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SEASONAL PARASITIC STUDY ON INDIAN MACKEREL, *RASTRELLIGER KANAGURTA* (CUVIER, 1817) ASSOCIATED WITH A SECONDARY BACTERIAL INFECTION IN KARWAR, UTTARA KANNADA, KARNATAKA

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ABSTRACT : Parasites in Indian Mackerel, *Rastrelliger kanagurta*, have not been studied well, especially in the Uttara Kannada district of Karnataka. *R. kanagurta* is an important marine food fish and is available throughout the year, on the west coast of Uttara Kannada. In this aspect, it is important to study the different parasites, their prevalence and the severity of the infestation in different seasons on *R. kanagurta* in this region. The present study revealed the morphological identification, prevalence, severity of the infestation and seasonal study of parasites in *R. kanagurta* from the Karwar coast from January to December 2022. The present study host acted as a vector associated with secondary bacterial infection (*Vibrio alginolyticus*) without any external symptoms in the month of July 2022. The current study investigated and found *Norileca indica* and *Nerocila phaiopleura*, *Trichodina* spp. and developmental stages of helminths in *R. kanagurta* during this period. A total of 746 fish were examined and the prevalence in seasons had no significant ($P > 0.05$, df = 2) effect on trichodinids and developmental stages of helminths. However, parasitic cymothoids (*Norileca indica* and *Nerocila phaiopleura*) varied significantly ($p<0.05$) according to the season. They showed greater prevalence during the pre-monsoon and least in monsoon due to fluctuations in environmental parameters like salinity (28.75±2.37) and temperature (28.5±0.9). Rainfall and flood water releases into the sea also play a key role during monsoons due to this water temperature and salinity come down. Finally, *Trichodina* spp., Isopods and developmental stages of helminth parasites were causing major histological changes in the infected tissues.

Key words : Parasitic study, co-infection, prevalence, severity, histopathology.

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INTRODUCTION

The Indian mackerel, *Rastrelliger kanagurta* is an important fishery along the west and east coasts of India observed throughout the year. Enormous data is available on food and feeding habits. The information related to brief descriptions and reports of single or rare parasite species belonging to metazoans (Madhavi and Lakshmi, 2011), monogeneans, Crustacean, and isopods (Ramesh and Ravichandran, 2010) were published from different geographic areas. Parasitic infestation in wild fish requires immediate attention, especially those that infect locally available important food fishes, which affect their visual quality, palatability and market value.

Marine fishes are a good source of quality protein, and other nutrients, however various diseases including parasitic infestations and bacterial infections pose a threat to the consumers (Park *et al*, 2009), in addition to the economic loss to the fisherman or farmers (Mokhtar *et al*, 2014; Yooyen *et al*, 2006). The World Health Organization (WHO) has estimated the risk and many more are at risk (WHO, 1995).

As per the data available, it is found that no work/ very less work has been done on the identification and the overall status of occurrence of the marine parasites in the Karnataka coastal region. Similarly, no efforts have yet been taken to carry out the water quality of the different areas of the Coastal Karnataka region in relation to parasitic communities. Available reports on Marine fish parasitology (especially isopods) may be doubtful and often published in local journals, sometimes not accessible easily from publishers (Trilles *et al*, 2011). Hence, this work will be certainly a primary database for the future steps taken in this direction.

The purpose of this study is to estimate the present status of parasite incidences in this region and to provide parasitological information for further study. Besides, the relevant occurrence of Marine fish parasites in selected fish species depending on the seasonal fluctuations will also help to understand the probable levels of infections in different seasons. In this regard, the present study revealed the prevalence of each parasite and the severity of the infestation in different months and seasons have been studied.

In the present study, an analysis was made on parasite communities of Indian mackerel, *R. kanagurta* of Karwar, Uttara Kannada coast. This paper deals with the various parasite species found in *R. kanagurta* of the Arabian Sea (from January-December 2022) and along with co-infection with *Vibrio alginolyticus*.

MATERIALS AND METHODS

The work was carried out for a period of 12 months between January to December 2022 on *R. kanagurta*. These samples were collected from the wild, Karwar, West Coast, Uttara Kannada, Karnataka India. The fish samples were collected once in every month along with water samples regularly. In every sampling, around 50-70 live/freshly dead fish were collected for the study.

The live fish were kept in the buckets with aeration and taken to the workplace/dissection room and then the total length of the fish, and body weight were noted. The organs like the kidney, liver, fins, skin, gill and intestine were observed for the presence of different parasites. Pooling and preservation of parasites were done in this study as per the techniques recommended by Soota (1980), Kennedy (1979), Ramudu *et al* (2016). For the ciliates, the gill was separated and transferred to Petri dishes having sterile PBS solution and slightly scuffled to free parasites from the gill lamellae. Then these were transferred onto glass slides with a dropper in a drop of sterile saline and covered with a cover slip for microscopic observation. In the case of the endo-parasites, the isolation technique followed as described by Akter *et al* (2007) and Ramudu *et al* (2016). Morphological identification of isopods was done by using reports of Bruce (1990). *Norileca indicia* and *Nerocila phaiopleura* were identified based on the original description done by H. Milne Edwards (1840), Bleeker (1857), respectively. The terms prevalence/PFI (%) were used by Margolis *et al* (1982), Bush *et al* (1997), the same terminology and methods were followed in the present study.

Photos were taken under a Carl Zeiss Microimaging phase contrast microscope (37081 Gottingen, Germany) with an in-built camera (ProgRes C3) and software. The Parasitic Frequency Index (PFI) was calculated as per Margolis *et al* (1982), this is further divided into rare (0.1–9.9%), occasional (10–29.9%), common (30–69.9%) and abundant (70–100%) as per Srivastava (1980). The severity of infestation was suggested by Lightner (1993), however, these methods were slightly modified and followed for the present study. The infested tissues were fixed in 10% NBF for further study. Histopathological techniques and analyses were followed as described by Roberts (2001). The pathogen was isolated from the vital organs such as kidney and liver, and identified as *Vibrio alginolyticus* based on the morphology of the colony, and biochemical and molecular characterization (Kimura, 1980; Felsenstein, 1985; Krupesha Sharma, 2012; Kumar *et al*, 2016).

Two-way ANOVA was done in Excel by using a data analysis tool. Further the Critical Difference (CD) was also calculated to study which month and source differed significantly (Snedecor and Cochram, 1962).

RESULTS AND DISCUSSION

Totally 756 samples of *R. kanagurta* were observed to isolates and identification of the isopod parasite. *N. indica* were recovered from the branchial cavity of the gill, body surface area. Similar observations were encountered by Aneesh *et al* (2016), Jemi *et al* (2020) in the same host. These parasites were found throughout the year, however, show highest in the pre-monsoon season (PFI, 59.2%) and lowest in the post-monsoon season (PFI, 36.88%), which are common in these seasons. A similar kind of study was done by different authors (Akter *et al*, 2007; Ramudu and Dash, 2013, 2015; Ramudu *et al*, 2016 & 2020; Gopalakrishnan *et al*, 2010; Jemi *et al*, 2020) in different regions of different fishes, however, the present study is on *R. kanagurta* and this is region specific study. Jemi *et al* (2020) authors have reported the highest infestation in the month of August 2018 followed by July 2018, these results differ from the current study. This may be due to the availability of the host and the breeding season of the host (Yohannan and Nair, 2002).

N. phaiopleura was observed maximum in the pre-monsoon season (PFI, 63.2%) and least in the post-monsoon season (PFI, 36.06%) which is also 'common' in all seasons (Fig. 3). A similar seasonal variation was found by Raja *et al* (2014) in Whitefin wolf-herring (*Chirocentrus nudus*). The prevalence of infestation also depends on the trawl ban (during monsoon), after the trawl ban is lifted that may lead to more availability

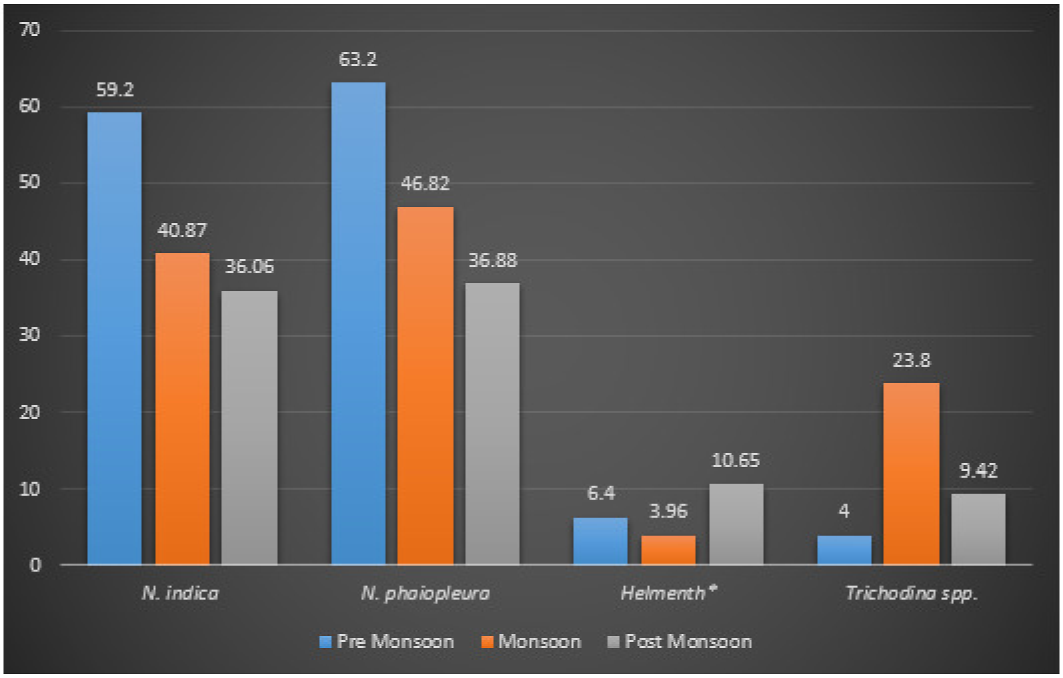

Fig. 1 : Prevalence (PFI%) and seasonal occurrence of parasites.

Table 1 : ANOVA - Statistical analysis among the parasites and seasons.

Source of variation	S S	df	MS	F	P-value	F crit
Seasons	198.985	2	99.49251	0.871699	0.465221	5.143253
Parasites	4274.38	3	1424.793	12.48326	0.005453	4.757063
Error	684.8177	6	114.1363			
Total	5158.182	11				

of host fishes (Raja *et al*, 2014; Rameshkumar, 2010).

During the study period developmental stages of helminth were found in the gut of the Indian mackerel, however, these parasites recorded peak condition in post-monsoon season (PFI, 10.65%) specified as occasional, however, these parasites were not found in a few months (April, May, July, August and September). The least prevalence was noted in the monsoon season (Table 1 and Fig. 1).

Trichodina spp. parasites were saucer-shaped, and found in gill (Lom, J. 1970; Table 1). The present study revealed that they were increased from pre-monsoon to monsoon (PFI, 4 to 23.8%) followed by reduced in post-monsoon (PFI, 9.42%) season. The infestation was higher in monsoon and post-monsoon (Fig. 1, Table 1) due to lower water temperature. These parasitic infestations were increase with low water temperatures and low salinity. These were occasionally found in monsoon and rarely observed in pre-monsoon and post-monsoon. The current study is supported by Abdel-Fattah (2020).

Statistical analysis

Statistical analysis (Table 1) revealed that the P value is lesser than the significant value ($P<0.05$, df=3), which indicates there is a significantly different in PFI values of each group/among the parasites (*N. indica*, *N. phaiopleura*, *Helmenth*, *Trichodina* spp.). However, the season's P value is greater than the significant value, which indicates there are no significant variations of prevalence in seasons ($P>0.05$, df = 2).

Associate infection with *Vibrio alginolyticus*

A total of 72 fish samples were taken from the site Karwar coast in the month of July for the parasitic study, among the five were infected with the secondary bacterial disease with a prevalence of 7.5%, which indicates as common as per Srivastava (1980). All the bacteria were isolated from the kidney and liver. Initial identification was done in the lab by morphology and biochemical characterization and further pure isolated colonies were purified. Cultures were maintained in broth medium at 29 or 34°C at the Microbiology lab for further studies.

Molecular characterization and Phylogenetic analysis

Pure single colonies were isolated from the liver and kidney, and pure single colonies were sent to Eurofins Genomics India Pvt Ltd., Bangalore for further sequencing study. The PCR amplified products were sequenced, their

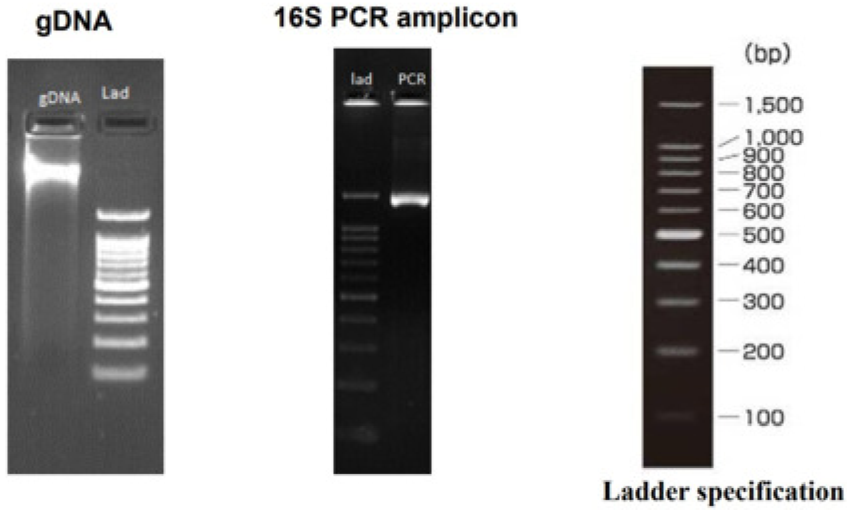

Fig. 2 : gDNA,16S amplification and ladder specification.

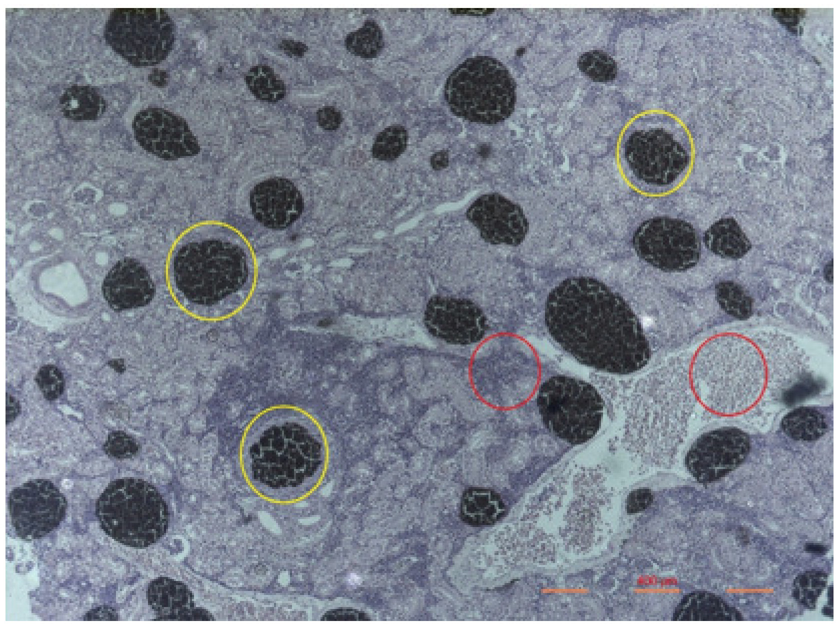

Fig. 3 : Kidney tissues showed necrosis (Yellow circles) and haemorrhage (Red circles) (H&E,100x).

quality was estimated with 1% Agarose Gel and a distinct band (Fig. 2) has been detected. The fragment of the 16S rDNA gene was amplified by 27F and 1492R primers. 1500 bp band was observed on Agarose gel. The purified DNA was subjected to automated DNA sequencing on the PCR amplicon and the sequence was purified. The amplified PCR product was purified with BDT v3.1 Cycle sequencing kit then, the concentration of the purified DNA was subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer. The 16S rDNA gene consensus sequence was generated from forward and reverse sequences by using aligner software.

The present sequences were subjected to standard nucleotide BLAST (www. ncbi.nlm.gov/BLAST). ClustalX (Thompson *et al*, 1997) and MEGA11 (Tamura *et al*, 2021) software are used for data analysis and multiple alignments, respectively. Then the phylogenetic tree was constructed using MEGA 11 (Tamura *et al*, 2021; Kumar *et al*, 2016) and also distance matrix was also prepared by using the same.

The evolutionary history was concluded by using the Neighbor-joining method (Kimura, 1980). The bootstrap consensus tree concluded from 100 replicates (Felsenstein, 1985), based on this the evolutionary history of the taxa was analyzed. During this, all gaps and missing data were eliminated. MEGA11 (Kumar *et al*, 2016) was used for evolutionary analyses. As per the history and results pathogen showed very high similarity with *Vibrio alginolyticus* based on molecular phylogenetic analysis with 99%. The pathogen was also identified and confirmed as *Vibrio alginolyticus* based on biochemical characterization without any external symptoms. The nucleotide sequence generated in the present study has not been deposited in the Genbank database.

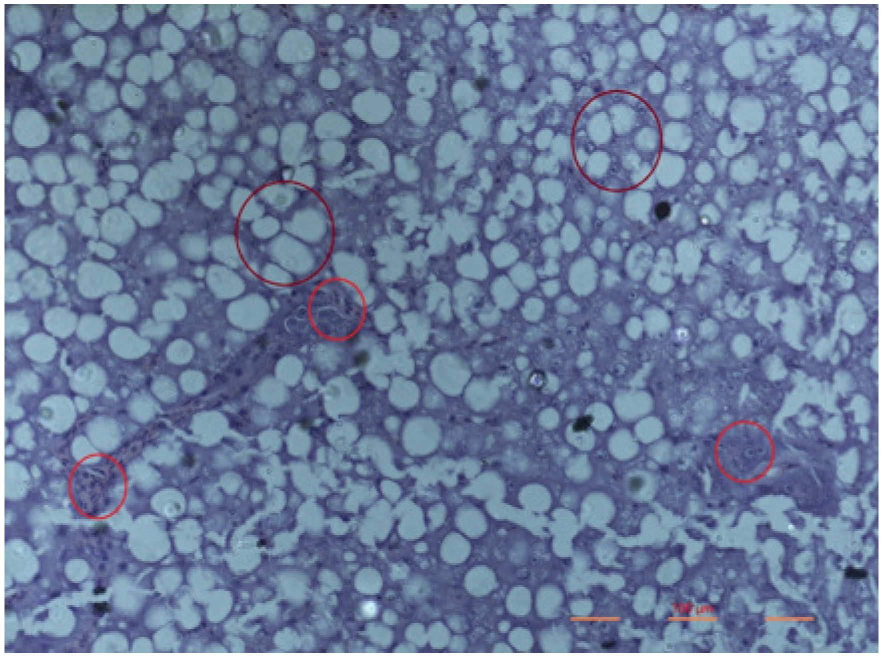

Fig. 4 : Liver tissues showed vacuole formation (Dark red circles) and hemorrhage (Red circles) (H&E, 400x).

As Ravichandran *et al* (2001) reported *Vibrio parahaemolyticus* and *Vibrio anguillarum* were isolated from the wounds of *Chirocentrus dorab*, which was also infested with the isopod parasite *N. phaiopleura*, however, the present study found similar observations in *R. kanagurta* with parasitic nfestation associated with secondary bacterial (*Vibrio alginolyticus*) infection.

Histological examination of the liver and kidney tissues shows necrosis, congestion and hemorrhage (Fig. 3) without any external symptoms, these results are corroborated by Krupesha Sharma (2012) and Tilusha *et al* (2023) in different fishes, however, vacuole formation has been observed in liver tissues (Fig. 4).

CONCLUSION

The current parasitic study associated the co-infection of *Vibrio alginolyticus* without any external symptoms on *R. kanagurta*, which indicates host act as a vector for transferring the disease. Vibrio bacteria is a commensal, opportunistic pathogen, that poses a great threat to food fishes. The prevalence of each parasite and the severity of the infestation in different months and seasons were studied well and all these reported parasites were causing pathological lesions in their vital organs need to study for further information. This work is the primary study in the Karwar coast and will be definitely a primary database for future further studies.

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