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**Egg Production, the Oncomiracidium and Larval Development
of *Benedenia seriolae*, a Skin Parasite of the Yellowtail,
Seriola quinqueradiata, in Japan**

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Abstract Observations were made on the rate of egg assembly in the capsalid monogenean *Benedenia seriolae*, a skin parasite of the yellowtail, *Seriola quinqueradiata*. An adult specimen measuring about 7 mm in length after flattening and preservation, laid 27 eggs in 1h at 20°C. It is suggested that forces exerted on the tetrahedral egg capsule by water currents generated by host swimming, contribute to the stretching of the still soft appendage of a freshly made egg, while it is still tethered to the parasite by the proximal end of the appendage lodged in the uterus. The anatomy of the oncomiracidium is described and an account is given of larval development, based on experimental infection of the related host *S. aureovittata*. The two anterior attachment discs are absent in the oncomiracidium, but are beginning to develop in post-oncomiracidia recovered one day after infection. The adhesive pads of the oncomiracidium persist on the discs throughout life and their subdivision into three zones is detectable in post-oncomiracidia and in adults. *B. seriolae* reached full sexual maturity on day 14 post infection (at 22°C).

Introduction

The yellowtail, *Seriola quinqueradiata*, is an important food fish cultured extensively in marine fish farms in southern Japan and, in these farms, the skin is commonly infected with the capsalid monogenean *Benedenia seriolae* (Yamaguti, 1934) Price 1939. In spite of the damage done by this parasite in fish farms, its biology is poorly understood. Hoshina (1968) made some observations on egg production, on the anatomy of the oncomiracidium and on larval development, but his drawing of the oncomiracidium is incomplete and there is no information on the development of the anterior adhesive discs of the parasite. During visits by the two senior authors

to the Seto Marine Biological Laboratory of Kyoto University at Shirahama and to the National Research Institute of Aquaculture at Nansei, opportunities arose to study living oncomiracidia and juvenile and adult specimens of *B. seriolae*.

Materials and Methods

Living specimens of *Benedenia seriolae* were removed with a scalpel blade from the skin of cultured yellowtail (Japanese name: *burai*), *Seriola quinqueradiata*, at fish farms belonging to the Inari Fish Culture Company and the Katada Fishermen's Association in Shirahama and to the Sazara Fish Co-operative in Mie Prefecture. The parasites were transferred to small Petri dishes containing filtered sea water and observed in a constant temperature room maintained at about 20°C. Some eggs were collected from these adult parasites but most eggs were gathered by suspending a piece of nylon netting with a mesh size of about 1.5 mm in a tank containing infected yellowtail at the National Research Institute of Aquaculture at Nansei. The appendages of the eggs became entangled in the netting which was then transported to Shirahama and maintained at 23°C in filtered sea water changed twice daily. Living oncomiracidia were restrained with slight coverslip compression and observed using phase contrast equipment. Photomicrography was used to record the shapes of the hooks. In order to study the larval development of the parasite an infection experiment was conducted at Shirahama. No specimens of *S. quinqueradiata* were available but three specimens of *S. aureovittata*, the host from which *B. seriolae* was originally reported by Yamaguti (1934), were obtained from the Fisheries Laboratory of Kinki University in Shirahama. These fishes, each of which measured about 28 cm in length, were immersed in fresh water for a few minutes in order to kill any attached specimens of *B. seriolae* and then placed in about 300 l of sea water. Large numbers of oncomiracidia were added to the tank and, after a three hour exposure period, the fish were maintained in running sea water, the temperature of which remained fairly constant at about 23°C throughout the course of the experiment. After 24 and 48 h and thereafter at 2-day intervals, the skin of the fishes was gently scraped with a scalpel blade and parasites collected by searching the scrapings with a stereomicroscope. The anatomy of the living parasites was studied as described above. Measurements were made with an ocular micrometer.

Results

1) Egg production and egg laying

Adult parasites attached by the haptor to the bottom of a glass dish readily assemble and lay eggs. Each egg has a tetrahedral capsule with a side length of 144 (130–150) μm ($n=6$) and a single long appendage which, in eggs laid by parasites detached from the host, is about 1 mm in length (but see below). This appendage bears no adhesive droplets and does not adhere strongly to the substrate.

The following observations were made on an adult parasite measuring 7.2 mm in length (after flattening and preservation in 5% formaldehyde) and possessing anterior hamuli with an average length of 426 μm . The parasite assembled 27 eggs in 1 h at 20°C and the average time required to assemble an egg in the ootype, measured from the moment of release of the oocyte (or zygote) from the germarium to the cessation of ootype contractions, was 52 (30–70) s ($n=28$). On average, 40 ootype contractions were involved in the assembly of a single egg. After the cessation of ootype movements the egg capsule remained in the ootype for a further 80 (25–165) s ($n=27$) and was then expelled along the uterus and out of the genital opening, but

was invariably retained for a minute or so in this position with the appendage still within the uterus. No egg capsules were retained within the uterus. The egg appendage was released before the next egg was laid, so that, at any time, no more than one egg capsule was found tethered by the appendage to the genital opening. On no occasion did this or any other parasite with a tethered egg, raise and lower the head region in the manner described by Kearn (1986) in *E. soleae*. The parasite began the assembly of each new egg as soon as the first egg left the ootype, i.e. the ootype was rarely empty for more than a few seconds. After a period of continuous egg assembly during which 30 or more eggs were made one after the other, the parasite ceased to make eggs. After resting with the ootype empty for about 10 min, egg assembly was resumed.

Some additional observations were made on egg laying in a young, 14-day-old adult about 4.4 mm in length (anterior hamulus length 324 μm) (see also p. 358). The assembly of each new egg did not begin in this parasite until about 9 min after ejection of the previous egg from the ootype. However, the time spent by each egg capsule in the ootype was comparable with that of larger adults and eggs were laid soon after assembly when still water-white.

It was noticed that the eggs assembled and released by parasites in a dish of still sea water had relatively short appendages, measuring about 1 mm in length or less. The appendages of eggs laid by parasites *in situ* on the skin of the host and entangled in nylon netting suspended in the tank with the swimming hosts appeared to be significantly longer. Because of their entanglement with the netting and with the appendages of other eggs, it proved difficult to obtain accurate length measurements and attempts to disentangle the appendages usually led to breakages. Nevertheless, these appendages were at least 2 mm long and possibly as long as 4 mm. It was also observed that the length of the proximal tubular region of the ootype where the appendage is assembled is about 500 μm .

Most partly-laid eggs, attached to the parasite by the appendage lodged in the uterus, are water-white, although occasionally the brown colour indicative of egg shell tanning is just detectable. An attempt was made to dislodge one of these eggs with a needle and it was found that the appendage was still soft, pliable and inelastic, stretching like toffee to produce a longer slender appendage about 2 mm in length.

2) The oncomiracidium

The eggs of *B. seriolae* began to hatch after incubation for about 5 days at 23°C and when exposed to the natural cycle of illumination, the larvae emerged mainly during the hours of daylight (Kearn, Ogawa & Maeno, 1992). The following account of the anatomy of the oncomiracidium is based on the study of at least fifty larvae. There is a general resemblance between the oncomiracidium of *B. seriolae* (Fig. 1) and those of monogeneans of the genus *Entobdella* (see Kearn, 1974). There are four conspicuous eyes with lenses, three transverse zones of ciliated cells, a ventral mouth between the eyes leading to a spacious cavity containing the pharynx, two lateral unbranched gut caeca and a disc-shaped haptor bearing 14 peripherally-located

Fig. 1. Oncomiracidium of *Benedenia seriola* (ventral view). ah, Anterior hamulus; ap, adhesive pad (openings of gland ducts); as, accessory sclerite; b, bladder; bg, body gland; ce, ciliated epidermis; e, eye; f, flame cell; ga, gland cells associated with alimentary canal; gc, cell with granular cytoplasm; i, intestine; lg, lateral head gland; mh, marginal hooklet; p, pharynx; pg, posterior median head glands; ph, posterior hamulus; s, sensillum; t, tendon.

marginal hooklets, each with a domus, and three pairs of median sclerites, namely accessory sclerites, anterior hamuli and posterior hamuli.

It is difficult to obtain an accurate measure of the total length of the living oncomiracidium. With just sufficient coverslip pressure to prevent swimming the larva is still capable of contraction and expansion, but the average length of such a larva is about 330 μm and the body width at its widest point is about 105 μm . The lenses associated with the large eyes frequently have a shallow median furrow, per-

haps indicating that they are secreted in two separate halves. Lyons (1972) found a pair of spherical bodies which she presumed to be ciliary photoreceptors in electron micrographs of sections through the head region of the oncomiracidium of *E. soleae* and, using phase contrast equipment, these bodies can be seen in living larvae lying between the pigment-shielded eyes and the lateral border of the head (Kearn, previously unpublished observation). However, in spite of a careful search of the corresponding region of many larvae of *B. seriolae*, no similar structures were located, although an irregularly-shaped cell (?) with granular cytoplasm was invariably present between each of the large eyes and the body margin.

The pharynx of *B. seriolae* contains papillae projecting into the pharynx lumen and each of these has a gland cell opening at the apex. Eleven of these papillae were counted in several oncomiracidia. There are also glands lying outside the pharynx with ducts opening at the junction between the pharynx and the gut caeca.

There are gaps in the cover provided by the ciliated epidermal cells and in these gaps there are projecting sensilla. Most flattened larvae are seen in dorsal or ventral view, so the most conspicuous sensilla are those that project from the lateral edges of the body and these are as follows: two sensilla on the anterior extremity of the head; a bunch of several sensilla on each side of the body just behind the large eyes; two other sensilla on each side, one anterior to and the other posterior to the bunch of sensilla; two sensilla on each side at the posterior end of the body.

The gland cells supplying secretion to the anterolateral areas of the head region are located in two sites, in the lateral region of the larva near the posterior end of the pharynx and in the head region just anterior to the eyes. The four, possibly five, lateral head gland cells are relatively small and their ducts follow an anterior course close to the lateral border of the head. The outlines of the cells anterior to the eyes (posterior median head glands) are not visible, but the extent of the glandular area can be identified by the distribution of the needle-shaped secretory inclusions. There are two groups of these cells, one on each side of the mid-line. No anterior median head glands, like those recorded in larvae of *Entobdella* spp. (Kearn, 1974), were observed in *B. seriolae* oncomiracidia.

There are two gland cells opening ventrally in each posterolateral region of the body and there are also gland cells associated with the haptor. However, it has proved difficult to trace fully the paths of the haptor gland ducts because of overlying features such as the haptor sclerites and excretory system. There is at least one gland cell on each side of the mid-line in the posterior region of the body and the ducts from these cells enter the haptor. There are two duct openings on the ventral surface of the haptor on each side, one in the anterolateral region near the accessory sclerite and the other in the posterolateral region near the anterior hamulus.

Three pairs of flame cells were found in the head region, one pair anterior to the eyes and two pairs lateral to the pharynx. The main longitudinal canals anterior to and posterior to the bladders contain flagella. The excretory canal system in the haptor is asymmetrical with four pairs of flame cells.

In the free-swimming oncomiracidium, the lateral regions of the disc-shaped

haptor are folded ventrally, but in compressed larvae the circular haptor is partly or fully unfolded. Examination of the unfolded haptor revealed the presence of a peripheral marginal valve. There is a pronounced notch in the posterior end of the accessory sclerite and a tendon from a (developing?) muscle in the body passes through the haptor peduncle, threads its way through this notch, changing direction as it does so, and then runs outwards, apparently becoming attached to the ventral tegument of the haptor.

3) Larval development

The following descriptions of post-oncomiracidia collected at each sampling interval from experimentally infected *S. aureovittata* are each based on at least two individuals. Parasites recovered from the skin of the experimentally infected hosts 24h after exposure to oncomiracidia were similar to oncomiracidia in size (appro-

Fig. 2. Larval development of *Benedenia seriolae* (ventral view). A. 1-day-old parasite. B. 2-day-old parasite. ad, Anterior attachment disc; ag, anterior median head gland; ap, adhesive pad; pg, posterior median head glands; sp, sensory pit (?).

ximate total length 332 μm ; anterior hamulus length about 38 μm) and in anatomy (Fig. 2A). However, differentiation of the anterior adhesive discs of the adult was already underway in the form of a flap of tissue, projecting in a ventrolateral direction from each anterolateral border of the head region. The lateral and posterior median head glands were visible and their duct openings were arranged in three adjacent zones forming a glandular pad on each anterolateral border of the head region. Two anterior median gland cells, absent in the oncomiracidium, opened on the anterior median border of the head and between each of these gland openings and the adjacent glandular pad was a ventrally-located circular pit, the rim of which was observed opening and closing. The eye lenses were still present.

Two-day-old parasites were significantly larger in size (total length 405 μm ; anterior hamulus length 52 μm) and the anterior discs appeared fully differentiated (Fig. 2B). In some individuals the lateral head glands and their ducts were present but relatively small, but in others they were not evident. The anterolateral glandular pads, each comprising three adjacent zones, occupied the anterolateral regions of the anterior discs. The anterior median gland cells and the sensory (?) pits were prominent and eye lenses still persisted. The pharynx was significantly larger with a lobed profile and the intestinal caeca had pouches projecting in anterior and lateral directions. New flame cells were present in the head region, in the posterolateral regions of the body and in the haptor.

Further developments in the adhesive region of the head involved mainly a progressive increase in size of the discs. Visualization of the gland cells became correspondingly more difficult and the lateral head glands and their ducts were not seen in larger parasites; however, the three adjacent zones of each pad could be detected by their apparently microvillous surface and not only persisted in older parasites but increased progressively in area. Flame cells continued to multiply and lateral diverticula developed from the intestinal caeca. In 4-day-old parasites (total length 664 μm ; anterior hamulus length 90 μm), the outer ring of 45 or 46 small papillae in the pharynx was recognised, in addition to the inner ring of 10 or 11 larger papillae (Fig. 3A). In 6-day-old parasites (total length 1.1 mm; anterior hamulus length 120 μm), the primordia of the testes and the penis sac were visible and the gut diverticula were beginning to develop subsidiary diverticula (Fig. 3B). In 10-day-old specimens (total length about 3.1 mm; anterior hamulus length 211 μm), the testes, penis sac, vagina, germarium and vitelline reservoir were identifiable. Spermatozoa were present in the testes but the germarium was not fully developed. In one specimen, unicellular body glands with openings on the ventral surface were widespread. There was little detectable change in the level of development of the reproductive system of 12-day-old parasites (total length about 2.9 mm; anterior hamulus length about 243 μm). The vitelline reservoir and vagina were empty. In one of these individuals, pigmented body cells were detected for the first time. Four symmetrically arranged cells, containing a diffuse, pale purple pigment were observed, two just anterior to the testes and two in the posterior region of the body. In some, but not all, adult parasites, these pigmented cells were numerous and widespread

Fig. 3. Larval development of *Benedenia seriola*. A. 4-day-old parasite. B. 6-day-old parasite (haptor omitted). ad, Anterior attachment disc; ag, anterior median head gland; ap, adhesive pad; b, bladder; dp, developing penis sac; i, intestine; t, developing testis.

throughout the body, apparently with the exceptions of the anterior discs and the haptor.

Parasites collected after 14 days, measuring between 3.8 and 4.4 mm in total length (anterior hamulus lengths between 292 μm and 324 μm), were found to be fully sexually mature and laid eggs (see p. 353). In these adults, the posterior median head glands and their ducts were by no means conspicuous but the triple nature of the adhesive pads was detected. A few body gland cells were identified in some of these adults and four gland duct openings were found in most specimens on the ventral surface of the haptor, a slit-like opening on each side anterolateral to the points of the accessory sclerites and an opening on each side near the hook of the anterior hamulus.

DISCUSSION

The observations recorded in the present paper show that *Benedenia seriolae* develops rapidly on *Seriola aureovitatta* at about 23°C, reaching sexual maturity on day 14, and achieves a high level of egg output. An adult measuring about 7 mm in length produced 27 eggs in 1h at 20°C. Hoshina (1968) studied larval development on *S. quinqueradiata* and his figures indicate somewhat slower development, the parasites reaching maturity in 18 days at an average temperature of 23.5°C. Maeno (unpublished) has also raised *B. seriolae* on *S. quinqueradiata* and it is interesting that, like Hoshina, he recorded slightly slower development, his parasites reaching sexual maturity in 14 days but at a higher temperature (25°C). However, Maeno found that the eggs laid by the smallest adults did not develop. The rapid development and high fecundity of *B. seriolae* are surprising when we compare it with the smaller parasite *Entobdella soleae*, which takes as long as 85 days (at 12°C) to reach sexual maturity (Kearn, 1990) and produces less than 3 eggs/h (Kearn, 1985). These features of *B. seriolae* may partly account for the problems it creates in enclosures where yellowtail are reared intensively.

In *B. seriolae*, the average time for egg assembly is 52 s, while *E. soleae* takes 4–6 min (see Kearn, 1985), but this is not the only factor contributing to the difference in the rate of egg production. In *B. seriolae*, the egg spends, on average, a further 80 (25–165) s in the ootype after the cessation of ootype movements, while in *E. soleae* the corresponding period ranges from 8 to 107 min. In large adult specimens of *B. seriolae*, the assembly of a new egg usually begins as soon as the previous egg has vacated the ootype and, even in a small, recently-mature adult the ootype remained empty for only about 9 min. On the other hand, the ootype in *E. soleae* usually remains empty for periods ranging from 2 or 3 min up to 1 h.

After leaving the ootype, each egg of *B. seriolae* is immediately propelled through the uterus and out of the body, whereas in *E. soleae* some egg capsules spend as long as 168 min in the uterus before leaving the body (Kearn, 1985). In both of these parasites, only the tetrahedral capsule is expelled from the body initially, the appendage remaining in the uterus and tethering the egg to the body. In *B. seriolae* each egg appendage is released, setting the egg capsule free, before the next egg is propelled along the uterus, so that only single eggs are tethered to the parasite by the appendage. In *E. soleae*, tethered egg bundles may consist of two, three or more eggs because of the accumulation of eggs in the uterus (Kearn, 1971a). Kearn (1971a, 1986) reported that specimens of *E. soleae* with tethered egg bundles performed vigorous lifting movements of the head region and he suggested that these movements may assist the attachment of the sticky droplets on the egg appendages to sand grains beneath the flatfish host. This suggestion gains support from the observation that specimens of *B. seriolae* with single tethered eggs do not perform similar movements; each egg is simply released from the body, presumably permitting the egg to drift away from the active round-bodied host and become entangled by its non-adhesive, filamentous appendage around a suitable projecting anchorage.

An interesting difference was found in *B. seriola* between the lengths of the appendages of eggs laid by detached adults in still sea water and eggs laid by adults attached to living hosts. Appendages from detached parasites measured about 1 mm in length or less, while those from attached parasites were at least 2 mm long. It was also observed that the proximal tubular region of the ootype where the egg appendage is assembled is about 500 μ m in length and that the appendages of freshly-made eggs, still tethered to the parasite, were soft, ductile and inelastic, stretching to a permanent length of at least 2 mm when pulled gently. It is suggested that there are two stages in the elongation of the egg appendage in *B. seriola*, first, a doubling in length while the egg and appendage are being expelled from the ootype and propelled down the uterus, and, secondly, a further doubling or trebling in length as a result of forces exerted on the free egg capsule of a tethered egg by water currents generated by host swimming. If this interpretation is correct, then the parasite is taking advantage of the forces generated by the host's swimming activity to increase the length of the egg appendage and the longer the appendage the better its chances of ensnaring a suitable anchorage as the egg drifts. Eggs failing to become anchored may drift away from areas inhabited by potential hosts. This exploitation of the host is possible because the eggs are expelled from the ootype and uterus soon after assembly, before the egg-shell material of which the appendage is formed, has become tanned, toughened and inextensible.

Hoshina (1968) illustrated (his Fig. 2) but did not describe the oncomiracidium of *B. seriola*. He provided little information on the glandular system. The feature which he labels as "primordium of anterior sucker" is, in fact, a group of swollen gland duct terminations opening on the anterolateral border of the head region. There is no trace of the "anterior suckers" (=anterior discs) in the oncomiracidium. Similarly, Jahn & Kuhn (1932) and Kearns (1971b) found that the anterior discs of *Epibdella* (= *Neobenedenia*) *melleni* and the related capsalid *Trochopus pini* respectively, are absent in the oncomiracidium. In *B. seriola* these anterolateral regions of the head receive ducts from lateral head gland cells and posterior median head gland cells, as they do in the oncomiracidium of *Entobdella* spp. (Kearns, 1974) and it seems likely that this secretion serves as cement for attachment of the adhesive pads.

Hoshina (1968) illustrated the main excretory canals and the bladders of the oncomiracidium of *B. seriola* but he did not record any flame cells in the body. In the present study three pairs of flame cells were identified in the head region. Hoshina correctly reported four pairs of flame cells in the haptor but erroneously represented the duct system as symmetrical.

Hoshina (1968) illustrated short median diverticula projecting from the two lateral gut caeca in the oncomiracidium but these were not found in the present study.

The shapes of the haptor sclerites of the oncomiracidium were somewhat crudely represented in Hoshina's figure. In particular, the well-developed hook of the posterior hamulus was not illustrated. Hoshina did not illustrate the notch at the proximal end of the accessory sclerite or the tendon passing through the notch, run-

ning in a peripheral direction and apparently becoming attached to the ventral tegument of the haptor. The haptor tendon of the oncomiracidium of *T. pini* follows a similar course (Kearn, 1971b) but in the oncomiracidium of *Entobdella* spp. the tendon attaches to the anterior end of the anterior hamulus after passing through the notch in the accessory sclerite (Kearn, 1974). Kearn (1971b) highlighted the same difference in the tendon termination between adult specimens of *Benedenia* (= *Neobenedenia*) *melleni* and *Pseudobenedenia nototheniae* on the one hand and adult *E. soleae* on the other, and this may be a fundamental feature distinguishing *Benedenia* and its relatives like *Trochopus* from *Entobdella* spp.

Hoshina's account of larval development of *B. seriolae* lacks observations on early post-oncomiracidia. His reference to degeneration of the eyes in 1- or 2-day-old parasites has not been confirmed in the present study and, indeed, the lenses as well as the pigment cups persist in post-oncomiracidia. In the 1-day-old post-oncomiracidia of *B. seriolae* recovered in the present study, it is clear that the openings of the anterior adhesive glands on each side are grouped in three zones. The triple nature of the pads was observed in juveniles and some adults of *E. soleae* by Kearn (1974) and, although this arrangement was not detected with the light microscope in the oncomiracidium of *E. soleae*, ultrastructural work by El-Naggar & Kearn (1983) revealed that the ducts open into three sacs on each side of the head.

The present study confirms the observation of Hoshina (1968) that the anterior attachment discs appear very early in larval development in *B. seriolae*. The discs are already partly developed in 1-day-old parasites, but an important discovery is that the anterior adhesive pads also persist throughout larval development. The pads are situated on the ventral surface of the anterolateral region of each disc and their microvillous surface reveals their triple nature in post-oncomiracidia and in adults. The area occupied by the pads increases progressively in size as the parasite grows but the lateral head glands do not appear to grow. They were detected in 2-day-old individuals but not in older specimens. As in *E. soleae* (see Kearn, 1974), it is not clear whether these cells disappear or whether they are obscured by surrounding tissues in older, bigger parasites and although the pads of *B. seriolae* continue to increase in area, the posterior median head gland cells and their ducts are relatively inconspicuous compared with those of *E. soleae*.

No anterior median head gland cells, like those found in the oncomiracidia of *Entobdella* spp. (Kearn, 1974), were observed in the oncomiracidium of *B. seriolae*, but a pair of such cells was observed in 1-day-old parasites and persists in older individuals. Moreover, many unicellular gland cells, similar to the body glands found along the lateral margins of the body in *E. soleae* (see El-Naggar & Kearn, 1983), were found opening on the ventral surface of the body in a 10-day-old specimen of *B. seriolae*, but fewer cells were found in older individuals. As in *Entobdella* spp., gland cells were associated with the haptor of oncomiracidia, post-oncomiracidia and adults, but their arrangement was a little obscure in the oncomiracidium. However, in adult *B. seriolae* four duct openings are located on the ventral surface of the haptor in similar positions to those reported by Kearn (1974) in the adult haptor of *E. soleae*.

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