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Outbreak of *Argulus quadristriatus* infestation in cultured Asian seabass (*Lates calcarifer*) in marine cages, Karnataka, India

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Original Article

Abstract

This paper presents an outbreak of *Argulus quadristriatus* infestation in cage-farmed Asian seabass in Uttara Kannada, Karnataka, India. A total of 46 Asian seabass (*Lates calcarifer*) specimens were collected from three distinct farming sites (Naganathwada (N); Sunkeri (S) and Kumta (K)) of which 13 were found to be infected with a total of 22 fish lice. Infected fish (n=13) exhibited clinical signs such as erratic swimming, rubbing, and haemorrhage. The highest prevalence of infestation was observed in the Naganathwada (N) location (PFI, 50%), compared to Sunkeri (S) and Kumta (K). The severity of the infection was found to be greater in Kumta. The mean intensity of *A. quadristriatus* infection was 4 males and 3 females per infected fish. Specific morphological differences between male and female *A. quadristriatus* included the presence of elongated, creamy-white testes with sperm in the males and round spermathecae in the females. Host tissue responses to the infestation included hyperplasia and necrosis of the epidermal and dermal tissue, leukocyte infiltration, and haemorrhage at the attachment sites. The study determined that the distribution and abundance of male and female *A. quadristriatus* varied across different sites, exhibiting distinct pathological alterations.

Keywords: *Argulus quadristriatus, Lates calcarifer, prevalence, intensity, histopathology*

Introduction

The genus *Argulus*, occurring as ectoparasites of freshwater, marine, and estuarine fishes, belongs to Branchiura which contains a single family, Argulidae. These are commonly called fish lice (Boxshall, 2005). In India, about 12 species of *Argulus* have been described (Natarajan, 1982). The three widely studied species of *Argulus* are *A. foliaceus*, *A. japonicus*, and *A. coregoni*, which infect freshwater fishes. In marine fishes, the term 'sea lice' refers to the numerous species of copepod crustaceans of the family Caligidae that are externally parasitic. The most intensively studied fish lice species in marine fishes are *Lepeophtheirus* spp. and *Caligus* spp. Argulus can cause significant damage to the host through their aggressive attachment and feeding behaviours (Mikheev *et al*., 2015). The infestation of *Argulus quadristriatus* in cage-farmed cobia can lead to significant economic losses due to reduced fish growth, increased mortality, and compromised marketability from clinical signs like haemorrhage and tissue damage. Estimated losses in Indian aquaculture from argulosis including *A. quadristriatus*, reach approximately ₹ 300 crores annually, straining small-scale farmers (Kumar and Kumari, 2022). They can easily attach to and detach from their hosts causing damage to epithelial tissue, leading to secondary infections. Argulus may also act as a vector in transmitting diseases from one host to another (Cusack and Cone, 1986; Boxshall, 2005; Poly, 2008; Subburaj *et al.*, 2018).

There is a great potential for cage farming of Asian seabass (*Lates calcarifer*, Bloch) in Karnataka, India because of its fast growth rate, high market demand and availability of hatchery-produced seeds. Asian seabass cage farming is carried out both in brackish and marine water in India. Worldwide, infestation of cage-farmed Asian sea bass with sea lice has become a great threat affecting the economy of farming. Although a few researchers have reported *A. quadristriatus* infestation in India (Devaraj and Ameer, 1977; Uyeno *et al.,* 2017; Subburaj *et al.,* 2018), no such reports exist from Karnataka. To the best of our knowledge, there are no documented cases of *A. quadristriatus* infestation in cage-farmed Asian seabass in India before the year 2019. During the routine health monitoring of cage-farmed Asian seabass, ectoparasites were observed on the body of the fish. Hence, a study was designed to identify the ectoparasites through microscopic examination and study the prevalence, severity of infection, mean intensity, abundance, sex differences and host tissue responses of *A. quadristriatus* in Asian seabass.

Material and methods

Asian seabass (length: 21.5 ±35.9 cm; weight: 161.6±2.36g; n=46) were collected from three farming sites: Naganathwada (N; 14° 50′ 480′′ N 074° 08′ 967′′ E), Sunkeri (S; 14° 50′ 293′′ N 74° 08′ 683′′ E) and Kumta (K; 14° 29′ 308′′ N 074° 22′ 894′′ E) in the Uttara Kannada district of Karnataka, India. In the investigated farming systems, monoculture practices were employed, with farmers cultivating a single species, namely Asian seabass (*L. calcarifer*). The culture system utilized cages with a diameter of 6 m, maintaining a stocking density of 1,500 individuals per cage. Feeding was conducted twice daily using trash fish. During the study period, physicochemical parameters of the water, including salinity, temperature, pH, dissolved oxygen, ammonia, nitrate, and nitrite levels, were maintained within optimal ranges. After recording the clinical signs, the fishes were examined grossly for the presence of any parasites. Wet smears of gills, skin, intestine and kidney were examined under a microscope (Zeiss, Germany) for the presence of parasites. For histopathology, normal tissues and representative tissues of skin and muscle from the sites of ectoparasite attachment were fixed in 10% neutral buffered formalin for 48 hours. Fixed tissues were washed and routinely processed in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections cut at 5μ thickness were stained with hematoxylin and eosin and observed under a microscope (Sharma *et al.*, 2013). Prevalence was calculated using the formula: total number of infected fishes/ total number of fish hosts examined. Prevalence was further classified as rare (0.1–9.9 %), occasional (10–29.9 %), common (30–69.9 %) and abundant (70–100 %) as per Srivastava, (1980). The severity of infection is expressed in numerical values (0 = Absent, 0.5 = Present, low grade, 1= present, High grade, 2= present, Very high grade) following the generalized scheme for grading infections (Lightner, 1993; Ramudu and Dash, 2013).

Results

A total of 22 ectoparasites were found on the fish body surface (Tables 2 and 3). The ectoparasites were confirmed as *A. quadristriatus* based on morphology (Fig. 9 and 10). The morphological features are depicted in Table 1. The lice as observed under a wet smear. The prevalence (PFI, 50%) of infestation was high in site N when compared to the other two sites such as S and K (PFI, 30% and 7%, respectively; Table 2). The severity of infection was found to be higher in site K (2= Present, very high grade) when compared to site N and S (both 0.5= Present, low grade).

Table 1. Morphology of *Argulus quadristriatus* compared with other reports

	Devaraj and Ameer (1977)		Subburaj *et al.* (2018)		Present Study	
Characters	Male	Female	Male	Female	Male	Female
Size	-	-	-		Small	Large
Longitudinal stripes	-	4	-	4	4	4
Body length	-	9.10 mm	-	6.59 -10.88 mm	6.1-10 mm	6.5 -11 mm
Carapace length	-	7.20 mm	-	-	6.7-7.0	6.9-7.10
Carapace width	-	4.75 mm	-	-	4.6-4.7	4.7-4.8
Anterior sinuses	-	Distinct	-	-	Distinct	Distinct
Cephalic area	-	2.80 mm	-	-	-	-
Lateral lobes of the carapace	-	6.25 mm	-	-	-	-

Characters	Devaraj and Ameer (1977)		Subburaj *et al.* (2018)		Present Study	
	Male	Female	Male	Female	Male	Female
Abdomen length	-	2.90 mm	-	-	1.2-2.7	1.5-3 mm
Abdomen width	-	2.55 mm	-	-	1.1-2.1	1.5-3 mm
Abdominal lobes	-	Pointed	-	-	Pointed	Pointed
Caudal rami	-	Absent	-	-	-	-
Sucking disc (mm diameter	-	0.78 mm	-	-	-	-
Sucking disc ribs (number)	-	58	-	50-60	56-60	55-60
Ribs of the suction cups are composed of imbricated plates	-	9 to 11	-	8-10	8-10	9-10
Position of smaller respiratory area	-	Anterior to larger respiratory area	-	-	Anterior to larger respiratory area	Anterior to larger respiratory area
Maxilliped	-	0.5 mm	-	-	-	-
Locality	Mandapam, Tamil Nadu (India)		Muttukkadu, Tamil Nadu (India)		Karnataka (India)	
Host (s)	*Psamnioperca waigiensis* (Cuvier)		*Rachycentron canadum*, (Cobia)		*Lates calcarifer*	
Site of infection	Body surface		Body parts		Body surface, dorsal fins, caudal fins, Head region	
Culture/ Wild	Wild fish		Earthen pond		Cages	
Clinical signs	-		Erratic swimming, rubbing		Excessive mucus, Haemorrhage, scale loss	

Table 2. Prevalence (%) and severity of infection of *A. quadristriatus*

Locations	No. of samples	No. of Infected fishes	*Prevalence (%)	Site of infection	Severity of infection
Naganathwada (N)	12	6	50[c]	Body and Fins	0.5
Sunkeri (S)	20	6	30[c]	Body and Fins	0.5
Kumta (K)	14	1	7[a]	Body and Fins	2

*Prevalence & severity score: 0 = Absent, 0.5 = Present, low grade, 1=present, High grade, 2= present, Very high grade

*Prevalence (%) further classified as a=rare (0 – 9.9%); b=occasional (10 – 29.9%); c = common (30 – 69.9%); d = abundant (70 – 100%).

Clinical signs

The prominent clinical signs observed were rubbing against the cage net and erratic swimming. Grossly, haemorrhages and skin erosions at the site of attachment by the lice were observed. Histological changes in tissues (Fig. 1 and 2) due to lice infestation included epithelial degeneration, fibrinoid necrosis (Fig. 3), haemorrhage and infiltration by leucocytes (Fig. 4 and 5). In many places, hyperplasia of epidermal and dermal tissues was observed. In both epidermal and dermal tissues, haemorrhages (Fig. 6 and 7) at the site of attachment and chronic inflammation leading to the formation of fibrinoid necrosis (Fig. 8) were also recorded.

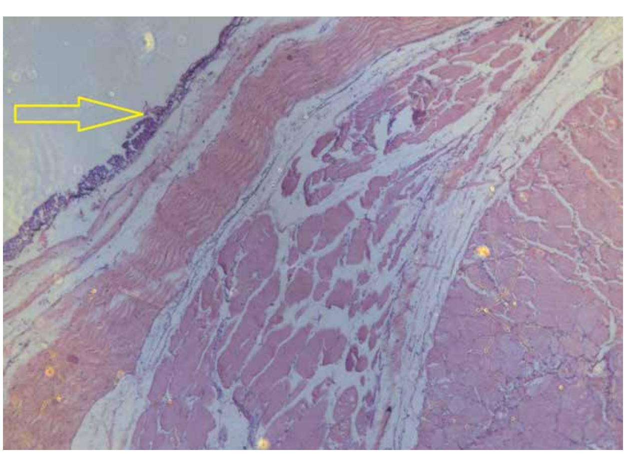

Fig. 1. Normal structure of skin (H&E, 50x)

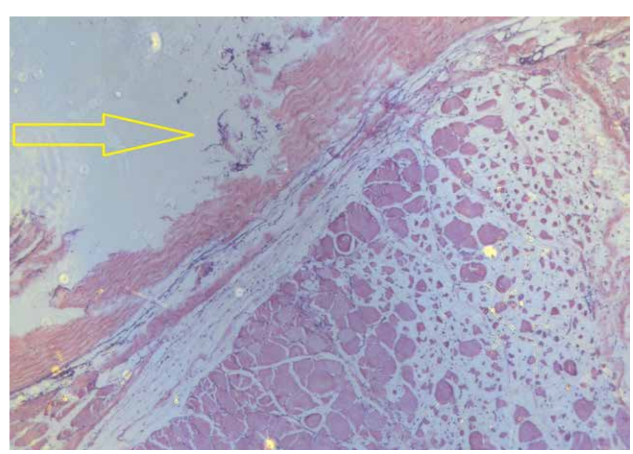

Fig. 2. Damage of external barriers (skin & tissue; arrow) due to feeding of *A. quadristriatus* (H&E, 50x)

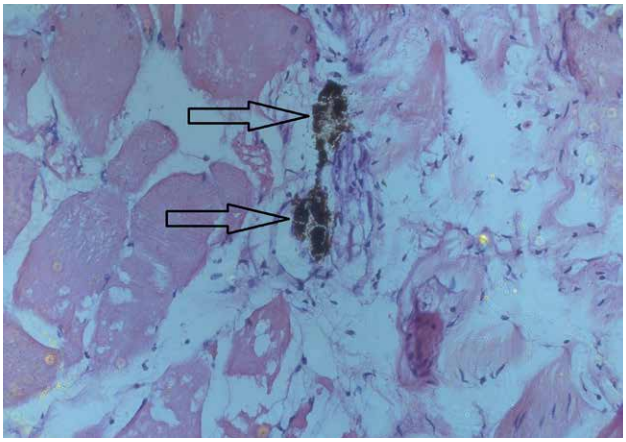

Fig. 3. Degenerative and necrotic changes (arrow) in the epithelial cell with hypertrophy of epidermal and dermal tissue (H&E, 200x)

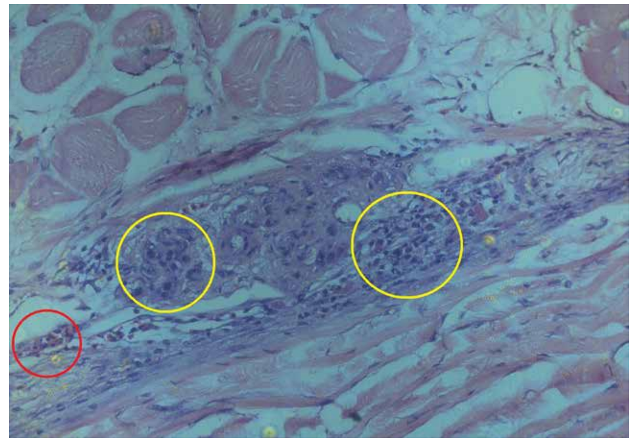

Fig. 4. A high degree of leukocyte infiltration (Yellow circles) in epidermal tissue tissues (H&E, 400x)

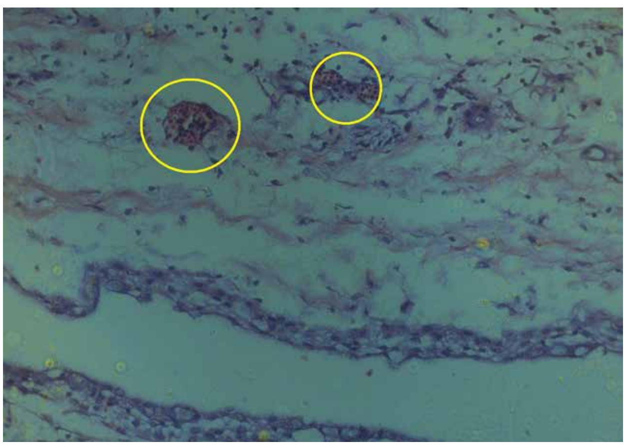

Fig. 5. A high degree of leukocyte infiltration (Yellow circles) in hypodermal tissue tissues with hypertrophy of RBC (Red circle) & haemorrhage (H&E, 400x)

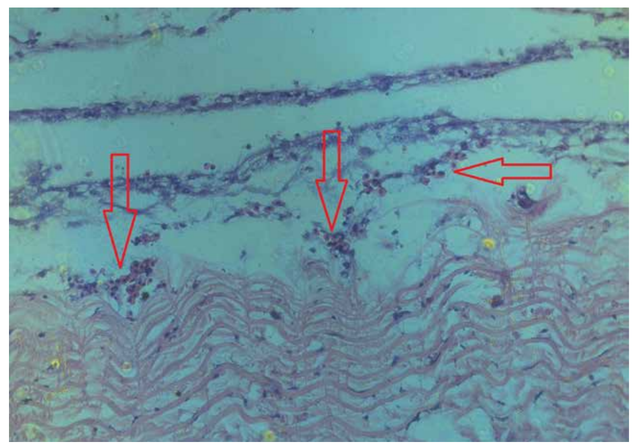

Fig. 6. Epidermal tissues with haemorrhage (arrow; H&E, 400x)

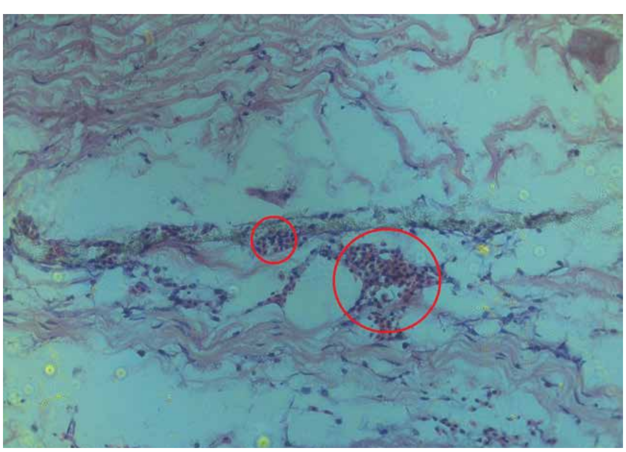

Fig.7. Hypo-dermal tissues with haemorrhage (circles; H&E, 400x)

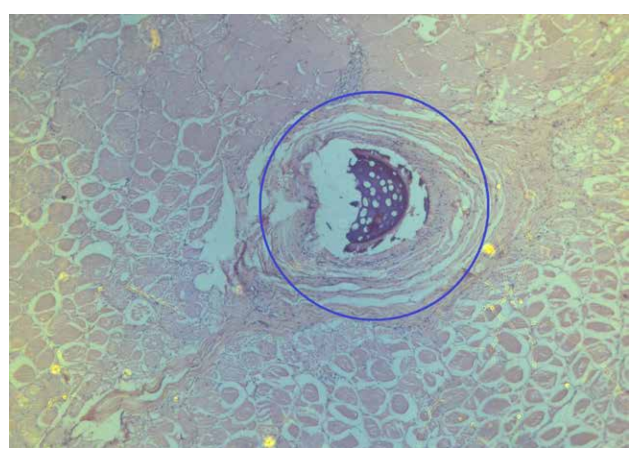

Fig. 8. Chronic inflammation leads to the formation of fibrinoid necrosis (Dark blue circle; H&E, 400x)

Parasite description

Phase contrast microscopy (Carl Zeiss MicroImaging Gmbh 37081, Axio-Germany) revealed prominent morphological features of (Fig. 9 and 10) *A. quadristriatus* like the presence of four longitudinal stripes, pointed abdominal lobes and presence of a smaller respiratory area anterior to the larger respiratory area (Table 1). Four males and three females each were recovered from sites N and K while four males and four females from site S (Table 3).

Mean intensity

The overall mean intensity of male and female *A. quadristriatus* was 4 and 3 respectively per infected fish with a very high infestation on the head region of fish sampled from the K site. Mean intensity was 0.6 for the male and 0.5 for the female for fish samples from site N, while for samples obtained from site S, it was 0.6 for both the male and female (Table 3).

Morphological features

The male has a body length of 6.1-10 mm; length of abdomen 1.2-2.7 mm; width of abdomen 1.1-2.1mm; number of longitudinal stripes 4. Abdominal lobes pointed, with 56-60 sucking disc ribs, 8-10 imbricated plates and a smaller respiratory area positioned anterior to the larger respiratory area. The female has a body size larger than males, 6.5-11 mm in length; abdomen 1.5-3 mm in length and 1.5-3 mm in width; with four longitudinal stripes. Abdominal lobes pointed, with 55-60 sucking disc ribs and 9-10 imbricated plates. Morphologically, specific differences were observed between male and female *A. quadristriatus*. Males possessed creamy white, elongated pairs of testes in the abdomen, while the abdomen of the female contained a pair of round spermathecae which were dark in colour.

Discussion

A. quadristriatus, was originally described by Devaraj and Ameer (1977) in wild female cuvier, *Psamnioperca waigiensis* collected from Palk Bay, off Mandapam, Tamil Nadu, India. Later, similar specimens were isolated from two species of goatfish and goby from Japan (Uyeno *et al*., 2017). Recently, Subburaj, *et al.* (2018) reported a closely similar specimen isolated from pond-cultured cobia, *Rachycentron canadum*, from India. In the present study, the morphological features of the parasites were found to be similar to the previously published studies (Devaraj and Ameer, 1977; Uyeno *et al*., 2017; Subburaj, *et al*., 2018). Accordingly, all the isolated parasites were confirmed as *A. quadristriatus*.

Though slight morphological dissimilarities were observed, they did not exceed the limits of natural variations, typical of populations or species. Small discrepancies in the morphometry of the parasites are influenced by different factors such as location, ecology and age of the hosts (Moshu and Molnar, 1997).

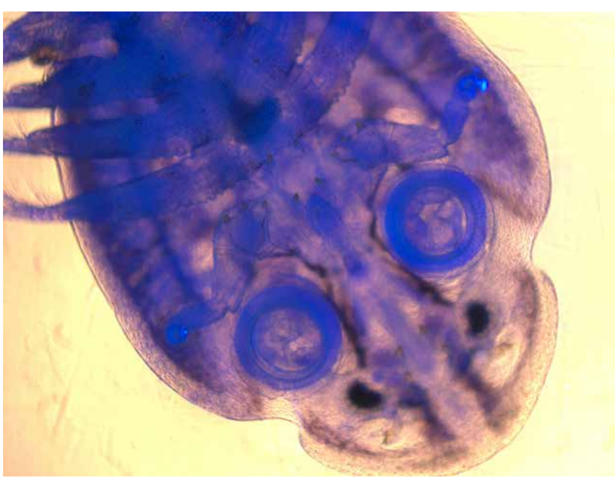

Fig. 9. Dorsal view of *Argulus quadristriatus* attached to the head region of Asian seabass (*Lates calcarifer*), illustrating key morphological features. (50x)

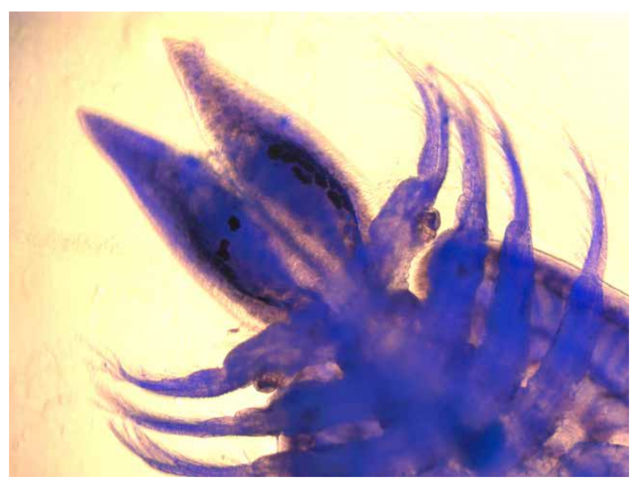

Fig. 10. Ventral view of the tail portion of *Argulus quadristriatus* attached to the head region of Asian seabass (*Lates calcarifer*), highlighting diagnostic morphological characteristics. (50x)

Table 3. Sex differences, mean intensity and abundance of *A. quadristriatus* on Seabass, *Lates calcarifer* in three different locations of cage culture

Locations	Gender status	No. of parasites isolated	Mean intensity	Abundance
Naganathwada (N)	Male	4	0.6	0.3
	Female	3	0.5	0.2
Sunkeri (S)	Male	4	0.6	0.2
	Female	4	0.6	0.2
Kumta (K)	Male	4	4	0.3
	Female	3	3	0.2

The clinical signs observed such as rubbing against the net of the cage and erratic swimming could be an effort to dislodge the attached parasites. Grossly, haemorrhages and erosions at the site of attachment by the lice were observed. The gross lesions observed could be inflicted by continuous breaking and feeding by *A. quadristriatus* on the skin and also by rubbing against cage nets due to irritation. Darwesh *et al.* (2014) reported similar lesions in the Goldfish (*Carassius auratus*) infested with *A. japonicus*.

A. quadristriatus feeds on the mucus layer of the skin and tissues below the skin (Fig. 2). Mucus and skin are important, both as external barriers to infection and as part of the osmotic system. Lesions and wounds form on the skin due to the feeding activity of *A. quadristriatus* and it may lead to osmotic imbalance, lowering the fitness of the host thereby providing a pathway for other infections and similar signs were reported by Bjørn and Findstad (1997) and Frazer (2008) due to sea lice infestations.

Histological changes in tissues due to lice infestation included epithelial degeneration and infiltration by leucocytes. In many, hyperplasia of epidermal and dermal tissue was also observed. Darwesh *et al.* (2014) observed similar lesions in *C. auratus* infested by *A. japonicus*. Fibrionoid necrosis with haemorrhage at the site of attachment of the lice recorded in the present study can be corroborated with earlier studies (Kabata, 1970; Rahman, 1996; Taylor *et al.*, 2005). Hyperplasia of the epithelium and congestion of blood vessels may lead to hemorrhagic and ulcerative lesions in infected fish. *A. quadristriatus* feeds on blood and other body fluids by piercing the host tissue with the pre-oral stylet and injecting cytolytic toxins, thereby causing the pathologies (Noga, 2010; Noaman *et al.*, 2010; Ahamad *et al.*, 2016). No mortality was observed due to *A. quadristriatus* infestation in Asian seabass during the culture period in any of the farms from where sampling was done. Infestation of cultured Asian seabass with *A. quadristriatusis* to be viewed with caution as these parasitic crustaceans may act as vectors for disease transmission (Noga, 2010; Aalberg *et al.*, 2016) and intermediate host for the nematode, *Mexiconema cichlasomae* (Moravec *et al.*, 1999).

Conclusion

In conclusion, the present study reports the infestation of cage-farmed Asian seabass with *A. quadristriatus*. Many species of fish lice cause epizootics with reduced growth and mortality in freshwater fish farms. However, in marine culture conditions, reports on such epizootics caused by sea lice are rare. In this context, the present study should merit further research attention considering the potential of cage farming of marine edible fishes. Also, the role of wild predatory fishes as a source of infestation in marine cage-farmed fish needs further investigation.

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Author contributions

Conceptualization: KRR; Methodology: KRR; Data Collection: KRR, SS; Writing Original Draft: KRR; Writing Review and Editing: SNK, KSSR, SBPP, AA, TH; Supervision: JL, KC.

Data availability

The data supporting this study are partially/publicly available at the https://eprints.cmfri.org.in

Conflict of Interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of samples/ protected environments.

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