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***Cymothoa indica* Schioedte and Meinert, 1884 (Crustacea: Isopoda: Cymothoidae) infestation in cage-cultured Asian seabass *Lates calcarifer* (Bloch, 1790) from the south-west coast of India**

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ABSTRACT

Infestation of the isopod parasite *Cymothoa indica* in Asian seabass *Lates calcarifer* (Bloch, 1790) fingerlings stocked in low volume cages is reported here. Hatchery produced seabass fry, reared to fingerling size (average size, 7 ± 1 g and 63 ± 12 mm) were used for stocking cages in Ashtamudi Lake, Kerala, south-west India. Instances of mortality were first observed on the 25th day of culture (DOC). By the 35th DOC, cumulative mortality of 84.6% was recorded. Intensity of parasitic infestation was found to be one parasite per fish. Parasites were observed to be latched to the tongue of the host fish. Reddening of buccal cavity, dark pigmentation, tongue degeneration/replacement were observed in the affected fish. On the 35th DOC, surviving fish in the cages were examined and the prevalence of *C. indica* was 53.08% in the remaining live fish. Though experimental infestation of Asian seabass by *C. indica* has been reported previously under laboratory conditions, this forms the first report on infestation of *C. indica* in cage-cultured Asian seabass.

Keywords: Asian seabass, Cage culture, *Cymothoa indica*, Isopoda, *Lates calcarifer*

The Asian seabass *Lates calcarifer* (Bloch, 1790) is one of the alternative species for promoting diversity in farmed brackishwater species. In India, cage culture in brackishwater is a relatively recent practice and its adoption has seen a gradual increase in the past decade through low volume cage culture of *L. calcarifer* (Vijayan *et al.*, 2015) and pearlspot, *Etroplus suratensis* (Prasad Kiran *et al.*, 2014). Pro-active initiatives for addressing the issues related to fish health is important for sustainable aquaculture development (Bondad-Reantaso *et al.*, 2005).

Cymothoid isopods comprise about 40 genera and over 380 accepted species (Smit *et al.*, 2014). Among these, 45 species infesting fishes have been reported from India (Rameshkumar *et al.*, 2016; Aneesh *et al.*, 2017). *Cymothoa indica* infestation in Asian seabass larvae was first reported under laboratory conditions by Rajkumar *et al.* (2005b). Cymothoid infestation was also reported in the long whiskered catfish *Mystus gulio* cultured in cages at Vellar Estuary in the south-east coast of India (Rajkumar *et al.*, 2005a). The present report is of *C. indica* parasitising and causing significant mortality in Asian seabass cultured in low volume cages in the south-west coast of India.

Fish were cultured in five cages off Kochuthuruthu Island, Chavara, Kollam in Ashtamudi Lake ($8^{\circ}57'28.4''N$;

$76^{\circ}32'38.7''E$), Kerala, India. Double-walled, low volume HDPE net cages (1.5 x 1.5 x 1m) of mesh size 12 mm were fixed in shallow area adjoining Kochuthuruthu Island in Ashtamudi Lake. *L. calcarifer* fry stocked were produced at finfish hatchery, Muttukadu experimental station of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai and reared to fingerling size in net cages at Pulicat Lake, Tamil Nadu. Fingerlings of average initial size 7 ± 1 g and 63 ± 12 mm (total length, TL) were stocked at 200 individuals per cage at a stocking density of 89 nos. m^{-3} . Fishes were initially fed on pellet feed and gradually weaned (within 3-4 days) to feed on trash fish, sardines and anchovies at 20% of the average fish body weight per day (twice daily). Size grading was done 10 days after stocking as a management measure to minimise cannibalism.

The water quality parameters were regularly monitored during the culture period. Temperature and pH were measured using a digital meter (Lab India, India), salinity was measured using refractometer (Atago, India). Ammonia-N and nitrite-N were analysed following standard methods (APHA, 1995). All the fish were routinely sampled for assessing growth, health status and presence of parasites. Various parameters of growth as well as mortality were calculated using the following formulae:

Weight gain (%)	= (Final weight - Initial weight)*100/Initial weight.
Specific growth rate (% day ⁻¹)	= ln (Final weight of fish) - ln (Initial weight of fish) x 100/ Days of culture.
Fulton's condition factor	= Weight of fish (g)/ [Length of fish (cm)] ³ × 1000 (Nash <i>et al.</i> , 2006).
Mortality rate (%)	= (No. of fish stocked - No. of fish survived) x 100/No. of fish stocked

Instances of mortality were first observed on the 25th day of culture (DOC) and on the 35th DOC severe mortality due to infestation by cymothoid parasite was recorded. Surviving fish were examined for the presence of external parasites or lesions. Body surface, gill filaments and buccal cavity were also examined for the presence of parasitic infestation. The weight (g) and total length (mm) of the fish were recorded. Live cymothoids were fixed in 5% formaldehyde and identified using taxonomical keys of Trilles and Bariche (2006) and Martin, *et al.* (2016). The prevalence (P) and intensity (I) of the recovered isopods were calculated as per the methods described in Margolis *et al.*, (1982) and Bush *et al.* (1997).

Mortality was first observed in seabass fingerlings in all the five cages (Table 1) after 25-28 days of culture (DOC). During the initial three days, mortality (%) ranged between 2-5%. By the 35th DOC, a cumulative mortality of 84.6% was recorded and a total of 81 out of 154 surviving fish were found to be infested with isopod parasites. The isopods collected were identified as *Cymothoa indica* Schioedte and Meinert, 1884. This species has been widely reported from south-eastern Pacific and Indian Ocean, from Bangkok (Schioedte and Meinert, 1884); Beirut (Trilles and Bariche, 2006); India (Chilton, 1924; Veerapan and Ravichandran, 2000; Rajkumar *et al.*, 2004, 2005a, b; Trilles and Bariche, 2006; Ravi and Rajkumar, 2007; Trilles *et al.*, 2011; Aneesh, 2014); Australia (Hale, 1926; McNeill, 1926; Jones *et al.*, 2008; Martin *et al.*, 2016); Indonesia (Nierstrasz, 1931; Trilles, 2008) and Vietnam (Trilles, 1975). *C. indica*, has

been reported from a wide range of fish species belonging to the family Carangidae, Cynoglossidae, Sillaginidae and from an unknown eel (Martin *et al.*, 2016). The parasite has also been reported to infect long whiskers catfish, *Mystus gulio*; spot tail needlefish *Strongylura strongylura* (Rajkumar *et al.*, 2004), Bloch's gizzard shad *Nematalosa nasus*; maned gobi *Oxyurichthys microlepis* (Ravi and Rajkumar, 2007), goby *Glossogobius giuris* (Chilton, 1924), larvae of barramundi *Lates calcarifer* (Rajkumar *et al.*, 2005a), streaked spinefoot *Siganus javus* (Rajkumar *et al.*, 2005b), common pandora *Pagellus erythrinus* (Trilles and Bariche, 2006), yellowstripe barracuda *Sphyrnaena chrysotaenia* (Trilles and Bariche, 2006), obtuse barracuda *Sphyrnaena obtusata* (Veerapan and Ravichandran, 2000; Trilles and Bariche, 2006), snakefish *Synodus myops* (Veerapan and Ravichandran, 2000; Trilles and Bariche, 2006), *Oreochromis mossambicus* (Rameshkumar and Ravichandran, 2010) and *Etroplus suratensis* (Aneesh, 2014).

The intensity of parasitic infestation was found to be 1 parasite per fish. Parasites were observed to be latched to the tongue of the host fish (Fig. 1). Most of the affected fish were observed to be emaciated with dark pigmentation. Reddening of buccal cavity, degeneration and loss of tongue were also observed in a few of the infested fish.

The water quality parameters recorded during the initial period of culture were: salinity - 25‰, temperature - 31°C, pH - 6.3, ammonia-N - 0.03 mg l⁻¹ and nitrite-N 0.08 mg l⁻¹. On the 35th DOC, the water quality parameters recorded were: salinity - 21‰, temperature - 30°C, pH - 6.25, ammonia-N - 0.02 mg l⁻¹ and nitrite-N - 0.07 mg l⁻¹. After 35 days, the fingerling had grown from an average size of 7±1 g and 63±12 mm (total length, TL) to 3.78±0.3 - 38.03±0.47 g and 75.87±3.21 - 116.96±1.95 mm (TL) in different cages. A condition factor below 1; of 0.88±0.02 and 0.87±0.05 were observed in cages 4 and 5, where the incidence of parasites was relatively more (72-75%) in the surviving fish population (Table 1).

Previously *Cymothoa* sp. and *Aegothoa* sp. were reported from aquaculture systems in Thailand (Leong and Wong, 1990). A study of parasitic fauna of *L. calcarifer* under mariculture in Indonesia recorded nineteen parasite

Table 1. Total length (mm), body weight (g), condition factor, mortality (%) and prevalence (%) of isopod (*C. indica*) infestation in Asian seabass stocked in five cages on 35th day of culture. Values are Mean±SE

Cage	Weight (g)	Total length (mm)	Mortality (%)	Prevalence (%)	Condition factor
1	38.03±0.47	116.96±1.95	86	31.82	2.45±0.09
2	23.16±0.40	94.40±1.51	81	57.90	2.86±0.12
3	11.91±0.54	82.95±1.35	89.5	28.57	2.10±0.09
4	5.00±0.59	82.80±0.58	70.5	72.13	0.88±0.02
5	3.78±0.30	75.87±3.21	96	75.00	0.87±0.05

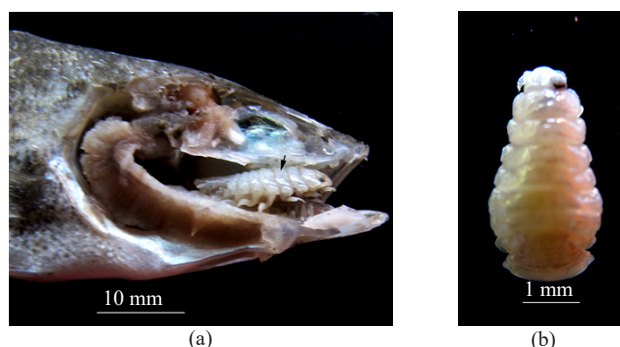


Fig. 1. (a) *C. indica* ovigerous female in the buccal cavity of of Asian seabass fingerling, (b) Dorsal view of *C. indica*

species, but no isopod parasites were observed (Ruckert *et al.*, 2008). Mortality of cage-cultured *L. calcarifer* in south-west coast of India due to parasitic infestation of the isopod *Cirolana fluviatilis* has been reported by Sanil *et al.* (2009). Infestation of *L. calcarifer* larvae (28 day old) by *C. indica* under laboratory conditions was reported by Rajkumar *et al.* (2005a). However, occurrence of *C. indica* in *L. calcarifer* from either wild or aquaculture systems has not been reported so far. In *L. calcarifer*, parasitic isopods; *Nercila barramundae* from Australia (Bruce, 1987) and *Rocinela latis* from Calcutta, India (Southwell, 1915) were listed by Hutson (2013) while reviewing the pathogens affecting captive and wild Asian seabass.

Previous reports of parasitic isopod infestations inflicting mortalities in fish reared in aquaculture systems in India include, *C. indica* infestations in *Mystus gulio* from Vellar Estuary which led to 100% mortality within 10 days (Rajkumar *et al.*, 2005b) and infestations with the isopod, *C. fluviatilis* infesting *L. calcarifer* in Cochin backwaters leading to cumulative mortality of 45% in six months (Sanil *et al.*, 2009). The productivity and economic viability of any finfish aquaculture enterprise can be severely compromised by outbreaks of parasitic diseases (Shinn *et al.*, 2015). Globally, development of cage culture has witnessed the emergence of various parasitic diseases owing to higher host population density (Kent, 2000; Murray and Peeler, 2005). Often, the dominant parasite species infecting fish in captivity may be rare or absent in their wild counterparts or even when present, their deleterious effects may be relatively less evident (Horton and Okamura, 2001; Nowak, 2007). Experimental infestation of seabass larvae by *C. indica* showed the site of infestation as branchial and antero-dorsal region of the fish (Rajkumar *et al.*, 2005a). In the present study, *C. indica* were latched to the tongue of seabass fingerlings. The intensity of parasitic infestation observed during the present investigation (1 parasite per fish) was similar to

that observed in seabass juveniles and blue spot mullet (1-2 parasite per individual) by Leong and Wong (1990) and Al-Zubaidy and Mahaisen (2014) respectively. In *M. gulio*, 1-3 numbers of *C. indica* were found to be lodged on the floor of buccal cavity, clinging firmly to tongue of the host (Rajkumar *et al.*, 2005b).

Experimental infestation of seabass larvae with *C. indica* led to haemorrhagic skin lesions and eroded scales with abundant mucus (Rajkumar *et al.*, 2005a). Gross lesions in the buccal cavity and callus like thickening on the gill arch and filaments were observed in *O. microlepis* infested with *C. indica* (Ravi and Rajkumar, 2007). In the present study, majority of the affected seabass fingerlings were found to be emaciated with dark pigmentation. Reddening of buccal cavity, degeneration and loss of tongue were also observed in infected fish.

Condition factor, an index of fish health has been used to indicate the effect of parasites on their hosts (Horton and Okamura, 2001). Initially, the fish were graded based on size and distributed into different cages based on size to minimise cannibalism. The condition factor for the five different cages ranged from 0.87 ± 0.05 to 2.86 ± 0.12 . The smallest fishes stocked separately in two cages (cage 4 and 5) had the lowest condition factor of 0.88 ± 0.02 and 0.87 ± 0.05 respectively and had the highest prevalence of parasites *i.e.*, 72.13 and 75.0% respectively, indicating correlation between prevalence and poor condition of the fish. Growth rates, energy reserves and longevity were reported to be severely compromised due to cymothoid infestation in five-lined cardinal fish *Cheilodipterus quinquelineatus* (Fogelman *et al.*, 2009). Relatively lower body weight in males, up to 20% and females, up to 32% were recorded in gobiid fish due to *C. indica* infestation (Ravi and Rajkumar, 2007). Barber *et al.* (2000) and Rameshkumar *et al.* (2013) also reported intra-buccal obstruction causing reduced food intake in *L. calcarifer* infested with cymothoan parasites and disturbed respiration (Parker and Booth, 2013). In the present study, relatively lower condition factor was observed in seabass fingerlings with higher prevalence of *C. indica*.

In an open water cage system, an effective anti-parasitic treatment may be both challenging and expensive (Nowak, 2007). No other specific control measures other than optimum culture practices have been suggested for limiting the parasite (Jithendran *et al.*, 2008). Use of fine mesh nets around the cages has been suggested as a preventive measure against transmission of cymothoan larvae (Bragoni *et al.*, 1984). In India, cage culture of finfishes is currently in its nascent stages. Devising efficient management strategies to optimise the

health of fish and preventing disease outbreaks will help expansion of sustainable cage fish farming in India.

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