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Histopathological changes in the skins and gills of some marine fishes due to parasitic isopod infestation

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PEER REVIEW

Peer reviewer

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Comments

This is a well-written study about the parasitized fishes. The results are interesting, and it is concluded that the infection studies of parasite that attaches or settles on the host body, at first, causes localized inflammatory changes, but with time, assuring a different or diffused character. This article is very useful for parasitologists and zoologists.

Details on Page 16

ABSTRACT

Objective: To study the histopathological symptoms owing to cymothoid isopod that were categorised as gross lesions.

Methods: Nature of damage fish tissues and gills were taken from the parasite attachment area of infested and uninfested fishes which were cut out in fresh condition fixed in 10% buffered neutral formalin. Fresh and recently preserved tissues and gills were washed in tap water and dehydrated using alcohol series. The tissues gills were then cleaned in methylbenzoate and benzene and embedded in paraffin wax. The serial sections cutting 4 to 5 m thickness, were stained with Erlich's haematoxylin and Eosin for histopathological analysis.

Results: In normal muscle tissue, the tensile strength of muscle fibers with extra cellular matrix collagen was extensively tight associated. This gave a rigid musculature pattern to the tissues. Infested fish exhibited histopathological anomalies such as tissue reactions, primarily associated with the formation of granulomas consisted of macrophages and epitheleioid cells, which were occasionally surrounded by a thin rim of fibroblasts. The infestations such as lipofibrosis, hyperaemia, haemorhagic lesions and penetration of dactylus usually pressure atrophy often accompanied by the presence of parasites. Lesions had well developed granulomas that underlined in the muscle or overlying subcutaneous tissue, form these spread to underlying organs.

Conclusions: It could be concluded that the infection studies of parasite that attaches or settles on the host body, at first, causes localized inflammatory changes, but with time, assuring a different or diffused character. The changes always begin with hyperaemia in the angles between adjacent sides at the site of attachment and then move towards deeply situated area.

KEYWORDS

Cymothoid isopod, Histopathology, Gill, Body surface, Parasitic infestation

1. Introduction

Isopod parasites are usually very large and fierce looking and the damage caused to the host fish is considerable. Fish share considerable evolutionary commonality with mammals, including man. Most of the basic pathological conditions that are recognized in mammals, *e.g.* hyperplasia, necrosis, inflammation, septicemia, metaplasia and neoplasia, are also observed in fish. Fish diseases and pathologies have also been used as indicators of environmental stress[1]. The consequences of the parasitization can cause the death of the host[2], increase the sensitivity of fish to pollutants like crude oil[3], and reduce the host growth even in the case of small intensity of infection[2].

Cymothoid isopods are a widespread family of blood

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feeding crustaceans that parasitize both marine and freshwater fishes in India and Malaysia[4-8]. The cymothoid isopod, *Nerocila accuminata* attaches to the skin of a variety of marine fish species[9]. The pathogenocity of isopods stated that the effect of the destruction of host tissue was mainly due to the pressure exerted by the parasites body[10]. Histology of the female reproductive system of a parasitic isopod *Nerocila serra* from the fishes of Waltair coast[11]. Although there are brief descriptions of the gross pathology of infestation on this Crustacean[12], apparently no histopathological details have been published. Records of infestations have been made for some members of the family Pandalidae[13,14]. Indian cymothoid fauna is still incompletely known despite several studies have already been published or reported[15-19]. However, there are few reports confirming histopathlogical changes which are infested by isopod parasites on tissue damage caused by them[20]. At present, there is no accurate assessment of the infestation of isopod parasites in relation to their histopathological studies. Hence the present was attempted to study the infestation and histopathology of infection by the isopod parasites in Carangids fishes.

2. Materials and methods

The damaged fish tissues and gills were taken from the parasite attachment area of infested (Figure 1) and uninfested fishes were cut out in fresh condition fixed in 10% buffered neutral formalin. Tissues and gills were considered for histopathological study. Fresh and recently preserved tissues and gills were washed in tap water and dehydrated using alcohol series. The tissues gills were then cleaned in methylbenzoate and benzene and embedded in paraffin wax. The serial sections cutting 4 to 5 m thickness, were stained with Erlich's haematoxylin and Eosin for histopathological analysis.

Figure 1. Gross lesions by *Nerocila depressa* on *Selaroides leptolepis* (*S. leptolepis*).

3. Results

3.1. Gross lesions

The histopathological symptoms owing to cymothoid isopod *Nerocila depressa* (*N. depressa*), *Nerocila phaeopleura* and *Ryukyua circularis* were categorised as gross lesions (Figure 2). On the surface of the body, small pinholes due to the penetration of the dactylus of pereopods and skin lesions due to the attachment of *N. depressa* were observed as gross pathological symptoms. Although there was no obvious bleeding, petechiae occurred over the entire surface of the lesion, especially where the pereopods and mouth parts of parasites attached to the skin. The anterior portion of the parasite was closely apposed to the host body surface, the posterior end raised from it, so as to face the upstreams of water currents. In the case, the host was infested by two parasites, the first located on the body surface and the second on the base of the opposite positions of body surface region. At the site of parasitic attachment, skin lesion was observed in the parasitized fishes. The cymothoid isopod tore the epidermis layer of the fish host using their mouth parts and fed on the muscle tissue beneath (Figure 1). The lesioned sites were pale red colour indicating anemia (Figure 2). It was not known whether isopods ingest host tissue or if damage was caused by abrasion. The severe damage would suggest that the host tissue is being eaten. Parasitized fish may be less able to escape predators compared to unparasitised fish due to altered hydrodynamics and reduced energetic resources.

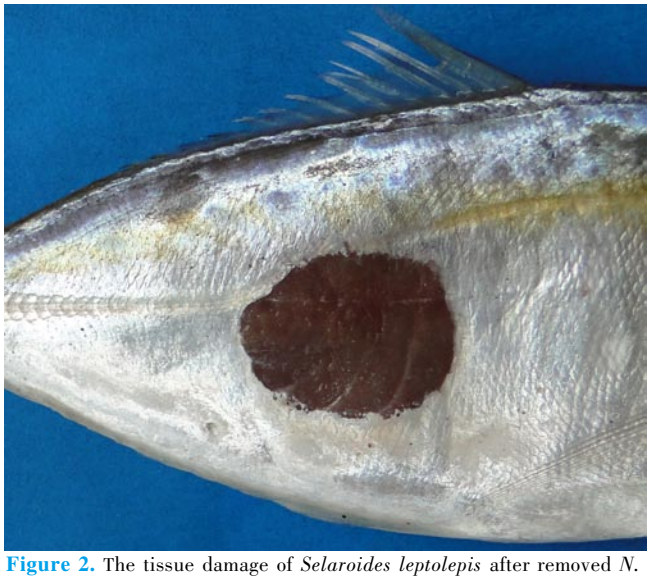

Figure 2. The tissue damage of *Selaroides leptolepis* after removed *N. depressa* parasite.

3.2. Histopathological anomalies

The tissue reactions primarily associated with the formation of granulomas consisted of macrophages and epitheleioid cells, which were occasionally surrounded by a thin rim of fibroblasts. Lesions were never encapsulated but appeared to follow myomeres. Lesions had well developed granulomas that under laid in the lost epidermis. Lesions appeared to originate in the muscle or overlying subcutaneous tissue, from these spread to underlying organs. There was heavy lymphocyte infiltration as well.

The structural details of the normal undamaged of tissue are shown in Figure 3-5.

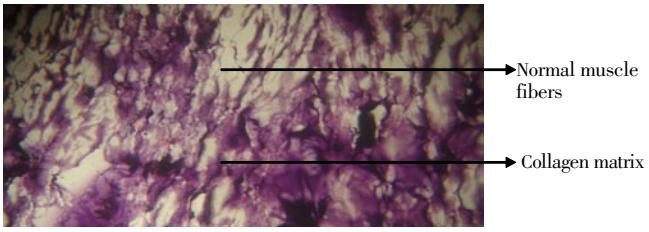

Figure 3. Histopathology of the normal tissue of *Selaroides leptolepis*.

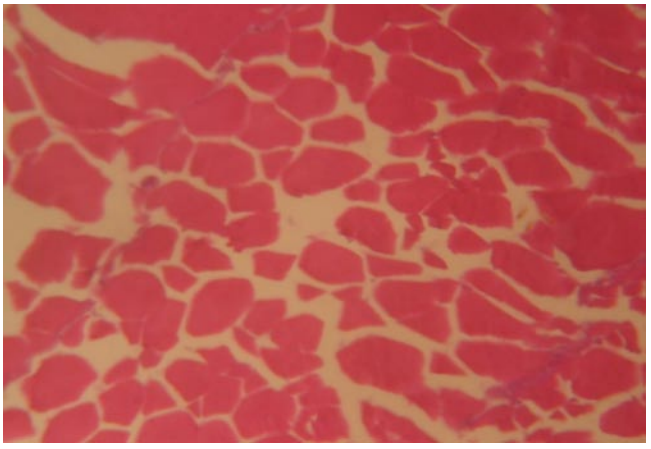

Figure 4. Normal tissue of *Sardinella gibbosa*.

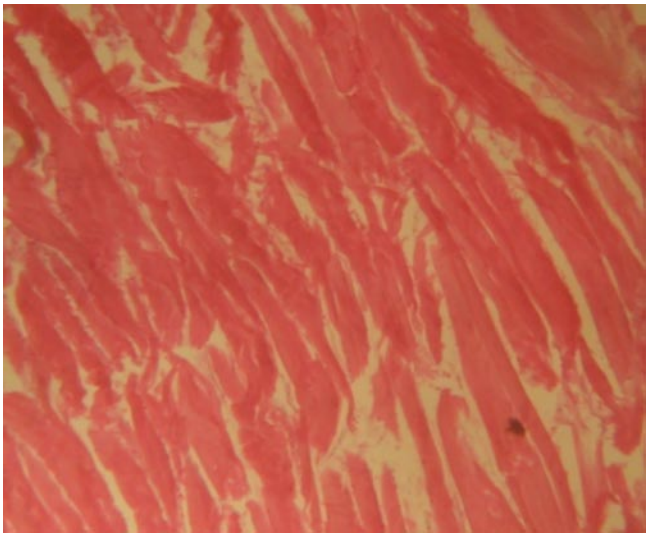

Figure 5. Normal tissue of *Dusumeria acuta*.

It could be observed clearly of the histological changes associated with isopod infestation of the tissue of body surface around the site of attachment, which were seriously damaged (Figure 6-8).

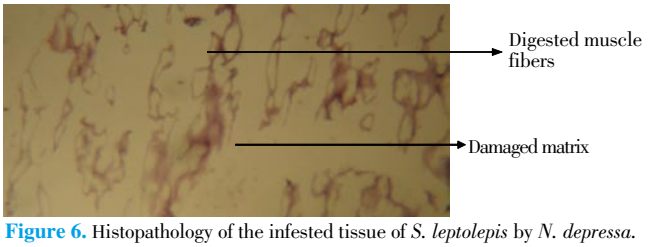

Figure 6. Histopathology of the infested tissue of *S. leptolepis* by *N. depressa*.

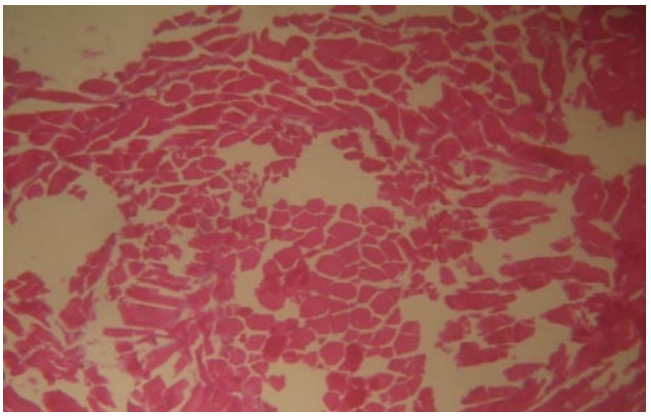

Figure 7. Infested tissue of *Sardinella gibbosa*.

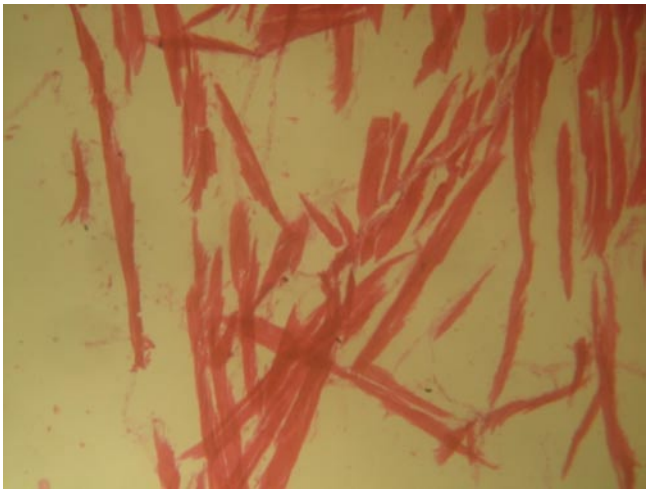

Figure 8. Infected tissue of *Dusumeria acuta*.

The lesions on the body surface involed only the tissue. In normal muscle tissue, the tensile strength of muscle fibres with extra cellular matrix collagen was extensively tight associated. This gave a rigid musculature pattern to the tissues. Histopathological section of normal fish tissues denoted this extensive normal muscle fiber arrangement with the extracellular matrix of collagen (Figure 3).

The tissue damage to host by cymothoids was often impressive, but this damage was caused by crypting (a necrotic eroding reaction of host tissues pressed against the parasite) or deformation (host growing against the parasite) (Figures 7 and 8). Cymothoid piercing-sucking mouth parts seemed more suited to body fluids.

In the case of this study, isopod infested fish tissues showed that there was a vigorous and damaged muscle fibers, and diminished collagen matrix was recorded (Figure 6-8). This indicated that the isopod infection with terms of their attachment in their body surface of fishes leaded to extensive tissue damage and the body surface acted as a good habitat for isopod prevalence.

Cymothoid isopod *Ryukyua circularis* was parasitic on *Amblygaster sirm* in normal gill filaments (Figure 9).

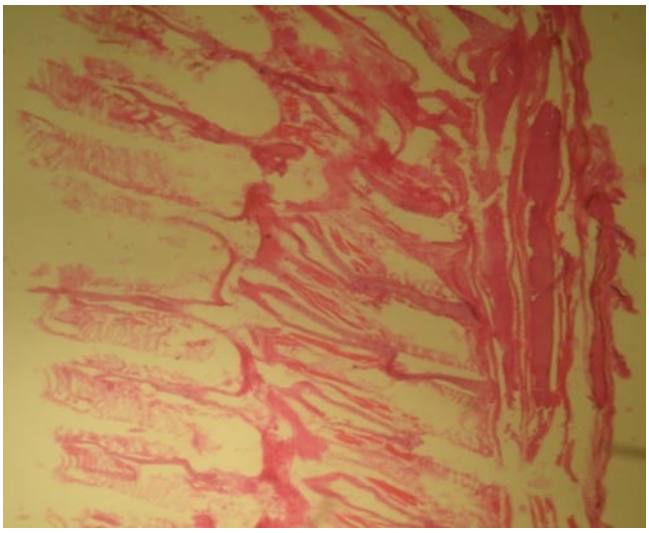

Figure 9. Nature of thickening between the gill lamellae in *Amblygaster sirm*.

These parasites were found very loosly attached to the gill filaments. Nature of damage, observed in the gill remained the same, but the degree of damage varies, as the closely opposed gill arch observed a higher damage (Figure 10). Secondary gill lamellae uneven clubbed and showed fusion. Bifurcation was noticed at the tip of lamellae and the cartilaginous support of the gill arch was twisted. Parasitized fish were reported to have larger erythrocytes containing less haemoglobin compared with unparasitized fish.

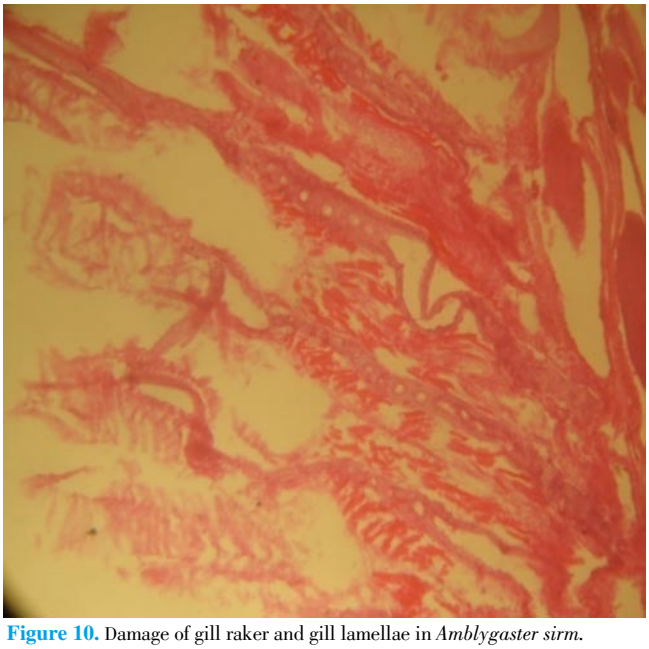

Figure 10. Damage of gill raker and gill lamellae in *Amblygaster sirm*.

4. Discussion

The parasites may be attached on the surface, fins, gills of the fish or in the internal organs without harmful consequences to the host. Histological examination on the tissue which was settled by the isopod showed that the actions of the parasites meet with a characteristic reaction on the host tissue, a reaction which is surprisingly uniform regardless of the stimulus applied. Earlier workers indicate the presence of hyperplastic and hypertrophied reaction as function of infection due to isopod parasites[21,22].

Paranthias furcifer infested with four specimens of *Nerocila acuminata*, for attachment of that isopod used the distal portion of claw-like prehensile pereopods, which are inserted into host dermis[23]. Penetration of dactylus inflicts tissue damage at the regions of attachment, the deep U- or Y-shaped skin depressions due to the penetration of dactylus was lined with glaucomatous tissue. However, in this study, host tissues were vigorous and damaged muscle fibers and diminished collagen matrix was recorded. The parasite infested on the body surface cause damage and disruption of epidermis of underlying tissues, due to attachment of pereopods. Presence of melanophores in the epidermis and dermal layers of fishes infested by *Nerocila acuminata*[23]. At the site of attachment, the parasites erode the epidermis, dermis and muscular tissue of the *Sardinella*. Such pathology may cause mortality in many fish[24]. In the present study, similar distinct melanophores were not accounted due to infestation of *N. depressa*. Healthy tissue were absent at the pereopod attachment sites. Infested tissues appear to be deteriorated and are irregular in structure. The effects may be due to the stress excerted by the parasite to the underlying tissues.

Generally, most pelagic fish within a population are not infected by isopod parasites. Furthermore, infected fish were found to be lighter than uninfected fish, indicating the histological effect of infection on the host. Several authors have reported histopathological changes on the body tissue of fish infested to isopod[23-25]. In most cases, histological changes were characterized by damage in the epithelial cells and/or hyperplasia, lamellar swelling, telangiectasis and aneurysm.

The gill filaments of Hawaian morey eel, *Gymnothorax eurostus*, were missing in the anterior or posterior-most region of the gill arches owing to the attachment of *Lironeca puki*[26]. The smaller males and juveniles of isopod *Olencira praegustator* parasite on Gulf menhaden caused changes on the gill filaments through feeding[12]. Infested gill rakers were seriously last, apical edges demaged and gill lamellae heavily destroyed. Gill lamellae of the first and second gill arches were eroded due to the isopodparasites *Joryma tartoor*[10]. But in the present study, secondary gill lamellae uneven clubbed and fusion.

Histological changes associated with *Peniculisa wilsoni* infestation are hyperplasia of the soft tissue of the fin around the site of attachment and damage to the affected bone lamellae[12]. Infested fish exhibited histopathological anomalies such as tissue reaction, primarily associated with the formation of granulomas consisted of macrophages

and epitheleoid cells, which are occasionally surrounded by a thin rim of fibroblasts[27]. The anamnesis that the places where parasites infected the individuals, presented a whitish colour and also small lesions in some of the fish specimens[28]. Histopathological lesions of gill and skin associated with external parasites in two common aquarium fish, Oscar (*Astronotus ocellatus*) and Discus (*Symphysodon discus*) fish[29]. Histopathological lesions of *Gyrodactylus* sp. on gill and skin are the same as *Dactylogyrus* sp[30]. The skin lesions are common in fish and the fish skin is constantly exposed to the water environment and external environment, is susceptible to the toxins, environmental pollution and pathogens[31]. Histo-pathological changes were observed mainly in the liver which were represented by loosening of hepatic tissue, eccentrically situated nuclei of hepatocytes and necrosis[32].

Observed destruction of host tissues as a result of the pressure exerted by the parasite body[33]. In this study, *N. depressa* was most frequently found on the body surface area. The position of attachment area might depend on the host's body movement. In undulatory swimming, a backward-travelling wave is generated by the sequential activation of the segmental myotomes from head to tail[34]. Secretion of mucus on the ligament surface as a reactive response of the host against infestation[22]. Penetration of dectylus pereopods resulted in small pinholes in the cartilaginous support of gill arches. Penetration of pereopods also caused bulging of gill arches at the affected parts.

Several studies have emphasized the connection between pollutant exposure and parasitic load in fish[35-38]. The muscular growth has been identified as lipofibrous nodule developed in response to irritation caused by the isopod *Joryma tartoor*[10]. In this study, parasitological investigations were differentiated between normal fish tissue lesions and infested tissue lesions by isopod parasites. In normal fish, tissues denote this extensive normal muscle fiber arrangement with the extracellular matrix of collagen. Histopathological studies reviewed the damage caused by the cymothoid isopod on the infected fish host. The damage caused to the tissues and their subsequent exposure could have caused the death of fish, thus causing the fish population to decline[24]. This could be one reason for the large decline in the sardine catch during that time. A positive linear relationship between the length of parasite and its host suggested that the fish were infected early in their lives and that they and their infecting parasites had grown together. *Ceratothoa imbricatus* on native fish from the south coast of Australia caused a reduction in weight when more than two parasites are found per fish[39]. Other studies have found that parasitic infection may reduce or interfere with the ability of the host to feed[40,41]. However, our data indicated that infection by *N. depressa* had little effect on the body of fish host reduced length and weight.

Erosion and thickening are the two unique morphological changes noticed owing to infestation. These changes are mainly due to the heavy pressure exerted by the parasite and also by their feeding nature. But in the present study, apart from muscle fibers, and collagen matrix reaction was also diminished. In any way, these muscle fibers formation is also a host reaction in response to the irritation caused by the isopod *N. depressa*. No doubt it is also a host response to withstand the attack of the parasite. Hyperplasia in some situations represent an adaptation by the organism to protect the underlying tissues form any irritant[42,43]. The pathological effects of cymothoid isopods on their hosts cluster at two extremes. Some associations are quite severe and can even cause host death[44,45], In fish, some infections that induce mortality are age and temperature dependent. Environmental change, especially habitat degradation by anthropogenic pollutants and oceanographic alterations induced by climatic, can influence parasitic-host interaction[46], whereas others are comparatively benign[47]. It could be concluded that the infection studies of parasite that attaches or settles on the host body, at first, causes localized inflammatory changes, but with time, assuring a different or diffused character. The changes always begin with hyperaemia in the angles between adjacent sides at the site of attachment and then move towards deeply situated area.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to Department of Science and Technology (Grant No. SR/FF/LS-088/2007) and Ministry of Environment & Forest, Government of India (Grant No. 22-18/2008-CS-I) for providing financial support and Director of Centre of Advanced Study in Marine Biology for providing facilities and encouragement.

Comments

Background

Isopod parasites are usually very large and fierce looking and the demage caused to the host fish is considerable. The histopathological symptoms owing to cymothoid isopod *N. depressa*, *Nerocila phaeopleura* and *Ryukyua circularis* were categorised as gross lesions. The cymothoid isopod tore the epidermis layer of the fish host using their mouth parts and fed on the muscle tissue beneath. The histological

changes associated with isopod infestation of the tissue of body surface around the site of attachment were seriously damaged. This indicates that the isopod infection with terms of their attachment in their body surface of fishes leads to extensive tissue damage and the body surface acts as a good habitat for isopod prevalence.

Research frontiers

This study aims to study the histopathological symptoms owing to cymothoid isopod that were categorised as gross lesions. In normal muscle tissue, the tensile strength of muscle fibers with extra cellular matrix collagen is extensively tight associated. The infestations such as lipofibrosis, hyperaemia, haemorhagic lesions and penetration of dactylus usually pressure atrophy often accompanied by the presence of parasites. Lesions had well developed granulomas that underlined in the muscle or overlying subcutaneous tissue, form these spread to underlying organs.

Related reports

On the surface of the body small pinholes due to the penetration of the dactylus of pereopods and skin lesions due to the attachment of *N. depressa* were observed as gross pathological symptoms. Many related works have been reported. Eller and Romestand *et al*. indicate the presence of hyperplastic and hypertrophied reaction as function of infection due to isopod parasites. Rand, Printrakoon *et al.*, and Radhakrishnan *et al*. have reported histopathological changes on the body tissue of fish infested to isopod. In most cases, histological changes were characterized by damage in the epithelial cells and/or hyperplasia, lamellar swelling, telangiectasis and aneurysm.

Innovations & breakthroughs

The innovative outcome of this paper to histopathological changes in some marine fishes due to parasitic isopod infestation.

Applications

The anterior portion of the parasite is closely apposed to the host body surface, the posterior end raised from it, so as to face the upstreams of water currents. In the case, the host was infested by two parasites, the first located on the body surface and the second on the base of the opposite positions of body surface region. At the site of parasitic attachment, skin lesion was observed in the parasitized fishes. The study is very useful for the researchers who work in parasitology field.

Peer review

This is a well-written study about the parasitized fishes. The results are interesting, and it is concluded that the infection studies of parasite that attaches or settles on the host body, at first, causes localized inflammatory changes, but with time, assuring a different or diffused character. This article is very useful for parasitologists and zoologists.

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