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Case Reports

A CASE OF GILL COPEPOD (*Lernanthropus latis*) INFESTATION IN AN ASIAN SEABASS (*Lates calcarifer*) NET-CAGED CULTURE IN KELANTAN

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SUMMARY

Lernanthropus latis (L. latis) is a parasitic copepod that attaches to gill filaments of fish. The parasitic copepods feed on blood, mucus and skin of the host causing mechanical injuries which may serve as a vector for primary viral and bacterial infections resulting in fish mortality. In this report, *L. latis* infestation was found in an Asian seabass net-caged culture site in Kelantan since early September 2017. The main complaint of farmer was that numerous elongated reddish parasitic organisms were seen attaching onto the gills filaments. A series of diagnostic workups were performed to identify the parasite and other disease conditions that may affect the fish due to the parasitic infestation. Gills, kidney, liver, and spleen were taken for histopathology, bacteriology and parasitology examinations. Morphological examination of the parasites revealed the presence of *L. latis.* Cultures of kidney, liver and spleen on thiosulfate-citrate-bile-sucrose agar yielded pure growth of *Vibrio* species but there were no clinical signs and histopathological lesions which were indicative of vibriosis. Water quality test indicated low dissolved oxygen of 2 ppm. The case was confirmed to be *L. latis* infestation. Treatment options were discussed with the farmer but logistically it is difficult to implement. The underlying problem was identified to be organic matter sedimentation and overload in the lagoon bottom due to unsustainable aquaculture practices since the operation of this culture site in 2003. Thus, good aquaculture practices were recommended as a long term strategy to overcome the parasitic copepod infestation in this culture site.

Keywords: Asian seabass, Gill copepod, Lernanthropus latis, net-caged culture, sustainable aquaculture, Kelantan

INTRODUCTION

Asian seabass, *Lates calcarifer* (*L. calcarifer*) (Bloch, 1790), is a catadromous teleost that spawns in marine water and undergoes parts of its juvenile development in the freshwater. It is euryhaline fish which can tolerate a wide range of salinity during its growing stage. It has become one of the most promising fish species for aquaculture due to its high fecundity and ability of its juveniles to wean to pelleted feed (Jerry, 2014).

Lernanthropus latis (Yamaguti, 1954), is one of the most common gill copepods of the family Lernanthropidae that is found in Asian seabass in Malaysia (Sarimah and Shaharom-Harrison, 2008 and Khalid and Shaharom-Harrison, 2014). The parasitic copepods attach to the gill filaments of the fish by using their piercing antennae assisted by its maxillipeds and 3rdlegs (Khalid and Shaharom-Harrison, 2014). Parasitic copepods are known to be potential disease causing agents and responsible for many primary disease outbreak such as vibriosis in marine fishes. (Stewart, *et al.*, 2004).These parasitic copepods feed on blood, mucus and skin of the host which may cause mechanical injuries and serve as a vector for primary viral and bacterial infection resulting in fish mortality. The prevalence of *L. latis* infestation in net-caged Asian seabass culture was reported to be as high as 100% in Malaysia (Kua *et al.*, 2012) and ranged between 80 -100% in Australia (Brazenor and Hutson, 2013). However, its prevalence was 0% in land-based cultured fish in Australia as the eggs require a salinity of at least $22^0/_{00}$ to hatch. The optimal salinity and optimal temperature for the parasite to complete its life cycle are $35^0/_{00}$ and 22°C to 34°C respectively (Brazenor and Hutson, 2013). Hence, manipulation of salinity and water temperature are strategies used to prevent and control the infestation of this parasitic copepod in brackish water culture fish.

This paper reported a case of gill copepod (*L. latis*) at the net-caged culture site in Sri Tujuh Lagoon, Tumpat, Kelantan in early September 2017 and discussed the treatment options as well as the environmental and husbandry factors that may affect its abundance in brackish water fish culture.

CASE REPORT

History

The affected Asian seabass net-caged culture is located in Sri Tujuh Lagoon, Tumpat, about 30 km from Kota Bharu, Kelantan. The site was established in 2003 that is 15 years ago with a total area of 24 hectares. There are around 2000 cages owned by about 98 farmers with an average production of about 120 to 150 tonnes per annum that worth RM 1.5 million (Ong *et al*., 2017). Since the beginning of September 2017, the farmers in this area have been complaining about parasites infesting on the gills of the Asian seabass. However, no apparent increased of fish mortality was observed.

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Table 1 Average reading of water quality testing in affected net-caged culture site

Parameters	Average Readings	Optimal range (Ong *et al.*, 2017)
Dissolved oxygen (DO)	2 ppm (low)	5 ppm
pH	7.52	7.5 – 8.5
Salinity	16 $^{0}/_{00}$	20 – 30 $^{0}/_{00}$
Temperature	30 °C	26 – 30°C
Turbidity	46 cm	30 – 45 cm

On-site Investigation and Laboratory Findings

Culture site investigation on the affected net cages showed a lot of Asian seabass swimming near the water surface of the cages. Water quality tests on dissolved oxygen (DO), pH, salinity, temperature, turbidity were taken from several culture cages and the average readings were tabulated in Table 1. Monthly average water quality records were available in this culture site as it was monitored regularly by a graduate intern under a knowledge transfer programme grant from the Ministry of Higher Education for the last two years. From the records, it was noted that salinity in this cultured sites ranged from 16-22 $^{0}/_{0}$ in the months of February to September 2017. This water salinity was conducive for the lifecycle of parasitic copepods except during the monsoon season between the months of November 2017 to January2018when the salinity of the water will drop until 0-5 $^{0}/_{0}$. Hence, copepods were normally not seen during the monsoon season when the water salinity is low. From the records, water temperature in this culture site ranged between 22-30^{o}C throughout the year.

A total of six fishes of approximately 15 cm in length / 60 g each were collected from the affected net-caged culture sites for post-mortem examination. Grossly, there was no visible lesion on the body and fins of the fishes. On opening the operculum of the fishes, the gill filaments appeared pale, with numerous reddish elongated parasitic organisms measuring approximately 0.3 – 0.6 cm long attaching parallel to the gill filaments (Figure 1). The intensity of infestation ranged from 6 to 20 parasites per host. Microscopic examination of the gills revealed that the parasite has two strings of egg sacs attaching to its trunk (Figure 2). The parasites were then removed from the gill filaments and observed under microscope at a higher magnification. From the morphology of the parasites' antennae, millipedes, 3rd and 4th legs and two strings of yolk sacs with eggs within (Figure 3), the parasite was confirmed to be *Lernanthropus latis* (Kabata, 1979). Histopathological lesions of affected gills revealed fusion of primary and secondary gill lamellae, gill filament erosion and hyperplasia of the epithelia cells (Figure 4).

There was no visible lesion on the kidney, liver and spleen. Bacteria culture from the kidney, liver and spleen on thiosulfate-citrate-bile sucrose agar (TCBS) agar yielded *Vibrio* species by biochemical tests. The colonies appeared yellowish in colour, measuring about 2–3 mm in diameters with translucent periphery and a darker centre. However, there were no apparent clinical sign and histopathological lesion which were indicative of septicaemia due to vibriosis in the fish. From the

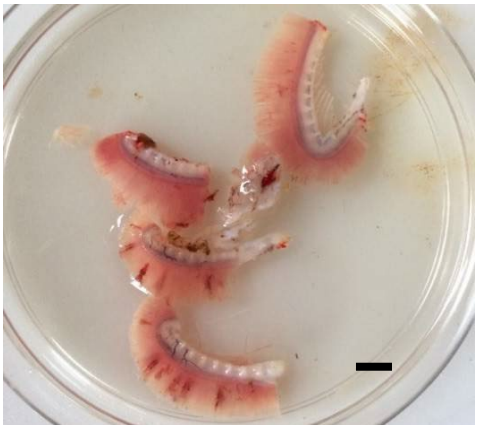

Figure1. Pale gill filaments with numerous reddish elongated parasitic organisms attaching parallel to the gill filaments. (Scale bar 0.5 cm)

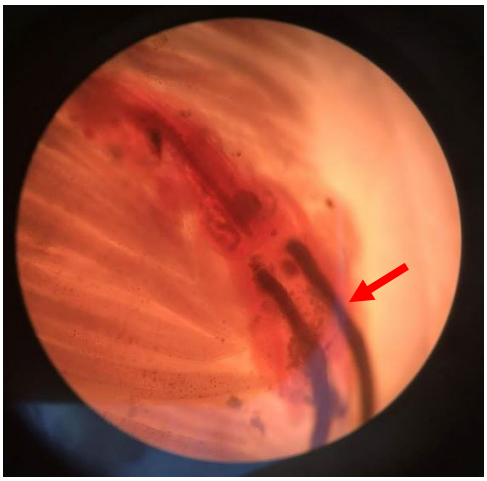

Figure 2. Appearance of *Lernanthropus latis* with egg-strings (arrow) (Mag. 40x)

diagnostic workups and morphology of the parasite, it was confirmed that the parasitic gill copepod infestation on the Asian seabass was *L. latis.*

DISCUSSION

Lernanthropus latis attaches to the gill filament by using its antennae, maxilla and maxillipeds assisted by its 3rd and 4th legs. The attachment of these appendages and feeding activities of the parasitic copepods have caused the histopathological changes as seen under histopathology in this case. Heavy infestation can cause severe damages which resulted in co-infections with viral and bacterial diseases. Parasitic copepod can be a vector to other disease causing agents which may result in mass

mortality. The mean intensity of infestation of *L. latis* was 3.8 parasites per host in Malaysia (Kua *et al.*, 2012), about 5 per host in Australia (Brazenor and Hutson, 2013) and 2 to 24 per host in India (Sethi and Sas, 2017). However, this level of intensity was not severe enough to cause mass mortality in fish (Kua *et al.*, 2012). In this case, the intensity 6-20 per host is considered not high enough to cause death in fish. Furthermore only mild hyperplasia of epithelia cell with fusion of some primary and secondary lamellae and some erosion of gill filaments were seen under histopathology (Figure 4).

From the water quality readings, it was noted that the average DO at 2 ppm was too low for the fish while all the other parameters were within the optimal range for the fish. The low DO would further aggravate the gill copepod infestation and the fish will become more susceptible to mass mortality due to hypoxia.

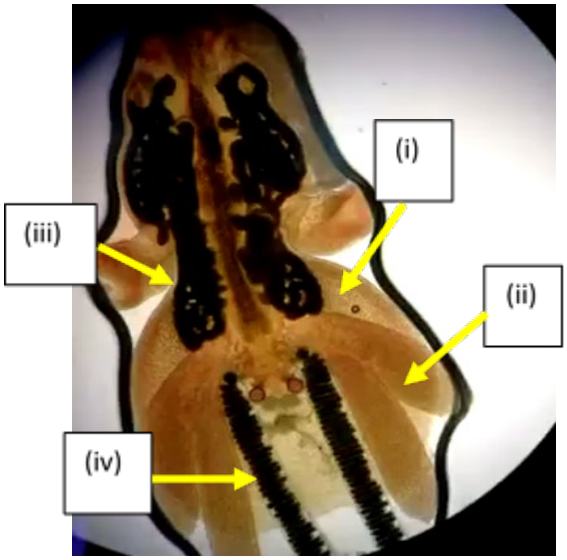

Figure 3. Ventral view of adult female of *Lernanthropus latis* with (i) 3rdleg, (ii) 4th leg, (iii) genital organs, (iv) two string of yolk sacs

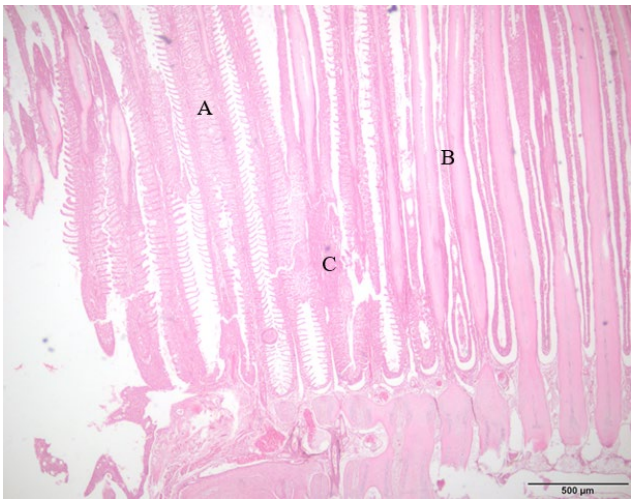

Figure 4. Histopathological lesions on the gill filament infested with *Lernanthropus latis*. A. Fusion of primary lamellae; B. Gill filament erosion; C. Hyperplasia of cells. (H&E 40X).

Some treatment options were discussed with the farmers. These included manually removing the gill copepods by using forceps or giving freshwater bath to the fish for 5-20 minutes. Manual removal of parasites was logistically impossible when it comes to treating the whole school of fish in the cages. There was also an issue with the water bath treatment as there was no freshwater piping to the cage site and to bring sufficient freshwater from the shore to the cage was a problem. Advice on adding Oxytetracycline into feed at 0.5g/kg of fish pellets for seven days as a prophylaxis was also proposed to prevent vibriosis, particularly in September, the non-monsoon month because the water salinity is conductive for parasitic copepods infestation. During monsoon season as the water salinity dropped to 0-5 $^{0}/_{00,}$ the parasitic copepods just disappear because the eggs will not hatch due to low water salinity. Chemical treatment of copepods using formalin and dichlorvos was not recommended since this a food fish culture site. Chemical such as formalin is only used in hatchery at 110 ppm for 45 – 60 minutes or dichlorvos at 0.75 ppm for 30 minutes bath during severe infestation. This is because we cannot reduce salinity during the eggs hatching stage (Sethi, 2015). However, all these treatment options may not necessary solve the real underlying problems. Our investigation has revealed that, poor practices accumulated in net-cage farming since the last 15 years were the root cause. There were evidences of overstocking and overfeeding by the farmers who also used trash fish as a supplementary feed for the fishes. This has resulted in the accumulation and sedimentation of organic matters in the lagoon bottom. The lagoon bottom has never been dug since its operation and this has resulted in poor water circulation. Many cage cultured sites were also very shallow which resulted in poor water quality particularly low DO during the early morning time as there is no photosynthesis taking place at night.

Since gill copepods infestations are unavoidable in net-caged culture in this site, the farmers were advised to practice good aquaculture practices as a long term strategy to remedy the problem. They were told not to overstock and over-feed the fish. Dead fish must be brought to shore for proper waste disposal. Paddles wheels were provided by the Department of Fisheries (DOF) to improve DO. DOF has also planned to deepen the depth of the lagoon by digging out the organic matters from the lagoon bottom regularly.

CONCLUSION

Seabass in net-caged culture in Sri Tujuh Lagoon was diagnosed with infestation of gill copepod, *Lernanthropus latis* based on the morphology of the parasite and the post-mortem pathologies. Treatments options can be suggested but are usually not very practical. A long term prevention and control strategy should include good aquaculture practice to overcome the environmental problems and to achieve sustainable aquaculture.

CONFLICT OF INTEREST

None of the author has any conflict of interest in this case report**.**

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