width. Male copulatory organ 11–16 in diameter; cirrus 3.5–5.5 long guarded by a single row of 4–7 minute spines.

Remarks: This parasite is unique in having inwardly bent root of hamuli. Similar structure was reported in *Gyrodactylus* species parasitic on *Noemacheilus* and its closely related genera of freshwater fish hosts (Ergens and Kartunova, 1991). The present new species, together with the following *G. pardalidis* sp. n., seems to form an independent species group within the genus *Gyrodactylus*.

Gyrodactylus pardalidis sp. n. (Figs. 3 and 4)

Habitat: fins of panther puffer, *Takifugu pardalis* (Temminck et Schlegel).

Type locality and date: Hagi, Yamaguchi Prefecture, Japan (34° 25' N, 131° 23' E); Apr. 23, 1987.

Additional record: fins of finespotted puffer, *Takifugu poecilonotus* (Temminck et Schlegel); Nomo, Nagasaki Prefecture; June 17, 1986.

Specimens deposited: Meguro, Pasasitological Museum, Tokyo, M. P. M. Coll. No. 19697–19698.

Description (based on 11 specimens): Body 0.33–0.67 mm long. Hamuli 35–43 long excluding folded part of root; base 28–35 long, point 19–24 long, root folded inwardly in middle. Ventral bar 17–24 long by 3.5–5.5 wide; ear-shaped processes 6.5–8.5 long; membrane 9–12 long, with rounded margin. Dorsal bar 15–22 long by 1–2 wide, slightly swollen in middle. Marginal hooks 18–21 in total length; handle 14–17 long; domus 8.5–10 long; hook proper 4–4.5 in length by 3.5–4.5 in distal width and 3–3.5 in proximal width. Male copulatory organ 9–14 in diameter; cirrus 3–5.5 long guarded by a single row of 4–6 minute spines.

Remarks: *G. pardalidis* sp. n. is most similar to *G. rubripedis* sp. n., but different in smaller size of hamuli and shape of the marginal hook proper.

Occurrences of parasites

In the two preliminary monitorings, no parasites were found from fish in the hatchery in May (no. of fish examined=16), but 3 fish were already infected with *Longicollum pagrosomi* in the rectum among 12 fish examined in July, a few days after the arrival at the farm.

A total of 13 species of parasites were found in or on tiger puffer during the regular survey period from July 29, 1986 to Nov. 26, 1987 (Table 1). Among them,

Figs. 2–4. Hard parts of *Gyrodactylus* species from the fins and body surface of puffers of the genus *Takifugu*. 2, *Gyrodactylus rubripedis* sp. n. from tiger puffer, *Takifugu rubripes*; 3, *Gyrodactylus pardalidis* sp. n. from *Takifugu pardalis*; 4, *Gyrodactylus pardalidis* sp. n. from *Takifugu poecilonotus*. a, hamuli and bars; b, marginal hooks; c, male copulatory organ. Left scale for a and c, 20 μm; right scale for b, 10 μm.

Table 1. List of parasites recovered from cultured tiger puffer *Takifugu rubripes* in this study

Ciliata	
<i>Trichodina fugu</i> Imai, Inouye, Kotani et Ogawa, 1997	(gills)
<i>Trichodina jadrana</i> (Raabe, 1958)	(gills)
<i>Cryptocaryon irritans</i> Brown, 1951	(fins, body surface)
Myxozoa	
<i>Kudoa shiomitsui</i> Egusa et Shiomitsu, 1983	(heart)
Multivalvulida gen. sp. (brain)	
syn. <i>Septemcapsula yasunagai</i> Hsieh et Chen, 1984	
Monogenea	
<i>Gyrodactylus rubripedis</i> sp. n. (fins, body surface)	
<i>Heterobothrium okamotoi</i> Ogawa, 1991	(gills, branchial cavity wall)
Trematoda	
<i>Tetrochetus</i> sp. (rectum)	
<i>Maculifer pacificus</i> Yamaguti, 1938	(rectum)
Acanthocephala	
<i>Longicollum pagrosomi</i> Yamaguti, 1935	(rectum)
Copepoda	
<i>Caligus fugu</i> Yamaguti et Yamasu, 1959	(buccal cavity wall)
<i>Pseudocaligus fugu</i> Yamaguti, 1936	(body surface)
<i>Neobrachiella hugu</i> (Yamaguti, 1939)	(buccal cavity wall)
syn: <i>Clavellopsis hugu</i> Yamaguti, 1939	

Cryptocaryon irritans, Multivalvulida gen. sp., *Tetrochetus* sp., *Maculifer pacificus*, *Caligus fugu* and *Pseudocaligus fugu* were only occasionally found, and seasonality on the occurrence was not clearly demonstrated. Thus, infections of the remaining 7 species of parasite were examined in detail (Fig. 5).

1. *Trichodina fugu* and *Trichodina jadrana*

As is shown in Fig. 5, all the fish examined were infected with *Trichodina* spp. throughout the study period. Identification of the two trichodinids revealed that *Trichodina fugu* was always a dominant species on the gills (data not shown).

2. *Kudoa shiomitsui*

The myxosporean was found all the time, with varying prevalences of infection. Seasonality of infection was not clear.

Fig. 5. Seasonal changes in prevalences of infection of tiger puffer with some parasites during the survey period.

3. *Gyrodactylus rubripedis* sp. n.

Although not all parts of fins and body surface were examined, the monogenean was sometimes found in large numbers, the highest number per fish being 50. The parasite tended to be abundant in winter.

Fig. 6. Amphipod, *Caprella acutifrons*, found in the intestine of tiger puffer. a, major part of body of the amphipod with two cystacanth larvae (arrowheads) of *Longicollum pagrosomi* in the body cavity; b, enlargement of a, taken under dark-field illumination. Probosci are indicated by arrows. Scale, 1 mm.

4. *Heterobothrium okamotoi*

This monogenean was first recovered on the gills in Nov. 1986, about 5 months after introduction of the puffers to the farm, and found also on the branchial cavity wall in Jan. 1987. The prevalence of infection stayed high (75–100%) until the end of the survey. Parasites on the gills were always immature, while those on the branchial cavity wall were mostly adult. There was no correlation between the number of parasites on the two habitats of the host (data not shown). The highest number of parasites per fish was 56.

5. *Longicollum pagrosomi*

In one of the three fish infected with the acanthocephalan in the preliminary survey in July 1986, the amphipod, *Caprella acutifrons*, containing two cystacanth stage larvae of *L. pagrosomi* in the body cavity, was found in the intestine (Fig. 6a, b). It was demonstrated that the amphipod acts as an intermediate

Fig. 7. Seasonal changes in frequency distribution of body length of *Longicollum pagrosomi* from the rectum of tiger puffer. White and black bars represent male and female specimens, respectively.

host for the acanthocephalan.

Seasonal succession of the frequency distribution of parasite body length (Fig. 7) suggests that the parasite has a one-year life cycle and that invades the fish in summer. Largest worm measured 12.8 mm long, and only a few fully formed eggs were observed in the female body cavity.

6. *Neobrachiella hugu*

This species was a very common parasite, and found throughout the survey period. The mean intensity of infection stayed low (0.4–2.8) in the first year, but it rose to 5.6–6.7 from July to November in the second year. No clear seasonal change was noticed in the prevalence of adults with egg sacs or of immature forms. These results suggest that copepodids, the infective stage

of the parasite, infect the fish all the year round with a peak period in warm water months.

Discussion

Thirteen species of parasites were recorded from tiger puffer in this study. Among them, 5 species, including one new species, were first reported from tiger puffer; *Gyrodactylus rubripedis* sp. n., *Tetrochetus* sp., *Macurifer pacificus*, *Longicollum pagrosomi* and *Pseudocaligus fugu*.

Since no parasite was found from juvenile puffers reared in the hatchery, it was thought that tiger puffer had mostly been infected with the parasites after arriving at the farm. However, *Kudoa shiomitsui* may have invaded the fish in the hatchery, for the myxosporean was already recovered from fish only about one month after the arrival. One-year old tiger puffer and several other fish species, i. e. yellowtail, *Seriola quinqueradiata*, and red sea bream, *Pagrus major*, had also been cultured in the same area, when juvenile puffers were introduced there. It is likely that the older puffers and other species of fish contributed as the source of infection of the young puffers with a possible exception for *K. shiomitsui*.

Okamoto (1963) described the life cycle of *Heterobothrium tetrodonis* (= *H. okamotoi*). A very unusual observation in his description was that the oncomiracidium, after settling down on the gills or branchial cavity wall of tiger puffer and shedding the cillia, encysted for a while in the host tissue. We sometimes observed encapsulation of this parasite on the branchial cavity wall. This was a host response to the parasite, and most probably he erroneously thought that the encapsulation was part of a developmental stage of the parasite.

In the present study, adult parasites of *H. okamotoi* were found only on the branchial cavity wall, while parasite on the gills were immature. This difference in the habitat of adult and immature parasites on the host strongly suggests that the monogenean migrates from the gills to the branchial cavity wall in the life cycle. The observation in this study will be described in more detail separately (Ogawa and Inouye, 1997a; 1997b).

Acanthocephalan infections of vertebrates are closely related to the feeding habits of the hosts. It was demonstrated that puffers not only were fed minced fish but also spontaneously ingested small invertebrates like amphipods propagated on the net meshing, and that the amphipod, *Caprella acutifrons*, is an intermediate host for *Longicollum pagrosomi*. Yasumono and Nagasawa

(1996) experimentally demonstrated that amphipods *Carpella equilibra*, *C. californica* and *Jassa* sp. aff. *marmorata* act as the intermediate hosts for *L. pagrosomi*, though they did not specify any of the three amphipods harbored the infective stage of the parasite. The acanthocephalan has been reported from many species of marine fishes, but mature worms were recovered only from red sea bream, *Pagrosomus unicolor* (= *Pagrus major*) (Yamaguti, 1935). This is the first record of the acanthocephalan from tiger puffer, but it seems that tiger puffer is not a suitable host for the acanthocephalan; specimens collected from puffers were considerably smaller than those from red sea bream (Yamaguti, 1935), and poorly matured.

Cryptocaryon irritans, *Gyrodactylus rubripedis* sp. n., *Heterobothrium okamotoi* and *Pseudocaligus fugu* sometime heavily infect cultured tiger puffer, and are considered pathogens. In the present survey, however, they appeared only occasionally, except for *H. okamotoi*. *Trichodina* spp. were ubiquitous parasites in this study. Although no quantitative study of the infection was conducted, fish infected with *Trichodina* did not show any sign of disease. *Longicollum pagrosomi* and *Neobrachiella hugu* provoked local hyperplasia of host tissue at the attachment site in the rectum and on the buccal cavity wall, respectively, but the intensity of infection with the two parasites never reached a state to cause disease. On the other hand, *H. okamotoi* occurred almost on all tiger puffer examined in the second year. The pathogenicity of *H. okamotoi* will be described separately (Ogawa and Inouye, 1997b).

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