PATHOLOGICAL RESPONSE IN GILLS OF MARINE FISHES DUE TO THE PARASITIC STRESS AND ITS RELATIONSHIP WITH WATER QUALITY

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Received on: 02.11.2023
Accepted on:13.02.2024

ABSTRACT

The term fish pathology in this paper applies only to Indian Mackerel, *Rastrelliger kanagurta.* Fish pathology deals with diseases caused by parasites, bacteria, fungi, and other pathogens that affect fish's lifestyle. In the present study, we observed significant pathological changes due to parasitic infestation/infections and their relation with water quality. Firstly, targeted isolation and identification of the parasites have been done from the *R. kanagurta.* In this investigation, we found adult isopods (*Norileca indica* and *Nerocila depressa*), developmental stages of isopods, *Caligus* spp., *Trichodina* spp. And *Dactylogyrus* spp.during this work period. A total of 750 fish were examined in this study, and we found a relationship between water quality and parasitic infestation. Salinity (28.70±2.30) and temperature (28.50±0.9) might have favored the proliferation of the parasites during this period. This may be the optimum range of water quality parameters for the growth and feeding of the parasites. As per our observations, salinity and temperature play an essential role in causing the parasitic infestation in *R. kanagurta*. The prevalence of the parasites had no significant (P>0.05, df=2) effect in all seasons. However, parasitic isopods differ significantly (P<0.05) according to the season. They showed maximum prevalence in the pre-monsoon (January to April). Finally, we observed that reported parasites were causing major histological changes in the gill tissues.

Keywords: Pathological anomalies, water quality, parasitic infestations, Indian mackerel

Introduction

Most of the parasite species are host-specific/tissue-specific, at least to some extent they are capable of infecting one or a restricted number of host species. Individual parasites may affect differently on different host species. The great diversity of parasites was reported by different authors on different fishes, and the detailed taxonomic classification was provided in detailed reviews on parasitology (Brusca and Bursca, 2003; Woo 2006; Eiras *et al.,* 2008). This has given an idea to study the diversity and host-parasite relationship. There are evident studies are available on parasitic infestations with pathological anomalies in freshwater fishes (Ramudu and Dash, 2013; Ramudu and Dash, 2015) and marine cultured fishes (Ramudu *et al.,* 2020), however, there were no studies on water quality in relation to parasitic infestations on wild collected fishes from marine water.

Many external parasites were living outside the host and preferred to infect the gill; monogeneans, copepods that can extremely proliferate and are found on gills (Kearn, 2004). Marine isopods are found in the buckle cavity of the gill, tongue, and surface of the body. Some marine leach, gnathiid isopods are mostly external and feed on blood (Grutter, 1994). Sucking and piercing mouthparts and clawed limbs of adult isopods are used for clinging onto their hosts (Ruppert *et al.,* 2004; Shields, 2014). Many other endo parasites like developmental stages of the helminth parasites are also found on the surface of the epithelium of the gut (Sanil *et al.,* 2011). A total of 47 species, belonging to the Platyhelminthes, Nematoda, Acanthocephala, and Copepoda; parasites were recorded, undoubtedly there are many more species of parasites yet to be discovered than the 47 species (Cortés, 2009). Many of the commercially important species still have not been examined for parasites.

Usually, parasites will try to avoid killing their hosts for their survival, this leads to chronic infestations on the host. Infested fish/hosts are also under stress in this circumstance. The hypothalamus/brain will release stress hormones to combat the infestations. Infested hosts/fish will try to develop immunity to fight against parasitic infestations under stress conditions. This leads to ultimately a few pathological changes in the vital organs of the infested fish. Environmental factors (salinity, temperature, and dissolved oxygen), weather conditions, and floods are important factors, which influence parasitic infections in wild fishes in open sea waters. Availability of the host, breeding season, and climate change are also important factors in the host-parasite relationship. With this background of work, we targeted to study the prevalence of the parasites, and pathological anomalies in vital organs of Indian mackerel, *Rastrelliger kanagurta* in relation to water quality have been studied from the Karwar region.

Materials and Methods

Collection of fish samples

Rastrelliger kanagurta samples were collected from the open sea (wild), Karwar, Karnataka, India from January to December 2022 with the help of local fishermen. A total number of 750 Indian mackerel fishes were brought to the laboratory during the year 2022 on a regular basis for the investigation. These identified fishes were for subjected to parasitological studies. All were measured in total length and body weight, further, these fishes were taken for a standard necropsy study (Blazer *et al.,* 2018). The important organs like gills, skin, muscles, fins, gut, liver, spleen, brain, and kidney were observed under a binocular microscope for the presence of parasites.

Water quality analysis

Water samples were also collected every month along with fish samples to study the parasitic communities and their relation with water quality. The Water quality parameters of water (Temperature, 28.50±0.9; Salinity, 28.70±2.37; DO, 5.60±1.06; pH,7.20±0.40; Ammonia, Nitrite, and Nitrate, 0 mg/L.) was estimated according to procedure framed by APHA (1998). In addition, all histopathological changes/anomalies of major organs were also observed and noted.

Histopathology

The vital organ gills were stored in 10% Neutral buffered formalin (NBF) for 24 hours, then processed as per standard procedures described by Roberts (2001). Further tissues were stained with hematoxylin and eosin stain, the stained sections were observed under the microscope, histopathological anomalies analysis were recorded as described by Roberts (2001) and summarized pathological anomalies further classified into grades (- absent; + low frequency; ++ frequent; +++ very frequent). These descriptions were given by Shah and Praveen (2022) and followed in this study. Microphotographs were taken by using Carl Zeiss Microimaging phase contrast microscope (37081 Gottingen, Germany) with an in-built camera (Prog Res, C3).

Statistical analysis

The data were analyzed statistically by analysis of variance (ANOVA) followed by Critical Difference (CD) was also calculated to study which month and source differed significantly (Snedecor and Cochran, 1962). The significance was noted at *P*d"0.05 and all the results of water quality are presented as mean, standard error of the mean.

Results and Discussion

The work was carried out for a period of one year (12 months) from January to December 2022 on *R. kanagurta*. These samples were collected from the wild, Karwar coast, Uttara Kannada, Karnataka, India. The fish samples were collected once every month along with water samples regularly. In every sampling, around 55-70 live or freshly dead fish were collected with the help of local fishermen for the study.

In the present study, we found that *R. kanagurta* infested with adult isopods (*Norileca indica* and *Nerocila depressa* (Fig. 1B), developmental stages of isopods (Fig. 1A), *Caligus* spp. (Fig.1 C&D), *Trichodina* spp. (Fig. 2C), and *Dactylogyrus* spp. (Fig. 1E) during this period.

Analysis: Relation of water quality with parasitic infestation

In the present study, we found that there was a relationship exist between water quality and stress by parasitic infestation. The prevalence of the parasites in different seasons (pre-monsoon, monsoon, and post-monsoon) had no significant (P>0.05, df=2) effect. However, parasitic isopods differ significantly (P<0.05) according to the season. They showed maximum prevalence in the pre-monsoon (January to April). Salinity (28.70±2.30) and temperature (28.50±0.9), might have favored the proliferation of the parasites during this period. This may be the optimum range of water quality parameters for the growth and feeding of the parasites. As per our observations, salinity and temperature play an important role in causing the parasitic infestation in *R. kanagurta*. Temperature is one of the critical abiotic factors that affect fish or fish behavior. These results are corroborated by different stakeholders from different countries (Fry, 1958). The maximum number of fishes, can't generate sufficient internal heat to maintain a body temperature from their external environment because of rapid heat exchange across the gills and the body surface (Stevens and Sutterlin, 1976).

As per the findings of Schulte (2011), temperature directly impacts the different chemical reactions, functions of the protein, and biological reactions reported and published. In our findings, high temperatures, favor the infestations by the pathogens. Parasitic infestation and thermal stress both can affect the normal physiology of the body, growth, reproduction, and other activities in order to survive in the water body. David *et al.* (2017) authors reported similar kind of findings in their book.

The stress response of the host

Indian mackerel is the most available marine fish in the Arabian Seaon the west coast. When there is a unfavourable weather condition, a bad environment, thermal stress, pollution, global warming, and low immunity of the host may favor the entry of parasites into the body of the Indian mackerel. Parasites are opportunistic pathogens when favorable conditions are available in a water environment, this causes primary infestation. External parasites can cause mechanical damage to the host surface; through this, there is a chance of getting bacterial and fungal diseases. However, fish will respond against the infestation mentioned in Fig. 1F. Response can be classified into three stages/responses i.e first response, secondary response, and third response. Most of the parasites are host-specific and tissue-specific and cause mechanical damage in a specific tissue; however, these mechanical damage can directly or indirectly affect the functioning of all other organs of the host fish. All these pathological anomalies can be seen during the secondary response (Fig. 1F). In the present study, we found that many pathological changes were observed in most of the vital organs of the captured fish from the Karwar coast, Karnataka, India.

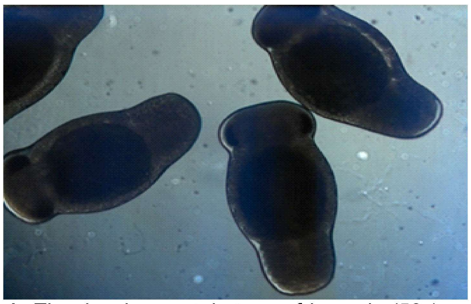
If fish are under stress due to parasitic infestations or bad weather conditions they will try to fight with the parasites/conditions for their survival during the first and secondary response. Under severe conditions of the infestations, sometimes fish fails to fight against these parasites. Under these circumstances, fish will try to flee from the hard situations/infestations. This process can be called a fight-flight model. This is supported by Cannon (1932); Selye (1956); McCarty (2016).

Pathological anomalies in gills

In the present study, our findings were distinguished clearly between normal fish tissue and infested fish tissue anomalies by different parasites. Significant pathological changes were observed in the gills due to parasitic infestation. These pathological anomalies were due to parasitic infestation and are directly or indirectly dependent on the water quality parameters. Change in water quality has a negative impact on the histology and functioning of an organ. Present findings are also corroborated by the observation made by Shah and Praveen (2022) in freshwater fishes.

Normal gill of the un-infested fish had no changes/anomalies in primary and secondary lamellae which could be easily understood by seeing in Fig. 2A. Gill arch, filaments, and lamellae (primary and secondary) were seen as well-structured and arranged with epithelial cells, and pillar cells

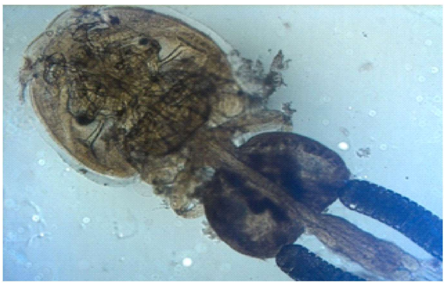
Fig.1: Different typesof parasites recovered from the *Rastrelliger kanagurta* during the study period from January to December 2022



A. The developmental stage of Isopods (50x)

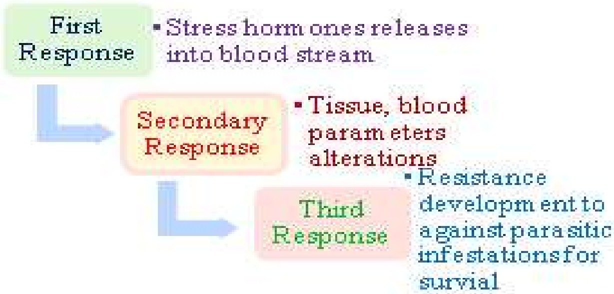
B. Dorsal view of *Nerocila depressa*

C. Wet mount of Male *Caligus* spp. (50x)



D. Female of *Caligus* spp. with egg sac (50x)

E. Wet mount of gill filament with *Dactylogyrus*spp. (50x)



F. Model of response by the Indian mackerel, *Rastrelliger kanagurta* during parasitic infestation

(Fig. 2A).

Infested fish gill (Table 1; Fig. 2&3) depicted the complete loss of secondary lamellae due to the attachment of fluke parasites, *Dactylogyrus* spp. (Fig. 2B), these flukes cause irritation in the gill, which leads to many pathological changes. The gill also shows the presence of *Trichodina* spp. attached to the secondary lamellae (Fig. 2C). Pathological changes like telangiectasia, curling of lamellae, necrosis, and degenerative changes in epithelial cells of gill filaments observed in the gill of infected tissues (Fig. 2C&D).

Blood congestion with hyperplasia of mucus cells (Fig. 2F) in primary lamellae, loss of secondary lamellae (LSL) on the other side completely fused, curling of lamellae with dilatation of blood was also recorded in section of infested fish gill (Fig. 2E). Hypertrophy of mucus cells (Fig. 3C), edema, inflammation, degeneration of respiratory epithelial cells, and proliferation of epithelial cells of primary and fusing secondary lamellae are also noted in during higher parasitic infestations (Fig. 3E).

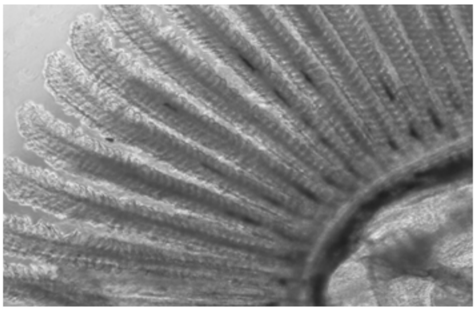
The reported isopod/developmental stage of isopod parasites was found attached to the gill filaments. The nature of the damage and degree of damage varies according to the prevalence, and intensity of the parasites, as per the present finding gill arch, filaments, and lamellae observed greater damage (Fig. 3A). Secondary gill lamellae show randomly clubbed and showed fusion and sometimes completely lost (Fig. 3D). Bifurcation (Fig. 3B) was also seen at the tip of the lamellae along with degeneration of respiratory epithelial cells, and hemorrhages in gill tissue are also depicted clearly in the gill histopathology section (Fig. 3B). These results are corroborated by the findings of Ganapathy and Ravichandran (2013). Infested gill tissue has shown local hemorrhages, which indicates a larger accumulation of blood cells (due to the blood congestion) in the gill arch, hypertrophy of mucus cells, and degenerative changes in the gill arch (Fig. 3F).

Anomalies like telangiectasia of lamellae, curling, loss of secondary lamellae, and degenerative changes in epithelial cells of gill filaments are the most common anomalies found in the gill of infested fish. These kinds of changes were observed by Ramudu and Dash (2015); Vankara *et al.* (2022) in freshwater-cultured fish infested with parasites. Cell necrosis seen in the gill lamellae can be identified from the pyknosis (shrinkage of a nucleus) and this leads to karyolysis. These findings are supported by Woo and Buchman (2012).

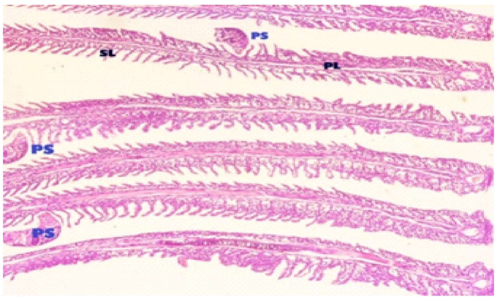
Table 1: Summarized histopathological variations in the gill of the host

Gill Anomalies	Normal gill	Infested tissue
Fusion of gill lamellae	-	+++
Loss of secondary lamellae	-	+
Hyperplasia of gill lamellae	-	+
Necrosis, Degenerative changes in epithelial cells of gill filaments	-	+
Oedema	-	+
Bifurcation of secondary lamellae	-	+
Vacuole formation in gill tissue	-	+
Hemorrhages in gill tissue	-	++
Epithelial lifting in gill tissue	-	+
Telangiectasia of lamellae	-	+++
Blood congestion in gill	-	++
Dilatation in primary lamellae	-	+
Hypertrophy of Mucus cells in gill tissue	-	+
Hyperplasia of mucous cells		+
Hypertrophy of Blood cells in gill tissue	-	++
Curling of secondary lamellae	-	+
The proliferation of epithelial cells of primary and secondary lamellae	-	+
Degeneration of respiratory epithelial cells	-	+
Inflammation	-	+

Fig. 2: Pathological alterations in the gills due to the parasitic infestations

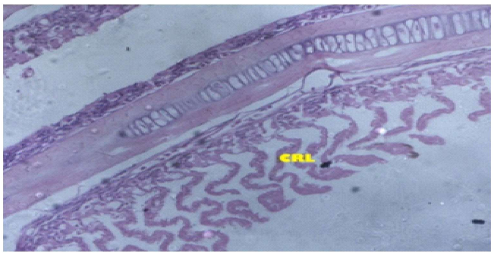


A. The normal gill structure of *R.kanagurta is* depicted with a gill arch, primary lamellae, and secondary lamellae (H&E, 50x)

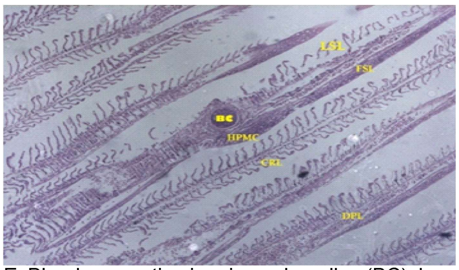


B. *Dactylogyrus* spp.(PS) attached to the primary lamellae (PL) by causing damage to the secondary lamellae (SL) (H&E,100x)

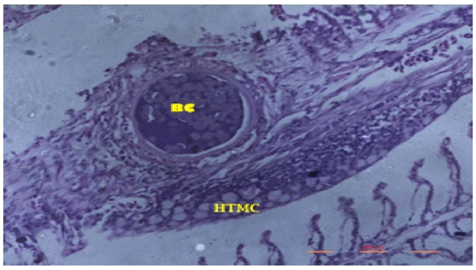
C. Gill shows the presence of *Trichodina* spp.(T) with pathological changes like telangiectasia (TG) of lamellae, necrosis (N), degenerative changes in epithelial cells (DC) of gill filaments (H&E,400x)



D. Gill of pathological anomaly shows curling of secondary lamellae (CRL) (H&E,400x)

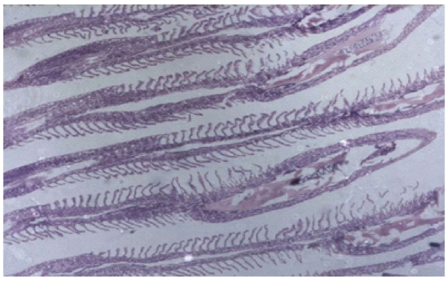


E. Blood congestion in primary lamellae (BC), loss of secondary lamellae (LSL) on one the other side completely fused (FSL), Curling (CRL) of lamellae with dilatation of blood (H&E,100x)

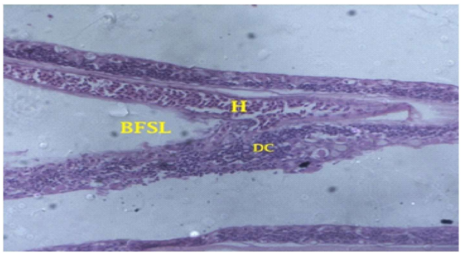


F. In higher magnification gill shows Blood congestion in primary lamellae (BC) with hypertrophy and hyperplasia of mucus cells (HTMC) (H&E,100x)

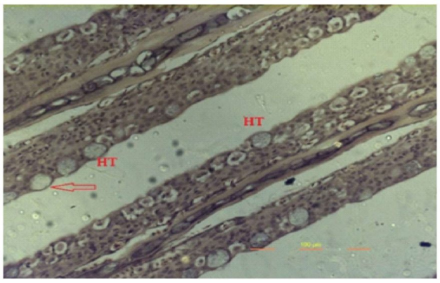
Fig.3: Pathological changes in the gill due to the parasitic infestations



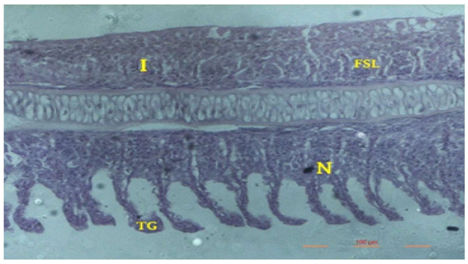
A. The gill structure is completely damaged due to the parasitic infestation (H&E,100x)



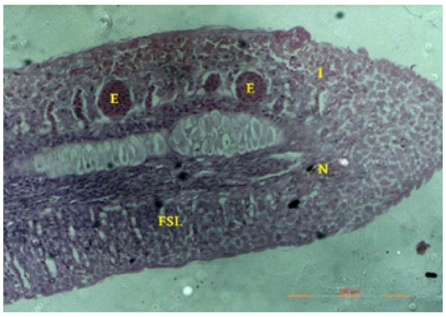
B. Bifurcation of secondary lamellae (BFSL), degeneration (DC) of respiratory epithelial cells, and haemorrhages (H) in gill tissue are shown clearly in the gill histopathology section (H&E,400x).



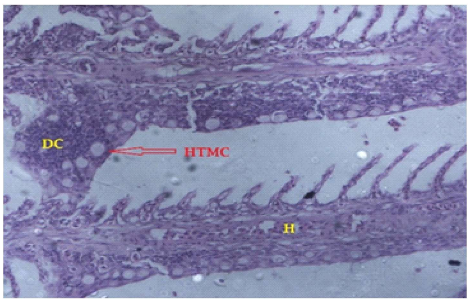
C. Gill of pathology depicted hypertrophy of mucus cells (HT, Red arrow) (H&E,400x)



D. Pathological anomaly shows fusing of secondary lamellae (FSL), necrosis (N), inflammation (I), and telangiectasia (TG) of lamellae (H&E,400x)



E. Edema (E), inflammation (I), and necrosis (N) were depicted in secondary lamellae along with complete fusing (FSL) of secondary lamellae (H&E,400x)



F. In higher magnification gill shows hypertrophy and hyperplasia of mucus cells (HTMC), degenerative changes in the gill arch, and hemorrhages (H) in primary lamellae (H&E,400x)

Conclusion

Parasites, often host and tissue-specific, inflict mechanical damage on fish gills, potentially leading to pathological abnormalities. Opportunistic in water environments, they cause primary infestation, leading to secondary bacterial and fungal infections. Our study highlights significant pathological changes in gills due to parasitic stress. While acute infestations can cause mass mortalities in aquaculture, the role of parasites in wild-caught Indian mackerel mortality remains unclear. Environmental factors like salinity and temperature influence parasitic infections, it is also evident in our findings. Despite extensive gut content analysis, little research focuses on parasitic infestations and their effects on Indian mackerel in the Karnataka coastal region. Our work establishes a vital database linking parasitic stress in fish gills to water quality parameters, provide future research directions in coastal Karnataka.

Acknowledgments

The authors are thankful to the Director, CMFRI, Kochi; Scientist in Charge, KRS of CMFRI, Karwar; and Head of the Division of Marine Biotechnology, Fish Nutrition, and Health Division, CMFRI, Kochi for their kind support, and encouragement.

Conflict of interest

Authors have no conflict of interest to declare.

References

APHA (1998) Standard Methods for the Examination of Water and Waste water. 20th Edition, American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC.

Blazer VS, Walsh HL, Braham RP, Smith C (2018) Necropsy-based Wild Fish Health Assessment. *J. Vis. Exp.* **11**(139): 57946.

Brusca RC, Brusca GJ (2003) Invertebrates, 2nd ed. Sinauer Assoc, Sunderland MA, 936.

Cannon WB (1932) The Wisdom of the Body. New York, NY: W. W. Norton & Company, Inc.

Cortés J (2009) Marine Fish Parasites. 86. https://doi.org/10.1007/978-1-4020-8278-8_47.

David HE, James BC, Suzanne C (2017) The Physiology of Fishes, Fourth Edition, CRC Press, Taylor & Francis Group, London, New York.

Eiras J, Segner H, Wahli T, Kapoor BG (2008) Fish Diseases, Vol 1-2. Science Publishers, Enfield, NH.

Fry FEJ (1958) Temperature compensation. *Ann. Rev. Physiol.* **20**: 207-224.

Ganapathy R, Ravichandran S (2013) Histopathological changes in the skins and gills of some marine fishes due to parasitic isopod infestation. *J. Coast. Life Med.* **1**(1):12-18.

Grutter AS (1994) Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. *Mari. Eco. Prog. Ser.***115**: 21-30.

Kearn GC (2004) Leeches, Lice and Lampreys. A natural history of skin and gill parasites of fishes. Dordrecht: Springer.

McCarty R (2016) The Fight-or-Flight Response: A Cornerstone of Stress Research. In Stress: Concepts, Cognition, Emotion, and Behavior. Cambridge, MA: Academic, 33-37.

Ramudu KR, Dash G (2013) Toxins released by various parasites/ Pathogens and their effects: Alternative remedy for good health: Areview. *SEBA newsletter* **5**(1): 4-8.

Ramudu KR, Dash G (2015) Histopathological alterations in the vital organs of Indian Major Carps with parasitic infestation in fish farms West Bengal, India. *Drug. Dev. Ther.* **6**: 38-43.

Ramudu KR, Saloni Shivam, Sanil NK, Krupesha Sharma SR, Vijayagopal P, Suresh Babu PP, Anuraj A, Navanath K, Madhusudhana RB and Jayasree Loka (2020) Histopathological Changes in the Gills Associated with *Dactylogyrus* spp. Infestation in Orange-spotted Grouper, *Epinephelus coioides* reared in Sea Cages. *Int. J. Curr. Micro. App. Sci.* **9**(08): 01-08.

Roberts RJ (2001) The parasitology of teleost. In: Roberts, R.J. (Ed.), Fish Pathology, W.B. Saunders, London. 254-296.

Ruppert Edward E, Fox Richard S, Barnes Robert D (2004) Invertebrate Zoology (7th ed.). Cengage Learning, 661-667.

Sanil NK, Asokan PK, Lijo J, Vijayan KK (2011) Pathological manifestations of the acanthocephalan parasite, *Tenuiproboscis* sp. in the mangrove red snapper (*Lutjanu sargentimaculatus*) (Forsskål, 1775), a candidate species for aquaculture from Southern India, *Aqua.* **310**(3-4): 259-266.

Schulte PM (2011) Responses and adaptations to the effects of temperature: An introduction. In: Farrell AP, Cech JJ, Stevens ED, Richards JG, Eds. Encyclopedia of Fish Physiology: From Genome to Environment. Academic Press, San Deago, CA, 1688-1694.

Selye H (1956) The Stress of Life. New York: McGraw-Hill.

Shah ZU, Parveen S (2022) Oxidative, biochemical and histopathological alterations in fishes from pesticide-contaminated river Ganga, India. *Sci. Rep.* **12**: 3628.

Shields J (2014) Epicaridea: The parasitic isopods of Crustacea". Virginia Institute of Marine Science.

Snedecor GW, Cochran WG (1962) Factorial experiments. In: Statistical Methods Oxford and IBH publishing Co. Kolkata 339-380.

Stevens ED, Sutterlin AM (1976) Heat transfer between fish and ambient water. *J. Exp. Biol.***65**: 131-145.

Vankara A, Thummala C, Khateef R, Rafeeq AP (2022) Histopathological evaluation of *Wallago attu* (Bloch & Schneider, 1801) infested by ectoparasites. *J. Basic Appl. Zool.* **83**: 32.

Woo PTK (2006) Strategies against piscine parasitosis: the 'Cryptobia model'. In Innovations and Technologies in Oceanography for Sustainable Development (eds. S.M. Phang, Siti Aisyah, V.C. Chong, M. George, Siti Aisyah & S.C. Ho), Maritime Research Centre, University of Malaya, Kuala Lumpur, Malaysia, 17-28.

Woo PTK, Buchman K (2012) In: Woo PTK, Buchmann K (eds) Fish parasites: pathobiology and protection. CABI, Cambridge