A study on ectoparasites in Indian Mackerel, *Rastrelliger kanagurta* (Cuvier, 1817) of Thiruvananthapuram coast, South India

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INTRODUCTION
Parasitic fish diseases are one of the most serious issues in the fisheries sector (Sethi *et al.*, 2013). The fish parasites were ranging from microscopic protozoans to easily visible crustaceans and annelids. Some of them are parasitic in the external surface of fish, and others are parasitic in the internal organs. The effects of parasites on different host species may differ (Roberts, 2012). Parasites interfere with the nutrition, metabolism, and secretory function of the alimentary canal, damage the nervous system and upset the host's normal reproduction. Besides direct losses, parasites may have a considerable impact on the behaviour of fish, reduced fecundity, their resistance to other stress factors, susceptibility to other infections, the potential legislative burdens, and their presence may also reduce the marketability of fish (Paladini *et al.*, 2017).

Ectoparasite infestation is one of the most hazardous threats to fish health. It causes low body weight gain, high mortality, and poor marketability due to skin and gill abrasions that promote opportunistic microorganisms invasion (Eissa, 2002). The Indian mackerel, *Rastrelliger kanagurta*, consti-
tutes an important commercial fisheries species in countries bordered with the Indian Ocean, Indonesia, Pakistan, India, Sri Lanka, Bangladesh, Myanmar, Thailand (Jayabalan et al., 2014). The Indian mackerel constitutes a prominent group in the landings of both the Arabian Sea and the Bay of Bengal (Goutham and Mohanraj, 2015), with a significant increase in the annual landings of Indian mackerel along the Indian coast. An average annual catch in the country was estimated to be 0.16 million tonnes (CMFRI, 2019).

The available information deals with reports of parasite species in R. kanagurta belonging to Monogenea, Digennea, and Crustacea (Madhavi and Triveni Lakshmi, 2011; Rameshkumar and Ravichandran, 2010). Madhavi and Triveni Lakshmi (2011) reported the metazoan parasite fauna of the fish from the Visakhapatnam coast, Bay of Bengal and discussed its role as a host different species of metazoan parasites. Later, Madhavi and Triveni Lakshmi (2012) studied the community ecology of the metazoan parasites of the R. kanagurta. Recently, seasonal variation in the prevalence of cymothoid isopod, Norileca indica, was studied by Jemi et al., 2020). There have been no studies on the distribution and infestation of ectoparasites in R. kanagurta. Accurate identification and changes in the occurrence of ectoparasites in these fishes are important due to their food value, consumer preference and availability in the market. Hence, this study aimed to identify major ectoparasites in Indian mackerel, R. kanagurta collected from fish landing centres of Thiruvananthapuram coast, South India.

MATERIALS AND METHODS

Fish species and sampling
Samples of Indian mackerel, Rastrelliger kanagurta were collected from major harbours of the Thiruvananthapuram coast – Perumathura (Longitude: E. 76°48’0.03”, Latitude: N. 8°37’30.59”) and Vizhinjam (Longitude: E. 76°59’15”, Latitude: N. 8°22’30”) during the period from October 2017 to September 2018 for parasitological analysis. The fish were collected from commercial fishing harbours caught by local fishermen to investigate parasite infestation. Thus, the animals used in this study (fish/parasites) did not require ethical committee approval for the present work. The collected fishes were transported to the laboratory and were examined immediately. A total of 240 fish with an average total length of 13 to 16 were used for this study.

Seasonal analysis
Monthly collection of 20 fish each was done for each pre-monsoon (February-May), monsoon (June-September), and post-monsoon (October-January) season. Prevalence and mean intensity of infestation of Trichodinids, Cymothoids and digenea cysts in relation to the month of the collection were calculated (Margolis et al., 1982).

Parasitological examination
Each fish was examined thoroughly and carefully for the presence of parasites after measuring length and weight. The methods suggested by Kennedy (1977) were adopted for parasitological studies. The location of the site of interaction and the number and types of parasites in each site of interaction was noted. Skin scrapings from different regions of the body were examined under the high power of Transmission Light Microscope (TLM) (Optika Microscope; Optikam B5 Digital Camera) for the possible presence of ectoparasites. The buccal cavity was also subjected to thorough microscopical examination. After completing the external examination, the operculum on either side was taken out, and their inner sides were thoroughly examined. Gills from the blind and ocular sides were excised and transferred to separate Petri dishes containing 0.65% saline.

Processing of protozoans
Thin slides were prepared for microscopic ciliate protozoans parasites from the skin and gill scrapings of the fish. The slides were stained according to Klein’s dry silver impregnation method (Klein, 1958). The slides were air-dried and covered with a 2% aqueous solution of silver nitrate (AgNO₃) for 8 minutes. After that, rinse the slides thoroughly in distilled water and exposed to direct sunlight for 1-2 hr. The slides were then allowed to dry and mount with DPX mountant.

Processing of digeneans
Gill examinations for the presence of digenean cysts were carried out under Stereo Dissecting Microscope (SDM) (Carl Zeiss Microscopy; GmbH Stemi 508) according to the methods suggested by Madhavi (2006). Encysted digeneans were carefully dissected under TLM to release the contained digenean parasite. After that, they were stained in Gower’s Carmine (Roberts, 1978). Stained materials were mounted in D. P. X.

Processing of cymothoids
Cymothoids encountered during the examination were carefully isolated and cleaned off mucus and other debris adhering to their bodies and preserved in 10% neutral buffered formalin. The general morphological features of the parasites were studied by using a hand lens and cleared in 50% lactic acid. Taxonomically important body parts such as pereopods, pleopods, uropod and mouthparts were carefully dissected out under SDM according to the techniques described by Aneesh et al. (2019).
Photographs
The photomicrographs of trichodinids and digeneans were taken under TLM. Taxonomic drawings of isopods were made using a drawing tube attached to TLM and the computer programme CorelDraw Version 14. The photographs of isopods were taken using Canon EOS 800D with a 35mm macro lens. Trichodinids, Digeneans and Cymothoids (Norileca indica and Nerocila phaiopleura) were identified using the taxonomic keys of Reichenbach-Klinke and Elkan (1965); Chauhan (1953); and Bruce (1987 and 1990), respectively. All the linear measurements are represented in micrometres for protozoan parasites. For metazoan parasites, the measurements are represented in micrometres as well as millimetres.

RESULTS

In the present study, it was observed that the Trichodinid ciliates were present in the scrapings from the body surface, buccal cavity, and gills of R. kanagurta. Digenean cysts were observed attached to the gill epithelium. Cymothoids were found to be present on the body surface, mouth, opercular cavity and gills.

Trichodinids (Mobilidae: Trichodinidae)

Taxonomic description
(8 specimens studied, all measurements are in microns)
A flat disc-shaped body having a diameter of 275.76 μm with rows of cilia at the circular periphery. Boarder membrane 19.37 μm long. Adhesive disc concave, surrounded by fine striated border membrane. The lightly stained central area of the adhesive disc presents, which helps attach firmly to the fish skin or gills. Adhesive disc 148.80 μm in diameter. Denticular ring present with denticles. Denticular ring 98.10 μm in diameter. The denticle spans 31.18 μm in width. The denticle blade had a length of 11.55 μm, Central area and ray were visible. The central part was 37.92 μm wide and Ray 9.49 μm long (Fig. 1B-D).

Cymothoids (Isopoda: Cymothoidae)

Norileca indica

Taxonomic description of the female
(10 specimens studied): Body asymmetrical, oblong, pale creamy, the dorsal surface highly convex, eyes dark and distinct. Body about 2.4-2.5 times wide, twisted to one side; widest at pereonite 4; dorsum moderately convex.

Fig. 1. R. kanagurta parasitised by Trichodinid ciliates. A) Gill filaments of the infested fish showing increased mucus production and paleness. B-D) Photomicrographs showing Klein’s silver-impregnated specimens of Trichodinids from the gill smears- Magnification X 100, X 40 and X 10 respectively.
Cephalon not deeply immersed in pereonite 1, interior margin sub truncate; eyes 0.47-0.52 width of the cephalon. Pereonite 1 with posterolateral angles weakly produced. Coxae of pereonite 2 as long as the segment; coxae of pereonites 3-7 all about 0.8 as long as the respective segment. Posterior margin of pereonite 7 weakly concave. Pleon 0.66-0.74 width of pereonite 4, with all segments about as wide as pereonite 7, pleonite 5 as wide as pleonite 1; lateral margins posteriorly directed. Pleotelson triangular, anteromedial surface vaulted. Antennule extends to pereonite 1, bases set wide apart, composed of 8 articles; antenna is slightly longer than antennule, with 9 articles (Fig. 1B).

Mandible with large palp, article 2 flattened, expanded; article 3 0.35 as long as article 2. Maxilliped article 3 with 4 large recurved spines. Pereopods 1-5 with weak swelling on anteroproximal margin of dactylus; pereopod 7 with 2 spines on posterior margin of propodus. Uropod slender, tapering exopod, 1.7-2.1 times longer than endopod; endopod apex narrowly rounded or obliquely truncate (Figs. 3 C-H).

**N. phaiopleura Bleeker, 1857**

**Taxonomic description of the female:** Eyes large, about 0.5 widths of the cephalon. Pleonites 1 and 2 with ventrolateral margins weakly times longer than article 2. Lateral margin with about 22 stout setae. Maxilliped article 3 with 4 recurved spines. Pereopods 1-5 with weak swelling on anteroproximal margin of dactylus; pereopod 7 with 2 spines on posterior margin of propodus. Uropod slender, tapering exopod, 1.7-2.1 times longer than endopod; endopod apex narrowly rounded or obliquely truncate (Figs. 3 C-H).

**Digenean cysts (Trematoda: Heterophyidae)**

**Metacercariae of Centrocestus spp. (Looss, 1899)**

Metacercariae of *Centrocestus* spp. were released from the cyst attached in the gill epithelium of *R. kanagurta* (Figs. 4 A-C).

**Taxonomic description**

Body (0.308-0.328 x 0.08-0.12) somewhat elongate, narrower in the extreme anterior end, pitcher shaped and covered with small cuticular spines up to the extreme posterior end. Oral sucker (0.026 x 0.04) large and terminal. Spines on the outer surface surround these suckers. The posterior sucker (0.0187) was small round and situated in the posterior half of the body. Oe-

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**Fig. 2.** *N. indica* infestation on *R. kanagurta*. A) Parasite attached on the buccal cavity of the fish. B) *N. indica* Female C) Male, D) Mandible, E) Maxilla, F) Maxillule, G) Maxilliped.
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sophagus (0.051-0.0457) long, slender. Pharynx small, at about ¼ distance from anterior end. Bifurcation of ceca at about one-third distance from anterior end. Ceca long (0.194-0.205), extending up to the posterior end of the body. Reproductive organs were rudimentary and situated at the extreme posterior end. Excretory bladder nearly ‘X’ shaped (Fig. 4D).

**Nature of infestation of the ectoparasites in R. kanagurta**

There was no seasonal variation in the prevalence of Trichodinids and digenean cysts. These parasites showed 100% prevalence in all the seasons. Eighty R. kanagurta specimens were examined for each season, and all of them were infested with Trichodinids and digenean cysts. Trichodinid parasitizing the gills of the fish showed increased mucus production, paleness in the gills, and multifocal whitish areas (Fig. 1A). The parasites were in permanent rotation while attached to a host, which irritated the epithelial cells. Seasonal variations in the rate of infestation of N. indica were observed (Table 1). The prevalence of parasitic Cymothoids fluctuated significantly according to the season. Out of 80 specimens of R. kanagurta examined, 36 were infested during pre-monsoon, 20 fishes were infested during monsoon, and 26 were infested during post-monsoon. A significant increase was observed in Isopods during pre-monsoon (45%) and showed a decrease during monsoon (25%). The highest prevalence of the parasite was observed during March (50%) and the least in September (20%). The mouth and gills (Fig. 2A) were the major sites of attachment for N. indica. In the parasitized fish, lesions with the erosion of the epidermis and underlying dermis were observed at the site of parasite attachment. Juveniles; males (Fig. 2C); transitionals; non-brooded ovigerous females; and Brooded ovigerous females carrying marsupimites (Fig. 2B) of N. indica were recovered from the fish. The posterior body surface was the preferred site for N. phaiopleura (Fig. 3A and B), and it showed site-specificity in the host. Large haemorrhagic wound/ulceration was observed at the parasite attachment site (Fig. 3C). Out of 80 specimens of R. kanagurta examined, 30 were infested by N. phaiopleura. Brooded ovigerous females and transitionals of N. phaiopleura were recovered during the present study.

**DISCUSSION**

The study based on the examination of R. kanagurta collected as monthly samples from the Thiruvananthapuram coast (Arabian Sea) revealed the fish’s protozoan and metazoan parasite fauna was fairly rich, comprising of trichodinids, digeneans, and Isopods. Trichodinid ciliates are geographically a widely dispersed group of ectoparasites in all the aquatic environ-
ments—freshwater, marine and euryhaline environments. Trichodinids are probably the most common protozoan parasites found in many other fishes from different parts of the world (Tantry et al., 2016).

Isopods reported from *R. kanagurta* in this study belonged to the family Cymothoidae (Leach, 1814), which represent the largest family of parasitic isopods infesting several fish species of economic importance, as reported by Ravichandran et al. (2019). Digeneans are also a common group of fish parasites and are reported from *R. kanagurta* so far from different parts of the world (Al-Zubaidy and Mhaisen, 2014; Madhavi and Triveni Lakshmi, 2011).

In the present study, the seasonal analysis revealed that the trichodinids and digeneans cysts showed a 100% prevalence over other parasites. This higher occurrence of these species may be due to the selection of larger sized fishes for the present study. According to Ozer and Erdem (1998), the severity of most parasitic infections increases with the age of the host fish, possibly due to the greater accumulation period and the larger space for feeding and breeding of the parasite. In a study of *Trichonia* spp. on the ornamental fishes of West Bengal, Saha and Bandyopadhyay (2017) stated that the biological factors, as well as environmental factors of the host, may play a pivotal role in the outbreak of protozoans. Xu et al. (2002) reported that the factors like pollution and stress, which lowered the host’s immune response, may also result in the proliferation of the parasite.

In the present study, the seasonality effect on trichodinid infestation and digenean cysts had no significant impact on parasite prevalence, similar to the work of Suliman et al. (2021) who reported the higher prevalence of trichodina on *Oreochromis niloticus* in the fish farms of Saudi Arabia in all seasons. Bo-pling and

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**Table 1.** Seasonal occurrence of Cymothoids (*N. indica*) in *R. kanagurta* during October, 2017-September, 2018.

| Season        | Pre- monsoon | Monsoon |
|---------------|--------------|---------|
| Month         | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. |
| No. of fish examined | 20   | 20   | 20   | 20   | 20   | 20   | 20   | 20   | 20   | 20   | 20   | 20   |
| No. of fish infected | 6    | 6    | 7    | 7    | 8    | 10   | 9    | 9    | 6    | 5    | 5    | 4    |
| Prevalence (%) | 30   | 30   | 35   | 35   | 40   | 50   | 45   | 45   | 30   | 25   | 25   | 20   |
| Average       | 33%  |      |      |      |      | 45%  |      |      |      |      |      | 25%  |

**Fig. 4 (A-D).** Digenean infestation on *R. kanagurta*. A) Digenean cyst in the gill of the fish (Magnification X 8). B) Digenean cyst (Magnification X 40). C) Metacercaria of Centrocestus (Looss, 1899) releasing from the cyst (Magnification X 40). D) Metacercariae of Centrocestus (Magnification X 40).
Xianghua (2000) report that the prevalence, mean intensity and relative density of metacercarial cyst infrapopulation of Centrocestus formosanus on the gills of grass carps Ctenopharyngodon idellus were not obviously correlated with the seasonal change of water temperature. In a study of metacercarial distribution of C. formosanus (Digenea: Heterophyidae) among fish hosts in the Guadalupe River drainage of Texas, Fleming et al., 2011 found that these digenean cysts showed 100% prevalence in the fishes, Cyprinella lutrensis, Diodon nigrotaeniatu and Ameiurus natalis. Season dependent variation was noticed in the intensity and prevalence of parasitic Cymothoids from R. kanagurma during the present study period (Table 1). Norileca indica showed greater prevalence during the pre-monsoon and least in monsoon season in this study. The prevalence could be dependent on environmental parameters like rainfall, salinity, and temperature. According to Aneesh et al. (2013), parasitic cymothoids’ prevalence and mean intensity depend on the environment’s seasonal variation. Higher prevalence in pre-monsoon during the present investigation may be due to the effect of increased salinity. Our findings support the work of Kottarathil et al. (2019), who suggested that higher salinity favours the isopod parasitization of fish which might be favourable for the parasite to infect its host fish. The low prevalence observed during the monsoon period in our study may be due to the weak salinity resulting from the heavy rainfall, inducing an unfavourable environment for the parasitic infestation. In a study on Nerocila spp. parasitized marine fishes of the Malabar Coast, India, Aneesh et al. (2013) corroborated with our findings. An increase in the infestation of the parasites from post-monsoon to pre-monsoon was observed during the study. The gradual increase in salinity during the post-monsoon season seems to facilitate the more parasitic infestation. Still, the exact mechanism by which the salinity plays a role in the parasitization is unclear, and a detailed and systematic experimental study is needed to confirm this. 

In the present study, the skin of trichodinid parasitized fish showed a change in body colouration and excessive production of mucus. The body of the host fish was observed dull with a thin film of mucus. Woo (2006) suggested that the clinical signs of trichodinid infestation most commonly observed were a mottled/grey appearance on the skin (caused by the excessive production of the mucus) in a study of Trichodina infestation on the skin of Tilapia. In the present study, Trichodinid parasitizing gills of R. kanagurma showed increased mucus production, paleness in the gills along with multifocal whitish areas (Fig. 1A). Valladao et al. (2013) found mucous production, paleness and multifocal whitish areas in the severely infested gills of Nile tilapia by Trichodinid, Paratrichodina africana. Complete fusion of the secondary lamellae, proliferation of mucous cells, mononuclear and eosinophilic inflammatory infiltrate, multifocal areas of necrosis, congestion, and desquamated cells may be the reason for these changes observed. These parasites attack the fish and cause massive destruction of the skin and gill epithelium (Sterud et al., 2008). Outbreaks and mass mortality of Atlantic cod (Gadus morhua) infested with Trichodina murmannica infection was reported in a coastal embayment of Newfoundland (Khan, 2004).

In the present study, digeneans were observed as cysts attached to the gill epithelium, and changes were noticed in the gill epithelium of the host (Fig. 4A). The changes that occurred in the gills could be due to epithelial hyperplasia and fusion of the filaments. Mitchell et al. (2000) studied heterophyid trematode infecting the gills of an endangered fish, the Fountain darter, Etheostoma fonticola, in two Central Texas spring-fed rivers. They found that digenean cyst infected gill filaments of the fish were shortened, thickened, and often distorted. Ravichandran and Rameshkumar (2012) studied the gill-infecting Didymozoid in fishes of Pazhayar, India and reported that the most common effect of digenean cyst on affected fish are epithelial cells on gill lamellae, damage to gill epithelium and thus respiration is affected. They also suggested that heavily infested digenean parasitized fish were often weak, thin, inactive, and fed poorly. According to Mitchell et al. (2005), metacercariae encysted in gills causes pathological alterations related to developmental delay and death, giving rise to economic losses in the fish farming industry.

Cymothoid infested fishes in this study showed damage to gills (Fig. 3A), skin (Fig. 3C), and tongue. During the study, lesions with the erosion of the epidermis and underlying dermis were observed at the site of parasite attachment in the N. indica parasitized fish. The result of the present study is corroborated with the study of Rameshkumar et al. (2013), who reported localized destruction of the epidermis and an inflammatory response around the site of attachment of Cymothoid, Catoessa bosci in Carangoides malabaricus from Tamil Nadu, India. In the present study, the gills of R. kanagurma were damaged due to the attachment of Cymothoid N. indica (Fig. 3A). While studying the effect of the Cymothoid, Joryma hilsae, on the branchial area of Stolephorus commersonii, Ravichandran and Rameshkumar (2014) suggested that erosion of gill lamellae, damage to gill rakers, and pale gills were the significant gross lesions found as a result of isopod infestation. In our study, large haemorrhagic wound ulceration was observed on the body surface of R. kanagurma infested by N. phaiopleura (Fig. 3C). It is also reported that N. phaiopleura cause skin damages in a
The Indian mackerel constitutes a prominent group in the landings of both the Arabian Sea and the Bay of Bengal (Goutham and Mohanraju, 2015). Different types of parasites found on the fish R. kanagurta during the present study indicate that Indian mackerel from the Thiruvananthapuram coast appear to be a potential host for these parasites. Considering the wide geographical distribution and host range of the trichodinids (Tantry et al., 2016), Heterophyid Digeneans (Gamit et al., 2018) and Cymothoids (Nagasawa and Isozaki, 2017b), these parasites have the potential to become a threat to coastal farming also. The Cymothoid, N. phaiopleura, is also reported to facilitate secondary microbial infections in fishes (Ravichandran et al., 2016). Fishborne Heterophyid Digeneans are known to have a zoonotic potential (Chai and Jung, 2017). Thus, adequate management measures, including chemical Indian mackerel prophylaxis, are needed to control the infection of these parasites to some extent in coastal farming.

Conclusion

The present study revealed that the Indian mackerel R. kanagurta is a potential host for three major parasitic groups, namely Trichodinids, Digeneans cysts (Centrocestus spp.) and Cymothoids (Norileca indica and Nerocila phaiopleura). The Trichodinids and digeneans showed 100% infestation in all the seasons. In contrast, seasonal fluctuation was observed in the prevalence of parasitic isopods. The Trichodinid infestation resulted in increased mucus production, paleness in the gills, and multifocal whitish areas. The encystment of digeneans in the gills of the fish also resulted in significant changes. In the Cymothoid infested fish, lesions with the erosion of the epidermis and underlying dermis were observed at the site of attachment. The parasites may induce damages to the attachment sites, thus harming the host’s physiological status. A heavy infestation can affect the commercial value of fish. The Trichodinid ciliates and heterophyid digenean cysts of Centrocestus spp. are reported for the first time from the gills of R. kanagurta from the world.

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Conflict of interest

The authors declare that they have no conflict of interest.

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