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Biochemical and histological alteration of edible fishes due to infestation of parasitic isopods

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Abstract
Proximate composition is one of the most significant aspect in fish nutrition. The effect of parasitic isopod on edible fishes showed a significant decrease in proximate composition and considerable histopathological alterations. Among the parasitized fish species, the maximum protein content was found in *Parastromateus niger* (18±0.96 µg/g). Whereas, the carbohydrate level was found maximum in *Arius maculatus* (0.9±0.32µg/g) and minimum in *Thryssa mystax* (0.3±0.28µg/g). In case of lipids, maximum value was found in *Rastrelliger kanagurta* (0.9±0.2µg/g) and minimum in *Trichiurus lepturus* (0.2±0.1µg/g). The present findings demonstrate the variations in the proximate composition of parasitized and unparasitized fishes. Histopathological observations on the parasitized host displayed several changes on the morphology of the muscle tissue and gill lamellae. The investigation revealed that parasitic isopods have ability to affect the health of edible fish species.

Keywords: isopods, parasites, infestation, biochemical, fishes

1. Introduction
Isopods are obligate fish parasites, occurring in all oceans with the exception of polar waters (Ahyong *et al*., 2011) [1]. Parasitic isopods are typically marine and usually inhabit the warmer seas and occur very commonly in commercially important food fishes (Kumar *et al*., 2017) [2]. Isopods are blood-feeding; attached in the buccal cavity of fish, others live in the gill chamber and present on the body surface including the fins (Rameshkumar *et al*., 2013; Rameshkumar and Ravichandran, 2014) [3,4]. From India 47 nominal species corresponding to 36 valid species infesting hosts belonging to 34 families were until now recorded (Trilles *et al*., 2011) [5]. A considerable variation were noticed in the effect of isopod parasites on the marine food fishes (Ravichandran, 2007; Fogelman and Grutter, 2008) [6, 7]. The skin lesions are most common and the fish skin is constantly exposed to various environmental stress (Noga, 2010) [8].
Hemorrhagic anemia in fish may be associated with trauma, cutaneous ulceration, parasitism and leads to nutritional deficiencies such as Vitamin K and Vitamin B (Groff and Zinkl, 1999; Campbell and Ellis, 2007) [9 10]. Parasitic isopod cause damages in their host (Ravichandran *et al*., 2010) [11] and the histopathological changes were observed in different organs, mainly in the liver. The infected fish liver showed loosening of hepatic tissue, eccentrically situated nuclei of hepatocytes and also necrosis (Kaur *et al*., 2012) [12]. The pathological effects of cymothoid isopods on their hosts are quite severe and can even cause mortality in fish (Papapanagiotou and Trilles, 2001) [13]. Parasitic disease of the fish are of greater concern in terms of economy and health. It is one of global problem but still they are poorly focused area of research. Understanding the disease pathology through biochemical changes and histopathology is very much essential for diagnosis as well as it will provide better insight in understanding the pathogenesis of the parasites. Though enormous reports are available on the aspects of parasitic diseases of fishes and the nature of isopod fish parasitism. The reports related to the histological and biochemical changes is scanty. The present study was aim to evaluate the occurrence of histological alterations and biochemical variations in marine fishes due to parasitic infestation. There are very limited literature review available in aspects of the histopathology of this parasitic infestation. This study will aid in full fill that lacuna.

2. Materials and Methods
2.1 Biochemical Estimation
Standard methods were followed for the estimation of protein, carbohydrate and lipid constituents from the 20 parasitized fish species (Dubois, 1956; Folch *et al*., 1968; Raymont *et al*., 1968) [14, 15, 16]. Host nomenclature and fish taxonomy was identified according to Fish Base (Froese and Pauly, 2013) [17]. Among each species, ten individuals were taken (5 parasitized and 5 unparasitized specimens with same weight) for estimating the biochemical content, whereas the unparasitized were treated as control samples.

2.2 Histopathological Examination
Tissue samples from parasitized and unparasitized fishes were dissected and fixed in formalin. The tissues were dehydrated with series of alcohol treatment in the order of methyl, ethyl and absolute alcohol and the tissues were embedded in paraffin wax. The paraffin tissue blocks were sectioned by microtome at a thickness of 4 µ. Tissue sections were collected on clean glass slides and left in the oven at 40ºC and deparaffinized in xylene and then immersed in descending series of alcohol, stained with hematoxylin and eosin stain for histopathological examination. Stained sections were examined under high power objective in light microscope and imaged.

3. Results and Discussion

The present findings revealed that the infestations of isopod parasites on body surface fins and gill raker of collected fish species. The observed symptoms on the infested fish species were skin lesions, little pinholes, and penetration of dactylus; pereopods on their body surface (Fig.1 & Fig.2).

3.1 Biochemical composition in relation to infestation

Biochemical compositions were estimated in the control and parasitized fish species in order to determine the variations. Isopod parasites inhibiting the growth of fish species caused significant decrease in the concentrations of biochemical constituents. Protein was one of the dominant biochemical constituents as they form the chief nitrogenous constituents in the body tissues. The protein content was found to be maximum in *Parastromateus niger* (18±0.96 µg/g) and minimum in *Trichiurus lepturus* (7±0.54µg/g) as shown in Fig.3. *Parastromateus niger* that were devoid of parasitic infestation showed maximum protein content (24±0.64µg/g).

In the isopod parasitized samples, the carbohydrate level was found maximum in *Arius maculatus* (0.9± 0.32µg/g) and minimum in *Thryssa mystax* (0.3±0.28µg/g), whereas the control *A. maculatus* showed maximum carbohydrate content of 1.3±0.34µg/g and minimum in *Otolithes ruber* (0.8±0.28µg/g) as shown in (Fig.4). The maximum value of lipid content in the infested sample was found in *R. kanagurta* (0.9±0.2µg/g) and minimum value was observed in *T. lepturus* (0.2±0.1µg/g). Lipid content in the control sample was found to be higher in *Eubleekeria splendens* (1.5±0.2µg/g) and lower in *O. ruber* (0.6± 0.3µg) (Fig.5).

3.2 Histopathological Studies

Tissues of host species parasitized with isopod parasites were sorted for histopathological examinations. The tissue of control fish *Carangoides malabaricus* was shown in Fig.6A. The pathological alterations on the infested tissue of *C. malabaricus* was primarily associated with the formation of macrophages and epithelioid cells, which were occasionally surrounded by a thin rim of fibroblasts (Fig.6B). Tissue of normal fish *Sardinella gibossa* shows compactly arranged cells and often form maize like patterns or extensively branched (Fig.7A). The infested tissue of *S. gibossa* depicted mosaic pavements of irregular polygonal epithelial cells with varied dimensions. The micro ridges of the cells appeared normal, uniform, smooth width and sinuous (Fig.7B). Histopathological section of control, *Hemirampus far* showed normal muscle tissue, fair strength and extensive and tight associations with extracellular matrix collagen (Fig.8A). The parasitic infested gills of the host fish showed considerable histopathological changes, mainly deterioration of mucous cells, blood cephalic cells, lifting and fusion. Tissues also showed desquamation, breakage of secondary lamella; lifting and proliferation of goblet cells (Fig.8B).

Despite the fact that the parasites are much smaller than the host species, their infestations often have noteworthy consequences on the host morphology. Thus, the harm caused to the host is the concept central to the many definitions of parasitism that have been proposed. However, there are reports where *Cymothoas pinipalpa* showed no obvious effects of parasitism on its host. Comparison of the biochemical constituents were made from the tissue sections of the parasitized and unparasitized fish species. Hence, the present study makes it evident that the isopod parasite shows an inordinate effect on the biochemical content of the fish species. Increase in the intensity of parasite infestation was found to be proportional to the decrease in the levels of biochemical constituents. The results of depletion of proximate composition due to parasitic infestation in five different fishes *Anabas testudineus, Channa orientalis*, *Lebistes reticulatus*, *Macropodus cupanus* and *Tilapia mossambica* (Nair, 1981) [18] were in accordance with the present study.

Findings from the present study showed that isopod parasites inhibit more amounts of carbohydrates and lipids than the proteins. The impact of the parasites on the carbohydrate content in infested fishes was found to be lower than the unparasitized fishes. Glycogen depletion in the infested fishes might be due to the feeding of isopod parasites on host blood, where the blood sugar serves as a source of energy reservoir. Owing to the loss of blood in the host species, they become anemic, which is macrocytic-hypochromic in nature. This in turn delays the fish growth leading to the reduction in the body weight due to the lack of food intake (Baker *et al*., 2005; Barber *et al*., 2000; Ravichandran *et al*., 2001)[19,20,21]. Decrease in the protein content may be due to the effect of isopod parasites, which feed on the gut content of the host species.

Histological examination of the isopod infested tissue showed some characteristic changes in the host tissue, with hyperplastic and hypertrophied reaction as seen in previous records (Kaur *et al*., 2012) [12]. Infested fishes exhibited histopathological anomalies such as tissue reaction, primarily associated with the formation of granulomas consisted of macrophages and epithelioid cells, which were occasionally, surrounded by a thin rim of fibroblasts. Lipofibrous nodule was developed in response to the irritation caused by the isopod parasite *J. tartoor* (Ravichandran *et al*., 2007) [22]. Erosion of gill lamellae, damage of gill rakers and pale gills were the severe gross lesions observed as a consequence of isopod infestation (Rameshkumar and Ravichandran, 2014) [4]. In most cases, histological changes in the infested tissue were characterized by damage in the epithelial cells, hyperplasia, lamellar swelling, telangiectasia and aneurysm. In this study considerable histopathological changes were observed in the infested body tissues. Damages in gill lamellae of *H. far* was observed. Tissue reaction, primarily associated with the formation of granulomas. These granulomas consisted of macrophages and epithelioid cells, which were occasionally, surrounded by a thin rim of fibroblasts.

Generally, most pelagic fish within a population were not infested by isopod parasites. Numerous reports are available on the histopathological changes on the body tissue of fish infested with isopod parasites (Printrakoon and Purivirojkul, 2011) [23]. The anamnesis of parasitic infestation was seen in the individuals with whitish colored small lesions in some fish specimens as reported earlier (De-Carvallio-Souza *et al*., 2009) [24].

Fig 1: Infestation of isopod parasite *Nerocila phaeopleura* on the body surface of *Rastrelliger kanagurta*

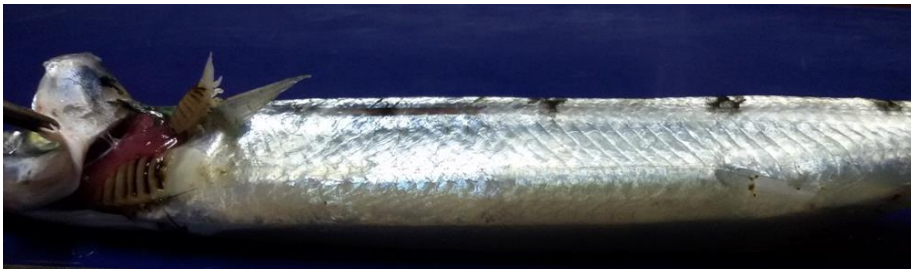

Fig 2: Infestation of isopod parasite on the gill raker of *Hemiramphus far*

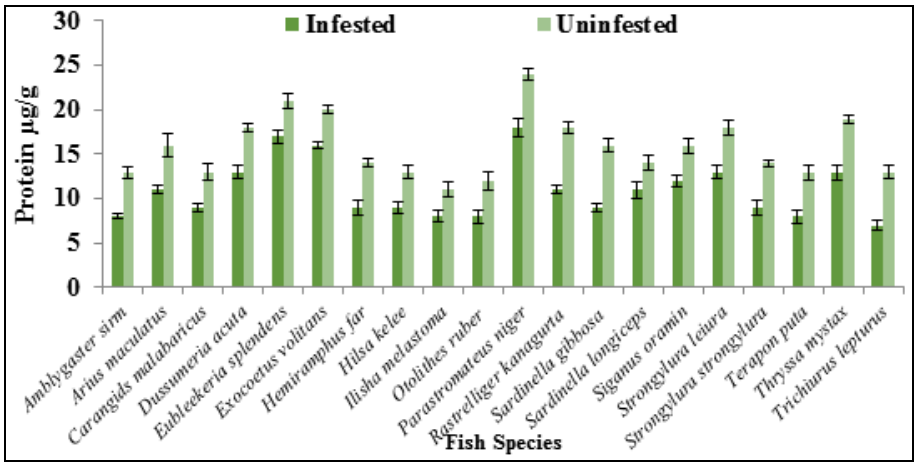

Fig 3: Variation of protein content in relation to infestation

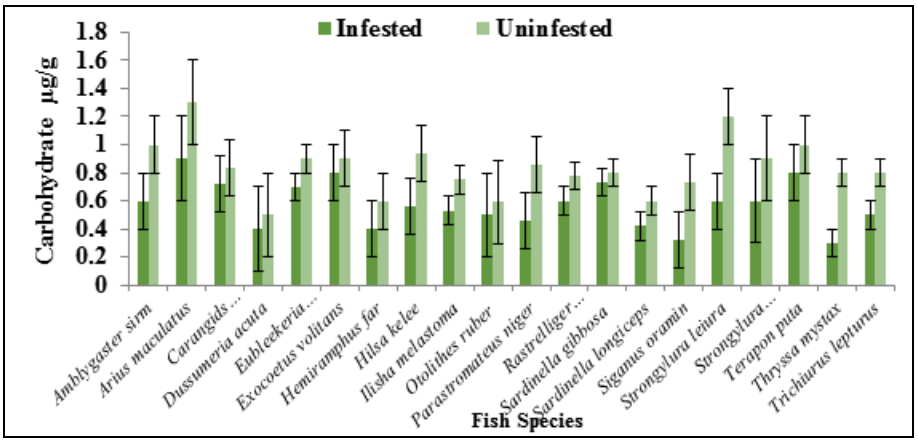

Fig 4: Variation of the carbohydrate content in relation to infestation

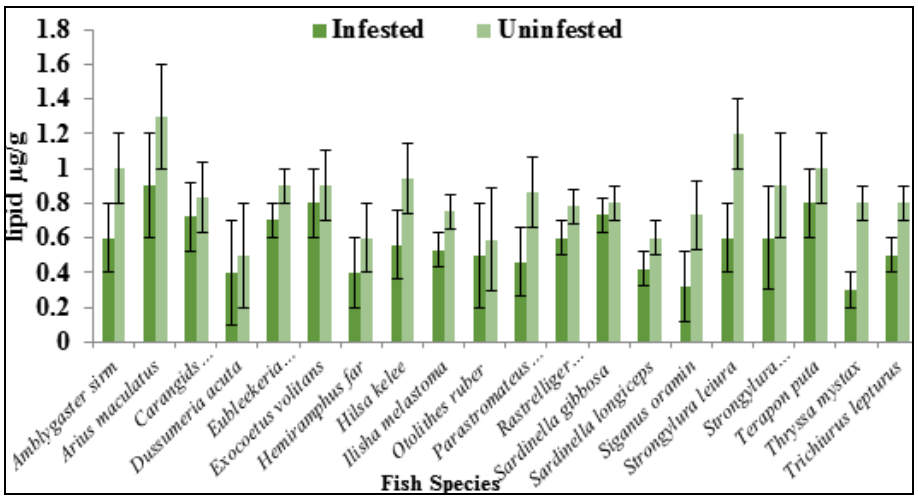

Fig 5: Variation of the lipid content in relation to infestation

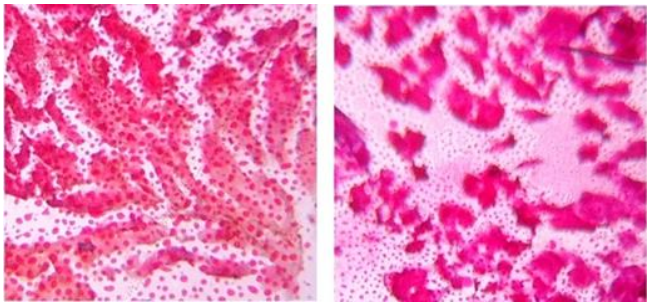

Fig 6: Histopathological studies of *Carangoides malabaricus*
a. Normal tissue of *Carangoides malabaricus*
b. Infested tissue of *Carangoides malabaricus*

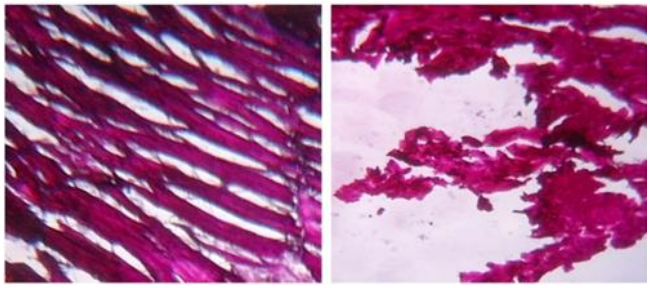

Fig 7: Histopathological studies of *Sardinella gibbosa*
a. Normal tissue of *Sardinella gibbosa*
b. Infested tissue of *Sardinella gibbosa*

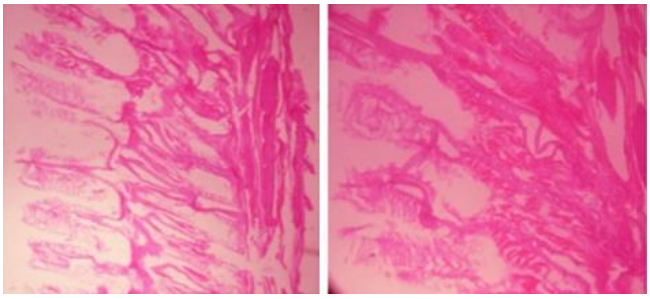

Fig 8: Sectioning of gill lamellae in *Hemiramphus far*
a. Nature of thickening between the gill lamellae in Normal *Hemiramphus far*
b. Damage of Infested gill raker and gill lamellae in *Hemiramphus far*

4. Conclusion

The present study confirms that parasitic isopods could affects the fish behavior, health, reproduction and nutritional status of parasitized fishes. The pathological signs of the parasitized fishes showed characteristic changes in morphology of the muscle tissue and gill lamellae. Parasitism lead to severe economic loss in the commercially important fish species.

5. Acknowledgment

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