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First report of histopathological alterations by an isopod parasite in Indian mackerel, *Rastrelliger kanagurta* (Cuvier, 1817)

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Original Article

Abstract

Cymothoid isopods are parasitic crustaceans that cause serious impact on marine fishes, which might lead to fish mortality and economic losses. This study aims to report the histopathological changes induced by Cymothoid, *Nerocila phaiopleura* Bleeker, 1857 in the skin of Indian mackerel, *Rastrelliger kanagurta* (Cuvier, 1817). Histopathological examination of processed skin tissues showed changes caused by *Nerocila phaiopleura* such as skin depression, erosion of skin layers, epithelial hyperplasia, epidermal spongiosis, necrotic lesions in the muscles, oedema, muscle degeneration and fibrosis. The host response included the appearance of lymphocytes, eosinophils and macrophages. The cymothoid *N. phaiopleura* are serious marine fish parasites that can cause severe economic loss in the commercially important fishes. The present study represents the first record of the histopathology of parasitic cymothoid *N. phaiopleura* on *R. kanagurta*.

***Keywords**: Parasitic cymothoid, Nerocila phaiopleura, Rastrelliger kanagurta, histopathological alterations, host response*

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). *Nerocila* is a large genus of the family Cymothoidae, Isopoda, including at least 65 species living attached to the skin or the fins of fishes. They possess prehensile pereopods through their powerful curved claws and highly modified buccal parts that help them to remain attached to their hosts. Blood feeding and parasitic attachment cause a variety of pathological effects on their host fish. The fish's impaired swimming ability 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). *Nerocila* spp. was reported to be 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).

Nerocila phaiopleura has been recorded from about 20 different fish species from India (Trilles *et al*., 2011). The Indian mackerel, *Rastrelliger kanagurta,* is one of the hosts for *N. phaiopleura* (Seth *et al*., 2013*).* The Indian mackerel constitutes a prominent group in the landings of both the Arabian Sea as well as in the Bay of Bengal (Goutham and Mohanraju, 2015), with a significant increase in the annual landings of Indian mackerel along the Indian coast (CMFRI, 2019).

The effect of the isopod parasite on host fish is considerably diverse and poorly studied (Cuyas *et al.*, 2004). In India, only few reports are there on the effect of parasitic isopods on host fishes (Jalajakumar, 1988; Lailabeevi, 1996). These studies have indicated that the harmful effects of parasites varied from tissue damage at their site of attachment to the mortality of the hosts. Though there are few reports of the infection of isopods in *R. kanagurta*, so far, there is no histopathological study to reveal the effect of isopods in this species. In this study, an attempt is made to elucidate the pathological changes in *R. kanagurta* due to infestation by the cymothoid *N. phaiopleura*.

Material and methods

A total of 120 fish samples of Indian mackerel, *R. kanagurta,* were collected from Neendakara (Lat. 8^{0}56'19" N, Long. 76^{0}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 *N. phaiopleura* were examined and identified according to the taxonomic keys of Bruce (1987) and Ravichandran *et al.* (2019) using a stereo dissecting microscope (SDM) (Carl Zeiss Microscopy; GmbH Stemi 508). Photographs were taken using Canon EOS 800D with 35 mm macro lens.

Ten specimens of fish skin with muscle tissue from infested host fish skin and exactly the corresponding uninfested fish skin were 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 (Leica: Histocore Biocut). Tissue sections on the glass slides were dewaxed in xylol, 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) (Optika Microscope; Optikam B5 Digital Camera).

Results

N. phaiopleura were found attached firmly to the posterior third of the body, overlying the lateral line and facing the head of *R. kanagurta* (Fig. 1a & b). Out of 120 fishes examined, 24

Fig. 1. (a & b) Parasite, *N. phaiopleura* attached to the posterior body surface of *R. kanagurta* (c & d). The site of parasite attachment showing large haemorrhagic wound (arrowhead) and small pin-holes with the formation of epidermal plaques (circle)

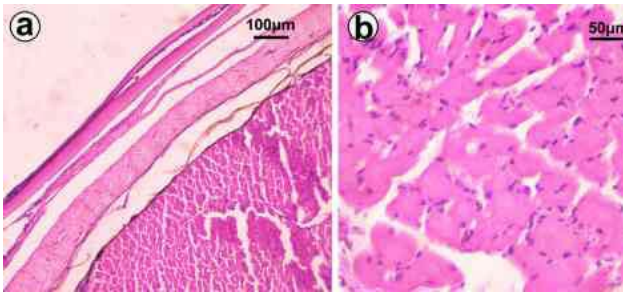

Fig. 2. Transverse sections of normal skin of *R. kanagurta*. (a) Compactly arranged cells in the skin layers (b) Normal muscle tissue shows compactly arranged muscle fibres.

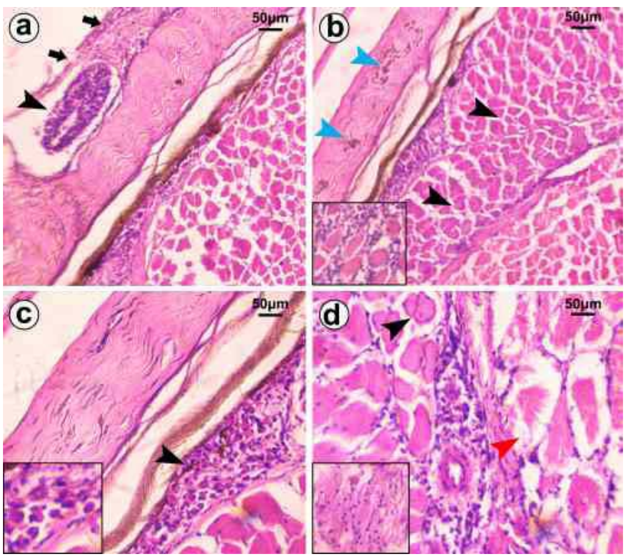

Fig. 3. Histopathological sections of posterior body surface of *R. kanagurta* infested by *N. phaiopleura.* (a) Depression at the site of parasite attachment along with massive lymphocyte infiltration (black arrowhead). Epithelial hyperplasia along with epidermal spongiosis (black arrows) (b) Severe degenerative changes in the dermal layers with proliferation of melanomacrophages (blue arrowhead). Degenerating muscle fibres (black arrowhead) along with macrophage infiltration (insert). (c) Infiltration of immune cells (black arrowhead) such as eosinophils and lymphocytes (insert). (d) Oedema (black arrowhead) and muscle tissue undergoing necrosis (red arrowhead). Muscle fibrosis along with severe prolife

were found to be infested with ovigerous females. Discrete alterations such as haemorrhages, loss of scales, extensive skin erosions/ skin ulceration were observed at the attachment site of the parasite (Fig. 1c arrowhead).

On the body surface, small pinholes with the formation of epidermal plaques (Fig. 1d circle) and large, round-shaped haemorrhagic wounds/ulcers (no fish skin was present on the wounds, where the muscle was exposed) were observed as gross pathological symptoms observed under SDM.

The parasitic isopod, *N. phaiopleura,* collected from the host's body surface, was identified by the key taxonomic characteristics: Lack of posterolateral processes on the pereonites, pleotelson curving smoothly to a distinct point, narrow and straight uropod rami. Uropod exopod straight, elongate about eight times longer than proximal width.

Histopathology of normal skin from the uninfected fish showed 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 *N. phaiopleura* was undergoing degenerative changes. At the insertion site of pereopods of the parasite, there was deep skin depression (Fig. 3a) along with massive lymphocyte infiltrations. The epidermis around the pereopod attachment site was hyperplasic. Within the most extensive hyperplastic tissue, epidermal layers were spongiose with the clear manifestation of epidermal spongiosis (Fig. 3b). The epidermal and dermal cell layers were infiltrated with inflammatory cells, mostly macrophages (Fig. 3b), lymphocytes (Fig. 3c) and eosinophils (Fig. 3c insert).

Muscle degeneration, along with oedema and tissues undergoing necrosis, were observed in the muscle tissues (Fig. 3d). Muscle fibrosis (the muscle tissue is replaced by degenerative fibrotic tissue) along with severe proliferation of inflammatory cells were evident (Fig. 3d insert).

Discussion

In the present study, the parasite is specifically attached to the posterior third of the body, overlying the lateral line and facing the fish's head, indicating that this is the most preferred attachment site of *N. phaiopleura*. According to Brusca (1981), all known species of *Nerocila* reside on the surface of their hosts. The preference of attachment area might depend on the host's body movement, the needs of the parasite and the limitations exerted by the morphology and habits of the host (Rameshkumar and Ravichandran, 2013). This position also makes accessible the streak of red muscle underlying the lateral line, which is highly vascular and contains much fat and myoglobin. It also allows the abdomen to be raised for pleopod ventilation without risking dislodgement when the fish's tail beats sideways (Bowman and Tareen, 1983).

Large haemorrhagic wounds were observed at the site of parasite attachment during the study. Raja *et al.* (2014) observed similar results in *Chirocentrus nudus* infested by *N. phaiopleura.* Isopod feeding activities involving perhaps either the rapidly repeated thrusts of the stylet of their piercing proboscis, or the rasping action of mandibles and/or toxic digestive secretions (Rand, 1986); penetration and movement of their claw-like prehensile appendages; and frequent shifts in position on the host causes a serious wound (Ravi and Rajkumar, 2007). The isopods inhabiting the body surface also causes severe injuries through pressure exerted by the parasite. The extent of damage is proportional to the duration of settlement and the size of the isopods (Romestand, 1979). The infested area showed white halo or epidermal plaques during the study. According to Rand (1986), the penetration of the dactylus of pereopods of the parasite results in small pinholes.

Histopathological examination of the infected skin revealed significant changes in all layers. Deep skin depression, together with massive lymphocyte infiltrations, was noticed at the insertion sites of pereopods. Rand (1986) reported 'U' shaped skin depression along with massive infiltration of inflammatory cells in *Paranthias furcifer* due to *Nerocila acumunata* infestation. The parasite infestation on the skin of the fish caused damage and disruption of the epidermis along with hyperplasia. Hyperplastic changes are either a response to regenerate the sloughed epidermal cells (Ravichandran *et al.*, 2007) or protect the underlying tissues (Meissner and Diamandopoulose, 1977).

In the present study, host response as a result of parasitic infestation includes increased infiltration of lymphocytes, macrophages and eosinophilic granulocytes. Ravichandran *et al.* (2007) reported perfuse infiltration of tissues with lymphocytes and granulocyte as a common feature in isopods infestation in fish as a defense mechanism. Similar histopathological alterations were accounted due to the infestation of *N. accuminata* (Rand, 1986) and *N. bivittata* (Elgendy *et al.*, 2018).

The significant histopathological changes in the fish tissues caused by *N. phaiopleura* are probably due to attachment, movement and feeding. Furthermore, localized loss of osmoregulatory skin function may occur at the lesions. Finally, the isopods may mechanically impede swimming performance, making infested fish more susceptible to predation and indirectly causing an increase in mortality in wild fish populations.

Overstreet and Howse (1977) reported that the hemorrhagic lesions produced by isopod parasites become the sites for secondary infections by microorganisms. According to Raja *et al.* (2014), secondary bacterial infection occurs in skin lesions caused by *N. phaiopleura* in Indian fishes. The damage caused by the isopods to the tissues and their subsequent exposure could cause the death of fish, thus causing the fish population to decline. The parasitic infestation and the visible external damages also will lead to consumer rejection. Isopod parasitic infection can lead to severe economic loss in commercially important fishes.

N. phaiopleura is reported from 50 species of fishes belonging to 14 families including Clupeidae, Engraulidae, Carangidae, Scombridae, Dussumieriidae, Chirocentridae, Pristigasteridae, Mugilidae, Sphyraenidae, Leiognathidae, Plotosidae, Polynemidae, Ariidae and Istiophoridae from the Indian ocean and Pacific ocean (Nagasawa and Isozaki, 2017; Rameshkumar *et al.,* 2013; Pillai, 1958; Raja *et al.*, 2014; Veerapan and Ramanathan, 1997). This clearly shows that this species is least host specific and can be a potential threat for farming of fishes. Almost all host fishes including *R. kanagurta* are very important and forms a significant catch in commercial ladings of many countries including India. Some of the hosts like *Tenualosa ilisha, Carangoides malabaricus, Parastromateus niger, Scomberomorus guttatus, Chelon parsia, Thunnus orientalis, Konosirus punctatus, Trachurus japonicas* are already identified as potential species for mariculture and lot of research is going on for seed production and farming of these species (Ranjan *et al.*, 2017; Nagasawa and Shirakashi, 2017). Crustacean parasites including isopods which are generally unnoticed as a major problem in the wild can be a serious problem in confined conditions of farming (Kabata, 1985). *N. phaiopleura* is already reported to facilitate secondary microbial infections in fishes (Ravichandran *et al.*, 2001). Considering its wide geographical distribution and host range, *N. phaiopleura* has the potential to become a threat for coastal farming. Adequate management measures including chemical prophylaxis can control the infection of *N. phaiopleura* to some extent in farming (Rameshkumar and Ravichandran, 2014; Jithendran *et al.*, 2008; Smit *et al.*, 2014).

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