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Comparitive Surveillance of Parasitic Grouping and Infestation in *Barytelphusa Cunicularis* and *Poecilia Reticulata* Influenced by Physico-Chemical Parameters

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Keywords: *Endoparasites; Barytelphusa Cunicularis; P. Reticulata, Anisakis sps.; Procamallanus sps.; Noyyal River*

Abstract

Background

Parasite is an organism which lives in or on the body cavity of the host, obtaining nourishment and protection. They are used as a bio-indicators of heavy metals in aquatic ecosystems. Crustaceans are free living aquatic organisms and few are even parasitic. Freshwater ornamental fish trade is a multimillion dollar industry. The vast majority of freshwater ornamental fish trade is of freshwater origin and farm raised.

Results

Parasitic grouping and infestation rate in *Barytelphusa cunicularis* and *Poecilia reticulata* were determined. Mean body weight of male, female crab was 15.50±6.48g and 13.71±1.05g respectively. Identified parasites were *Anisakis sps., Hirudo sps., Rotifer sps., Procamallanus sps.,* and

Ciliated protozoan. Morphometric analysis was carried out in the *B. cunicularis.* The incidence of infection (40.00%) of protozoan and nematode were higher in male crab. The highest incidence is observed in protozoans (60.00%) of female crab. In *P. reticulata* the incidence was high with arthropoda (33.33%). Among the two selected aquatic species, the intensity of trematode species (14.00) were found to be high in male crab. The lowest incidence was recorded in P. retiulata of rotifer *sps.* (6.67%). The water quality parameters like temperature 24±1°C, pH 7.60±0.32, dissolved oxygen 1.40±0.012, salinity 0.10±0.001, phosphates 0.50±0.15 and nitrites 0.24±0.03 were recorded for pond water medium of the host species. The water quality parameters like temperature 27±1°C, pH 7.75±0.19, dissolved oxygen 2.86±0.01, salinity 2.17±0.87, phosphates 1.30±0.13 and nitrites 0.39±0.08 were recorded for Noyyal river water medium of the host species.

Conclusion

Noyyal river water is reported to be a medium for host parasitic prevelance.

Background

Fresh water systems in tropics host a diverse endemic fauna including fresh water crabs with 1280 species representing one fifth of all the world's brachyurans [1]. In India, a total of 96 species under 41 genera in 6 families have been recorded [2]. Freshwater crab plays a significant role in nutrient cycle, water quality monitoring and fishery wealth as they are consumed in many parts of the world. The crustaceans are highly sensitive to pollution and any disturbances in physicochemical parameters may cause immune vigour. Freshwater Crab (*Barytelphusa cunicularis*) has always been a nutritional food commodity, rich in proteins, carbohydrates, vitamins, having great medicinal properties. The crab species, *B. cunicularis* is hardy to withstand without water in moist and muddy burrows and can air breathe and remain alive without food for 3-4 days. The freshwater crabs, *B. cunicularis* are widely distributed in the region and consumed by socio-economically poor people to obtain their energy requirements [3]. *Poecilia reticulata*, commonly called as "guppy" is one of the most famous tropical ornamental fish in the world. *P. reticulata* has had mixed success in controlling mosquito populations. In some areas it is regarded as beneficial as a control agent [4], but in other areas it is reported to have had minimal effects on mosquito populations [5]. Parasites are harmful and limiting factors in breeding and rearing ornamental fish industry [6]. Economic losses due to parasitic infestations not only result from direct harm to fish but also from disfigurement that inflict a big loss to aquaculture [7]. Crustaceans serve as hosts for a wide range of protozoan and metazoan parasites, some of them cause considerable pathogenicity by affecting the growth and reproductive performance of the hosts [8]. The incidence and rate of infestation of parasites are associated with physico chemical parameters of water and fish health [9]. The present work was carried out to investigate the different parasitic communities and to find out the infestation rate in freshwater crab *B. cunicularis* and *P. reticulata* influenced by the physicochemical parameters.

Materials and Methods

Collection and transportation of specimen

A total of 20 freshwater crabs, *B. cunicularis* were collected from the Noyyal River, Marudurai and 15 guppy fish (*P. reticulata*) from the semi-natural pond, PSG college of Arts and Science campus site. Collected samples were placed in plastic container and transported to the lab. Analysis of specimens for parasites was done within 24 hrs of collection.

Determination of morphometric indices of *B. cunicularis*

5 males and 5 female freshwater crab, *B. cunicularis* was selected among the collected specimens and was subjected to morphometric indices study. Weight was measured in gm. Length of major chelates, Length of minor chelates, Carapace width, carapace length was measured in cm.

Examination of specimen for parasites in *B. cunicularis*

The collected specimen was immediately examined for ectoparasites with the aid of hand lens and the unaided eye. Then the carapaces were cut open and the internal organs were placed in the petridish containing saline solution (0.85% NaCl). The scrapings from the intestine, gut, gills and hepatopancreas were taken on the clean slide to examine the endoparasites. The smeared slides were air dried, fixed in acetone free methanol and stained with Giemsa (HiMedia, Mumbai). The prepared slides were examined under the microscope using 10X, 40X and 100X objectives.

Examination of specimen for parasites in *P. reticulata*

The specimen was examined immediately for ectoparasites using hand lens. Skin samples were taken from different spots. The skin mucus from the dorsal fin, pectoral fin, pelvic fin, anal fin and caudal peduncle of the fish were scraped using cover glass. Mucus was then placed on a glass slide with a drop of distilled water and examined under the microscope for observing the presence parasites in the mucus. Incision was made on the mid-ventral line and the intestine were taken out and placed in the petridish containing saline solution. Gills were removed using the forceps, placed on the glass slide and viewed for the parasitic presence. Contents from the intestine were removed and viewed under the microscope.

Examination of specimen for parasites in *P. reticulata*

$$Incidence\ of\ infection = \frac{Infected\ host \times 100}{Total\ host\ examined}$$

$$Intensity\ of\ infection = \frac{No\ of\ parasites\ examined\ in\ one\ host}{No\ of\ infected\ host}$$

$$Density\ of\ infection = \frac{No\ of\ parasites\ collected\ in\ a\ sample}{Total\ host\ examined}$$

$$Index\ of\ infection = \frac{No\ of\ host\ infected \times No\ of\ parasite\ collected}{Total\ host\ examined}$$

Physico-chemical parameters of water sample

The Water samples were collected from Noyyal river and semi-natural pond were tested for the physico-chemical parameters such as pH, Temperature, Dissolved Oxygen, phosphate, nitrite and salinity. The water samples were collected in a plastic container and brought to the lab for analysis.

Statistical Analysis

The results of the present work were analyzed statistically by using SPSS statistics version 20 package software [11]. Data were obtained as Mean±S.D of five to eight individual

Results

During the present study parasitic communities infesting *B. cunicularis* and *P. reticulata* were studied.

Morphometric indices of *B. cunicularis*

Carapace width, Carapace length, Length of major chelate, Length of minor chelate and weight were examined and their results are represented as mean ± standard deviation of morphometric indices of male and female crabs (table 1).

Table 1: *Morphometric indices of B. cunicularis*

Sex	Carapace Length	Carapace Width	Major Chela	Minor Chela	Weight
Male	2.58±0.15	3.6±0.53	6.92±0.89	5.26±0.87	15.50±6.48
Female	2.52±0.08	3.5±0.16	5.82±0.42	5.16±0.32	13.71±1.05

The results are mean value of five individuals ± standard deviation.

Examination of specimen for parasites in *B. cunicularis* and *P. reticulata*

Protozoan, nematode, trematode, arthropod and rotifer occurrence were represented as mean ± standard deviation (table 2). In male crab the mean trematode *sps.* (14.00±0.00) occurrence is high when compared to

the other parasitic groups. The mean rotifer *sps.* (6.00±0.00) is high in female crab. In *P. reticulata* the mean protozoans (3.50±2.38) are high.

Table 2: *Mean values of parasitic group occurrence in B. cunicularis and P. reticulata*

Parasitic groups	Males	Female	*P. reticulata*
Protozoan	4.50±2.65	2.17±1.17	3.50±2.38
Nematode	10.25±8.85	1.50±0.71	2.25±0.50
Trematode	14.00±0.00	---	----
Rotifer	2.67±1.53	6.00±0.00	1.50±0.71
Arthropoda	---	------	1.20±0.45

Parasitic infestation in male crab *(B. cunicularis*)

The incidence, intensity, density and index were calculated. During the study period, the highest number of 41 nematode species and lowest of 8 rotifer species were collected from the infected male crab. The highest incidence 40.00% was found to be similar in protozoan and nematode species. Intensity of 14.00 in trematode *sps.* followed by nematode 5.75, whereas highest density 2.30 and index 16.40 is recorded for nematode *sps.* of male crab. Trematode parasites showing the lowest value for incidence 10.00% and index 01.40. Rotifera *sps.* showing the lowest value of intensity 1.33 and density 0.40 (table 3).

Table 3: *The fluctuations of overall incidence (%), intensity, density and index in male crab*

Parasitic groups	No of crabs examined	No of crabs infested	No of parasites examined	Incidence (%)	Intensity	Density	Index
Protozoa	10	4	18	40.00	1.75	0.70	7.2
Nematode	10	4	41	40.00	5.75	2.30	16.40
Trematode	10	1	14	10.00	14.00	1.40	1.40
Rotifera	10	3	8	30.00	1.33	0.40	2.40

Parasitic infestation in female crab (*B. cunicularis*)

The highest number of protozoan *sps.* 13 and lowest 3 of nematode species collected from the infected female crab. The highest incidence 60.00% and index 7.80 is seen in protozoan *sps.* Rotifera *sps.* showing the highest intensity 6.00 and density 0.60 in female crab. Index of nematode was the same as rotifera

which was also 0.60. In female crab there was no infestation by trematode *sps*. The intensity and density of protozoans are low when compared to other parasitic species (table 4).

Table 4: *The fluctuations of overall incidence (%), intensity, density and index of parasites in female crab*

Parasitic groups	No of crabs examined	No of crabs infested	No of parasites examined	Incidence (%)	Intensity	Density	Index
Protozoa	10	6	13	60.00	0.50	0.30	7.80
Nematode	10	2	3	20.00	1.00	0.20	0.60
Trematode	10	0	0	0	0	0	0
Rotifer	10	1	6	10.00	6.00	0.60	0.60

Parasitic infestation in *P. reticulata*

A total individual number of 31 parasites were examined include protozoans, nematode (larva), arthropod and rotifer. The incidence of arthropods was found to be high 33.33% followed by protozoans, nematodes and rotifers. Rotifer *sps*. shows the lowest value of density and index of same value 0.13. Intensity of rotifer *sps*. was high 2.00 and lowest range was seen in arthropod 0.40. Incidence 26.67% in both protozoan and nematode parasites (table 5).

Table 5: *The fluctuations of overall incidence (%), intensity, index, and density of parasites in P. reticulata*

Parasite groups	No of fish examined	No infested	No of parasites examined	Incidence (%)	Intensity	Density	Index
Protozoa	15	4	14	26.67	1.50	0.40	3.73
Nematode	15	4	9	26.67	0.75	0.20	2.40
Arthopoda	15	5	6	33.33	0.40	0.13	2.00
Rotifera	15	1	2	6.67	2.00	0.13	0.13

Physico-chemical analysis of water medium

Physico-chemical parameters like temperature, pH, dissolved oxygen, salinity, phosphate, and nitrite were estimated and their results are represented as mean ± standard deviation of triplicate analysis of semi-natural pond and Noyyal river water (table 6).

Table 6: *Estimation of water quality parameters*

Parameters	Units	Semi natural Pond water	Noyyal river water
Temperature	°c	24±1.00	27±1.00
P^{H}	Unit scale	7.60 ± 0.32	7.75 ± 0.19
Dissolved oxygen	mg/l	1.40 ± 0.012	2.86 ± 0.008
Salinity	mg/l	0.19 ± 0.001	2.17 ± 0.869
Phosphate	mg/l	7.45 ± 0.008	16.24 ± 0.005
Nitrite	mg/l	0.04 ± 0.002	0.05 ± 0.003

The results are mean value of five individual's ± standard deviation.

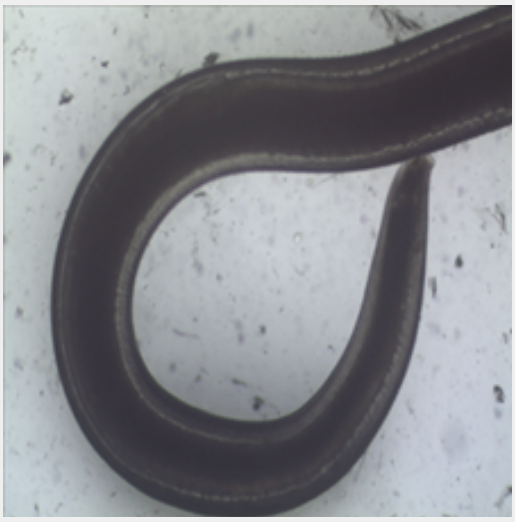

Fig 1: *Anisakis sps.*

Fig 2: *Hirudo sps.*

Fig 3: *Rotifer sps.*

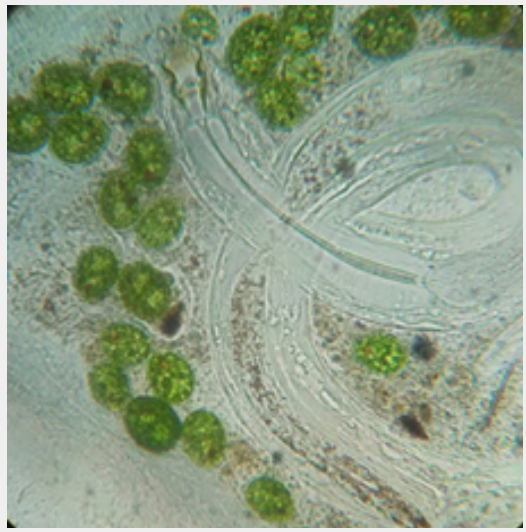

Fig 4: *Procamallanus sps.*

Fig 4: *Protist*

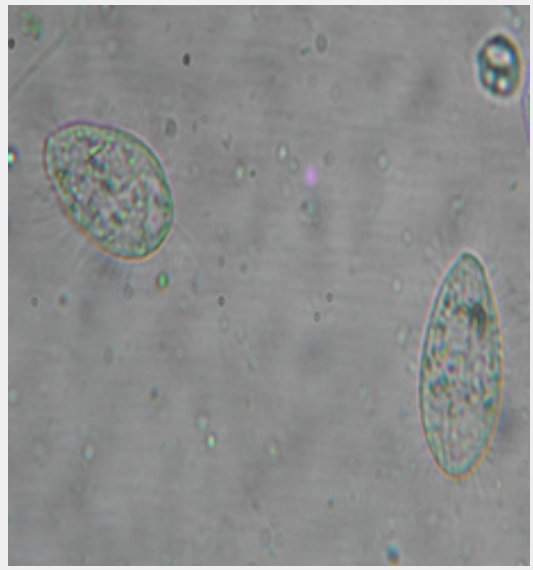

Fig 6: *Ciliated protozoan*

Discussion

Parasites are found everywhere and on every organism. The parasitic presence in the host is generally at equilibrium in aquatic organisms and the most common lifestyle on the planet [12]. When there is change in the environment, the state of balance of parasites between the host and the nature gets affected thus resulting in disease. The changes can be of temperature, climate or anthropogenic such as pollution and urbanization [13].

Parasites can cause mechanical damage (fusion of gill lamellae, tissue replacement), physiological damage (cell proliferation, immunomodulation, altered growth, detrimental behavioral responses) and reproductive damage [14]. Colonization of the gills by parasites often causes proliferative cell changes, including severe epithelial hyperplasia, hypertrophy, edema and interlamellar vesicle formation [15].

Parasitic infestation has harmful influence for fish health that inhibits the normal growth of the fishes and outbreaks high mortalities [16]. The declination of *Channa punctatus* is regarded as its susceptibility to Epizootic Ulcerative Syndrome disease [17] and over exploitation and habitat degradation [18]. Hameed *et al.,* (2000) reported that in India the culture of *M. rosenbergii* was developed few decades ago as an alternative to the *Penaeus monodon* and to compensate for the substantial losses due to the epidemics of white spot syndrome in penaeid shrimp farming, hypothesizing that *M. rosenbergii* is resistance to white spot disease [19]. Vogan *et al.,* (2002) reported that the Chitinolytic bacteria are the primary aetiological agents of shell disease syndrome in marine crustaceans [20]. The disease principally results from the breakdown of their chitinous exoskeletons by the shell disease pathogens, but pathogenicity may also manifest internally should a breach of the carapace occur. (Owens, 2017) reported that half a million farmed salmon died from

a sea lice outbreak in New Brunswicks Passamauoddy Bay [21]. More than 250,000 died directly from the parasites, which attach themselves to the fish and feed on their skin, blood, and mucus, while another 284,000 were euthanized to try to contain the spread. The outbreak, which affected two sites owned by Gray Group, a bankrupt aquaculture company that still had fish in its pens, was a "catastrophic loss,"

In fish species, the host age, behavior, physiological and immunological condition, location in the water column and feeding habits could affect the relationship while the parasites mode of entry, ability to evade its host defense, nutritional requirements and living in a site where the immune response is reduced and mimicking its hosts protein composition are factors that influence susceptibility and infectivity. The interaction can also be affected by water temperature, crowding and habitat changes [22]. Parasitic crustaceans are among the most harmful pests of fishes. Certain species cause mass infection and mortality in fish culture sometimes in nature, resulting in considerable economic losses [23].

Parasites can adopt to any changes in their surroundings, depending on the environment and host health condition. Parasites are expected to grow faster in host that are better at acquiring nutrient from natural ecosystem, it is possible that the most competitive hosts are better at countering infections, if they have an improved immune response [24]. In this present study, crab species were collected and parasites were examined. During the study of freshwater crab spp. various parasitic communities were identified including protozoan, nematode, trematode and rotifers. Similarly, Jithendran *et al.,* (2010) reported on wide variety of parasitic organisms such as blood parasite, nematode and ciliated protozoans in mud crab [25].

In the present study parasites were isolated from *B. cunicularis* and *P. reticulata*. The parasitic infestation between the *P. reticulata*, male and female crab *B. cunicularis* have been discussed. Protozoans are common freshwater parasites. In this study, the incidence of protozoan parasites was found to be in the order of female crab (60.00%) > male crab (40.00%) > *P. reticulata* (26.67%). The prevalence rate of nematode infestation was found to be higher in male crab (40.00%) followed by female crab (20.00%) and *P. reticulata* (26.67%). The incidence of arthropoda was recorded to be 33.33%, while no arthropods were recorded in the crab species. A prevalence of 10% was recorded for trematodes in male crab and no trematode were recorded in both female crab and *P. reticulata.* The rotifer infestation was found to be very low in *P. reticulata* (6.67%) when compared to the crab species. Similarly, Miah *et al.,* (2013) reported on the parasitic infestation in Channa punctatus, the incidence of protozoans was high (60.00%) [16].

In the present work, examination of the intestinal contents of crab and *P. reticulata* revealed the presence of two nematodes (*Anisakis sps.* and *procamallanus sps.*), trematode (*Hirudo sps.*), ciliated protozoan and *Vorticella sps.* Similar results were also recorded by Mandal *et al.,* (2015) [26]. Okaka *et al.,* (2002) recorded nematodes as the most common parasite, infecting 18.6% of the fish population [27]. Anisakiasis is a fish-borne zoonosis caused by the ingestion of third stage larvae of the genus *Anisakis* (Nematoda: Anisakidae), commonly present in the body cavity and muscle of many fish species and cephalopods [28]. It is a most common nematode to be affecting the fish. But in the recent study, this parasite was isolated from the gut of the crab spp. Larvae of *Anisakis sps.* was relatively high (incidence = 40.00%) in comparison with that reported in rockfish (Sebastes capensis, 2.9-4.8%) [29]. The mean abundance of *Anisakis sps.* was 12.7±9.07. Similarly, 15.0±3.9 results were also obtained by Rodriguez-Santiago, (2014) [30]. Consumption of raw or thermally inadequately treated fishery products represents a public health risk with the possibility of

propagation of live *Anisakis* larvae, the causative agent of the zoonotic disease anisakidosis, or anisakiasis [31]. In humans, larvae penetrate the mucosa of the stomach, small intestine or colon, causing eosinophilic granuloma formation with severe symptoms of disease [32]. The other clinical form is triggered by repeated consumption of thermally processed seafood that contains the whole nematode or its molecular traces, inducing hypersensitivity in the consumer and an array of allergic symptoms: acute urticaria and angioedema, bronchospasm, fatal respiratory arrest and anaphylaxis [33].

H. auctum is a common nematode occurring in many regions of the world in the sea and backrish water [34]. The parasites mostly seen in *P. reticulata* are *D. extensus, Gyrodactylus sps., Trichodina sps., Ichthyophthirius multifiliis, Capillaria sps., Chilodonella sps.*

Salinity is the total concentration of the dissolved ions in the freshwater. In this study the value of mean salinity is high (2.17±0.869) in Noyyal water and low in semi-natural pond water (0.19±0.001). Dissolved oxygen is the amount of oxygen that is dissolved in the water at given atmospheric pressure, water temperature and salinity, these affect the concentrations of dissolved oxygen in an aquatic ecosystem, as cold freshwater holds more oxygen than warmer and salty water [35]. In this study, the maximum mean dissolved oxygen concentration was recorded (2.86±0.008) in Noyyal river and minimum concentration of (1.40±0.012) in semi-natural pond water. Dissolved oxygen adversely affect the functioning and survival of the biological communities. Organisms in standing freshwater may have to tolerate low levels of oxygen because oxygen diffusion slows down considerably when water is not flowing.

The pH is an important parameter in water quality assessment as it influences biological and chemical processes within a water body [36]. The pH (or the hydrogen ion activity) indicates the intensity of the acidic or basic character of a medium. In this study maximum of pH (7.75±0.19) were recorded in Noyyal River when compared to the semi-natural pond water (7.60±0.32). Both are alkaline in nature. The study revealed that the phosphate presence was high in Noyyal river (1.30±0.133) than semi-natural pond water (0.50±0.152). Nitrite is an intermediate product of inorganic oxidation and of the bacterially mediated processes, nitrification and denitrification, which involve transformations of nitrogen in soil and water [37]. The toxic effects of nitrite result from impairment of oxygen transport and cause anoxia in fish. The nitrite value of Noyyal river and pond water was recorded 0.05±0.003 > 0.04±0.002. In this work, increase in concentration of nitrite was recorded in Noyyal river. The acceptable level of nitrite in freshwater fish pond ranges between 0.001 and 0.004 mg/L [38]. Similar results were obtained from Mohan *et al.,* (2013) recorded nitrite value to be 0.01-0.6mg/l in Noyyal river source [39]. Nitrite in water is either due to oxidation of ammonium compounds or due to a reduction of nitrate. It can be toxic to certain aquatic organism even at concentrations of 1 mg/L.

The temperature of surface waters is influenced by latitude, altitude, seasons, time of day, air circulation, cloud cover as well as the flow and depth of the water body [40]. The metabolic rate of aquatic organisms is also related to temperature, and in warm waters, respiration rates increase leading to increased oxygen consumption and increased decomposition of organic matter. Majumder *et al.,* (2015) investigated the effect of temperature on the prevalence of different parasites in *Cirrhinus mrigala* [41]. The temperature is high in Noyyal River (27±1°C) when compared to the semi-natural pond water (24±1°C). Chanda et al., (2011) reported that the intensity of infection is high in *Ichthyophthirius sps.* and the intensity of infection is high in

summer months when the temperature is high or moderately high [42]. Hossain *et al.,* (2008) reported that the parasitic prevalence is very much dependent on the temperature [43].

The term pollutant in a broad term refers to a wide range of compounds, from a superabundance of nutrients giving rise to the enrichment of ecosystems to toxic compounds that may be carcinogenic, mutagenic or teratogenic. Pollutants can be divided into two major groups, namely, those that affect the physical environment and those that are directly toxic to organisms, including human beings [39]. The deterioration in various water quality characteristics clearly indicates the possibilities of pollution due to industrial activities such as coffee vegetables oils, leather tanning, textiles and foundries in and around Coimbatore city. The population of Coimbatore has also a strong impact on the Noyyal River with regard to pollution and due to this Noyyal River acts as a carrier for the pollution. During the non-flow period of the river, water can be stagnated and the pollution may enter into the ground water. So the ground water quality also gets depleted [44].

A pond is a body of standing water, either natural or artificial, that is usually smaller than a lake. A river is a natural flowing watercourse, usually freshwater, flowing towards an ocean, sea, lake or another river. In some cases, a river flows into the ground and becomes dry at the end of its course without reaching another body of water. River water pollution is not only an aesthetic problem, but a serious economic and public health problem. Moving water dilutes and decomposes pollutants more rapidly than standing water, but many rivers and streams are significantly polluted. A primary reason for this is that all three major sources of pollution (industry, agriculture and domestic) are concentrated along the rivers. Mohan *et al.,* (2013)

reported that the concentration of TDS, sulphate, hardness, pH, chloride values are higher in Noyyal river source due to the sewage disposal, bathing of animals, utilization of soap for clothes washing [39]. Present study reveals that the physicochemical parameters like phosphorous, nitrite, pH are high in Noyyal river collected from Marudurai, erode when compared to the semi-natural pond collected from the campus site. Hence pollution might be a factor for the increase in pH, nitrite and phosphate of Noyyal river, which have attributed for the higher prevelance and infestation of parasites in Noyyal river [45].

Conclusion

The water quality plays an important role for the abundance of parasites and their ability to survive on the host. The physico-chemical parameters of semi-natural pond and Noyyal water with incidence, prevalence, index and density of different parasites were studied. From the above study it was revealed that the examined P. reticulata and B. cunicularis were found infected with more than one parasite, i.e. the mode of infection was multiple and there was no host specificity. This could be due to less host preferences and common habitat of the host. The difference of parasitic infestation in different zones could be due to differences in the climate, average rainfall, soil textures, which is revealed by the analysis of water quality parameters of temperature, pH, salinity, dissolved oxygen, phosphate and nitrite.

Abbreviations

SPSS: Statistical package of social science

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