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Infestation of gill copepod *Lernanthropus latis* (Copepoda: Lernanthropidae) and its effect on cage-cultured Asian sea bass *Lates calcarifer*

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Abstract. Twenty Asian sea bass *Lates calcarifer* from a floating cage in Bt. Tambun, Penang were examined for the presence of parasitic gill copepod, *Lernanthropus latis*. The prevalence of *L. latis* was 100% with the intensity of infection ranging from 1 to 18 parasites per host or 3.75 of mean intensity. Female parasites having oblong cephalothorax and egg-strings were seen mainly on the entire gill of examined Asian sea bass. The infected gill of Asian sea bass was pale and had eccessive mucus production. Under light and scanning electron microscopies (SEM), *L. latis* was seen grasping or holding tightly to the gill filament using their antenna, maxilla and maxilliped. These structures are characteristically prehensile and uncinate for the parasite to attach onto the host tissue. The damage was clearly seen under SEM as the hooked end of the antenna was embedded into the gill filament. The parasite also has the mandible which is styliform with eight teeth on the inner margin. The pathological effects such as erosion, haemorrhages, hyperplasia and necrosis along the secondary lamellae of gill filaments were seen and more severe at the attachment site. The combined actions of the antenna, maxilla and maxilliped together with the mandible resulted in extensive damage as *L. latis* attached and fed on the host tissues.

INTRODUCTION

The intensification of production in aquaculture often sees abnormalities in fish behaviour, changes in body colouration, lesions, haemorrhages and ulcerations of the cultured fish (Leong *et al*., 2006). Lack of health management measures for that condition could lead to mortality of the cultured fish. Parasitic infestations associated with other diseases have been responsible for many problems in fish reared in net-cage environment (Leong, 1994; Leong *et al*., 2006). Isopoda, Branchiura and Copepoda are the three major groups of parasitic crustaceans commonly found on fish hosts. Of these, parasitic copepods are the most common and cause increasingly serious problem in cultured fishes (Leong, 1994; Noga, 2000; Boxshall & Halsey, 2004; Johnson *et al*., 2004). In Malaysia, parasitic copepod infestations particularly of the genera *Caligus* spp. have been reported widely as compared to *Lernanthropus* spp. (Leong 1984, 1985, 1986; Venmathi Naran *et al*., 2009, Ho *et al*., 2011, Kua & Muhd-Faizul, 2010, Muhd-Faizul *et al*., 2012 and Leaw *et al*., 2012).

The genus *Lernanthropus* (Copepoda: Lernanthropidae) consists of more than 100 species of gill parasites and some species such as *Lernanthropus kroyeri* can cause high mortalities in smaller-sized European sea bass *Dicentrarchus labrax* (Kabata, 1979; Ho & Do, 1985; Athanassopoulou *et al*., 2001; Henry *et al*., 2009). As compared to other copepods infecting gills, copepods such as Lernanthropids are larger in size and can be seen with the naked eye. It usually feeds on the gill tissues and blood of its host which can seriously damage host tissues (Kinne, 1984). Normally they cause only minor harm

to their hosts when present in small numbers. However, heavy infections can cause severe damage to gill tissues and respiratory impairment accompanied by secondary infections and result in stress and osmoregulatory failure. Toksen (2007) reported that cultured *D. labrax* infested with *L. kroyeri* in the gill show signs of respiratory distress, increase in mucus production and swim in surface water. Lamellar necrosis, anaemia and secondary infection bacterial infection have also been reported in European sea bass infested with *L. kroyeri* (Manera & Dezfuli, 2003). *Lernanthropus* sp. is not host specific although some other species are strictly host specific (Sharp *et al.*, 2003). Infection of *Lernanthropus* species have been reported in marine teleosts and cultured species such as *Lernanthropus calcarifer* in the Gulf of Thailand (Humphrey *et al.*, 2006), *D. inbrax* in Australia (Sharp *et al.*, 2003) and yellowtail kingfish *Seriola lalandi lalandi* in New Zealand (Ho & Kim, 2004). In Malaysia, infestation by this group of parasitic copepods (Lernanthropidae) was observed in cage cultured seabass (*L. calcarifer*) in early 2004 in Penang waters during our on-going monitoring program on ectoparasites of marine fish farm.

However, the economic impact of *L. latis* infestation and its pathology on the Asian sea bass is not known. The only report was on the morphological and infection study of *L. latis* on the gills of cage-cultured sea bass in the east coast of Malaysia by Sabri & Shaharom (2008). Hence, the present study aims to investigate the pathological changes in Asian sea bass gill infected with *L. latis* based on light microscopy, histology and scanning electron microscopy.

MATERIALS AND METHODS

Twenty live cage-cultured Asian sea bass were obtained from a floating cage culture locality at Bukit Tambun, Penang, Malaysia. The live fish were transported to the wet laboratory in oxygenated tank filled with seawater and examined within 24 hours. The fish were euthanized with clove oil based on Cooke (2004) and weighed before undergoing necropsy process. The gills from each fish were removed, placed in a petri dish consisting of 0.85% normal saline and examined under dissecting microscope. The copepods were removed from the gills using fine forceps and fixed in 70% alcohol for further identification. The identification and prevalence of *Lernanthropus* species was based on Kabata (1985) and Bush *et al.* (1997) respectively.

For histology investigation, the gills with gill copepods were fixed in 10% buffered formalin for 24 hours. The samples were processed (Leica ASP 300) and embedded in paraffin wax. The embedded specimens were sectioned at 5 micrometers, stained with haematoxylin and eosin (H&E) and finally mounted with DPX before the sectioned samples were examined under compound microscope (Leica DM5000B) connected to a digital camera (Leica DFC 320) associated with computer software (Leica QWin). The histological techniques were based on Humason (1979).

For scanning electron microscopy gill samples (n = 5) with attached gill copepods were placed in 0.85% normal saline for further washing. The specimens were fixed in McDowell-Trump fixative at 4°C for 2-24 hours before washing in 0.1M Phosphate buffer (pH 7.2) for 10 minutes (repeated thrice) and post-fixed in 1% aqueous osmium tetroxide for 1-2 hours (McDowell & Trump, 1976). The specimens were further washed in distilled water for 10 minutes (repeated twice) before dehydrated in a series of ethanol (50%, 75%, 95% and 100%). Tissue samples were dehydrated in 1-2 ml of hexamethydisilazane (HMDS) for 10 minutes and air dried at room temperature. The dried sample was mounted onto a SEM specimen stub using a double-sided sticky tape and the sample was coated with gold before viewing under LeoSupra 50VP Field Emission SEM equipped with Oxford INCA400 energy dispersive x-ray microanalysis system at magnifications of 25x – 10Kx. They were processed using Hexamethyldisilazane to prepare soft insect tissues for scanning electron microscopy according to Nation (1983).

RESULTS AND DISCUSSION

A 100% prevalence was seen in the twenty Asian sea bass (average weight 56.1 ± 18.6 g) with the number of *L. latis* found on each fish ranging from 1 to 18 (Table 1 and Figure 1). Based on the morphology, the gill copepod was identified as *L. latis* Yamaguti 1954. Female *L. latis* with characteristic oblong cephalothorax and egg-string were seen on most of the gills of examined sea bass (Figures 2 & 3). The average body length of

Table 1. Summary of gross observation findings on the gills of Asian sea bass

Fish No.	Weight (g)	Gross observation of gills		Examination of gill copepods
		Pale in colour	Excessive mucus	*Lernanthropus latis* (number)
1	45	–	–	+ (2)
2	65	+	+	+ (5)
3	55	–	–	+ (1)
4	30	–	–	+ (2)
5	64	–	–	+ (1)
6	58	–	–	+ (2)
7	33	+	+	+ (9)
8	42	–	–	+ (2)
9	56	–	–	+ (1)
10	36	–	–	+ (2)
11	58	–	–	+ (2)
12	56	+	+	+ (5)
13	33	+	+	+ (7)
14	42	+	+	+ (7)
15	62	–	–	+ (2)
16	47	+	+	+(18)
17	88	–	–	+ (2)
18	73	–	–	+ (1)
19	86	–	–	+ (2)
20	94	–	–	+ (2)
Prevalence (%)	56.1 ± 18.6	30	30	100 (1–18), M.I (3.75)

Note: + (present), – (absent), Prevalence (number of individuals of a host species infected with parasite ÷ number of hosts examined) and Mean intensity (M.I)

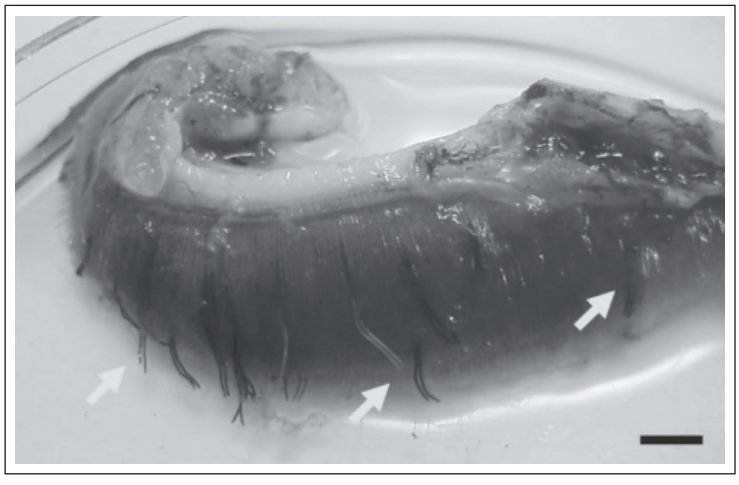

Figure 1. The gills of Asian sea bass infected with *Lernanthropus latis* (arrow). Scale = 0.26 cm

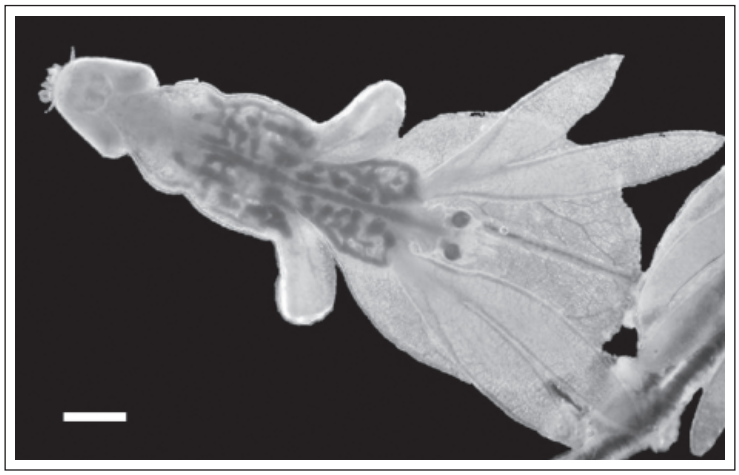

Figure 2. A live female *Lernanthropus latis* without the egg-strings under light microscopy. Scale = 2 µm

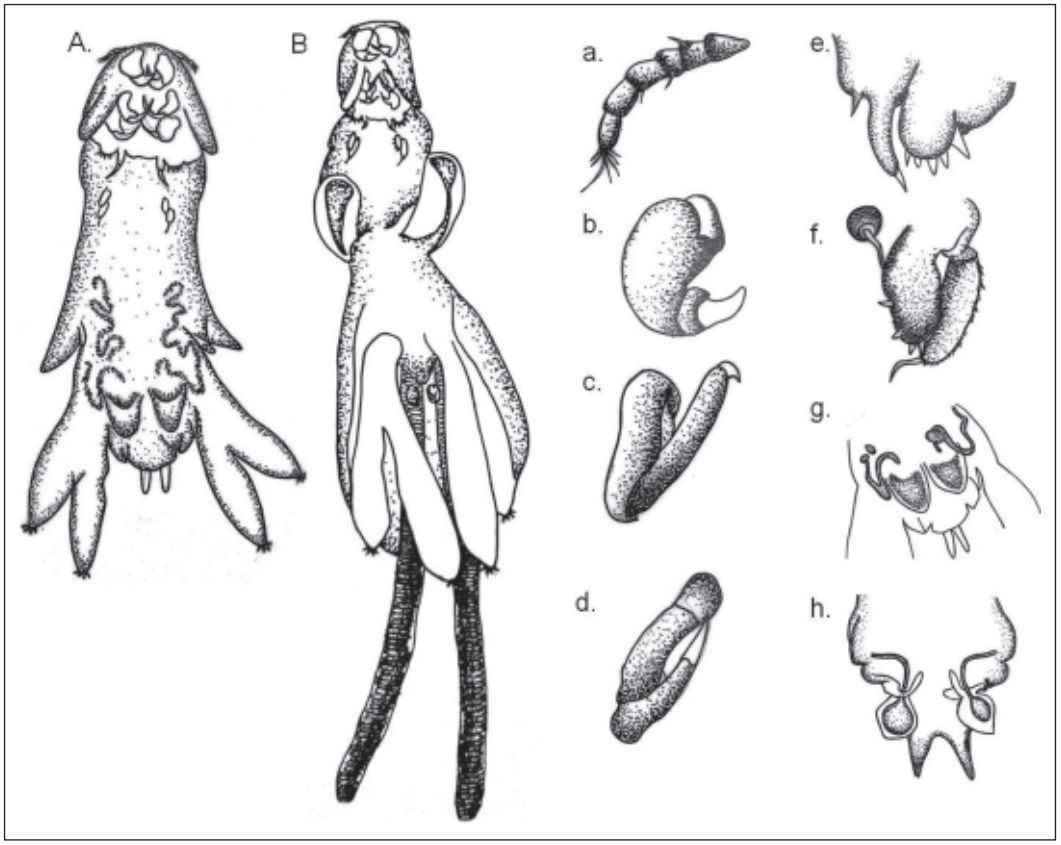

Figure 3. Schematic drawing of *Lernanthropus latis* based on the light microscopy and scanning electron microscopy. A. Male, ventral view; B. Female, ventral view; a. antennule; b. antenna; c. maxilla; d. maxilliped; e. leg 1; f. leg 2; g. posterior extremity of male, ventral view; h. posterior extremity of female, ventral view

the female parasite was 4.45 ± 0.37 mm. In the present study, the heavily infested Asian sea bass of *L. latis* showed pale gills and excessive mucus production which indicated anaemic condition. Similar symptoms have been reported in fish infested with *L. kroyeri* in European sea bass (Toksen, 2007). The present study also showed that fish infested with a few *L. latis* (1 to 2) do not show any sign of abnormality as compared to heavily infested fish (5 to 18). Generally, fish infected with *Lernanthropus* spp. showed pathological symptoms such as respiratory distress, lethargy, dark coloured skin, increase in mucus secretion and mortality in small fish (Henry *et al.*, 2009).

Under SEM and light microscopy observation, the antenna, maxilla and maxilliped of *L. latis* were able to grasp or hold tightly to the gill filament (Figures 4a & b). The structure of the antenna was also seen under SEM with characteristically prehensile and uncinate which are used by the parasite to attach or feed on the host tissue (Kabata, 1985). The damage was clearly seen under SEM as the hooked end of the antenna was embedded on the gill filament. Several rows of minute setae were seen on the antenna (Figure 4c). The mandible was long and thin with eight teeth on the inner margin (Figure 4d). Observation under light microscope by Ho *et al.* (2011) showed that lernanthropids used their prehensile antennae, maxillipeds and the third leg to attach on gill filaments. We further observed that only the antenna, maxilla and maxilliped play a role in the attachment to gill filaments under light microscopy. The possible reason could be due to the loose attachment by the first and second legs of *L. latis* to the host's gill filaments as compared to the antenna, maxilla and maxilliped.

Histopathological changes such as haemorrhages, hyperplasia, and necrosis along the secondary lamellae of gill filaments were seen with greater severity at the site of attachment (Figure 5). These findings were similar to the damage done by *Lernanthropus* sp. to the gills of sea cultured barramundi (*D. inbrax)* reported by Humphrey *et al.* (2006) with localised destruction of gill tissue with haemorrhages at the attachment sites. *Lernanthropus kroyeri* was known to cause histo-

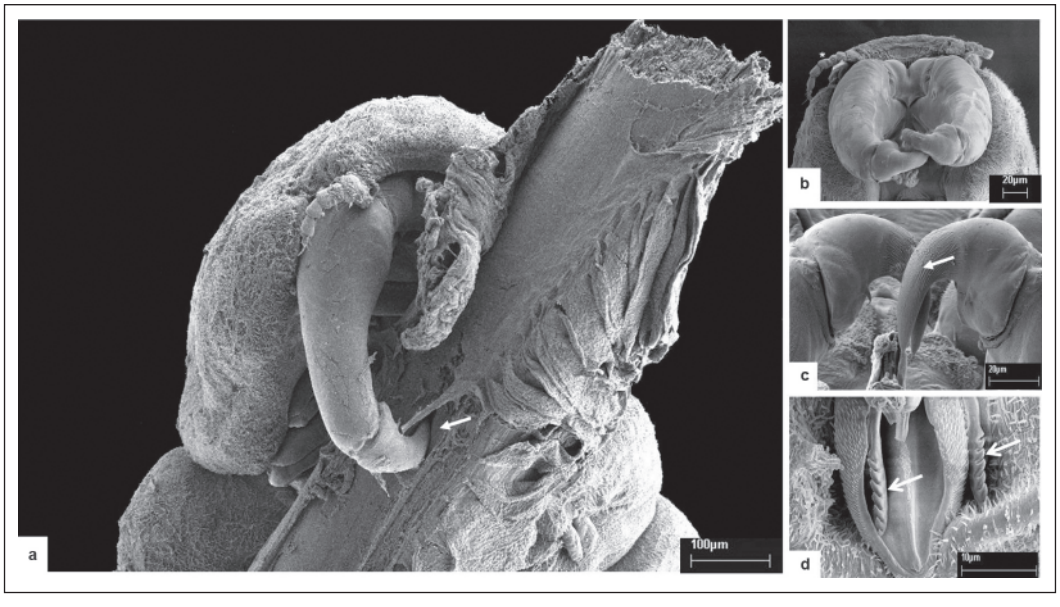

Figure 4. An Adult female *Lernanthropus latis* attached to gill filament observed under scanning electron microscope (a). The attachment of adult female *Lernanthropus latis* at the gill filament with the antenna (arrow), Scale = 100 µm, (b) Close view of the antenna of adult female *L. latis* without the gill filament, Scale = 20 µm, (c). Close view of the hooked end of antenna (arrow), Scale = 20 µm and (d). The mandible (arrow) with 8 teeth on inner margin, Scale = 10 µm

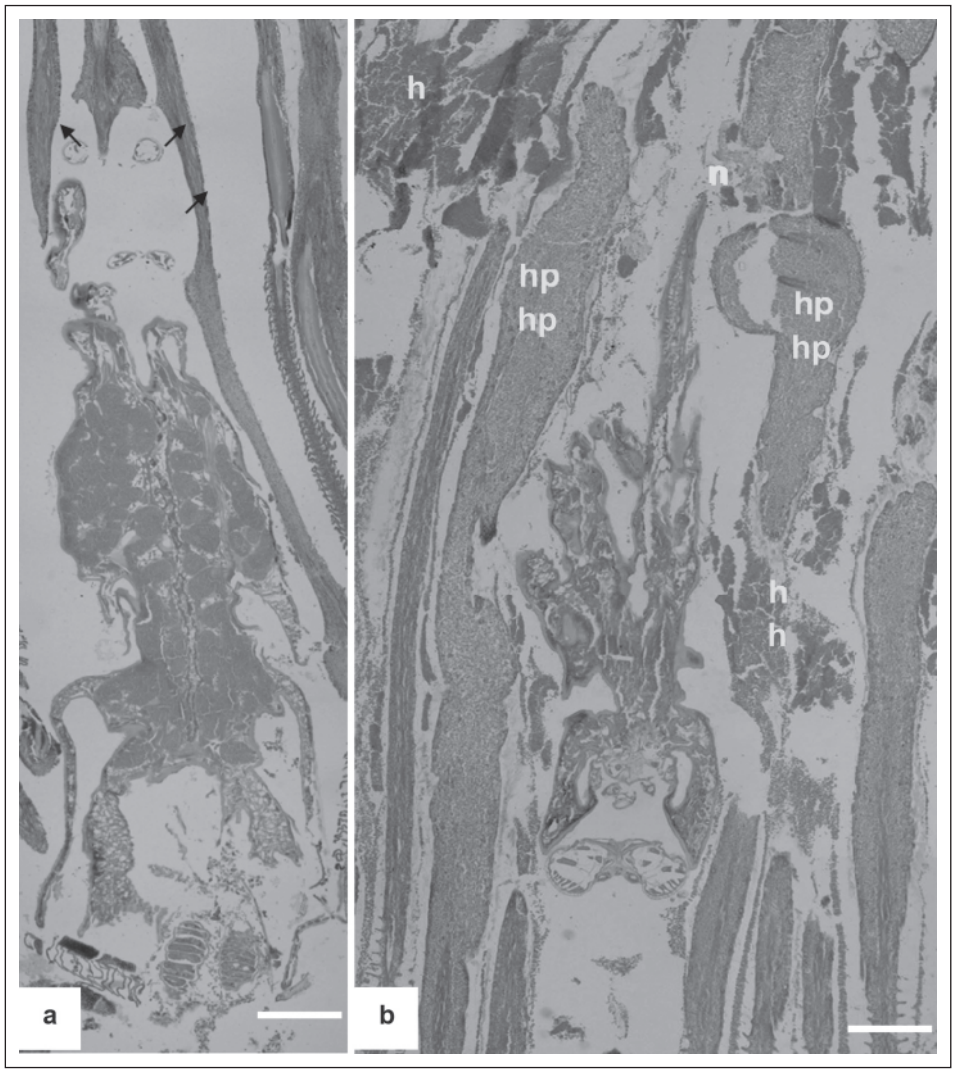

Figure 5. Histopathological studies on the gill filament infested with *Lernanthropus latis*. (a). erosion (arrows) at the gill filament caused by the attachment using the head of *L. latis* and (b). Close-up of another histopathological section of the gill filament infested with *L. latis* showing haemorrhages (h), hyperplasia (hp) and necrosis (n) along the secondary lamellae. H & E. a). Scale = 3 µm and b) Scale =2.5 µm

pathological damage that facilitates secondary infection (Manera & Dezfuli, 2003). Infestations of lernanthropids in mostly marine fish were reported to cause pathological symptoms such as desquamation and necrosis to the secondary lamellae near the site of attachment (Jithendran *et al.*, 2008). In their reports, lernanthropids were considered as serious pathogens in many species of wild fish and cage-cultured sea bass. Pathological changes such as necrosis in epithelial tissue and ligament, increase in mucus secretion and narrowing of capillary veins are commonly observed when lernanthropids attach to the gill filament with their third legs (Kinne, 1984). However, our present study shows that the damage by *L. latis* was caused by the combined actions of the antenna, maxilla and maxilliped together with the mandible when the parasite attached itself and fed on the host tissues. Despite the 100% prevalence of *L. latis* infestation in Asian sea bass, no mortality was reported by the fish farmer. We believe that the low

number of *L. latis* (mean intensity = 3.75) were not severe enough to cause mortality. However, extensive gill damage due to the attachment and feeding of *L. latis* could lead to loss of fish respiratory surface. The open wounds at the gills could also lead to secondary infections.

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