Histological observations on the testes of dealfish (Lampridiformes) stranded on the coast of Niigata District, Sea of Japan

Yoshiharu Honma^{1*} • Tatsuo Ushiki¹ • Hiroya Hashizume¹ • Masaei Takeda¹ • Akira Aoyagi² and Yukihiro Nakamura³

Abstract

The testes of two dealfishes, *Trachipterus ishikawae*, 1.43m and 1.53m in total length, stranded on the beach at Johetsu and Echizen-hama, Niigata Prefecture, Sea of Japan, respectively, were examined histologically. Both individuals had slender and undulate testes, being 7.7g and 15.4g in weight, respectively. The testes were surrounded entirely with a thick capsule of dense connective tissue (tunica albuginea) and a superficial serous membrane (mesothelium), which contained numerous vertically suspended cysts enveloped by delicate connective tissue septa. In each cyst, a number of relictual spermatozoa and spermatids were seen, in addition to a very low number of spermatocytes. However, no other early state spermatogenetic cells were encountered. Released sperms and sperm balls were detected in and near the spermiduct, indicating that the testes of both individuals were spent.

Key words: dealfish testis, Niigata coast, stranded dealfish, testis histology, Trachipterus ishikawae

Introduction

Gonadal histological observations of the rare and unusually-shaped-fishes belonging to Taeniosomi (Lampridiformes) have been made by the senior author (in collaborative studies from time to time, when comparatively fresh specimens of various species have become available) (Tamura and Honma 1971, 1972; Honma et al. 1973; Honma and Tsumura 1980; Honma and Mizusawa, 1981; Honma et al. 1999, 2002, 2004). In spring 2003, two male dealfishes were stranded on the coast of Niigata Prefecture, Sea of Japan.

Unfortunately, the gonads were not entirely suitable for histological study, since the specimens were first frozen for a considerable period after collection, and the gonads subsequently immersed in $5\sim10\%$ formalin solution.

In spite of this, examination of gonad and other

visceral organ histology was made in an attempt to elucidate the reproductive biology and life history of ribbonfishes. As shown in Table 1 of a previous paper (Honma et al. 2004), most of the specimens already studied were female, only one poorly preserved male having been investigated (Honma and Mizusawa 1981).

Accordingly, the following short report on the two male dealfishes was prepared.

Materials and Methods

Two medium size male dealfishes, *Trachipterus ishikawae*, 1.43 m and 1.52 m in total length, were collected on the beaches at Ara-hama, Johetsu City (on 23 March, 2003, specimen A), and Echizen-hama, Maki-machi (on 7 May, 2003, specimen B), Niigata Prefecture, Sea of Japan, and subsequently frozen. After defrosting, the testes

[「]新潟大学大学院医歯学総合研究科細胞機能講座顕微解剖学分野 〒951-8510 新潟市旭町 1 - 757;2 寺泊町立水族博物館 〒940-2502 新潟県三島郡寺泊町花立;3 上越市立水族博物館 〒942-0004 新潟県上越市西本町 4 - 19 - 27

¹ Division of Microscopic Anatomy and Bioimaging, Department of Cellular Function, Niigata University Graduate School of Medical and Dental Sciences, Asahimachi 1-757, Niigata 951-8510, Japan; ² Teradomari Town Aquarium, Hanadate, Teradomari-machi, Niigata Prefecture 940-2502, Japan

³ Johetsu City Aquarium, Nishihoncho 4-19-27, Johetsu, Niigata Prefecture 942-0004, Japan

of both specimens were removed and preserved in 5~10% formalin solution. The collection locations of both specimens are shown in Fig. 1.

Following a previous report (Honma et al. 2004), three pieces (anterior tip, middle portion and posterior tip) were cut from each testis, refixed in Bouin's solution for improvement of tissue texture and staining ability, and dehydrated through an alcohol series. After being embeded in paraffin, the blocks were sectioned at 5μ m, stained with hematoxylin-eosin (HE) double stain and Masson-Goldner (MG) associated with aldehyde fuchsin (AF) tetrachrome, and observed under a light microscope (Leitz Orthoplan, Wetzlar, Germany).

Results

Macroscopy

The testis of specimen A measured 230mm (length), 15mm (width) and weighd 7.7g (Fig. 2), that of specimen B being 280mm by 20mm and 15. 4g (Fig. 3).

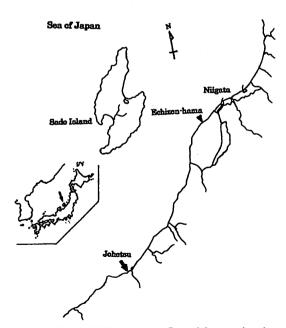


Fig. 1. Map of Niigata coast, Sea of Japan, showing stranding locations of dealfish, *Trachipterus ishikawae*. Specimen A (arrow) collected from Johetsu City; specimen B (arrow head) collected from Echizen-hama, Maki-machi.

The testis were covered entirely with a thin superficial serous membrane (mesothelium) and a thick fibrous membrane (tunica albuginea), which had the form of a remarkably elongated bilobate canal resembling a continuous undulation of lamellae. Similar to the structural pattern of eel testis, each lobe was suspended vertically and conjoined tightly with a thick fibrous connective tissue strand, with an elongate spermiduct in the dorsal portion. A cross-section of part of the undulating structure in the testes is diagrammatically illustrated in Fig. 4.

Microscopy

Both testes showed nearly identical architecture and microscopic images, although it was impossible to obtain good preparations of the cellular elements. Each testis comprised vertical series of (numerous) lobules, including a number of dilated cysts (Fig. 5). Most of the cysts, surrounded by a delicate connective tissue wall (septa), were occupied by a comparatively high density of spermiogenetic cells (spermatids) and sperms (spermatozoa), although spermatogenetic cells were very rare (Fig. 6).

Large spermatocytes, including a light round nucleus with distinct chromatin, were very seldom recognized (Fig. 6). Similarly, spermatids in transformed stages were seen only sporadically. Most of the (small rounded) spermatids with scanty cytoplasm, were gathered in masses, whereas relictual sperms were variously scattered (Fig. 7). Spermatozoan size was less than $1/2\sim1/3$ of that of a spermatid, the former resembling a tiny spherule (Fig. 8). It was difficult to demonstrate flagellae and associated structures in both spermatids and spermatozoa, at the light microscope level.

In the peripheral region of each lobule, cysts comprising swollen cells with dilated vacuoles were encountered (Fig. 9). Although the posterior portion of the spermiduct ran parallel to the rectum, the anterior portion of the spermiduct, located just dorsal to the testis, contained a small number and / or small masses (balls) of relictual sperms (Figs. 10, 11).

In spite of careful examination, it was impos-

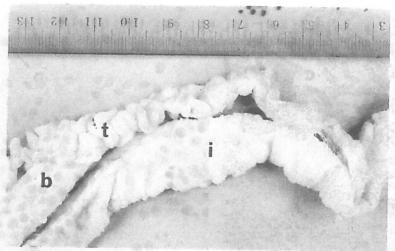


Fig. 2. Part of the testis (t) of specimen A, running parallel with the blind sac of the stomach (b) and intestine (i).

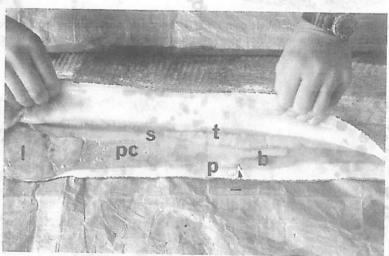


Fig. 3. Digestive organs associated with the testis (200mm in length) of specimen B. b, blind sac of stomach; l, liver; p, pylorus; pc, pyloric caeca; s, stomach; spleen (arrow head); t, testis.



Fig. 4. Diagrammatic cross section of dealfish testis made from specimen B showing bilobate components of lobules (1) and spermiducts (d). c: cyst.

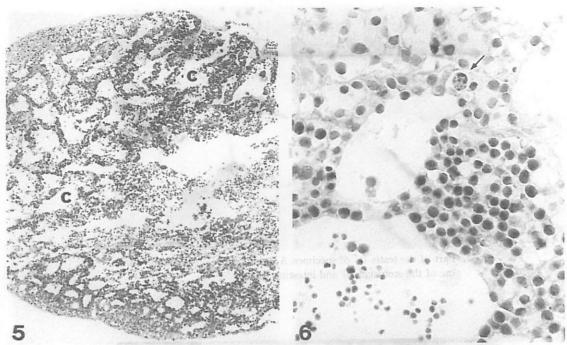


Fig. 5. Section of part of spent testicular lobule comprising numerous cysts(c). Low magnification. Hematoxylin-eosin stain (HE). ×100

Fig. 6. Spermatids and relictual sperms (spermatozoa) in the cysts. Note large spermatocyte (arrow). HE. $\times 400$

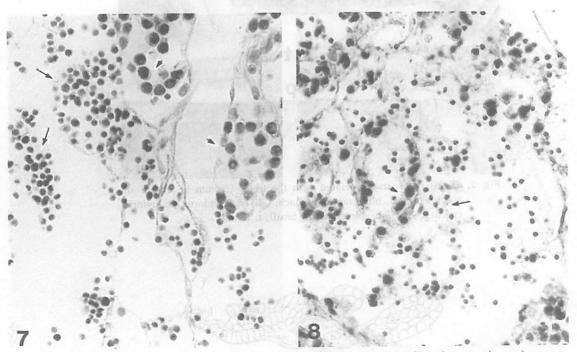


Fig. 7. Small masses of spermatids (arrow head) and Fig. 8. Note smaller relictual sperms (arrow) comparsperms (arrow) in the cysts. HE. ×400 ed with spermatid size (arrowhead). HE. ×400

Figs. 5~10. All microphotographs are made by specimen A.

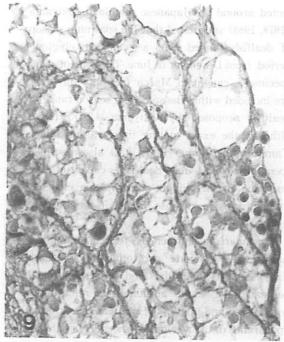


Fig. 9. Dilated cells with vacuoles located near tunica albuginea. HE. ×400



Fig. 10. Sagittal section of spermi duct, containing masses of sperms (arrow), running parallel with intestine (i).

Low magnification. HE. ×100

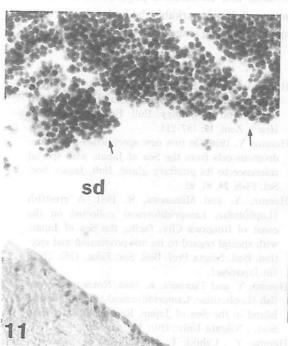


Fig. 11. Masses of relictual sperms (arrow) in posterior portion of spermi duct (sd). HE. ×400

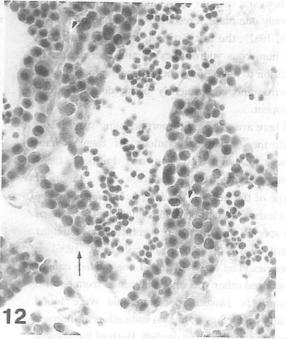


Fig. 12. No obvious Leydig cells between narrow spaces (arrow head) formed by segments of adjacent cysts. Note no detection of Sertoli cells near basal membrane (arrow) of cysts . HE. ×100

sible to detect the Leydig cells located in the interstitial tissue between the cysts. On the other hand, Sertoli (sustentacular) cells near the cystic wall were very occasionally encountered in both specimens (Fig. 12).

Discussion

It was noted that the shape and features of the dealfish testes were similar to those of anguilliform fishes, a likely result of the testes of both groups being confined to slender and elongated body cavities (Honma, 1958).

Previous reports on the reproductive organs of taeniosomate fishes include three female deal-fishes (*Trachipterus ishikawae*), one sex unknown king-of-the-herring (*T. trachipterus*), two (one male and one female) crestfishes (*Lophotus capellei*), three female oarfishes (*Regalecus russelli*) and one female unicornfish (*Eumecichthys fiskii*) (Tamura and Honma, 1971, 1972; Honma, et al. 1973, 1999, 2002, 2004; Honma and Tsumura 1980; Honma and Mizusawa 1981).

However, the nine individuals examined, included only one male (crestfish) (Honma and Mizusawa, 1981), the testis of that individual comprising numerous spermatocytes and spermatids, in addition to a small number of spermatogonia and sperms, and consequently being determined as nonspent.

There are as yet no reports dealing specifically with the testicular histology of taeniosomate fishes, although Harrison and Palmer (1968) mentioned briefly the location and cylindrical shape of the testis in *Radiicephalus elongatus*, the specimen exmined being designated as neotype of the species, and an oral presentation on testicular histology was given by Kobayashi et al. (1996) on 7 species. The latter included one oarfish caught at sea and other specimens obtained from several aquaria in Japan. Spermatogonia were found locally in each cyst, but no indication of intersex was recognized in the oarfish. Both of the present male dealfishes were spent, according to the histological examination.

Based on his observations of pelagic eggs col-

lected around the Japanese mainland, Okiyama (1978, 1988) speculated that the spawning season of dealfish ranged over a comparatively long period, from December to June. The present spent specimens, caught in March (A) and May (B), are included within the spawning season range of dealfish proposed by Okiyama (1978, 1988), although the exact mating time was unknown. Tamura and Honma (1971, 1972) also described spent ovaries in female dealfishes caught in May and June off the Niigata coast, further evidence for the inclusion of those months in the breeding season.

A comparison between the testicular architecture of taeniosomate and other allotriognath fishes, such as lampridids and veriferids, should be made, the adults all having a remarkably compressed and high body, whereas the external appearance of taeniosomates is slender, similar to that of cutlassfishes (Trichiuridae) (Honma, et al. 2002). Interestingly, however, the young of several species of taeniosomate fishes, such as Zu cristatus and Desmodema polystictum, are characterized by remarkable body height in their metamorphosing stages.

References

Harrisson, C. M. H. and Palmer, G. 1968. On the neotype of *Radiicephalus elongatus* Osorio with remarks on its biology. Bull. British Mus. (Nat. Hist.), Zool. 16: 187-211.

Honma, Y. 1958. On two new specimens of the catadromous eels from the Sea of Japan, with special reference to its pituitary gland. Bull. Japan. Soc. Sci. Fish. 24: 87-92.

Honma, Y. and Mizusawa, R. 1981. A crestfish (Lophotidae, Lampridiformes) collected on the coast of Itoigawa City, facing the Sea of Japan, with special regard to its ink production and ejection. Bull. Niigta Pref. Biol. Soc. Educ. (16): 21-26 (in Japanese).

Honma, Y. and Tsumura, K. 1980. Notes on a crestfish (Lophotidae, Lampridiformes) caught off Sado Island in the Sea of Japan. Rep. Sado Mar. Biol. Stat., Niigata Univ. (10): 11-16.

Honma, Y., Ushiki, T. and Takeda, M. 1999. Histology of the ink tube and its associated organs in a unicornfish, *Eumecichthys fiskii* (Lampridiformes). Ichthyol. Res. 48: 19-25.

Honma, Y., Ushiki, T., Takeda, M. and Ishibashi, T.

2002. Comparative anatomy and histology of the visceral organs of an oarfish, Regalecus russelli, and a razorback scabbardfish, Assurger anzac, caught off the coast of Fukui Prefecture, Sea of Japan. Bull. Kashiwazaki City Mus. (16): 71-86.

Honma, Y., Ushiki, T., Hashizume, H., Takeda, M., Misaki, H., Inagi, A. and Ishibashi, T. 2004. Histological observations on the ovaries of two species of taeniosomatous fishes (Lampridiformes) caught in waters adjacent to the Japanese Main Island. Publ. Seto Mar. Biol. Lab. 40: 113-120.

Honma, Y., Yasuda, T. and Kon, T. 1973. A record of the young of King-of-the-Herring, *Trachipterus iris*, from Tsuruga Bay, the Japan Sea. Ann. Rep. Sado Mar. Biol. Stat., Niigata Univ. (3): 1-10.

Kobayashi, K., Suzuki, K. and Shiobara, Y. 1996. Histological observations on the gonads of taeniosomatous fishes, especially oarfish. The 41st Meet. Aquar., Japan. Assoc. Zool. Gard. Aquar. 22 (abstract), (in Japanese).

Okiyama, M. 1978. Successful spawning of some holopelagic fishes in the Sea of Japan and zoogeographical implications. Proc. 7th Japan-Soviet Joint Symp. Aquaculture. pp.223-233.

Okiyama, M. (ed.) 1988. An Atlas of the Early Stage Fishes in Japan. 1154p. Tokai Univ. Press, Tokyo. (in Japanese).

Tamura, E. and Honma, Y. 1971. Notes on a collection of the dealfish, *Trachipterus ishikawai*, from northern part of Niigata Prefecture, facing the Japan Sea, with description of some of the endocrine glands. Ann. Rep. Sado Mar. Biol. Stat., Niigata Univ. (1): 17-27.

Tamura, E. and Honma, Y. 1972. Histological observations on another dealfish, *Trachipterus ishikawai*, caught off the coast of Sado Island in the Japan Sea. Ann. Rep. Sado Mar. Biol. Stat., Niigata Univ. (2): 9-22.

(Received April 26, 2004; accepted May 24, 2004)

新潟県沿岸へ漂着し、または沖合いで漁獲された サケガシラやリュウグウノツカイなど紐体類(異顎 類)の生殖腺をはじめとする内部諸器官の組織学的 観察は、ほとんど行われていない。

2003年3月23日に上越市荒浜海岸へ全長1.43mの雄サケガシラ(A個体)と、2003年5月7日に西浦原郡巻町越前浜へ全長1.52mの雄サケガシラ(B個体)が禊着した。冷凍個体より精巣を摘出し、10%フォルマリンで固定し剖検した。A個体の精巣は長さ230mm、幅15mm、重さ7.7g、B標本は280mm、20mm、15.4gであった。つぎに、精巣の前方、中央、後方の3箇所より小片を切り出し、プアン氏液で再固定してパラフィン切片を作成のうえ、ヘマトキシリン・エオシン(H・E)の二重染色と、Masson-Goldnerの三重染色にアルデヒドフクシン染色を加えて(MG-AF)、顕微鏡下で観察した。

精巣全体は延長性の資状器官で、表層の薄い中皮 (契膜)とその下の厚い白膜(結合組織)で覆われ、 背方に細長い精管が走っていた。精管からは、無数 の半円形層板(精細管相当)が襞打ちながら垂下し、 対(双葉性)をなしていた。層板は、多数の包嚢を 包含していた。A、B両標本とも、包嚢は残存精子 や精子細胞をもち、拡張したり精子を放出して崩壊 したりしていたが、精管中にわずかの残存精子がみ られた。しかし、セルトリ(支持)細胞、精原細胞、 精母細胞および間質中のライジッヒ細胞は認められ なかった。したがって、サケガシラの精子形成はほ ぽ完全同時発生型といえる。

観察した両個体の 報線は,ともに放精後のもので, いままでに検索した他個体生殖腺の組織学的所見を 加味すると,繁殖期は12月から6月にわたるかなり 長いものであることが推察された。