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Examining Ecto and Endoparasitism in Multicellular Organisms as the Emergence of Distinct New Microbiotopes

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Abstract

Fish is the primary source of nutrition for many people in India, especially Maharashtra. One of the many excellent freshwater fish resources is the Krishna River in the Sangli District. The fish used in the study were: *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, *Tilapia zilli*, *Gnathonemus cyrinoides, & Mormyrops deliciosus*. Using standard techniques and equipment, the lab checked 80 samples for Ecto & Endo parasites. Fishes' mean condition factors were calculated and analysed.*C. gariepinus* female parasite prevalence was 66.67 percent and male parasite prevalence was 60 percent out of 400 investigated fishes. Female prevalence of parasites in *Tilapia zilli* was 34.2%, whereas male prevalence was 66.6%. Female parasite prevalence for *C. nigrodigitatus* was 60%, whereas male prevalence was 72.72 %. For *G. cyprinoides*, female parasite prevalence was 60% while male prevalence was 63.63%; for *M. deliciosus*, female prevalence was 57.57% while male prevalence was 63.82%.

Keywords: Prevalence, parasite, endoparasitism, ectoparasitism

INTRODUCTION

Parasites' incredible variety and pervasiveness across ecosystems make their way of life one of the most successful on Earth [1]. Most parasites fall into one of two categories, ectoparasites and endoparasites, depending on whether or not they have any contact with the outside world [2]. To complete their life cycle, some parasites need only one host (direct life cycle), whereas others must use intermediate hosts (indirect life cycle) in which they undergo biochemical & morphological changes (mobility, reproduction) [2]. Multiple types of eukaryotic parasites, both unicellular and multicellular, have been documented in fish [3-6]. Fish gills and skin are vulnerable to ectoparasitic organisms due to frequent interaction with the surrounding water. Mucus on the skin and gills could be the initial line of defence against these potential infections [7]. Endoparasites are parasites that live inside of their hosts, typically in the thoracic cavity, muscles, & urinary and digestive organs [2]. Parasites are essential to population dynamics and ecosystem function [1,8-10] due to their impact on host fitness & host-environment interactions (competition, predation, behavioural changes).

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Labeo rohita (Hamilton) is a native diurnal resident of Bangladesh, India, Pakistan, Nepal, and Myanmar's rivers, streams, and canals. It is also common in ponds, ditches, lakes, and ox-bow lakes. Although it is typically a middle-dwelling carp, this species can be found everywhere in the water column. Early-stage crustaceans and insect larvae make up its diet [11]. The percentage composition of Rohu's diet may consist of 35% algae, 20% higher plants, 23% protozoa, 15% crustaceans, and 7% mud and sand [11]. The average fertility is between 2,000 and 3,000 eggs/kg body weight [13], with a fertility range of 2,26,000 to 28,00,000 eggs/kg body weight[12]. Never reproduces in ponds, but rather migrates to rivers to spawn during the monsoon (April-September). To the exception of hypophysation, to which it responds almost instantly [14].

As one of the most dangerous dangers to fish health, Ecto and Endo parasitic protozoa occupy a prominent niche. Fish are attacked by parasites, which destroy the epithelium lining their skin and gills. Because of their prevalence in fish populations worldwide, parasite infections are a major issue in the tropics. Parasites among the most diverse & common pathogens aquaculturists will encounter. Fish parasites can be either exterior or inside. Parasitic diseases frequently serve as an indicator of water quality, as parasites tend to proliferate and diversify in polluted waters [15]. Parasites can cause harm to any species of fish they infect, either by injuring tissues or organs during burrowing or food consumption, or by removing digested food from the fish's gut and secreting proteolytic enzymes. Parasites rarely kill their host, but some can place a major strain on fish populations, which can be both biologically and economically concerning. Parasites care about their host because they depend on it for survival. Occasionally, fish will perish when there is an abundance of parasites or when the fish is under a lot of stress for some other reason. Parasites can cause harm to a fish in many ways, including direct tissue injury, the draining of blood and other cellular fluids, the diversion of nutrients, and the development of secondary disorders. Fish parasites cause economic losses due to mortality, treatment costs, and reduced growth during and after disease outbreaks, which discourages the spread of aquaculture.

Fish containing protozoa parasites are efficient of transmitting the sickness to humans when consumed. Parasites known as protozoa are a major reason why fish population’s crash in tropical freshwater systems and pose a health risk to humans. One of the scientific benefits of correctly recognising a fish is the ability to assess the fish's health, as certain parasitic illnesses manifest with symptoms that influence the external therapy of the animal [16]. This investigation examined ecto & endoparasitism in multicellular organisms.

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Endoparasites

Most parasites infecting humans are already inside the host (endo- means internal). These can be helminthes (worms) or protozoa, or even the larval stages of insects or other arthropods (insects, mites, etc.) Parasites, whether helminthic or protozoal, can cause damage to a wide variety of human organs and tissues. Many different kinds of endoparasites live in or pass through the digestive tract after being ingested. Trichinella spp. & Toxoplasma gondii, for example, live in muscles; Echinococcus spp. and liver fluke larvae reside in the liver; Schistosoma hematobium affects the urinary bladder; and so on.

Ectoparasites

Infectious organisms that live on humans are called ectoparasites (ecto- means outside of). Fleas, lice, mosquitoes, insects, mites, ticks, & others all fall under this category. Ectoparasites are parasites that adhere to a host organism and feed off of it, but they often don't remain on the host for its whole life.

Fig.1: Pediculus humanus capitis (male)

Some of these species are ambiguous in terms of their classification, existing on a spectrum between endoparasites & ectoparasites; scabies mites, for example, are commonly categorised as ectoparasites despite the fact that the female scabies mite burrows into the skin. Larvae of some flies can infiltrate healthy tissue and feed on it, while those of others can only feed on dead tissue.

LITERATURE REVIEW

Ravichandra et al. (2014) [17] examined only about 3 percent of all nematode species. A single cubic foot of soil could house millions of nematodes from many different families. Plant-parasitic nematodes are worm-shaped creatures that are so small they are practically invisible when present in soil. Phytonematodes can inflict damage on plants ranging from relatively mild disruption to total plant tissue destruction. The severity of plant injury caused by nematode activity is dependent on a number of variables, including the species composition of the plant and nematode as well as the

prevailing environmental conditions, such as precipitation, soil type, land contour, and cultural practises.

Duneau et al. (2012) [18] investigated the function of moulting in parasite defence. The penetration of parasites into the host is crucial for a successful infection, and the epithelium serves as the first line of defence for the host. The shedding of this protective covering (moulting) is an essential part of the life cycle of numerous invertebrate & vertebrate taxa, and is widely thought to render hosts susceptible to parasites and predators. Here, we examined whether moulting increases the likelihood of infection by the castrating bacterium Pasteuria ramosa using the crab Daphnia magna. This parasite is reported to connect to the cuticula of its host before to entering its body. The possibility of effective parasite infection is drastically lowered if the host moults within 12 hours of exposure to the parasite. Therefore, moulting is advantageous for the host exposed to this parasite.

Rueckert et al. (2009) [19] examined metazoan fish parasites in Segara Anakan, an Indonesian lagoon with brackish water located on the southern coast of Java. Seven commercially significant marine fish species *Mugil cephalus, Siganus javus, Scatophagus argus, Caranx sexfasciatus, Lutjanus johnii, Eleutheronema tetradactylum, and Johnius coitor* were tested for the prevalence of metazoan parasites at two separate lagoon sample locations. 43 species/taxa of parasites were discovered, constituting a varied parasite zoo. Ectoparasites (31) were more numerous than endoparasites (31).In a tropical brackish water locale, and perhaps in other tropical and non-tropical marine ecosystems as well, the diversity of endoparasites and the ratio of ectoparasites to endoparasites can be used to characterise environmental health.

Rueckert et al. (2009) [20] investigated the ecto- & endohelminth parasites of *Epinephelus coioides* groupers from an Indonesian mariculture farm fed various diets. 13 parasite species/taxa plagued pellet-fed *E. coioides*, of which 6 had monoxenous life cycles and seven had heteroxenous life cycles. Fourteen parasite species/taxa, 4 with a monoxenous life cycle & 10 with a heteroxenous life cycle, were detected in waste-fed fish. 62 parasite species were isolated from Balai Budidaya Laut Lampung (39% ectoparasitic & 61% endoparasitic), four of which were also discovered in cultured *E. coioides*& fourteen in other groupers. These parasites were transmitted to aquaculture fish by the rubbish fish.

Koltai et al. (2002) [21] described 15,000 nematode species. Nematodes have been described as the most abundant and widespread multicellular organisms on Earth. The soil is one of the most significant nematode habitats. They are by far the largest component of the fauna in diverse soils, comprising > 90% of the total population and 10% of the total biomass. As principal eaters of plant tissues or decomposers, soil nematodes might be categorised as bacterivorous, fungivorous,

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predatory, or omnivorous. Clearly, the link between plant-feeding nematode species and decomposers is heavily dependent on the nature and condition of the vegetation.

METHODOLOGY

Study Area

The research area is the river Krishna in the Sangli District. The Krishna River contains a diverse fish life.

The studied fishes:

Clarias gariepinus

It averages 1–1.5 m in length as an adult. It has a maximum length of 1.7 metres & a max. mass of 60 kilogrammes. These fish are distinguished by their slender builds, flat skulls, and four-barbell teeth. Big modified gill arcs serve as a secondary respiratory system. Like many catfish, this species is nocturnal. It feeds on both living and decomposing animal materials. Due to its big mouth, it may ingest prey that is relatively huge. In order to escape drying waters, it may even crawl over dry land. Additionally, it may live in shallow mud for extended durations between rainy seasons. (Plate 1).

Plate 1:*Clarias gariepinus*

Chrysichthys nigrodigitatus

It is widespread across Africa and can be caught in Nigeria, where it is known as the silver catfish. They belong to the tribe of Bagridae. The Niger Delta is home to many of these fish since they are a great source of protein and make up the bulk of the commercial catch of the region's artisanal fishermen (Plate 2).

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Plate 2:*Crysichthys nigrodigitatus*

Tilapia zilli

It can grow to be 40 centimetres long, weighs up to 300 grammes, and sports anywhere from 13 to 16 spines along its back. T. zilli is olive-dark on top & olive-light to yellow-brown on the sides when it isn't breeding. One can see a bright green lip colour and a somewhat reddish breast (Plate 3).

Plate 3:*Tilapia zilli*

Mormyrups deliciosus

They are ubiquitous across the Afrotropical river basins and especially so in West Africa. Their non-visual electric organs are vital for nighttime movement & communication, and they have developed a wide variety of feeding strategies (Plate 4).

Plate 4:*Mormyrups deliciosus*

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Gnathonemus cyprinoides

They belong to the family Mormyridae and have two structurally dimorphic traits: the males have a longer anal fin ray bone & a depressed posterior ventral body wall (technically called anal fin indentation) than the females do (Plate 5).

Plate 5:*Gnathonemus. Cyprinoides*

Sample collection

There were a total of 400 fish investigated, 80 each species. Each fish's length & weight were recorded to the closest 1 cm and 0.1 g, respectively, using a top-loading metre balance and a measuring tape. Examining the papillae allowed for the determination of the sexes of the fish. Samples of pond water were taken in 250 ml sterilised glass bottles from three distinct pond locations 15 to 20 cm below the water's surface at each sampling.

Sample analysis

Separately analysing and averaging the physicochemical properties of the three water samples. The method developed by Emere and Egbe (2006) [22] was used to check for parasites on the gills, fins, & skin of each fish. All of the fish were analysed with a hand lens for ectoparasites on their skin, gills, & fins. Fish fillets were taken with the use of a scalpel. In a petri dish, tissue was stirred with a mounted pin after 3 ml of 0.9% saline solution had been introduced. The blended solution was collected in a few drops using a dropper, then put on a microscope slide & sealed with a cover slip. Then, a light binocular microscope was used to make observations. Each fish's gills were later removed using a dissection kit. All of the gills were submerged in 10 ml of sterile saline solution in a Petri plate. The fish's guts and stomachs were sliced open, and their contents were poured into the petri dish containing salt water. Intestinal lumen lining was also extracted & placed in the salt water. 1-2 drops of the solution were placed on a slide with coverslips & endo parasites were seen utilizing a light binocular Direct Res. J. Agric. Food Sci. 142 microscope. The procedures of Emere and Egbe (2000) [23] were used to collect Ecto parasite data from the fish's gills, fins, and skins, and Endo parasitic data from the fish's stomach and intestines. The parasites were identified by

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sketching them as seen through a binocular microscope and comparing the sketches to the visual guide on fish parasites by Pouder [24].

Alkalinity

Methyl orange was used to determine the overall alkalinity. Methyl orange indicator was used, and two or three drops were put to each 100 mL of water. Up until a faint pink colour formed, this was titrated against a water sample containing N/50 sulfuric acid. At this stage, the alkalinity of the water might be determined by recording and calculating the volume of titrant used [25].

Temperature

Using a thermometer with a mercury-filled bulb, the water's temperature was measured. The measurements were taken on location. Once between 8:00 am and 12:00 pm was repeated for each site visit.

Dissolved oxygen

Annes (1966) [26] utilised a modified version of the Winkler approach. Water samples from the location were collected using approximately labelled 300 ml BOD bottles. To prevent turbulence or bubbles from interfering with the measurements, water samples were obtained at a depth of 0.5 metres while the bottle remained submerged. The bottles were then set aside to slowly fill with water without being disturbed. The bottle's water level was held steady, and then it was gradually inverted and filled to its brim. Eight drops of manganese sulphate solution & eight drops of alkaline KI were added to the vial, & it was then turned upside down several times to ensure the solutions were completely mixed. The bottle was rapidly sealed, making sure no air was trapped inside, and inverted several times to achieve a thorough blending of the contents. In the presence of oxygen, a flocculent orange-brown precipitate formed. After waiting a few minutes for the flocculent to settle, the bottle was inverted many times to verify the reaction between the sample & reagent. The fixed sample was transferred to a burette from a graduated cylinder. To the zero spot on the burette, 0.025N of sodium thiosulfate was added. Slightly depressing the plunger caused 8 drops of starch indicator solution to be introduced to the sample, changing its colour from yellow-brown to blue. The titration must be continued until all traces of blue have vanished (end-point). Each reagent's volume was noted, as were the beginning and ending burette readings. These numbers were used to calculate the total volume of dissolved oxygen.

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pH

The pH level was measured with a British Milwaukee Smart Meter S2O4 pH metre. A 50 ml water sample was added to a 100 ml beaker, & the electrode for the metre was inserted in the water. One minute later [27], we took a reading. In-situ Secchi disc readings were taken to determine the turbidity levels. The turbidity of the water was determined by inserting a Secchi disc into the pond with the aid of a graded thread and observing when the white portion of the disc completely disappeared. The procedure was repeated three times, & the average was taken.

Biological oxygen demand

The same procedures were used to collect BOD samples as those outlined for dissolved oxygen. Two samples are collected for BOD analysis. The dissolved oxygen levels in the first sample were immediately measured, whereas the second sample was stored in the dark at 20.125°C for 5 days. The BOD, or the amount of oxygen used by bacteria to decompose organic matter in the sample bottle during the incubation time, can be estimated from the difference in oxygen levels between the first and second tests (mg/L) [28].

Conductivity

The American Phillips Hanna HI9813-0 conductivity metre was utilised for the measurements. The water samples were shipped off to the lab for further analysis. The conductivity metre has been adjusted to give the most precise readings possible. To measure in microhms (s), a range of 2000 was established, and the instrument's calibrated knob was adjusted until it read 1,000 s. While inserting the probe into the water sample, care was taken to fully submerge the probe's slot at the end. After agitating the sample with the probe for five to ten seconds to eliminate any bubbles that may have become trapped in the slot, readings were taken.

Statistical analysis

Results were analysed statistically using a chi-square test with significance levels of 0.05 or less, and the variance of the means and variables [29].

Condition factor K

The following formula was used to estimate the condition factor "K," or the fish's overall health, often reffered as the Ponderal index or the Fulton Coefficient of condition.

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

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Where K= Condition factor, W= Body weight of the fish (g), L= Total or standard length of the fish (cm).Individuals were categorised into low conditions & high conditions status on the basis of the condition factor [30].

Percentage prevalence of parasites

$$\text{Percentage prevalence} = \frac{\text{Number of fish infected}}{\text{Number of fish examined}} \times 100$$

RESULTS AND DISCUSSION

Four hundred (400) species were studied, including eighty (80) *C. gariepinus*, eighty (80) *C. nigrodigitatus*, eighty (80) *T. zilli*, eighty (80) *M. deliciosus*, and eighty (80) *G. cyprinoide*. *C. nigrodigitatus* had the highest percentage of parasite prevalence among male species, whereas *Clarias gariepinus* had the lowest % of parasite prevalence. *Clarias gariepinus* had the highest prevalence of parasites among female species, whereas *Tilapia zilli* had the lowest frequency. *Chrysichthys nigrodigitatus* had the maximum prevalence of parasites overall, whereas *Tilapia zilli* had the lowest prevalence (Tables 1-3).

Ecto & endo parasites of the fish used in the study are listed.

The Ecto parasites found were:

Flexibacter litoralis ambloplitis – Uvulifer

Arulus japonicas lavaretus –Salmincola

Diplostomulum Ichthyophthirius multifilis- flexicaudum

The Endo parasites found were

Lingual anatine lucii- Acanthocephalus

Clinostomum marginatum latum- Diphyllobothrium

Contraceacum spiculigerium tubifex- Eustrongylides

Table 1: Condition factor of the sampled fish

Species	**K -RANGE**	**K-MEAN**
C. gariepinus	1.30 - 2.78	2.675
C. nigrodigitatus	1.15 - 2.36	2.330
T. zilli	1.54 - 2.58	2.800
G. cyprinoides	1.67 - 1.84	2.560

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M. deliciosus	0.60 - 2.10	1.635

Table 2:Ecto and endo parasite prevalence in the genders of the fish population

Species	**Sex**	**Infected**	**Non Infected**	**Total**	**(% Prevalence)**
Clarias gariepinus	Male	30	20	50	(60.00)
	Female	20	10	30	(66.67)
	Total	50	30	80	62.5 %
C. nigrodigitatus	Male	40	15	55	(72.72)
	Female	15	10	25	(60.00)
	Total	63	27	80	78.75 %
Tilapia zilli	Male	30	15	45	(66.66)
	Female	12	23	35	(34.28)
	Total	44	46	80	55 %
G. cyprinoides	Male	35	20	55	(63.63)
	Female	15	10	25	(60.00)
	Total	56	34	80	70 %
M. deliciosus	Male	30	17	47	(63.82)
	Female	19	14	33	(57.57)
	Total	58	32	80	72.50 %

Table 3: The mean physicochemical parameters

WEEKS	**ALK (ppm)**	**BOD (mg/l)**	**COND. (u/cm)**	**DO (mg/l)**	**pH**	**TEMP. (°C)**	**TURB. (meters)**
1	124	1.18	122	7.58	10.12	25	3.2
2	140	2.02	110	7.00	8.57	27	2.4
3	152	1.90	100	8.28	7.56	28	3.4
4	134	1.30	98	7.50	9.75	26	2.2

CONCLUSION

As a conclusion, many parasites in fish have been discovered, but only a select few can infect humans. The average weekly physicochemical characteristics were as follows: total alkalinity

(137.5 ppm), BOD (1.595 mg/l), DO (7.59 mg/l), pH (9), temp (26.5°C), conductivity (107.5 cm), & turbidity (2.8 m). Parasite infection was found in 66.67% of the females & 60% of the males of the 400 C. gariepinus examined. 34.28% of female Tilapia zilli were infected with parasites, compared to 66.66% of males. 60% of female C. nigrodigitatus were parasitized, compared to 72.72 percent of males. The prevalence of parasites in female G. cyprinoides was 60%, while in males it was 63.63%; in female M. deliciosus it was 57.57%, and in males it was 63.82%. A fish's quality in terms of the presence and number of parasites can be directly affected by the method of capture, handling, & storage. Fresh, frozen, salted, or pickled fish, as well as other forms of processing (such as heading and gutting, candling, and trimming), can all help with the control of parasites in fish. The most efficient methods of parasite removal involve either freezing the parasites or inactivating them with heat.

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