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Life cycle of the marine leech (*Zeylanicobdella arugamensis*) isolated from sea bass (*Lates calcarifer*) under laboratory conditions

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ABSTRACT

Infestation of unidentified marine leeches in Malaysia was first reported in 1988 in grouper (*Epinephelus coioides*) cultured in floating cages with a prevalence of 0.4%. Recently (2004–2006), the marine leech *Zeylanicobdella arugamensis* was regularly isolated from marine fish cultured in cages. In May 2006, approximately 60% of moribund sea bass fingerlings reared in cages were infected with *Zeylanicobdella arugamensis*, which may also serve as a vector for the bacteria, *Vibrio alginolyticus*. The aim of the present study was to determine the life cycle of *Z. arugamensis* under laboratory conditions. A total of 105 adult leeches, 4.5–14.0 mm in length, from five trial experiments were sampled and brought to the laboratory. Leeches with average size exceeding 10.00 mm could deposit cocoons after 5–8 h of isolation. Seven days were needed for the new egg inside the cocoon to develop into juvenile under 27 °C at 28 ppt. It took another 9 to 10 days for the juvenile leeches to grow to mature adults. Overall, the *Z. arugamensis* took 16 to 17 days to mature. In the present study, we observed that a single leech can reproduce and this self-fertilization or direct fertilization has never been reported in a piscicolid leech.

1. Introduction

Infestation by unidentified marine leeches in Malaysia was first reported by Leong and Wong (1988) in grouper cultured in floating cages with a prevalence of 0.4%. Cruz-Lacierda et al. (2000) reported mortality within 3 days of adult cultured orange-spotted grouper, *Epinephelus coioides* due to heavy infestation of the leech, *Zeylanicobdella arugamensis*, following blood loss. Recently (2004–2006), *Z. arugamensis* has been regularly isolated from diseased and healthy marine fish cultured in cages. In Malaysia, the major species of marine fish cultured in cages were grouper (*E.* coioides, *E. lanceolatus*, *E. fuscoguttatus*), snapper (*Lutjanus johnii*, *L. argentimaculatus*, *L. stellatus*) and sea bass (*Lates calcarifer*). The leeches became pathogenic under certain circumstances. In May 2006, almost 60% mortality occurred in sea bass fingerlings reared in cages at Bt. Tambun, Penang with heavy infestation of these leeches. The infected sea bass were thin and anemic, showing clinical symptoms such as dark body discolouration, scale loss at abdomen and caudal peduncle, frayed fins and swimming restlessly at the water surface and also acting as carriers of bacteria, *Vibrio algniolyticus* (Kua et al., 2006). An estimated 70% of the sea bass fry placed in the cages after 2 weeks were infected with both leeches (*Z. arugamensis*) and bacteria (*V. algniolyticus*).

The first report of *Z. arugamensis* from Malaysian waters was from the seahorse *Hippocampous kuda* and unidentified eel species by De Silva and Fernando (1965). In our present study, we observed that it also infects the grouper *Epinephelus tauvina* and cobia *Rachycentron canadum*. Apart from the occurrence of *Z. arugamensis* in grouper (*E. coiodes*) and its role as a vector for trypanosomes, almost nothing is known about its biology (Crusz-Lacierda et al., 2000; Hayes et al., 2006). The present study aimed to determine its life cycle under laboratory conditions.

2. Material and methods

2.1. Source of adult leeches

A total of 147 adult leeches with a range of 10.9–12.5 mm in length from five trial experiments were sampled from the cages in Bukit Tambun, Penang, Malaysia (Latitude: 5.2° and Longitude: 100.4°). Adult leeches were isolated by hand and placed into glass container with 300 ml seawater before transporting to the laboratory.

2.2. Cocoon deposition and hatching

Active adult leeches were chosen and placed individually in petri dish containing 20 ml (90 mm in diameter) of filtered seawater at 28 ppt salinity obtained from the sites. A clean slide was placed in each petri dish to observe the cocoons development. The number of eggs laid on the slide by each individual adult leech was recorded

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Table 1
Number of adult leeches, cocoons deposited and hatched juveniles used in the present study.

Trial	Sampling date	Number of adult leeches	Average size (Mean ± std) mm	Reproductive system	Number of cocoons deposited	Number of leeches hatched (%)
1	29th Jan. 2007	16	12.5 ± 0.1	Ovary and testis present	58	29 (50)
2	28th Feb. 2007	25	10.9 ± 0.2	Ovary and testis present	393	253 (64.38)
3	24th July 2007	11	5.2 ± 2.1	Ovary and testis present	0	0
4	20th Aug. 2007	45	4.5 ± 1.0	Ovary and testis present	0	0
5	6th Sept. 2007	8	11.0 ± 3.0	Ovary and testis present	145	80 (55.17)
Total		147			596	362 (60.73)

under 4× and 10× magnification of a dissecting microscope (Leica Zoom 2000) connected to a digital camera (Leica DFC 320) linked with computer software (Leica QWin). The adult leeches were incubated at 27 °C. Water was exchanged 50–60% every 2 days with new sterile filtered seawater. During incubation, the cocoons were kept in an incubator at 27 °C. Observations were carried out for 20 days after which the adult leeches were removed. However, egg development in the same petri dish was observed for a month after the cocoon was deposited.

Twenty cocoons attached to the slide were sampled randomly for observation under dissecting or compound microscope (Leica DM5000B) at 4 h intervals. The embryonic morphology was recorded by using a stereomicroscope camera and the size of eggs was measured using image analysis software. The number of juvenile leeches hatched was also recorded.

2.3. Experimental design of juvenile transmission to uninfected sea bass fry

Uninfected sea bass fry (3–5 cm, $n = 200$) was purchased from a private hatchery and placed in 300 l aquaria with a bio-filter system. Fish were fed with commercial pellet twice a day with 10% of water exchange daily. Uninfected fishes were placed individually in 100 ml of sterile seawater (120 ml glass jar) and gently aerated. A total of 31 active juvenile leeches were sampled randomly under a dissecting microscope. The juvenile transfer was carried out under a dissecting microscope. Briefly, a glass rod with blunt end was used to bring active juvenile leeches from the petri dish into the 120 ml jar containing only one uninfected fish. The site of infection was on the caudal fin and only the juvenile which successfully attached to the fish was used for further observations. About 50% of water was changed throughout the experiment. Leech development was observed every 6 h under dissecting microscope and terminated when the juvenile reached the adult size. Leeches were removed when they reached the size ranging from 10 to 12 mm or a cocoon was seen on the wall or bottom of the glass jar. The leeches were placed individually in petri dishes containing sterile sea water (28 ppt) and incubated at 27 °C. Daily observation on egg laying was done under dissecting microscope.

2.4. Observation of direct fertilization of marine leech

A total of 3–5 juvenile leeches with a range of 1–2 mm in length from the newly hatched cocoon were chosen for fertilization observation. An individual juvenile leech was introduced into 120 ml jar containing only one uninfected sea bass fry. The transmission method was the same as described earlier. Development of leech from juvenile up to adult stage and able to produce cocoons was observed every 6 h under dissecting microscope. The experiment was terminated when a juvenile leech hatched from the cocoon in each jar.

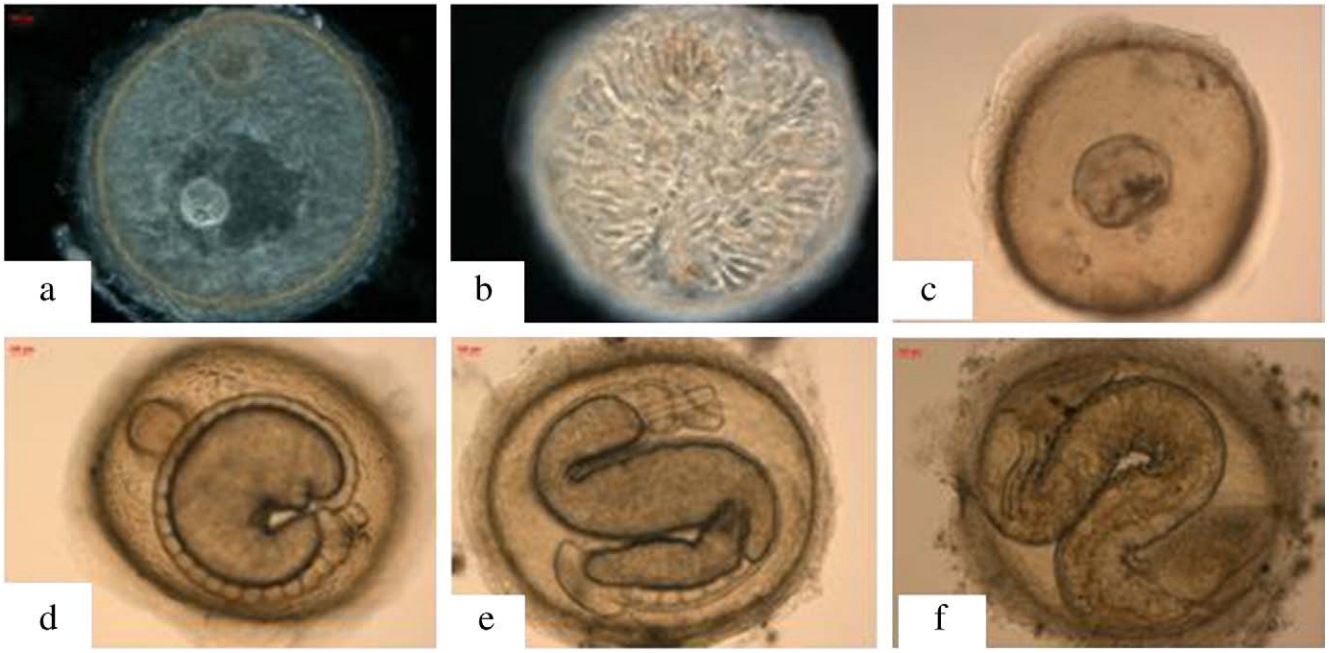

Fig. 1. Embryonic developement of *Z. arugamensis* at 27.00 ± 0.5 °C. (a) Newly produced cocoon; (b) Early blastula; (c) Early gastula; (d) Late gastrula; (e); Free embryo and (f) Juvenile. Scale bar, 1.2 cm = 100 μm.