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Ectoparasite infection of two commercially important fish species in the Negombo Estuary, Sri Lanka: Flathead grey mullet (Mugil cephalus) and Green chromide (Etroplus suratensis)

Udaya Priyantha Kankanamge Epa¹* and Ganga Dayananda¹

¹Department of Zoology and Environmental Management, University of Kelaniya, Dalugama 11600, Sri Lanka

*Correspondence: epa@kln.ac.lk

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factor

Abstract

This study aimed to assess the prevalence, diversity, and intensity of external parasitic infections in Mugil cephalus and Etroplus suratensis from the Negombo estuary, Sri Lanka, and to evaluate their potential impact on fish health and condition factors. External parasites were identified in 210 M. cephalus and E. suratensis collected from the Negombo estuary in Sri Lanka. Both species had high disease prevalence, with M. cephalus infected at 86% and E. suratensis infected at 78%, respectively. Fish parasites belonging to Phylum Apicomplexa, Phylum Protozoa, Phylum Nematoda, Sub Phylum Crustacea (copepods and isopods), and Class Trematoda (monogeneans) were found in both species. The mean parasitic intensity was significantly higher in *E. suratensis* than in *M. cephalus*. *Caligus* curtus, C. robustus, Dactylogyrus spp., Ergasilus boleophthalmi, E. parvitergrum, and Cryptocaryon sp. were recorded at a higher intensity in both species. According to the Shannon-Weiner diversity index, M. cephalus had a higher parasite diversity (1.37) than E. suratensis (1.25). Ectoparasites were more common in the gills of M. cephalus (84.6%) and E. suratensis (88.5%) than in the skin, fins, and operculum. The condition factor of infected E. suratensis (1.91±0.82) was slightly lower than that of uninfected fish (2.2 ± 0.76) . However, there was no difference in condition factor between (1.84 ± 0.41) and uninfected *M*. (1.87±0.61). The number of infected parasites per fish was unrelated to either length class or condition factor in either species (p>0.05). The findings of this study could be used to manage disease outbreaks in the wild and aquaculture development efforts in estuarine environments.

INTRODUCTION

Estuaries are a complex network of interconnected habitats (e.g., mangrove, seagrass) and sustain high primary and secondary productivity and diversified fish and invertebrate communities (Hsieh *et al.*, 2021). They have served as the foundation

for the growth of urban centers and cities, and significant fishing pressure is unavoidable when a substantial human population is near estuaries (Gagné *et al.*, 2008; Aladetohun *et al.*, 2013). The Negombo estuary, located on Sri Lanka's

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west coast, is heavily influenced by fishing, aquaculture, and human encroachment. It is one of the most popular tourist destinations in Sri Lanka. It has high fish productivity and is rich in other aquatic resources and biodiversity, including mangroves and seagrasses.

Estuarine fish communities include autochthonous species, allochthonous species that enter estuaries as migrants, and diadromous and amphidromous species. Flathead grey mullet (Mugil cephalus) is a commercially important euryhaline and eurythermal marine teleost of the Family Mugilidae (Özer et al., 2016). It contributes to estuarine and coastal fisheries in many temperate and tropical countries (Merella and Garippa, 2001; Aladetohun et al., 2013). It is a suitable candidate species for monoculture and polyculture since it feeds on detritus, periphyton, and microflora at the lower trophic level. This species is frequently cultured in brackish freshwater semi-intensive fishponds (Raja, 2021) and can be fed supplemental feed and natural food.

Etroplus suratensis is an estuarine fish species belonging to the Family Cichlidae and is also found in rivers, large streams, lakes, and reservoirs. It plays a significant role in the commercial fishery in Sri Lanka and India. Due to its remarkable taste, soft meat, and high nutritional content, E. suratensis is a highly sought-after and popular food fish in South Asian countries (Roshni et al., 2017). Because of their wide salinity tolerance, omnivorous feeding habits, breeding in confined waters, and high market value, E. suratensis is an ideal candidate for commercial aquaculture in brackish environments (Raja, 2021). It has also been introduced for ornamental and commercial aquaculture in Malaysia and Singapore.

In recent years, fishermen in the Negombo estuary have observed a considerable decline in catch and average size of *E. suratensis* and *M. cephalus*. According to Roshni *et al.* (2017), the catch of *E. suratensis* from Vembanad Lake, Kerala, India, has also recently decreased

dramatically. Therefore, it is essential to provide baseline scientific data on the wellbeing of these species in estuaries where intensive overexploitation of ichthyologic resources is observed. Fish parasites, either alone or in conjunction with other environmental stresses, may influence the health of the host and alter its population characteristics, affecting its economic importance (Merella and Garippa, 2001; Kayis et al., 2009; Özer et al., 2016; Aladetohun et al., 2013). Acanthocephalans, ciliates, copepods, flagellates, isopods, monogeneans, myxosporidians, as well as trematode adults and metacercariae, have been reported in the estuarine fish species (Lester and Halpern, 2008; Alvarez-Pellitero, 2000; Johansen et al., 2011; Özer et al., 2016; Raja, 2021). Identification of fish parasites in the estuaries will also be helpful as aquaculture activities in and around the estuarine environments have increased in the recent past. This study was conducted to identify ectoparasites of *E. suratensis* and *M.* cephalus in the Negombo estuary and to study the prevalence of parasitic species in these two fish species. The relationships between the parasitic infection and growth parameters were also investigated.

METHODOLOGY Ethical Approval

The procedures applied in this study followed the guidelines of the Ethics Review Committee of the University of Kelaniya (ERC/FS/UoK/06.2016) regarding the care and use of animals in research.

Place and Time

E. suratensis and *M. cephalus* sampling was conducted at the Katunayake, Negombo estuary (70 6'-7012'N; 79040'-79053' E), Sri Lanka, from February 2021 to June 2021. Negombo estuary has an average depth of less than 2 m and covers an area of around 32 km2 (Hsieh *et al.*, 2021).

Research Materials

Fish were caught using 2-3 m diameter cast-nets and 25 m long seines, with 01 cm

and 2.5 cm mesh sizes, respectively. The length and weight data of fish were collected using a measuring scale and an electrical balance (KERN KB 1000 – 2, Germany). Fish were anesthetized using MS 222 (Tricaine methanesulfonate; Sigma-Aldrich Chemicals Private Limited, India). Histological sections of the gills of infected fish were taken using a microtome (Leica microtome RM2235, Microscopical studies China). conducted using a dissecting microscope (Olympus CX21FS1, Philippines). chemicals and reagents used were Giemsa's solution, hematoxylin and eosin stains, Bouin's fixative, and ethanol (Sigma-Aldrich Chemicals Private Limited, India).

Research Design

A total of 210 fish of two selected species, 105 E. suratensis and 105 M. cephalus, captured from the Negombo estuary, were examined for external parasites using standard parasitological and histopathological methods. **Parasitic** prevalence, intensity, and abundance of each selected fish species were calculated using the following equations (Mergo and Crites, 1986).

Prevalence =

ind host species infected with a particular parasite species

number of hosts examined

total ind parasite species in host species sample Relative abundance =

ind parasite species in a sample of hosts

total no.of host species (infected+uninfected)

The fish condition factor (K) was calculated using the equation:

$$K = \frac{100W}{L^3}$$

W = weight (g) and L = total length (cm)

Work Procedure

Fish were sampled using cast and seine nets from the adjacent Katunavake fish landing site with the assistance of fishermen. Sampling was conducted 0.5-1.5 Km away from the coast in the morning hours. Fish were thoroughly observed to identify body necrosis and any abnormalities in the body and fins. A few specimens were anesthetized using MS 222 and monitored for parasitic

infections. All the other captured fish were euthanized immediately on ice and brought back to the laboratory. The wet weight, standard, and total lengths of each specimen were recorded to the nearest 0.001 g and 1 mm, respectively.

Wet mounts of mucous smear, fin, opercula, gills, and eyes were prepared and observed under the dissecting microscope (Olympus CX21FS1, Philippines) to identify and count the parasites (Tonguthai et al., 1999). For further identification, isolated parasites were stained using Giemsa's solution, and permanent slides were prepared. For histological observations, gills were preserved in Bouin's fixative for 24 hr and then immersed in 80% ethanol. After dehydration with a series of ethanol and benzene, they were embedded in paraffin and were serially sectioned at 5 μ m thickness (Leica microtome RM2235) and stained with hematoxylin and eosin. Parasites were identified using scientific keys up to the species level or the nearest taxonomic level (Fernando and Hanek, 1973; Tonguthai et al., 1999; Bijukumar, 1997a; Aladetohun et al., 2013; Merella and Garippa, 2001; Raja, 2021).

Data Analysis

The Anderson-Darling test was used to confirm the normality of the data. The Krusno. infected individuals of the host species sample kal-Wallis test was used to see whether the number of parasites varies with fish species and morphological features. The condition factors of infected and uninfected fish were compared using a student t-test. MINITAB 14 statistical software was used to run all the statistical tests.

RESULTS AND DISCUSSIONS Percentage Parasite Infection

Fish parasites belonging to Phylum Apicomplexa, Phylum Protozoa, Phylum Nematoda, Subphylum Crustacea (copepods isopods), and Class Trematoda (monogeneans) were isolated from operculum, skin, fins, and gills of E. *suratensis* and *M. cephalus* collected from the Negombo estuary. Out of 210 fish specimens

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used in the parasitological survey, only 37 (17.6%) were found uninfected. The percentage of infection was higher in *M. cephalus* than in *E. suratensis*, with values of 86% and 78%, respectively (Figure 1). The mean parasite intensity of *M. cephalus* collected from the Lower Kızılırmak Delta in Turkey was 99.61% (Özer and Kırca, 2015). Merella and Garippa (2001) discovered metazoan parasites in 100% of *M. cephalus* samples tested for parasites in Mistras lagoon, Western Mediterranean, and Özer *et*

al. (2016) examined 254 specimens and found only one specimen without any parasitic infection in the Lower Kzlrmak Delta in Samsun, Turkey, indicating that this fish species is more susceptible to parasitic infections. However, the results of the present study did not compare favorably with Aloo (1998), who indicated that ectoparasites were rare in *M. cephalus* in the coastal area of Kenya. They have found only a few parasites in the skin and mouth cavity of *M. cephalus*.

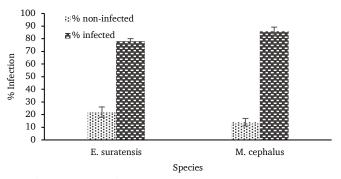
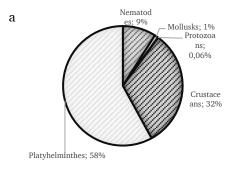


Figure 1. Percentage of parasitic infection (mean±SD) in *M. cephalus* and *E. suratensis* in the Negombo estuary, Sri Lanka.

Distribution of parasites in *M* cephalus and *E. suratensis*

Parasites belonging to the phylum

Platyhelminthes were prominent in *M. cephalus* (Figure 2a), while parasites belonging to the subphylum Crustacea were prominent in *E. suratensis* (Figure 2b).



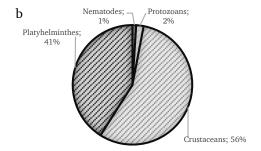


Figure 2. Percentage occurrences of parasites in M. cephalus (a) and E. suratensis (b).

The average number of parasites per fish was significantly higher in *E. suratensis* (9.25 ± 1.77) than in *M. cephalus* (3.52 ± 0.58) (p<0.05; t-test). The number of parasites per fish significantly varied

(p<0.05; Kruskal-Wallis test) with the length classes of both fish species (Table 1). Fish with higher length classes had a higher number of parasites.

Table 1. Ectoparasitic infection in different length classes of *M. cephalus* and *E. suratensis* in the Negombo estuary.

Species of fish	Mean numbers of parasites ± SE per length class of fish		
	<25 cm	25 – 30 cm	>30 cm
M. cephalus	0.8 ± 0.09	2.5 ± 0.12	3.0 ± 0.53
E. suratensis	0.5 ± 1.50	13.0 ± 0.9	18.8 ± 1.21

Parasitic diversity in M. cephalus and E. suratensis

Both *E. suratensis* and *M. cephalus* were infected by nine parasites, including nematode species, *Cryptosporidium*, *Caligus curtus*, *C. rotundigenitalis*, *Ergasilus rostralis*, *E. parvitergrum*, *E. boleophthalmi*, *Dactylogyrus* sp., and *Cryptocaryon*. The presence of *Cryptosporidium* in both fish species needs more attention as it is one of three pathogens that most commonly cause

diarrhea in children under the age of two in developing countries (Kotloff *et al.*, 2017). *Cryptocaryon* is a common parasite of the gills and skin that causes cryptocaryosis in several commercial and ornamental marine and brackish water fish species (Raja, 2021). Though parasitic species diversity was not significantly different between *E. suratensis* and *M. cephalus* (t-test; t tabulated > t calculated), all the diversity indices were comparatively higher in *M. cephalus* than in *E. suratensis* (Table 2).

Table 2. Diversity indices for ectoparasitic species in *M. cephalus* and *E. suratensis* in the Negombo estuary

Species of fish	Shannon-Weiner diversity index	Simpson's index	Margalef's index
M. cephalus	1.37	0.056	1.61
E. suratensis	1.25	0.046	1.24

The site of infection, prevalence, intensity, and abundance of external parasites in *E. suratensis* and *M. cephalus* in

the Negombo estuary are given in Tables 3 and 4, respectively. Skin infections were only observed in *E. suratensis*.

Table 3. Site of infection, prevalence, intensity, and abundance of ectoparasites in E. suratensis in the Negombo estuary (Mean \pm SD, Number of fish = 105).

saraters in the Negombo estuary (Mean ± 5D, Number of fish = 105).				
Parasite	Site of infection	% Prevalence	Intensity	Abundance
Caligus cybii	Fins	2.2	1.0 ± 0.0	0.02 ± 0.02
Cryptosporidium	Fins	2.2	1.0 ± 0.0	0.02 ± 0.02
Nematode species	Skin	4.4	1.0 ± 0.0	0.04 ± 0.03
Lamproglena sp.	Gills	6.7	1.0 ± 0.0	0.06 ± 0.03
Caligus rotundigenitalis	Gills	2.2	3.0 ± 0.0	0.06 ± 0.06
Ergasilus rostralis	Gills	6.7	1.0 ± 0.0	0.06 ± 0.03
Cryptocaryon sp.	Skin & Fins	2.2	7.0 ± 0.0	0.14 ± 0.14
Caligus robustus	Fins & Gills	8.9	4.2 ± 1.6	0.33 ± 0.19
Ergasilus parvitergrum	Gills	4.5	4.0 ± 1.0	0.15 ± 0.11
Caligus curtus	Gills	17.8	5.9 ± 0.9	0.9 ± 0.3
Ergasilus boleophthalmi	Gills & Operculum	8.9	31.5 ± 8.3	2.4 ± 1.3
Dactylogyrus sp. 1	Gills	48.9	7.0 ± 1.3	3.0 ± 0.7

In the present study, *Lamproglena* was only found in the gills of *E. suratensis*. *Lamproglena* is a fish parasite found in Asia, Africa, Europe, and South America that

attaches to the gills and body surface, producing wounds that create secondary infection sites for bacteria (Fahmy *et al.*, 2019).

Table 4.	Site of infection, prevalence, intensity, and abundance of ectoparasites in M .			
	cephalus in the Negombo estuary (Mean \pm SD, Number of fish = 105).			

Parasite	Site of infection	% Prevalence	Intensity	Abundance	
Nematode species	Skin	1.6	1.0 ± 0.0	0.02 ± 0.02	
Lironeca sp.	Gills	1.6	1.0 ± 0.0	0.02 ± 0.02	
Ergasilus rostralis	Gills	1.6	1.0 ± 0.0	0.02 ± 0.02	
Ergasilus parvitergrum	Gills	1.6	1.0 ± 0.0	0.02 ± 0.02	
Snail species	Gills	3.2	1.0 ± 0.0	0.03 ± 0.02	
Ergasilus sieboldi	Gills	1.6	2.0 ± 0.0	0.03 ± 0.03	
Dermoergasilus	Gills	3.2	1.5 ± 0.5	0.05 ± 0.04	
Caligus curtus	Gills	4.8	1.0 ± 0.0	0.05 ± 0.03	
Dactylogyrus sp. 1	Gills	3.2	3.5 ± 0.5	0.11 ± 0.08	
Caligus rotundigenitalis	Gills	4.8	2.7 ± 1.2	0.13 ± 0.09	
Cryptocaryon sp.	Skin, fins	9.7	1.7 ± 0.3	0.16 ± 0.07	
Cryptosporidium	Skin, fins	6.4	2.5 ± 0.9	0.16 ± 0.10	
Ergasilus boleophthalmi	Gills, operculum	17.8	4.5 ± 0.8	0.81 ± 0.26	
Dactylogyrus sp. 2	Gills	38.7	5.0 ± 1.4	1.94 ± 0.62	

Parasites were not isolated from the eyes of both fish species (Figure 3). The number of parasites recorded from the operculum was low, while a higher number was recorded from the gills. Gills are rich in oxygen, food particles, and fresh blood. Parasites can quickly grow and reproduce within the gills. So many parasites are attracted to the gills of fish. The most common copepod parasites identified belong to the families Ergasilidae and Caligidae (Figure 4). They were attached to the gill filaments of the fish. *E. rostralis*, *E. parvitergrum*, and *E. boleophthalmi* were

found in both fish species. Many females of E. sieboldi were isolated with egg sacs in M. cephalus, which were collected just after rainy days. The prevalence rate of copepod parasites in fish was also higher in the rainy season than in the dry season in the Lac Nokoue Lagoon in the Republic of Benin, West Africa (Aladetohun et al., 2013). Ergasilid copepod species, namely Dermoergasilus amplectens, E. sieboldi, E. parvitergum, and Sinergasilus major, were recorded in E. suratensis and M. cephalus in Valaichchenai lagoon, Sri Lanka (Harris et al., 2023).

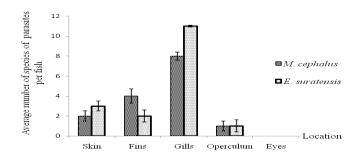


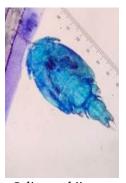
Figure 3. The average number \pm SD of ectoparasitic infections in the skin, fins, gills, operculum, and eyes in M. cephalus and E. suratensis in the Negombo estuary.

The Caligid copepod species recorded were *C. robustus*, *C. curtus*, *C. rotundigenitalis*, and *C. cybii*. The adult caligid body is divided into four distinct sections or tagmata: the cephalothorax, the fourth leg-bearing somite, the genital

complex, and the abdomen. The cephalothorax results from the fusion of the cephalon, the maxilliped-bearing somite, and the first three leg-bearing somites (Dojiri and Ho, 2013). Caligids have two suckers-like organs close to the anterior end of the

cephalothorax. *C. curtus and C. rotundigenitalis* were common to both fish species. *Caligus* spp. are known to have less host specificity (Yuniar *et al.*, 2007;

Jithendran *et al.*, 2008; Kazachenko *et al.*, 2014; Raja, 2021). In highly infected *E. suratensis*, *C. cybii* was found even attached to the pectoral fins.









Caligus cybii

Caligus robustus

Ergasilus boleophthalmi

Ergasilus sieboldi

Figure 4. Some external metazoan parasitic species were recorded from *M. cephalus* and *E. surantensis* from Negombo estuary, Sri Lanka (Wet mounts and Giemsa staining).

Dactylogyrus has a wide temperature and salinity tolerance and is a frequent parasite of fish gills in the Cyprinidae family (Shamsi et al., 2009). When several Dactylogyrus parasites were infected, dark color patches were noticed on the gill surfaces of E. suratensis. As Rattan and Parulekar (1998) indicated, in the wild, heavy infection of monogeneans in E. suratensis gill filaments causes mortalities. Shamsi et al. (2009) stated that Dactylogyrus infection increases with age because older fish have a bigger gill surface area and no permanent immunity to these parasites. Dactylogyrus sp. 2 was found in both fish species. This parasite has four three-segmented legs with four spines on the distal segment. However, Dactylogyrus sp. 1 had a body slightly narrowing to the front and towards the haptor, and was only found in M. cephalus. The cephalothorax of Dactylogyrus sp. 1 was large, flat, and fringed with an edge membrane. Its frontal plates formed well and had widely spaced lunules. Dactylogyrus sp. are highly host-specific. Lamproglena was recorded from the M. cephalus, while Lironeca was only found in E. suratensis. A snail species was found attached to the gills of M. cephalus. Aquatic mollusks use fish's gills for respiration and filter-feeding activity (Bijukumar, 1997b;

Raja, 2021).

Condition factor

The condition factor is an important index for measuring feeding intensity, age, growth rates, and health conditions in fish, and it is heavily impacted by both biotic and abiotic environmental conditions in which fish reside (Özer and Kırca, 2015; Epa and Narayana, 2016). The condition factor of *M*. cephalus ranged from 1.24 – 2.08, while the same in *E. suratensis* varied from 2.11 - 2.43. The condition factor of *E. suratensis* caught from the Meenachil River in Kerala, India, was 2.83, somewhat higher than the values observed in the current study (Mani and Sebastian, 2023). The infected E. suratensis condition factor (1.91 ± 0.82) considerably lower than the uninfected E. suratensis condition factor (2.2 ± 0.76) (Figure 3). Fish condition is linked to nutritional status, disease impacts, and hazardous chemical exposure. It causes greater than normal or less than normal weights of individual fish (Doyon et al., 1988: Fisher and Fielder, 1998). However, there was no difference between the mean condition factor of infected M. cephalus (1.84 ± 0.41) and uninfected M. cephalus (1.87 ± 0.61) . This may be attributed to the

comparatively low average number of parasites per fish in *M. cephalus* compared to *E. suratensis*. This result contrasts with Özer

et al. (2016), who indicated a lower condition factor in *M. cephalus* infected with external parasites.

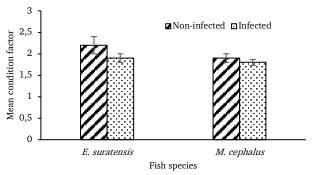


Figure 5. Mean condition factors of infected and uninfected *E. suratensis* and *M. cephalus* collected from the Negombo estuary, Sri Lanka

The high level of parasitic infection observed in *M. cephalus* and *E. suratensis* in the natural environment in the Negombo estuary could be attributed to its suboptimal environmental conditions. Negombo estuary is one of the most polluted estuaries, with hypoxia due to high levels of fertilizers, chlorophyll a, particulate matter, and dissolved carbon in the water (Hsieh *et al.*, 2021). This must be considered when developing fisheries management plans and aquaculture development activities in the Negombo estuary.

CONCLUSION

Ectoparasites recovered from suratensis and M. cephalus were from the Phylum Protozoa, Phylum Nematoda, Sub Phylum Crustacea (copepods and isopods), and Class Trematoda (monogeneans). In M. cephalus, parasites from the phylum Platvhelminthes were common. parasites from the subphylum Crustacea were abundant in E. suratensis. M. cephalus has significantly more parasite diversity than E. suratensis. M. cephalus had a higher percentage of infection than E. suratensis, with values of 86% and 78%, respectively. The average number of parasites per fish in E. suratensis was significantly higher than in M. cephalus. The number of parasites per fish did not differ significantly between length classes in either fish species. The number of parasites reported from the operculum and skin was minimal, whereas the number of parasites recorded from the gills was much higher in both fish species. The condition factor of infected *E. suratensis* was considerably lower than that of uninfected *E. suratensis*, whereas *M. cephalus* showed no such association.

CONFLICT OF INTEREST

All authors have no conflicts of interest upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

UPKE conceived and designed the study, wrote the core sections of the article, and assisted in data analysis and result interpretation. He was responsible for drafting the initial and final versions of the manuscript and actively participated in discussions on the article's content, incorporating feedback from the co-author. GD contributed to various aspects of the research, including data collection and histopathological studies, and assisted in data analysis. She also played a role in drafting the manuscript and provided revisions and feedback.

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