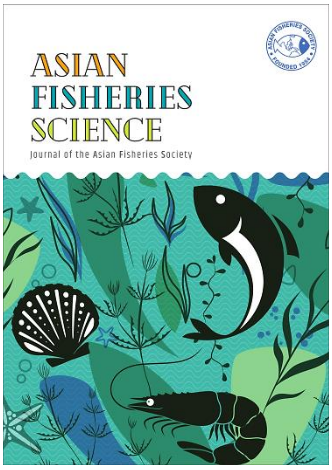

Histopathology of *Anilocra leptosoma* Bleeker, 1857 (Isopoda, Cymothoidae) Infestation on Its New Host *Nematalosa nasus* (Bloch, 1795) From India

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Abstract

Cymothoid isopods are parasitic crustaceans that cause serious impact on marine fish and might lead to fish mortality and consequently, economic losses. Histopathological alterations caused by *Anilocra* spp. have not been studied well. This study aims to report the histopathological changes caused by Cymothoid, *Anilocra leptosoma* Bleeker, 1857 in the skin of Bloch's gizzard shad, *Nematalosa nasus* (Bloch, 1795). Histopathological examination of processed skin tissues showed changes caused by *A. leptosoma,* such as hyperplasia and erosions of the epidermis associated dermal oedema and muscle degeneration. The host response also included an aggregation of subepithelial dense sheets of hemosiderin-laden macrophages within the dense mixed inflammatory cells. The cymothoid, *A. leptosoma* are serious parasites of marine fish that can cause severe economic loss in the commercially important fish species. The present study represents the first record of the parasitic cymothoid, *A. leptosoma* on *N. nasus* from India.

Keywords: parasitic cymothoid, epithelial hyperplasia, inflammation, hemosiderin-laden macrophage

Introduction

Parasitic isopods belonging to the family Cymothoidae Leach, 1814 are extremely serious fish parasites that adversely affect the health of aquatic animals with considerable economic losses (Aneesh et al., 2017). Fifty valid species of the genus *Anilocra* Leach, 1818 have been reported (Bruce, 1987). Only two valid species are known from India, *Anilocra dimidiata* Bleeker, 1857 (Rameshkumar et al., 2011), and *Anilocra leptosoma* Bleeker, 1857 (Aneesh et al., 2017). Species of *Anilocra* are known to prefer the external surfaces of marine fish hosts that inhabit subtropical, tropical, and temperate waters (Smit et al., 2014). Blood feeding and parasitic association cause a variety of pathological effects on their host fish. The impaired swimming ability of the fish tends to reduce their capacity to escape from the predator, and hence there is a greater risk of being eaten (Ostlund-Nilsson et al., 2005). The parasites are associated with many commercially important fish species worldwide and cause significant economic losses to fisheries either by killing, stunting, or damaging these fishes (Elgendy et al., 2018).

The Bloch's gizzard shad (*Nematalosa nasus* Bloch, 1795) is an anadromous fish that inhabits a wide range of marine environments (Mukherjee et al., 2016). There are several reports on *N. nasus* parasitised by isopods belonging to the genera, *Agarna* Schioedte and Meinert, 1884; *Cymothoa* Fabricius, 1793; and *Nerocila* Leach, 1818 (Trilles et al., 2011). Although there are reports on *Anilocra* infestation of fish from India, there is no record of the parasite from *N. nasus* and only one previous report of the parasite on *N. nasus* from Australia (Bruce, 1987).

The effect of the isopod parasite on host fish shows considerable variation (Cuyas et al., 2004). In India, few studies have reported the effect of parasitic isopods on host fishes (Rand, 1986; Jalajakumar, 1988; Lailabeevi, 1996). These studies have indicated that the harmful effects of parasites varied from tissue damage at their site of attachment to mortality of the hosts. A review of literature revealed that there exist only two previous reports on the effects of isopod genus *Anilocra* on host fishes. In this study, an attempt was made to elucidate the pathological changes in *N. nasus* due to infestation by the cymothoid, *A. leptosoma*.

Materials and Methods

A total of 120 fish samples of Bloch's gizzard shad, *N. nasus* were collected from Neendakara (Lat. 8°56'19" N, Long. 76°32'25" E), South-West coast of India. The fish were examined thoroughly for the isopod parasites. The mode of attachment of isopods to the host skin and the gross changes made by them were observed and photographed. The specimens of *A. leptosoma* were examined and identified according to the taxonomic keys of Bruce, 1987 and Aneesh et al. (2017) using stereo dissecting microscope (SDM) (Stemi 508, Carl Zeiss, Germany). Photomicrographs were taken using SDM and taxonomic drawings were made using CorelDraw software.

Ten specimens each for uninfested and infested host fish skin was excised and fixed in 10% neutral buffered formalin for histopathological analysis (Ananda Raja and Jithendran, 2015). The tissues were washed with distilled water for cleaning and dehydrated in a series of ethyl alcohol (50, 70, 90 and 100 %) cleared in xylene, embedded in paraffin wax and sections were made at 4 µm using a microtome (HistoCore BIOCUT, Leica, Germany). Tissue sections on the glass slides were hydrated in a series of ethyl alcohol (100 %, 80 % and 70 %) and stained with haematoxylin and eosin, cleared in xylene and mounted using DPX mountant (Carleton, 1980). The sections were examined and photomicrographed using transmission light microscope (TLM) and Optikam B5 Digital Camera (Optika, Italy).

Ethical approval

The animals used in this study did not require ethical approval.

Results

Anilocra leptosoma were attached posterodorsally to the head of *N. nasus* (Fig. 1a) and some of them recovered from the base of the dorsal fin, facing the cephalon towards the host mouth. Out of 120 fish examined, 13 were found to be infested each with a single ovigerous female. Discrete alterations such as skin depression and darkening were observed at the attachment site of the parasite (Fig. 1b).

The parasitic isopod, *A. leptosoma* (Figs. 1c-e) collected from the body surface of the host was identified by the key taxonomic characteristics: Pleotelson ovate, lateral margins converging smoothly to caudomedial point, and pleonite 1 lateral margin posteriorly produced (Figs. 1f-g).

Histopathology of normal skin from the head region of the uninfected fish showing compactly arranged cells and normal tissue architecture (Fig. 2a). Sections of muscle showed normal morphology and no specific lesion in the uninfested fish skin (Fig. 2b).

The histopathology of skin tissues infested with *A. leptosoma* was undergoing degenerative changes. At the point of parasite attachment, there was depression (Fig. 3a) and epidermal erosion (Fig. 3b) along with hyperplasia (Fig. 3b). The underlying epidermis and dermis were infiltrated with inflammatory cells (Fig. 3b), oedema (Figs. 3a, b, c,) and tissue undergoing necrosis (Figs. 3c, d). In addition, in the inflammatory cells large numbers of eosinophilic cells were seen (Fig. 3d inset).

The host response to *A. leptosoma* infestation also included an intense increase in subepithelial dense sheets of hemosiderin-laden clusters of macrophages (melanomacrophages) (Fig. 3b inset) within the dense mixed inflammatory infiltrate (Figs. 3b, c). These melanomacrophages (MMs) were enlarged and closely packed aggregates of melanomacrophage centres (MMCs). In skin tissues, most of the MMs appeared in yellowish-brown colour, but in some regions, they were darkly pigmented (Figs. 3b, c)

Discussion

Histopathological examination of the infected skin revealed significant changes in all layers. In the present study, the parasite was seen to be specifically attached to the fish's head region. According to Fogelman (2009) the body surface is the preferred attachment site for this parasite. *Anilocra* species are usually found just beneath either the eye or attached posterodorsally to the eye of their hosts. In the present study the parasite was attached mainly a little above the postereo-dorsal region of the eye after the operculum. The selection of the site of attachment may depend on the host's morphology, body movement or habitat (Rameshkumar and Ravichandran, 2013a).

The depression observed in the skin of the infested fish may be due to erosion of the body surface due to the attachment of the parasite for a long period. According to Mladineo et al. (2020) pressure is exerted by the isopod at the attachment site in turbulent sea conditions and the tissue reactions seen is caused by the feeding activities, in addition, there is skin darkening associated with parasite infestation as reported by Rojas et al. (2018) in rainbow trout, *Oncorhynchus mykiss* (Walbum, 1972) due to copepod infestation. This darkening may be due to the increased concentration of melanin-based pigmentation, which also forms a defence mechanism to resist the ectoparasite (Kittilsen et al., 2009).

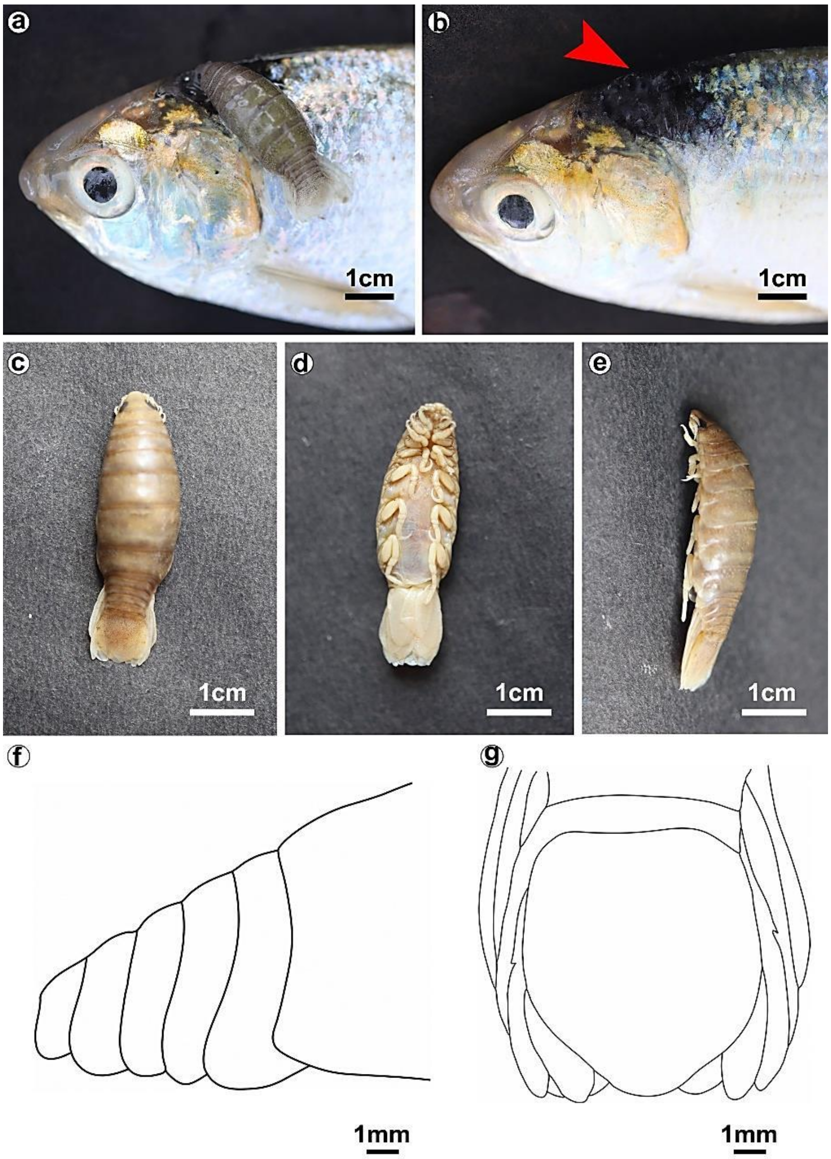

Fig. 1. (a) Parasite *Anilocra leptosoma* attached to the head of *Nematalosa nasus*; (b) The site of parasite attachment (red arrow) showing dark colour and depression in the skin. The parasite *Anilocra leptosoma* ovigerous female (4 cm): (c) dorsal view, (d) ventral view, (e) lateral view, (f) Pleon, (g) Pleotelson.

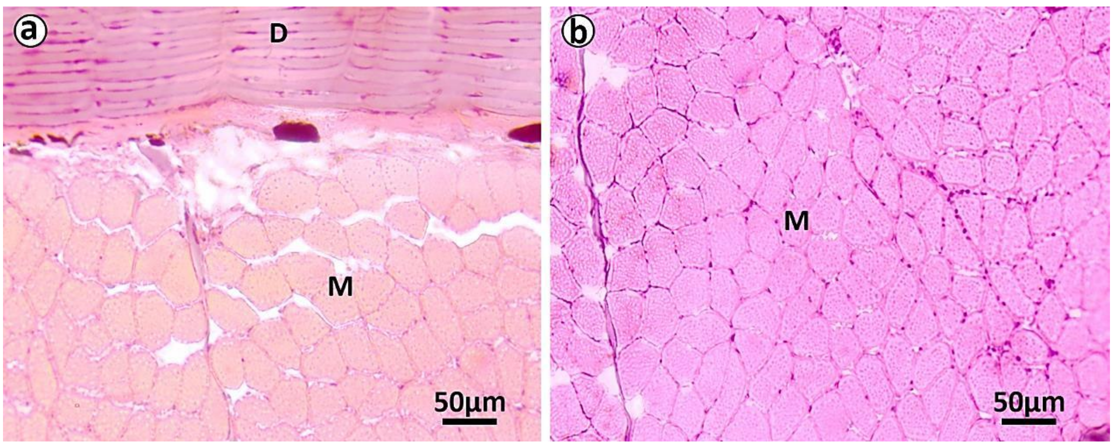

Fig. 2. Transverse sections of normal skin of *Nematalosa nasus*. (a) Compactly arranged cells in the skin layers; (b) Normal muscle tissue shows compactly arranged muscle fibres.

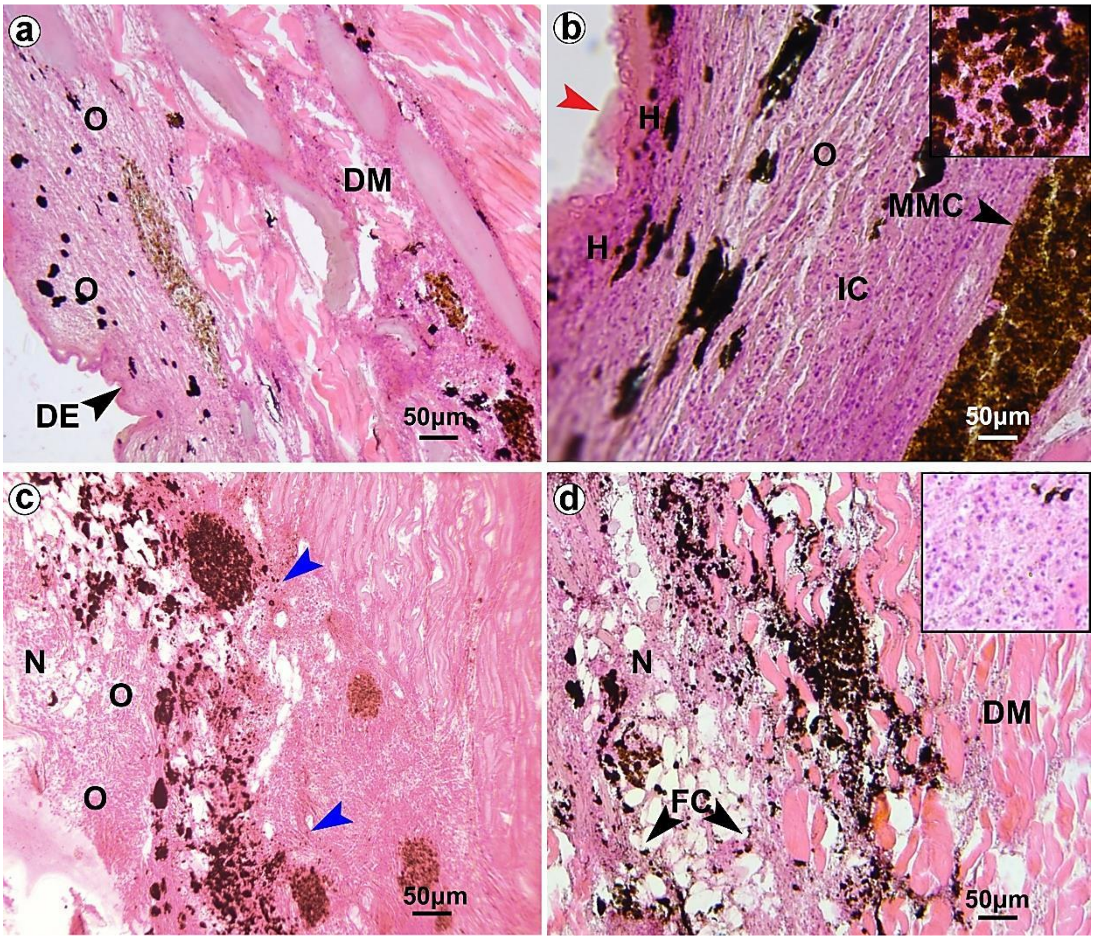

Fig. 3. Histopathological sections of head of *Nematalosa nasus* infested by *Anilocra leptosoma*. (a) Depression (DE) at the site of parasite attachment, with muscle degeneration (DM) and oedema (O) below the site of parasite attachment; (b) Epidermal erosion (red arrow), hyperplasia (H) in the epidermal layer, and severe degenerative changes in the dermal layers. Proliferation of melanomacrophage centre (MMC) and infiltration of inflammatory cells (IC). Melanomacrophages (inset); (c) Oedema (O) and muscle tissue undergoing necrosis (N). Severe proliferation of melanomacrophage centres within the mixed inflammatory infiltrate (blue arrows); (d) Infiltration of eosinophils and lymphocytes (inset), muscle degeneration (DM) and presence of fat cells (FC).

Histopathology of skin of *N. nasus* infested with *A. leptosoma* showed several variations than the normal tissue architecture. In the present study, the epidermis at the site of pereopod attachment was irregular and eroded. Purivirojkul (2012) also reported erosion of the epidermis and dermis at the attachment site of the isopod, *Nerocila depressa*. According to Rameshkumar and Ravichandran (2013b) the isopods use claw-like prehensile pereopods for attachment to the host skin and the insertion using the appendages, causing severe erosion in the dermis and the underlying muscular tissues at the site of attachment similar to the damages caused by *Catoessa boscii* in the buccal cavity of *Carangoides malabaricus* (Bloch & Schneider, 1801). The degenerative changes seen in the underlying tissue at the point of parasite attachment could also be due to the parasite feeding activity as reported by Ravichandran et al. (2007). He reported the necrotic lesions in *Parastromateus niger* due to the infestation of isopod, *Joryma tartoor*.

An increase in subepithelial dense sheets of hemosiderin-laden macrophages within the dense mixed inflammatory infiltrate was observed in *N. nasus* infected with *A. leptosoma*. Based on the type of injury made by the parasites and their depth of penetration, the cellular response to infestation may change. Dezfuli et al. (2011) reported an increase in granular leukocytes as an inflammatory response when the parasites adhering to the tissue, whereas the immune cells and MMCs increase due to deeper penetration of the pereopods into the tissues. The inflammatory infiltration of white blood cells is a common feature of the isopod infesting fish (Ravichandran et al., 2007) which serves as a defence mechanism against the parasite infestation (Rand, 1986). According to Roberts (1975) MMCs are seen involved in the innate and adaptive immune response. Large numbers of MMCs were noticed in all layers of the skin tissues of infested fish. An increase in MMCs due to similar infestations in fishes were also reported by Ziegenfuss and Wolke (1991) and Ananda Raja et al. (2020). These macrophage aggregations distort skin architecture and may develop necrotic regions (Guarner and Brandt, 2011). The excessive accumulation of the haemosiderin pigment in organs (haemosiderosis) represents a typical pathological process (Wolke et al., 1985). Haemosiderin is normally seen associated with lipofuscin granules (Agius and Agbede, 1984). In some regions of the skin layers, MMs were darkly pigmented. Agius and Roberts (2003) suggested that increased haemosiderin, lipofuscin and melanin pigments deepen the dark colouration of MMs.

Status of MMCs in parasite infested fish helps to monitor the health of wild fish populations (Wolke et al., 1985). MMCs are formed as a chronic inflammatory response to serious tissue damages. MMCs numbers also increase in the supportive tissues due to the extensive skin damages (Roberts, 2001). In the present study, the tissue damage was visible by the naked eye as a depression at the attached site of the parasite. The parasite does not penetrate into the host but the damage was seen in the underlying tissue with degeneration of the muscle fibres, along with infiltration of eosinophilic granulocytes. This indicates that the parasite was almost permanently attached to this location throughout its life (Overstreet, 2017). Williams Jr and Bunkley-Williams (2019) indicated this as a successful adaptation of isopod parasites in locations around the cephalic region of the host without making deep penetrations.

Conclusion

The present study revealed the deleterious effect of a cymothoid, *Anilocra leptosoma* on the marine fish, *Nematalosa nasus* through histopathological analysis. The parasite induced severe inflammatory responses, hyperplastic and degenerative changes in the skin and underlying muscles, thus can harmfully affect the physiological status of the host. A heavy infestation can affect the commercial value of fish. The report is a new host record from India and provides information on the histopathology of fish infestation by *A. leptosoma*.

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