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A Temporal Analysis of Parasitic Infections in Indian Mackerel, *Rastrelliger kanagurta* (Cuvier, 1817) along the Western Coast of Karwar Bay, Karnataka: Employing a Monthly Assessment with histopathology

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Abstract

The study showed the morphological identification, prevalence, and severity of the infestation of parasites in R. kanagurta from the Karwar coast, month-wise from January to December 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 755 fish were examined, and the monthly prevalence was estimated. Statistical analysis revealed that the P value is lesser than the significant value (P<0.05, df=3), which indicates there are significant differences in PFI (% prevalence) values of each group/among the parasites (N. indica, N. phaiopleura, Helmenth, Trichodina spp.) and also there is a significant difference of prevalence in monthly (P<0.05,df=11). Weather changes like temperature, salinity, flood water, pollutants, ballast water, and sudden rainfall all play a key role in the infestation of parasites in the selected fish. Availability of the host (R. kanagurta), breeding season, and ban period (west coast) were also important factors causing the parasitic infestation. In conclusion, Norileca indica and Nerocila phaiopleura, Trichodina spp., Isopods, and developmental stages of helminth parasites were causing histological alterations in the infected tissues.

Keywords: *Parasitic study, Marine fish, PFI (%), severity, Histopathology*

1. Introduction

Marine fish are a good source of omega-3 fatty acids, protein, and other nutrients. Among the marine fish Indian mackerel, *Rastrelliger kanagurta* is an important food fish and is available throughout the year. however various diseases including parasitic infestations and secondary bacterial, and fungal infections are a threat to consumers (Park et al., 2009). Whereas the effects of parasites on *R. kanagurta* sometimes leading to chronic mortalities are known much less, parasitic infestation with secondary bacterial infection is also studied much less on these wild hosts. In large-scale human activities also becomes more and more difficult to study the effects of parasites on *R. kanagurta* from the wild open sea. As per the records available, it is found that very little work has been done on the marine fish parasites in the Karnataka coastal region. Similarly, no data is available about water quality in relation to parasitic communities. Many authors isolated and identified different parasites from different fishes (Ramesh & Ravichandran 2010; Kayis & Ceylan (2011); Madhavi & Lakshmi, 2011; Rameshkumar et al., 2016). Trilles et. al., (2011); however, no data is available in relation to water quality and parasitic infestations/infections on Marine fish parasites (especially isopods). Hence it is important to study the parasites on *R. kanagurta* and this work will be certainly a primary database for the future steps taken in this direction.

The purpose of this study is to isolate, identify, and severity of the infestation of different parasites monthly and to study the cause of factors associated with the infestations in this region. This will

provide parasitological information for further study. In the present study, a month-wise analysis of parasites of Indian mackerel of Karwar, Uttara Kannada coast was done. This paper deals with the various parasite species found in *R. kanagurta* of the Arabian Sea from January to December 2022.

2. Materials And Methods

The work was done for a period of twelve months from January to December 2022 on *R. kanagurta*. Samples were collected from Karwar Bay (140 48′ 32″N 740 07′ 07″E) with the help of local fishermen. The fish samples were collected once every month along with water samples regularly. In every sampling, around 50-70 live/freshly dead fish for this study.

The fish were collected in buckets 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. Many methods were followed for this study shown in Table 1

Table 1. Methods followed for this study

Process used for	Method/Modified method followed/Proposed by	References
Isolation and preservation	Soota; Kennedy and Ramudu	Soota (1980); Kennedy (1979); Ramudu et al., (2016)
Ciliate parasites	Akter and Ramudu	Akter et al., (2007); Ramudu et al., (2016)
Morphological identification of isopods	Bruce; Milne Edwards and Bleeker	Bruce (1990); H. Milne Edwards (1840); Bleeker (1857)
Prevalence/PFI (%)	Margolis and Bush	Margolis et al., (1982); Bush et al., (1997)
Classification of Prevalence/PFI (%)	Srivastava	Srivastava (1980)
Severity of infestation	Lightner	Lightner (1993)
Histopathological techniques	Roberts	Roberts (2001)
Data analysis	Snedecor and Cochram	Snedecor & Cochram (1962)

Photos were taken under a Carl Zeiss Microimaging phase contrast microscope (37081 Gottingen, Germany) with an in-built camera (ProgRes C3) and software. 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 & Cochram, 1962).

3. Results and Discussion

Totally 755 samples of *R. kanagurta* were observed to isolate 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 Bruce (1990); Aneesh et al., (2016); Rameshkumar et al., (2016) and Jemi et al., (2020) in the same host. The Parasitic Frequency Index (PFI) of *N. indica (*Fig.1A) was noted maximum in February, March, and April (PFI, 64.5%, 66.1%, and 71.47%), which were stated as common in February and March, however, ‘abundant’ condition in April month. The occurrence of these parasites reaches the least in the month of August (PFI, 27.7%), and the condition was specified as ‘occasional’. 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 & Nair, 2002). A similar kind of study was done by different authors (Akter et. al., 2007; Ramudu & Dash, 2013, 2015; Ramudu et. al., 2016; 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. *N. phaiopleura* was observed during the entire study (Table.2); The *Nerocila* species were found on the fins, the caudal peduncle, and on the body surface, similar findings found by Kayis & Ceylan (2011); Rameshkumar et al., (2016). The highest prevalence was noted in April (PFI, 76.7%) month and followed by March (PFI, 73.5%) and December (PFI, 72.5%), however least in the month of November (PFI, 17.1%). These parasites were ‘abundant’ in April, March, and December months, on the other hand, ‘occasional’ in months of November. The prevalence of infestation also depends on the trawl ban, after the trawl ban is lifted that

may lead to more availability of host fish (Raja et al., 2014; Rameshkumar 2010). The large size/bigger fish and the aged host also may increase the chances of infestation with parasites. Larger fish eat more and come into contact with larger volumes of water, which is more exposed to parasites (Rohde, 1984). During the study period Developmental stages of helminth were found in the gut of the Indian mackerel, however, these parasites recorded peak condition in December (PFI, 32.2%) specified as 'common', however, these parasites were not found in a few months (April, May, July, August, and September).

Trichodina spp. parasites were found in the gill (Lom, 1970; Fig.1B, Table 2). The present study revealed that they were not found in the months of January, February, March, and June, however, the highest prevalence was observed in the month of May (PFI, 48.3%) and stated as common due to lower water temperature. These parasitic infestations were increased with low water temperatures and low salinity. The current study is supported by Abdel-Fattah (2020).

Table 2. Morphological identifications, prevalence, severity of the infestation:

Months	Number of Fish Examined	Parasites recovered	Number of fish Infested	PFI (%)	Site	Severity of infestation
JAN 22	64	*N. indica*	23	35.9[c]	#Gill & Body	1
		N. phaiopleura	20	31.2[c]	#Gill & Body	1
		*Helmenth**	5	7.81[a]	Gut	0.5
FEB 22	62	*N. indica*	40	64.5[c]	#Gill & Body	1
		N. phaiopleura	45	70.3[d]	#Gill & Body	1
		*Helmenth**	6	9.67[a]	Gut	0.5
MAR 22	68	*N. indica*	45	66.1[c]	#Gill & Body	1
		N. phaiopleura	50	73.5[d]	#Gill & Body	1
		*Helmenth**	5	7.3[a]	Gut	0.5
APR 22	56	*N. indica*	40	71.4[d]	#Gill & Body	1
		N. phaiopleura	43	76.7[d]	#Gill & Body	1
		Trichodina spp.	10	17.8[b]	Gill	0.5
MAY 22	62	*N. indica*	20	32.2[c]	Gill & Body	1
		N. phaiopleura	25	40.3[c]	Gill & Body	1
		Trichodina spp.	30	48.3[c]	Gill	0.5
JUN 22	64	*N. indica*	30	46.8[c]	Gill & Body	0.5
		N. phaiopleura	35	54.6[c]	Gill & Body	0.5
		*Helmenth**	10	15.6[b]	Gut	0.5
JUL 22	72	*N. indica*	38	52.4[c]	Gill & Body	0.5
		N. phaiopleura	40	55.5[c]	Body	0.5
		Trichodina spp.	20	27.7[b]	Gill	0.5
			5	7.3[a]	Kidney	0
AUG 22	54	*N. indica*	15	27.7[b]	Body	0.5
		N. phaiopleura	18	33.3[c]	Body	0.5
		Trichodina spp.	10	18.5[b]	Gill	0.5
SEP 22	58	*N. indica*	20	34.4[c]	Gill & Body	0.5
		N. phaiopleura	18	31.0[c]	Gill & Body	0.5
		Trichodina spp.	3	5.1[a]	Gill	0.5
OCT 22	54	*N. indica*	18	33.3[c]	Gill & Body	0.5
		N. phaiopleura	15	27.7[b]	Gill & Body	0.5
		*Helmenth**	3	5.55[a]	Gut	0.5
		Trichodina spp.	2	3.7[a]	Gill	0.5

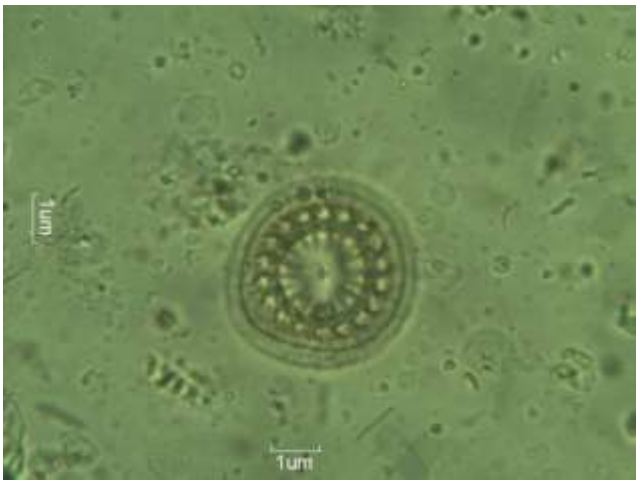
NOV 22	70	*N. indica*	10	14.2[b]	Gill & Body	0.5
		N. phaiopleura	12	17.1[b]	Gill & Body	0.5
		*Helmenth**	3	4.2[a]	Gut	0.5
		Trichodina spp.	3	4.2	Gill	0.5
DEC 22	62	*N. indica*	40	64.5[c]	Gill & Body	0.5
		N. phaiopleura	45	72.5[d]	Gill & Body	0.5
		*Helmenth**	20	32.2[c]	Gut	0.5
		Trichodina spp.	15	24.1[b]	Gill	0.5

**Developmental stages helminth, # branchial cavity*

PFI (%)-a=rare (0.1 – 9.9); b=occasional (10 – 29.9); c = common (30 – 69.9); d = abundant (70 – 100)

Fig.1. Parasites recovered from the host fish during the study

A. *N. indica* isolated from the the gill of *R. kanagurta*



B. *Trichodina* spp. isolated from the gill of *R. kanagurta* (200x).

Statistical analysis

Statistical analysis (Table 3) 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.) and also there is a significant difference of prevalence in monthly (P<0.05, df=11).

Table 3. ANOVA- Statistical analysis among the parasites and months

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Parasites	16956.92	3	5652.308	30.0646	1.47E-09	2.891564
Months	5888.201	11	535.291	2.84721	0.009842	2.093254
Error	6204.18	33	188.0055			
Total	29049.31	47				

Histopathology

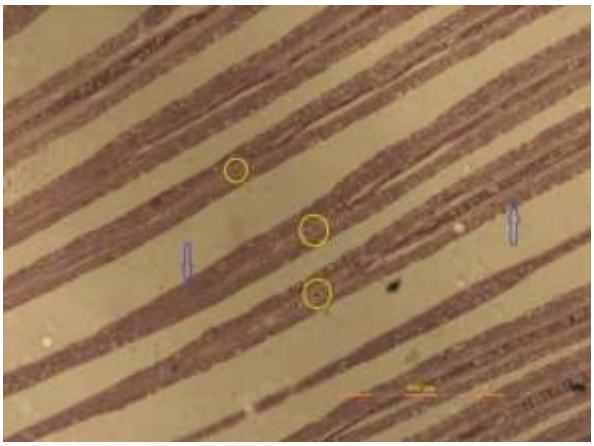
Trichodina spp. infecting gill of *R. kanagurta* showed higher mucus production, pale colour in the gill. Gill arches with loss/fusing of lamellae, and the arch shows hemorrhages and vacuoles sometime necrosis (Fig.2A). The symmetry of the arrangement of primary lamellae has been lost, inter digitations were damaged prominently, similar kinds of changes were observed by Ramudu & Dash (2013; 2015), Ramudu et.al., (2016) and exposure to some water pollutants was described by Perisetti (2011) in

freshwater fishes. These results are corroborated by the results of Ramudu et al., (2020) in Orange-spotted Grouper infected with flukes.

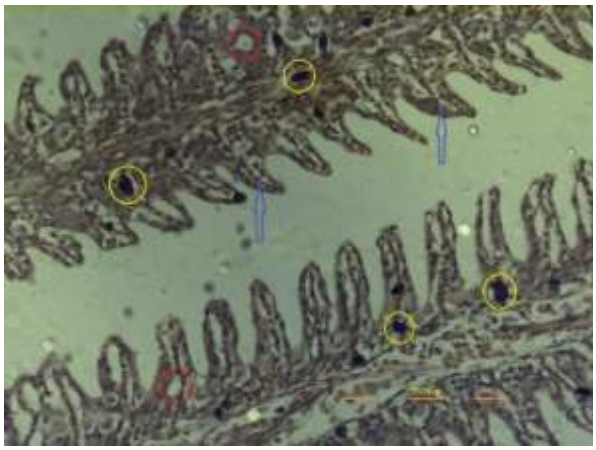
Very minor/no changes were observed in the skin/dermis at the site of attachment due to the Cymothoid/isopods infestation, however, Ganapathy & Ravichandran (2013) reported that infested fish exhibited many histopathological anomalies in their publication. Isopods were also attached to the gill, these were triggering significant necrosis and atrophy (Fig. 2B), this may be due to a parasite that attaches or settles on the branchial cavity of the gill, at first, causing localized inflammatory changes and then continuous damage of the gill by the isopod parasites. Similar changes were noted in marine fishes by Ganapathy & Ravichandran (2013). Finally, this will lead to damage to the gill structure with the swelling of the gill lamellae, also the same kind of observations reported by Nadia & Ibrahim (2018).

Developmental stages of helminth parasites were isolated from the gut of *R. kanagurta* and pathological changes were observed due to these developmental stages of parasites shown as hemorrhages (Fig.2C), the structure of the gut tissues was damaged (Fig.2D). In the intestine and ceca, most damage (mucosal erosion, fibrosis, and chronic inflammation, Fig.2D) was induced by the developmental stages of parasites, these results are supported by the Esch & Huffines (1973); Cristina et al., (2021).

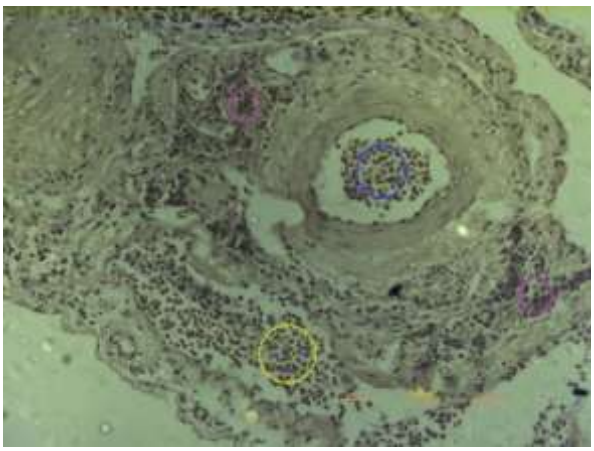
Fig.2. Histopathological alterations in the vital organs of the *R. kanagurta* due to the parasitic infestations



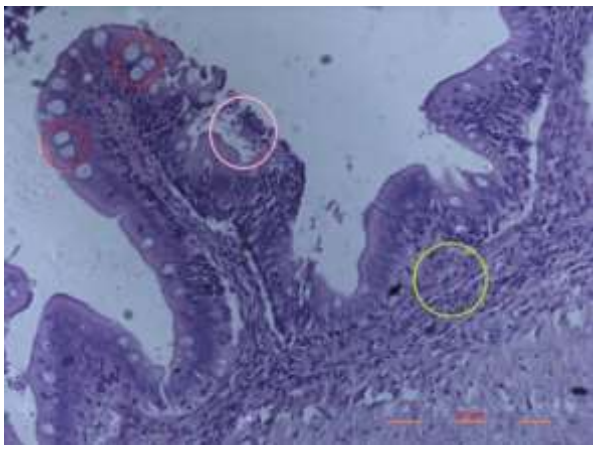
A. Gill arches observed with fusing of lamellae (Blue arrow), hemorrhages (Yellow circles) & vacuoles (H&E,50x)



B. Gill tissue with necrosis (Yellow circles), vacuoles (red circles), and atrophy (Blue arrow) of the cells (H&E,400x)



C. Gut tissue with hemorrhages (Yellow circle) and atrophy (purple circles) of the cells, Blood cells were found inside the parasite (Blue circles) (H&E,400x)



D. Gut tissue with Vacuole formation (Red circles), mucosal erosion (Rose circle), and chronic inflammation (Lime circle) (H&E,400x)

4. Conclusion

The prevalence of each parasite and the severity of the infestation in different months were studied well and all these reported parasites were causing pathological lesions in their vital organs. This work will be definitely a primary database for future studies.

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Conflict of Interest

The authors state that they do not have any competing interests.

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