Comparative study of Helminth community and their effect on *Xenentodon cancila* (Hamilton, 1822) and *Polynemus paradiseus* (Linnaeus, 1758)

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TO

ALMIGHTY ALLAH

AND

TO MY PARENTS

AND

TO MY HUSBAND

DECLARATION

I declare that the work reported in this thesis to the University of Dhaka for the degree of Doctor of Philosophy is based on personal investigation and data collection, under the supervision of Dr. Sharmin Musa, Professor, Department of Zoology, University of Dhaka and Dr. Hamida Khanum, Professor, Department of Zoology, University of Dhaka has neither being concurrently submitted in candidature for any other degree anywhere.

Yasmeen Sultana

CERTIFICATE

This is to certify that the dissertation entitled "Comparative study of Helminth community and their effect on *Xenentodon cancila* (Hamilton, 1822) and *Polynemus paradiseus* (Linnaeus, 1758)" submitted by Yasmeen Sultana for the degree of Doctor of Philosophy in Zoology (Parasitology), University of Dhaka, Bangladesh, based on the record of original investigation carried out by her under our supervision.

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CONTENTS

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ABSTRACT

In the present study, a total of 321 *Xenentodon cancila* and 321 *Polynemus paradiseus* were examined during the period of January 2017 to December 2018 (both from Swarighat, Dhaka) for the investigation of parasite infestation, DNA barcoding of parasites, proximate composition and pathological effects on the hosts. A total of 9 species of parasites were collected and identified from *X. cancila*, of which two were trematodes (*Bolbocephalus sp, Isoparorchis hypselobagri*); four nematodes (*Metaquimperia bagari*, L_3 larva of *Gnathostoma spinigerum, Camallanus ophiocephali*, *Porrecaecum trichuri*.) and three acanthocephalans (*Neoechinorhynchus prolixum, Acanthocentis nigeriensis, Pallisentis ophiocephali*). From *Polynemus paradiseus*, a total of 10 species of parasites were recovered and identified. Among them, four were trematodes (*Prosogonotrema bilabiatum, Uterovesiculurus hamati*, *Thaparotrema vittalani*, *Hypohepaticola callionymi*); two cestodes (*Nybelinia lingualis*, *Parachristianella trygonis*); two nematodes (L_4 larva of *Dujardinascaris sp*., *Metaquimperia bagarii*) and two acanthocephalans (*Neorhadinorhynchus aspinosum*, *Pallisentis ophiocephali*). Acanthocephalan parasites showed the highest infestation rate (57%) whereas no Cestoda was found in *X. cancila*. Trematode parasites showed the highest prevalence (68%) in *P. paradiseus*. Among the total helminth parasites recovered, the most numerically dominant and highly prevalent acanthocephala was *Pallisentis ophiocephali* (23% with mean intensity 1.14 in *X. cancila* and 4% with mean intensity 1.17 in *P. paradiseus*) and trematode was *Prosogonotrema bilabiatum* (23% with mean intensity 1.03) in *P. paradiseus.*

The prevalence of infestation of parasites was 60% in *X. cancila* (192 specimens) with mean intensity 1.14 per infested fish while in *P. paradiseus*, 49% was infested (158 specimens) with mean intensity 1.09. Regarding the organal distribution, most of the parasites were found to favour the intestine of both host fish. The prevalence of infestation in *X. cancila* was observed higher during winter while in *P. paradiseus,* it was higher during rainy season. The maximum intensity of parasites of *X. cancila* was recorded during rainy season and in *P. paradiseus*, it was recorded during summer.

The effects of modifying factors such as sex, season, length, climate and diet of the hosts on the abundance of parasites were also studied. Among the main food items, small fishes comprised the greatest proportion (37%) in *X. cancila*, whereas, it comprised only 19% in *P. paradiseus*; the crustacean food item was 28% in *X. cancila* while in *P. paradiseus*, it was 31%; the mollusks comprised the highest proportion (35%) in *P. paradiseus*, whereas, in *X. cancila*, it comprised of 5%. *X. cancila* and *P. paradiseus* also consumed aquatic insects, tadpoles, annelids as additional food. Presence of large variety of small fishes, crustacean and other invertebrates in the stomach and intestine indicated their possibility as "carrier host" of these parasites in both host fish.

Helminth parasites especially immature stages caused tissue damages through formation of tunnels by lysing and ingestion of tissues. This is followed by moisture accumulation, tissue destruction, hemorrhage and massive melanization in different parts and organs of the hosts. Infected liver and kidney showed incipient vacuolation and massive melanization. Massive pigmentation was also noted in viscera of *X. cancila* due to the infection of juvenile *Isoparorchis hypselobagri*. The present observation on biochemical analysis presented small variation in nutrient contents between *X. cancila* and *P. paradiseus*. Protein, fat, and ash level were higher in non-infected *X. cancila* and *P. paradiseus* than those of infected.

DNA barcoding is a widely spread technique for species identification. An attempt has been made to molecular identification of acanthocephalan parasites based on mitochondrial cytochrome oxidase I (COI) gene as marker. In this study, one acanthocephalan species (*Pallisentis ophiocephali*) was identified. A total of three sequences of one species was generated. The species was identified by adopting DNA barcoding of mitochondrial cytochrome c oxidase subunit I (COI) gene. Genetic divergence was observed 0% within species and 22% between species. Phylogenetic tree was established where individuals belonging to the same species were grouped under same clade. The species *Pallisentis ophiocephali* has been newly recorded. The study showed the efficiency in identifying acanthocephalan species which might work as a referral study for molecular identification of parasites in Bangladesh.

ABBREVIATIONS

Abbreviations	Illustrations
AFA…….	Acetic Formalin Alcohol
BBS…….	Bangladesh Bureau of Statistics
BLAST……	Basic Local Alignment Search Tool
BMD……	Bangladesh Meteorological Department
COI……..	Cytochrome c oxidase subunit I
DoF……..	Department of Fisheries
GDP…….	Gross Domestic Product
ICES……	International Council for the Exploration of the Sea
IPCC……	Intergovernmental Panel on Climate Change
MEGA…….	Molecular Evolutionary Genetic Analysis
Mt DNA……	Mitochondrial DNA
NCBI……	National Center for Biodiversity Information database
NOAA…….	National Oceanic and Atmospheric Administration
PCR……..	Polymerase Chain Reaction

CHAPTER-1

INTRODUCTION

INTRODUCTION

Bangladesh is the largest delta in the world. Her geology, climate, flora and fauna are mostly influenced by the Himalayas. Most of the territory of Bangladesh belongs to floodplains formed by silt carried down by the Himalayan River. The country indeed differs greatly from the Trans-Himalayan region- geology, geography, climate and biodiversity as far as concerned. Most of its area consists of plains which are raised a few meters above the sea level. Due to her unique location in the subtropical region along with the three great rivers- the Ganges, the Brahmaputra and the Meghna, warm climatic condition with sufficient rainfall (1200-1500mm) and nutritive soil, Bangladesh is very rich in aquatic resources (BBS 2020).

Fisheries Sector of Bangladesh: Prospect and Potentials

Many rivers, tributaries, canals, haor, baor, ponds, beels, flood plains, haors, baors, brackish water etc. are available in Bangladesh which make it the largest water resources in the world. Fish and rice both are interrelated words for Bengali people as fish is an indispensable and matchless food component for their daily meal. Fish is the leading source of protein in the country. It has been estimated that about 80% of the animal protein comes from fisheries sector and rest 20% from other resources like poultry and livestock (BBS 2020).

At present the number of inland fish species in Bangladesh includes 260 (Rahman 1989) and marine fish species includes 475 (Rahman *et al.* 2009, Hussain 1970, Shafi and Quddus 1982).

Sources of Fish Production in Bangladesh

In Bangladesh sources of fish production includes three types of major fisheries resources–

i) Inland capture (27.72%),
ii) Inland culture (57.38%) and
iii) Marine capture (14.90%).

Total inland water fishing area is 4,724,993 hector, while the marine area is 68903.37 sq. n. miles (DoF. 2020). So Bangladesh has a great potential for the progress of marine, estuarine and freshwater fishes.

Fish Production

The yearly fish production in Bangladesh was 4503371 metric tons and contributed about 60% to the country's animal protein consumption. Fisheries sector contributed 3.52% to national GDP, 26.37% to the agricultural GDP and 1.39% to foreign exchange earnings by exporting fish and fish products in 2019-20. (DoF 2020).

Source of Nutrients

The body of fish comprises mostly of water, lipid, ash and protein though little amount of carbohydrates and non-protein components are also there (Cui and Wootton 1988; Love 1980; Wootton 1990; Siddique *et al*. 2012; Azim *et al*. 2012). It is similarly a decent source of fat and certain necessary minerals such as iron, calcium, phosphorus, zinc, copper, sodium, potassium etc. Besides all dietary essentials, amino acids are present in fish flesh and about 80%-90% of fish protein is digestible (Nilson 1946). So fish protein is the best animal protein that is very much essential for human body development. However, the amount of components might change in intra and inter species and also with size, sexual condition, feeding, time of the year and physical activity (Weatherley and Gill 1987). The development of fish depends on a number of factors such as food, space, temperature, salinity, physical activity etc. (Weatherley and Gill 1987; Ahmed *et al*. 2012). Any kind of alteration in these factors may result in a change in the components of fish body (Kamal *et al*. 2007).

Fish is a very healthy source of protein for people in several places of the earth, specifically in emerging nations. People have been consuming aquatic animals to attain most of their protein requirements. Fisheries sector also plays a significant role in rural employment and poverty mitigation. Fisheries would definitely satisfy the demand for animal protein if only the already existing fish resources were well exploited through development (DoF 2020).

But recent scientific observation has been revealed that protein contents, the food value and nutritional amounts of fish body decreased to 60% due to parasitic infestation. Research on fish parasites is very important for fishery management. It is also necessary to restrain the spread of diseases to the humankind and domestic animals where fish act as carriers. The parasites have detrimental effect on fish (Cross 1933). This parasitic infestation ultimately interferes with the decrease of fish production.

There has to be programs such as fish parasitological investigations performed, fish maintenance and diseases should be controlled to achieve a good bond between fish and their surroundings. If these are maintained properly, a healthy fish stock can be attained.
So study of helminth parasites in this field is so important for human welfare.

Parasitism in fish

Parasite is an organism that survives on or in its host (which is a different and a larger species) and that nourishes itself at the cost of the host without destroying the host, quickly as a predator does its prey, but often imposing some extent of injury affecting its host's welfare (Cheng 1964).
Parasites hold a specific site in the animal kingdom for their extraordinary adaptations as well as destructive actions, to hosts, which are associated with the utilization of host's nutrients, blood, tissues and others (Hoffman 1967). Parasitism is said to be obligatory because the parasite cannot normally survive if it is prevented from making contact with its host.

In general, the parasitic life is extremely successful because it evolved without depending on others in almost every phylum of animals, from protestant phyla to arthropoda and chordates, as well as in many plant groups. Most parasites live in a specialized environment of which they live, in the course of evolution, taken full advantage (Dawes 1976). Once adopted to a host, however, they evolved with it, becoming more and more specific in their need for host substances. So, it is important to know in which species and under what circumstances these parasites, both ecto and endoparasites, are able to live and survive in hosts.

In the study of parasites, fish perform an important role as a host for maintenance of helminth parasite. Fish is the host of several parasites and also act as a carrier of numerous larval parasitic forms that ultimately mature and cause severe illnesses in numerous terrestrial vertebrates including man (Schmidt 1970).

In maximum tropical and subtropical countries of the world including South Asia, thousands of fish species are found, most of them carry heavy infection of helminthes (Sood 1989). Fish parasites are a common part of the ecosystem and are found in most water bodies, both salty and fresh. Consumers are unlikely to come across parasites due to the measures put in place by commercial fishing and processing to remove or minimize the presence of parasites.

Fish face numerous parasitic agents in their surroundings. Parasites are instinctively and functionally damaging for living beings plus have predatory exploitative impacts, feeding on the host's nutrients and causing distress in respiration. The demand of the infested fish in the market falls, resulting in large financial losses. The parasites may weaken and sometimes kill the fish by taking full advantage of their nutrients (Ekingen 1983). The aquatic surroundings may upset the host-parasite balance due to some unwanted natural or human-related activities. This may lead to the growth of a few types of parasite and huge losses of fish (Hoffman 1967).

Majority of people get their essential nutrients from fish but taking fish that lodges any type of parasites could be unsafe for their health. Thus, the fish that are sold in the market should be in a good condition to ensure proper health of humans. The accessibility of healthy, high-quality products increases their demand, leading to better income (Hoffman 1968; Grabda 1991). The atmosphere of aquatic region is quite suitable for the progress and continuance of the parasite’s life cycle. Despite the fact that infections due to parasites are usually very common in the normal habitat of fish, pathologies due to parasites are seldom faced by fish (Barber and Poulin 2002).

Pathogens and parasites are very common in most fish. Frequently, this affects the fish to some extent. If there is a huge expense, the effects can be considered as a disease. Disease is a vital negotiator of fish mortality, particularly at a young age. The impacts of pathogens and parasites can be reduced with behavioral or biochemical means of

certain fish. Certain Interacting factors may result in lower levels of infection which may become fatal diseases. More specifically, stressing factors like droughts, pollution or predators, may result in an outburst of disease. Disease may specifically cause problems while pathogens and parasites carried by introduced species affect inherent species.

Although some of the fish parasites are harmless to human beings and domestic animals, several species of parasites of various groups of helminths, in the larval stages infest the muscle and viscera of fish, and in turn, are capable of attaining adult stage in the alimentary canals of people and domestic animals. Fish helminthes, therefore, can be pathogenic to men and domestic animals also.

A variety of larval stages of parasites in fresh water teleosts have zoonotic potentials, if the host-fish is consumed raw or lightly cooked. These are mostly parasites with piscivorous mammalian carnivores, including men as their ultimate hosts as the parasites tend to infest men due to their low host distinctiveness at the adult stage.
The nematodes, essentially are bio-helminths which either spend one of their developmental stages or complete their development in fishes (Dogiel 1961).

Fresh water fish could perhaps be significant as sources of nematode infection to human. In larval form, the commonest among them, acaroid larvae are commonly present in the visceral cavity of countless species of fresh water and marine fish (Margolis 1970). The first intermediate hosts of these nematodes are crustacea while the final hosts may be fish, birds and mammals including men depending on the type of parasite species. The larval stages can encyst in the body of the fish and if these fish are eaten raw or undercooked, the larvae may in turn infest alive in men. The eosinophilic granuloma of the gut can be caused by immature stage of parasites. Other than the remedial importance they are very unappealing to customers. Ascaroid larvae have the ability of relocating themselves from one fish host to the other, causing diseases (Wootten and Smith 1976). Control of parasites can be dealt within the structure of the total communal as well as monetary improvement of the affected civilizations.

The ingestion of uncooked fish is engrained in the culture of many rural areas. It is difficult to introduce any effective measure of many of these parasites, without radical alterations in the communal framework of the regions concerned.

The acanthocephalans, with their fearsome proboscises, sometimes cause several local damage, as observed in the intestine of the fish. As these parasites are capable of transferring their sites of attachments in the host intestine, each individual can be responsible for initiating numerous necrotic hemorrhagic ulcers (Ronalds Roberts 1978).

So, the fish, a prime source of quality protein, are commonly attacked by helminth parasites, causing various diseases which incur considerable economic loss in the form of mortality, stunted growth and poor flesh quality. Unfortunately, people have only limited knowledge about the distribution, pathogenic effects and control of maximum of the diseases in natural population of fish.

The composition of the parasite fauna is affected even more seriously by the physiological and biological factors of the host. Among these, most important factor is the food of the host, which include numerous animals serving as intermediate hosts for parasites. Other important factors include the ability of the fish to develop immunity towards particular parasite species and the age of the host. All these features govern the final composition of parasite fauna of the fish.

There is an influence of hosts' age on their parasite fauna. In some fish there is a correlation in the rise of intensity and incidence of infestation with the age of the fish. Although evidences are rather a few at present, but indication do exist, that the intensity of the infestation of parasites tends to decrease again in the old and aging fish. Another general rule was proposed to be postulated for the parasites of the fishes that the earliest parasites to infest the fish that are without intermediate hosts. This is clearly demonstrated by Dogiel (1964) in the young eels.

Types of investigation done in Bangladesh

Systematics of parasites

The research works on fish parasitological analysis which were done in Bangladesh have been revised by reading the existing literatures. Reasonable workings, especially on taxonomy, type of infection as well as pathology of dissimilar groups of fish parasites have been performed. In Bangladesh, from freshwater and marine fishes, around 290 species of parasites have been documented. Only a limited number of efforts have been effectively taken to limit the parasites by the use of simple chemicals like salt, lime, formalin and few others. There has already been recommendation regarding the upcoming tasks on parasitology for viable yield of fish which will be in good health (Chandra 2006).

In Bangladesh, so far, research on the helminth parasites and biological aspects have been made mostly on edible fresh water and estuarine fish members under the families like Cyprinidae, Nandidae, Channidae, Anabantidae, Heteropneustidae, Notopteridae and Clupeidae. Only a few works have been done on the family Belonidae and Polynemidae. *Xenentodon cancila* (Hamilton Buchanan, 1822) is the only member belonging to Belonidae available in Bangladesh. And *Polynemus paradiseus* (Linnaeus, 1758) belongs to the family Polynemidae found in Bangladesh coastal and offshore waters and caught in large quantities from the shallow estuarine ground especially from Meghna, Chandpur and Bay of Bengal. The above mentioned two species are considered to be important fishes of Bangladesh because they are full of nutrients and delicious and have high market value.

Helminths: Helminths are a vast category of fish parasites belonging to trematodes, cestodes, nematodes as well as acanthocephalans; attacking the host both externally and internally.

Trematodes: Trematodes is the group that is widely analyzed among the groups of fish parasites in Bangladesh. In Bangladesh a series of works have been performed on helminth infestation and in different aspects of biology of the economic fish. Among the lot, Bashirullah (1972) explained *Isoparorchis hypselobagri* and recorded its life

cycle. Bashirullah and Hafizuddin (1973, 1974, 1976), Chandra (1983, 1984, 1994) and Chandra and Banerjee (1993a, 1993b); Golder and Chandra (1987), Golder *et al*. (1987) described the digenean parasites of various fishes and trematodes of estuarine fishes were recorded by Chandra (1993). Many digenean parasites of marine as well as freshwater fishes are also recorded by Bashirullah (1973).

Cestodes: In Bangladesh, fish cestodes, mainly their systematic have been examined by countless scientists. Fish histopathology, intensity of parasitic infestation and variants in seasons had been analyzed by numerous workers. Caryophyllaeid is a distinct group of cestode parasites, Caryophyllaeid cestodes of catfish (magur and singhi) were examined more closely (Ahmed and Sanaullah, (1977, 1979); Rashid *et al*. 1983, 1984, 1985; Ahmed *et al*. 1984; Chandra and Khatun, 1993 and Chandra *et al*. 1997). Khusi *et al.* (1993), D'Silva and Khatoon (1997) recognized few marine cestodes. Cestode *Dibothriocephalus latus* from Bombay duck (*B. loitta*) of Bay of Bengal was reported by Uddin *et al.* (1980), and Diphyllobothriid larva from meni fish of Mymensingh was reported by Chowdhury *et al.* (1982). Still, certain writers (Hoffman 1968; Moravec 1998) said that it's not likely to be present in Bangladesh.

Nematode: The nematodes, essentially are bio-helminths which either spend one of their development stages or complete their development in fishes (Dogiel 1961). Many nematodes were reported from marine as well as freshwater fishes. Some species of nematodes from marine water and fresh fish fish were observed by Bashirullah (1973). Chandra (1992b) organized the nematodes noted from freshwater fishes of Indian subcontinent. The systematics of several nematode worms were studied by Bashirullah (1972, 1973, 1974a, 1974b), Ahmed and Begum (1978) and Ahmed and Rahman (1977). Bashirullah and Ahmed (1976a, 1976b) observed development of *Camallanus adamsi* and *Spirocamallanus intestinecolisi* in the copepod intermediate host. The growth, movement and dissemination proficiency of the early stage larvae of *Procamallanus heteropneustes* in copepods was recorded by Chandra and Modak (1995). A small number of nematodes from lizardfishes of Bay of Bengal were reported by Mandal (1995). Out of a dozen fish species, nematode *Gnathostoma spinigerum* was addressed by Bashirullah (1973), Khanum *et al*. 1996 and Akhtar *et al.* 1997. This nematode is the causative agent of gnathostomiasis, a severe zoonotic disease of human.

Acanthocephala: Acanthocephala is a distinct little group of fish parasite which can cause severe damages to infected fish. Until now, it hasn't achieved much recognition from the scientists in Bangladesh. Ahmed and Rouf (1982), Ahmed and Begum (1978), Chowdhury *et al.* (1982), Chandra (1985, 1992a, 1993), Chandra and Rahman (1988) as well as others took part on the taxonomy of this group of fish parasite and explained quite a few species.

The works on helminth parasites of *Xenentodon cancila* in Bangladesh have been done by Bashirullah (1973), Ahmed (1981), Khanum *et. al*. (1989) and Sharmin *et al.* (2003). Altogether seven nematodes, viz. *Camallanus gaboes*, *Camallanus xenentodoni*, *Contracaecum sp*. *Gnathostoma spinigerum*. *Paragendria bagarii*, *Metaquimperia bagarii*, *Procamallanus cancilus*; one trematode, *Isoparorchis hypselabagri*; and one acanthocephalan, *Pallisentis ophiocephali* were reported by them.

The only work on helminth parasites of *Polynemus paradiseus* in Bangladesh have been done by Latifa *et al*. (2008). They studied the incidence of infestation of helminths parasites of *Polynemus paradiseus* and identified nine species of parasites of which four were trematodes, two cestodes, two nematodes, one Acanthocephala from the buccal cavity, oesophagus, stomach, intestine, caecum, liver, and body cavity of the host fish.

In Bangladesh rain falls almost throughout the year. The ponds, rivers, haors, bills and other water bodies get filled up during monsoon. The rainy season is favorable for helminth infestation. During this particular period, fish are infected by parasites quite often. Threadfin fish are also infected by several parasites at this time as well. *P. paradiseus* is infected by several parasites due to their voracious feeding habit. It is usually accepted that the parasite fauna in an aquatic environment is determined by the interaction of a number of biotic forces. Moreover, the ecological changes in habitat of the organisms' living will be reflected in the parasitic fauna. In this perspective, not much is known about the life history of the parasite to co-relate the occurrence of certain parasites with particular food items. But the correspondence of alteration in parasite incidents with change of incidence of food items analyzed shows, to some extent, the group of organisms that may serve as intermediate hosts for specific parasites (Scott 1975).

The food and feeding habit of some common fishes of Uttar Pradesh, India was examined by Das and Moitra (1955) and concluded that the food of the surface feeders mainly consisted of algae, rotifers, crustaceans' etc. The food of the middle-feeders composed of algae, aquatic flora, and various types of adults' crustaceans, insects, fish and fish scales. The food of the bottom feeder fishes composed of decomposed aquatic vegetation, fish scales, sand and mud.

Sometimes the activities of parasites may grow to be so intense that the mortality and morbidity of the brood fish significantly hamper initial breeding cycle (Kaneko *et al*. 1988). Certain parasites are severe nuisances in fish culture, while the others are possibly latent dangers to fish culture. Meanwhile, the achievement of execution of countless fisheries development programs relies on strengthening the fish parasitological studies. Researches on parasites of fish in Bangladesh are very recent and fragmentary.

The parasites play a critical role in maintaining the ecosystem balance with relation to all living creatures. Every living creature considered by parasitism either as a host or as a parasite (Combes 1995). Besides, the parasite is a very important constituent of global biodiversity (Poulin and Morald 2004). The parasitic copepods have a very significant biodiversity and parasitize practically all groups of marine animals. So they play a vital role in biodiversity, balancing and functioning of marine ecosystems. Researches on copepods biodiversity on coasts of Tunisia were done by Essafi (1984), Benmansour and Ben hassine (1997; 1998), Benmansour (1995; 2001), Yamak (2000), Djait (2009) and Souidenne (2011).

Several types of fish serve as intermediate host as well as definite hosts. Two of these hosts are from the family Belonidae and Polynemidae. *X. cancila* and *P. paradiseus* are intermediate hosts as well as definite hosts at the time of severe infestation. The parasite consumes internal tissues of the fish and parasitizes it. This parasite is a huge danger because there are shortages of host specificity to such a level that it can contaminate all fresh water fish and even frog tadpoles and salamanders (Baur 1962; Hoffman 1967).

''Like all animals, fishes have their full complement of disease and parasites and of disorders, both malignant and benign and there is no question that most fishes die from

such disorders, natural enemies other than men" (Lagler 1956). Helminthes parasites generally affect the internal organs of the host fish, particularly the gut, they can perforate the intestine heavily and inhibits host's growth. The usual growth of fish is hindered and repressed if they are heavily infested with endoparasite viz., trematodes, nematodes, cestodes and acanthocephalans. Just like other vertebrates these fish parasites depend on the nutrients of the host's tissue or intestine (Markov 1946). The irritating activities and damage of tissues lining the walls of the oesophagus, stomach, intestine etc. are responsible for microscopic lesions in their host's tissues which eventually become the location for secondary infection by bacteria (Cheng 1964. Each true fish parasite therefore uses the fish for its home and food and the total damage is related to the number of parasites present (Soulsby 1968; Olson 1974).

The fish parasite either may be external or internal. The endoparasite usually infest internal organs of fishes. If the parasites are in the digestive tract, they depend on either the nutrients of the host's intestine or its tissues. The influence of the parasite may result in extensive change in individual organs or tissues or it can exert a general effect.

Study of parasites is very modern and recent in Bangladesh. Parasitic fish helminthes are not human parasites, except a few (*Isoparorchis hypselobagri* and *Gnathostoma spinigerum*) and therefore are not directly concerned with human diet. However, the helminthes by their harmful activities may suppress the fish development and in intense cases can kill them which will result in huge economic loss to the fish stock. These fish could have been the major sources of animal protein (87%) to our people (Mannan 1977). The helminth fauna of this continent including Burma and Ceylon was first studied by Southwell (1913 and 1930) and then by Baylis (1939). Later on Srivastava (1936), Gupta (1951, 1953 and 1961), Gupta and Verma (1976) published a series of papers on fish helminthes.

Thomas (1964) worked on the population dynamics of digenetic trematode in vertebrates. The intra species competition of parasites was examined by Dobson (1985). Anderson (1976) studied the periodic difference in the population dynamics of *Caryophyllaeus luticeps*. Kennedy (1978) and Lawrence (1970) worked on availability

of modes of feeding, dispersal and environs of host, which impact the parasitic growth significantly. The parasite causes reduction of the nutritive substances in host's body which ultimately causes the low yield and financial damage in fish industry (Hiware 1999)

Mustafa and Ahmed (1979) worked on *Notopterus notopterus*, the species that is predominantly carnivorous and a column feeder from both pond water and jheel water. They found that the amount of protozoan, crustaceans and plants in the fish stomach of both habitats are almost the same but insects were found more in the jheel water fish stomach compared to pond water fish.

The periodic arrangements of feeding habit of freshwater fish *Colisa fasciatus* was examined by Mustafa *et al*. (1981). He observed that the fish prefers algae in winter, insect's larvae in summer, diatoms and protozoan in autumn. He also found that a high percentage of crustaceans were consumed by the fish in winter, spring and early summer.

Zaman and Seng (1986) found the caryophyllid cestode, *Djombangia penetrans* infecting catfish *Clarias batrachus* and *Clarias macrocephalus* in Kedah, Malaysia. At the point of penetration, the epithelium was destroyed completely. And in case of heavy infestation, the affected intestine enlarged, dilated and at the point of attachment, appears as a white dot.

Parasitic diseases of fish and their possible remedial measures are very important issue now a day. Some histopathological evidence provides information for these fish diseases. Chowdhury *et al*. (1986) studied the infestation of *Isoparorchis hypselobagri* in the swimbladder of *Mustus vittatus, M. cavasius* and *M. tengra*.
Nahar (1988) investigated the prevalence and intensity of helminth parasite in *Xenentodon cancila*.

In Bangladesh only a very few works have been done on the histopathological effects of parasites. Among the few, Ahmed and Sanaullah (1979), Ahmed and Rahman (1979), Sultana *et al*. (1992) worked on *Clarias batrachus*, goldfish and flatfish. Jahan (1971) thoroughly studied on the histology and histochemistry of *I. hypselobagri*. Very few studies have been done on parasitization, host parasite relationship and histopathology of *I. hypselobagri*. Siddiqui and Nizami (1978) reported incidents of these trematode from *W. attu*.

No comprehensive work has been done on histopathology of *Polynemus paradiseus* and *Xenentodon cancila*. Only a few mentionable work has been done in Bangladesh and some parasites have been identified through these works.

Khanum *et al*. (1989) reported on the surveillance on parasite infestation in relation to time of year and sizes of *Xenentodon cancila* (Hamilton). Among the four length groups of the fish, the intermediate size-groups presented the highest prevalence of infestation. The intensity of the parasites maintained an inverse relationship with the length of the fish. During the drier time of the year, relatively greater rate of infestation was observed.

Akhtar *et al*. (1989, 1990) examined the helminth infestation with regard to the seasons and the body's length of *X. cancila*, one acanthocephalan, three nematode sp. encysted ascaroid larvae, *Metaquimpera bagarii* and *Camallanus gaboes* were reported available from *X. cancila*. They discussed the incidence of helminthes parasites in this fish in relation to food items.

In Bangladesh, Islam (1970) observed the overall prevalence of parasitic infestation in various organs of *Rita rita*. The sample size was small and he indicate monthly or seasonal variation of infestation or the distribution of parasites according to size and sex of the host. Sex and age of the host are important factors in the epiodemiology of the parasites. The age or sex-prevalance and the age or sex- intensity can ascertain which sections of the community are most at risk. Therefore, the present investigation was undertaken to determine the parasite fauna in *R. rita* according to season, sex and length of the host and also to note whether there has been any recruitment of parasites in the last 44 years.

Farhana and Khanum (2013) reported the dispersal of helminth parasites in various organs of *Mystus aor* (Hamilton) and *M. bleekeri* (Day). They investigated the helminth infestation and their organal distribution, of *M. aor* and *M. bleekeri* during January 2004 to December 2005. In *M. aor*, the overall prevalence was 85.95% with a mean intensity of 46.26+-12.0, whereas in *M. bleekeri*, 72.95% prevalence with mean intensity of 56.49+-12.29 was recorded.

Biochemical constitution of biota exhibits many differences from species to species. Within the fish, the distinction ranges within interspecies, inside intra species in various parts of the body, which varies from time to time of the year, with respect to different phase of growth, size, development, etc. There are a few necessary components in fish; considering the level of extent, they are moistness, protein, fat and minerals. Usually 60-84% water, 15-24% protein and 0.1-2% fat are available in healthy fish. The amount of components varies from species to species but the major deviation observed in the content of fat. The fatty fish generally have more than 2% of fat, whereas the lean fish have less than 0.5% of fat (Haque 1975).

The major portion of aquatic biodiversity is characterized by fish parasites and accordingly becomes infected directly or indirectly through the surroundings or through their corresponding hosts. When analyzing upon a secretive approximation of a mean of 3 to 4 fish parasites in every living fish species and a present number of 31,400 clarified fish species, it can be estimated that there are around 120,000 fish parasites (both protozoans and metazoans) present. Associated with quite a few stages of life cycle which can infest every aquatic hosts, this wide range of biodiversity shows an extensively ignored tool for various ecology-based applications.

The species composition and fish parasite biodiversity in the water bodies survive on species abundance of the final hosts and their environment. The universal fish fauna includes greater than 31,400 species (Froese and Poulty 2010), around fifty percent of them (14,970 species) living in salty waters. Due to the long-standing constancy of saltwater ecosystems, fish parasite variety per host is greater in marine water than in freshwater.

Change in climate has direct as well as indirect effects on fish that are exploited for commercial purposes. Uninterrupted impacts act on physiology and activities and change growth, development, ability of reproduction, mortality and distribution. Secondary impacts change yield, arrangement and configuration of the environments on which fish depend for food and shelter.

All over the globe, the rising temperatures is the most common effect of global warming. The mean worldwide temperature has risen by about 1.4 degrees Fahrenheit (0.8 degree Celsius) during the last century, according to the National Oceanic and Atmospheric Administration (NOAA). Brander *et al*. (2003), ICES (2006) and Drinkwater (2005) recorded that the impacts of rising temperature on saltwater and freshwater ecologies are already apparent by this time. The quick pole ward movements in dispersal of fish and plankton in areas such as the North East Atlantic, where the alteration of temperature has been quick. A number of the variations are likely to have optimistic consequences for the production of fish (Brander and Mohn 2004), however, in different circumstances reproductive ability is hindered and stocks grow into becoming susceptible to the number of fishing that once had been sustainable (Friendland *et al*. 2003). Local extinctions are happening near the borders of existing ranges, specifically in certain fish such as Salmon and surgeon (Reynolds *et al*. 2005)

It has additionally been observed that variation in climate has deep influence on the intensity of rain (Wasimi 2009). Forecasts showed that fluctuations in weather might fortify monsoon circulation, rise temperature as well as make rainfall even more severe. Hence, weather change will surely bring changes to rainfall pattern. This will disrupt inland water provisions that is already becoming scarce due to the growing population and improved per capita consumption. This will cause even more trouble in predicting excessive rainfall events as there will not be a homogeneous series of values which might be extrapolated statistically. Still, it is predicted that dangerous events may happen more frequently than before (Linacre 1992).

The lives of organisms are significantly influenced by temperature and humidity. Temperature and humidity are dependent on sunlight. To continue life properly, living beings need an optimum temperature. If the temperature becomes too hot or too cold,

their bodies will not function properly. Living beings' physiological processes require an optimal temperature range.

Humidity, which is the amount of water vapor in the air, interacts with other climate variables. It is affected by both wind and rainfall. Humidity affects the energy budget and thereby influences temperature in two ways. Firstly, water vapour in the atmosphere contains "latent" energy. The latent heat is removed by transpiration or evaporation, cooling the earth's surface. Secondly, water vapor is the most abundant greenhouse gas. It absorbs the infrared energy emitted upward by the earth's surface, which is the reason that humid areas experience very little nocturnal cooling but dry desert regions cool considerably at night. This selective absorption causes the greenhouse effect. (Wikipedia, the free encyclopedia)

There has been considerable change in the environmental situation in Bangladesh since the 1980s. Hence, it may alter the parasite fauna of *Xenentodon cancila* and *Polynemus paradiseus* because of the excess use of inorganic fertilizer and pesticides in cultivated lands, discharge of industrial waste, insufficient waste disposal, etc. that cause changes in the aqua-environment indirectly. The host, the pathogen plus the environment are in a constant state of instability, having the ability of changing in any step with any variation may cause new infection of parasite in *X. cancila* and *Polynemus paradiseus.*

Parasites are very good material for investigations on the arrangement of communities by means of null models as hosts are habitats with well-defined boundaries and different communities, replicates of the same host species can be collected with relative family (Gotelli and Rohde 2002; Tello *et al*. 2008). In spite of the harmony that parasites of the same species have a tendency to aggregate distribution (Kennedy 2009), i.e., few hosts harbor many parasites, while some hosts harbor only a few parasites or are not parasitized. The arrangements of distribution of infra communities and component communities are still ambiguous Bush *et al.* (1997).

Morphological identification

Taxonomic identification of a species is the first and principle work for a researcher. Morphological analysis is based on the set of external characters of a species. It mainly deals with the body shape, size, body measurement, meristic count, sexes (not separate, i.e. hermaphrodite or separate), head end (head may provide with suckers, often with hooks or without hooks; may be with no suckers, no hooks), alimentary canal (it can be entirely absent, may be incompletely present with no anus or may be present and complete with anus) and also related to body cavity (absent or present) etc. Morphology of parasite is related to its habit and habitats mainly representing the feeding habit, locomotion and external environment.

Traditionally, fish parasites identification has always used morphological characteristics. It is the first and simple way to identify a parasite species. The morphometric and meristic study attempts to identify a parasite species correctly. But, these characteristics are not sufficient to recognize every single species, specifically for those rare and cryptic species.

Molecular identification

The identification of species of fish parasites has been a problematic one due to the sample's resemblance in morphology and mostly have had key diagnostic features removed due to poor collection and preservation method. Additionally, the morphology of the identical species may alter rapidly and considerably at the time of its developments from larvae to the young phase. (Matarese *et al.* 2011).

Molecular identification technique can increase the speed of the identification of an unknown organism. It implies identifying any species at a molecular level without any consequences of physical state of species. For molecular identification usually a marker is used which is a DNA based marker called molecular marker and characterization by using this molecular marker is called molecular characterization. Molecular markers especially mitochondrial DNA cytochrome oxidase I (mt-COI) is non-recombinant. Additionally, it does not depend on its surroundings; hence, they are regarded as the better option for distinction of the species (Layton 2014). Molecular markers are convenient tool for intricate taxonomic identification whereas morphological

characteristics are confusing (Douek *et al.* 2002; Westheide *et al*. 2003). The technique can be used as a speedy implementation to investigate numerous ambiguous species, composition of species, and cryptic species (Spies *et al*. 2006).

Barcoding of fish parasite with COI gene

Structurally identical species may have totally dissimilar genetics. And so, molecular procedures are suitable for classifying the species that are difficult to differentiate otherwise (Aksakal and Erdoğan 2007).

In recent years, the molecular approach has been able to give solutions DNA based identification is important to overcome these limitations.

Mitochondrial DNA

Mitochondria is a membrane-bound organelle found in most of the eukaryotic organisms and transmitted energy to the cell. In each of the eukaryotic cell there are about hundreds to thousands of mitochondria are found. Though DNA mostly packed in chromosome within nucleus, mitochondria also have some DNA of their own. Mitochondrial DNA mainly contain 37 genes of which 13 genes provide indication for making enzymes which are involved in oxidative phosphorylation. The other genes provide indication for making transfer RNA (tRNA) and ribosomal RNA (rRNA) which assemble amino acids into functional protein. Oxidative phosphorylation is a process of producing ATP by using enzyme to oxidize nutrient and release energy stored in nutrient. The usage of mt DNA for identifying species has been stated to have extraordinary amounts of success; maximum experiments have displayed inaccuracy rates of less than 5% (Waugh 2007).

Mitochondrial COI gene in species identification

A protein which is now a days established as one of the most genetic marker in molecular systematics is mitochondrial COI or cytochrome c oxidase I. COI is the prime subunit of cytochrome c oxidase complex which is a key enzyme used in aerobic metabolism.

Mitochondrial COI gene has the ability not only to identify the individuals belong to same species but also differentiate individual from different species because the

alteration rate of gene sequence is slow enough to identical to same species and fast enough to diverse between species. Most researchers use a section of COI gene (658bp)

for recognizing species in DNA barcoding because of several features; at first, presence of this region in every animal, secondly, in this region, insertion and deletions are occasional, finally, it holds sufficient sequence divergence which is enough to differentiate similar species (Hebert *et al*. 2003a; Hebert *et al*. 2004). Because of short

length and robust universal primers, it is not difficult to amplify and sequence (Folmer *et al*. 1994; Zhang and Hewitt 1997; Simmons and Weller 2001). This identification system is cost effective, reliable, also a digital solution for species identification problem).

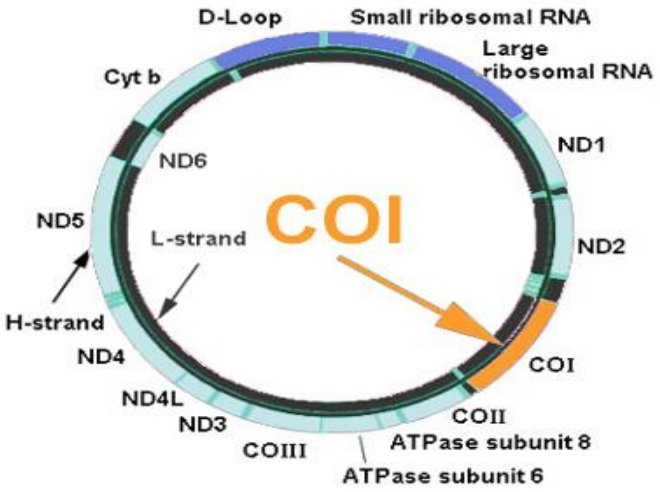

Figure 1. Position of COI gene in the mitochondrial genome

Mitochondrial DNA has some properties that make it unique compared to other nuclear genes. Its several special features are:

1. MT DNA shows fast mutation rate evolving 5 to 10 times faster than nuclear DNA and the structure of mitochondrial genome is simple to compare with nuclear DNA.
2. It is much easier to extract from the sample because a cell usually contains 100-1000 of mitochondria, each with more than one DNA molecule.

3. High nucleotide diversity provides variation to explore different parameters such as gene flow and subdivisions.
4. Mitochondrial genome rarely contains duplicate or non-coding sequence.
5. Mitochondrial genome lacks introns.
6. MT DNA is haploid. Its material inheritance pattern with the absence of recombination gives small effective population size and makes it more susceptible to the genetic drift.
7. MT DNA is more sensitive to detect genetic differences.
8. The evolution rate of this genome is fairly rapid and therefore, it exhibits a great potential as bar-coding gene.

Although these limitations, the COI gene of mitochondrial DNA (mt DNA) is becoming the standard barcode region as it can be amplified without much difficulty, applied through an extensive range of taxa and results can be found more quickly and cheaply.

The use of DNA barcode for species recognition has achieved worldwide support as an applicable tool for species identification, by the barcode of life. Several regions of mitochondrial DNA have proven a useful tool for population studies. The present study used sequence variations in the mitochondrial DNA COI genes (Figure1). Most of the researchers confirmed the use of COI gene as a perfect genetic marker for distinguishing and identifying the closely related species (Steinke *et al*. 2005; Ward *et al*. 2005; Lakra *et al*. 2011; Hubert *et al*. 2008; Ambili *et al*. 2014; Ratnasingham *et. al*. 2007) because it is considered first, second and third codon position.

DNA barcoding

DNA barcoding is a tool that utilizes a short length of DNA of a particular organism to identify its species (Hebert *et al*. 2003). Identifying an unknown organism and referring to its corresponding species is the key purpose of DNA barcoding (Kress *et al*. 2005). DNA barcoding is a powerful marker to detect genetic uniqueness of individuals, population or species and can correct field misidentification, reduces the uncertainty of species identification, makes identification of species more exact and expands the technical expertise of taxonomist.

The key purpose of DNA barcoding is not only to define the patterns of relationship but also to identify an unidentified sample in terms of an already existing taxonomy. DNA barcoding is an ever more fashionable and unique conception that has created enthusiasm in increasing biodiversity field. Still, this technique should be performed along with other procedures for effective conservation efforts.

Canada, Australia, Taiwan, Indonesia, India, USA, have already barcoded most of the marine water fish species (Bineesh *et al*. 2017, Prehadi *et al*. 2015, Sembiring *et al*. 2015, Hubert *et al*. 2015; Knebelsberger *et. al.* 2015). In Bangladesh Department of Zoology, University of Dhaka has established an update on DNA Barcoding Lab and barcoded over 100 marine fishes (Ahmed 2018). The application of DNA barcoding is very modern and recent in Bangladesh but no molecular study of fish parasites has been done yet.

DNA barcoding using approximately 655 bp of COI gene has been widely employed in different biological fields to differentiate closely related species in studies, ranging from molecular systematics to seafood product identification (Giuffra *et al*. 2010).
DNA barcoding (using the mitochondrial COI gene) is a powerful molecular and taxonomic method in the identification of acanthocephalan species.

A particular region of the mitochondrial COI gene is PCR-amplified and then sequenced and analyzed; resulting 4 different colors of bars that used to indicate A, T, G and C. Then following 650 bars, each representing a DNA base, appear like a grocery barcode, hence the name of DNA barcoding.

There are several benefits of parasite barcoding:

1. Fish parasite can be identified easily by taxonomists
2. Interspecies can be classified
3. Previously unidentified fish parasite can be identified
4. Proper Identification of species which cannot be classified by traditional taxonomy. It provides permanent tags unchanged during taxonomic revisions.

Importance of acanthocephalan identification by COI gene

Structural identification of acanthocephalans is still confusing because of their scarce morphological characters and their great intraspecific differences**.** Identification of acanthocephalan species by the sequence of COI is very efficient. Also, it is a fundamental part for the identification of different species to improve global biodiversity status. Sometimes, the traditional taxonomic system cannot able to identify several fish parasite species. As a result, proper identification of acanthocephalan species often becomes difficult. Identification of acanthocephalan species by COI gene is important.

Structural identification of acanthocephalans is still quite difficult because of their rare structural forms and their great intraspecific variants. DNA barcoding is a very good system for taxonomic task at the species level. During this investigation, new DNA barcoding data for Acanthocephala (obtained from host fish species) had been provided an important contribution to acanthocephalan taxonomy and distribution in gar fish. Nonetheless, the taxonomic task of the species should stay open. The investigation emphasizes the problems in handling dependable DNA barcodes and highpoints the significance of the establishment of such DNA barcodes to overcome these.

Very few literatures are available on fish parasites found in Bangladesh and most of them are based on morphological characteristics. Identification of fish parasite species by COI gene is necessary to characterize the acanthocephalan with a phylogenetic relationship.

Justification of the work

As there is no detail record of parasitological, histo-pathological and biochemical works on *Polynemus paradiseus* and *Xenentodon cancila*; so, the present investigation has been under taken to investigate the detail information regarding the parasitological aspects of *Polynemus paradiseus* and *Xenentodon cancila*.

The threadfish *Polynemus paradiseus* and the garfish *Xenentodon cancila* have been chosen for investigating the helminth parasites for the human welfare. Considering the above mentioned fragmented studies done on *P. paradiseus* and *X. cancila*, it is also

necessary to increase the production of these fish in the country primarily by culture, by knowing their parasitic information as well as their nutritional status. As no systematic study of the parasite of *P. paradiseus* and *X. cancila* has been done in our country, it was targeted to find out the diverse features of parasitic infestation and infection of the fish.

No comprehensive work has been done on histopathology of *Polynemus paradiseus* and *Xenentodon cancila*. To attain well-maintained fish stock, we have to execute programs such as fish parasitological investigation, limit fish illnesses, some biological aspects and conservation of healthy relationship between fish and their environments. Considering all these facts, it is felt that to increase the production of *Polynemus paradiseus* and *Xenentodon cancila*, a systematic study of the parasites and their impacts on the nutritional values of their host fishes should be thoroughly studied. For that, a fanatic survey of parasites in these two host fish is urgently needed. So, as a primary step, the present investigation is undertaken to identify the parasites of the host fish of Bangladesh.

Study on the DNA barcoding of fish parasite species has not yet been done in Bangladesh, although a few amount of work has already been done in many countries including Canada, Australia, Taiwan, Indonesia, India, and USA. This research is necessary to study the biodiversity of fish parasite because this work in Bangladesh have not been surveyed properly.

Bangladesh fisheries have a potential scope of development to strengthen the national economy. A comprehensive development plan is necessary for the overall development on this sector. For better planning accurate fisheries statistical information is essential.

Therefore, the present study was conducted for the following objectives.

Objectives of the research

1. To determine the parasite community, prevalence and pattern of infestation in *Polynemus paradiseus* and Xenentodon *cancila*
2. To find out the distributional pattern of different groups of parasites in different parts and organ of the host fishes
3. To prepare an update check-list of helminths parasites from freshwater garfish and brackish water threadfish
4. To find out the relationship of prevalence and intensity and occurrence of parasite infestation and their correlation with the host fishes
5. To determine the relationship between the parasitic infection in *P. paradiseus* and *X. cancila* in different months and seasons
6. To find out histo-pathological effects on host fishes due to parasitic infestation.
7. To determine variation in biochemical components like protein, fat, moisture in infected and non-infected host fishes
8. To overcome the setbacks of the morphological identifications where many cryptic and new species couldn't be identified

CHAPTER-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The purposes of review of literatures were to collect the information regarding the background, history and previous relevant studies. A remarkable number of national and international literatures were cited and reviewed which were closely related to the present study. The summerised information's/literatures are given below:

Fish Biology:

Moffett and Hunt (1943) worked on the winter feeding habit of the blue-gills. They noted a change in food habit of this fish with the change in season and recorded little food in the stomach in winter.

Mookherjee *et al*. (1946) worked on *Glossogobius guiris* and reported that animals and plants occurred in the ratio of 70:30 in the adults, but the ratio of plant materials was more in the juveniles.

Angelouplu (1947), Hossain (1997), Bolock and Koura (1959) worked on the food and feeding habits of *Clarias gariepinus* and stated that as a carnivore fish it has a small gut, body length ratio in comparison with other species as it lacked pyloric caeca.

Pillary (1952) stated that the proper diet of a fish may not be obtained by the analysis of the contents of the alimentary canal of various samples chosen randomly. However an extensive examination of the contents of the alimentary canal stretched across the most of the year, could produce valuable information for the growth and operation of fishery.

Nikolosky (1963) reported that in most cases fish did not show any considerable variation in their diet, except feeding intensity which differs due to their spawning season. He also realized the importance of three particular categories of food in the diet of fish.

Ahmed *et al*. (1980) worked on the food and feeding habits of the catfish *Clarias batrachus*, found that the food of adults and juveniles varied considerably, the adults

consumed mainly chironomid larvae, copepods etc. while the juveniles consumed mainly chironomid larvae, ostracods and dragon flies.

Siva and Rao (1983) did a detail observation on the food materials of 354 *Mystus vittatus* collected from Hussain Sagar lake and concluded that *M. vittatus* feed mainly on insect larvae (35.5%) and insects(16.3%) available there. The other food items include both plant and animal matter.

Ali *et al*. (1985) worked on the food and feeding habits of *P. pangasius* and stated that' this fish is fairly an omnivore and among their food macro-crustaceans occupied the highest position. Their investigation also reveals that *P. pangasius* does not compete with the plankton feeders and concluded that *P. pangasius* can be cultured in pond with major carp.

Kader *et al*. (1988) worked on the food and feeding habits *of Gobioides rubicundus* and observed that the main foods were shrimps, crabs, gobioid juveniles, chaetognaths, copepods and occasional items were algae and diatoms. He also reported that the number of mature male fish with empty stomach was highest in the pre spawning month while female was highest empty stomach during the spawning month.

Fish Parasites:

Kulasiri and Fernando (1956) recorded *Camallanus anabanis* in *Anabus testudineus, Ophiocephalus punctatus, Puntius filamentus* and *Rasbora daniconius. P. filamentus, R. daniconius* and *O. punctatus* are the new hosts for this spp. *P. planoratus* was recorded in *Clarias batrachus* and *Clarias* spp. They also recorded *C. sweeti* in *A. testudineus, C. batrachus and Clarias* spp.

Clay (1979) observed the youth and fecundity of African catfish (*Clarias gariepinus*) keeping a close watch on the reproducing behavior of the Nile catfish (*Clarias lazera*).

Hine (1980) worked on the nematode parasites and found both male and female *Spirocamallanus* species established in the mid intestine. Parasites most often found in

the mid intestine, but occurred throughout the intestine. He also noticed that the larger worms moved and were lost more rapidly than smaller worms.

Bhalerao (1932) wrote about the possibility of infestation of *I. hypselobagri* (Billet, 1898) in man and domestic carnivores. He listed the occurrence of *I. hypselobagri* from gas bladder of *W. attu.* He also obtained immature forms of this parasite from muscle lining the coelomic cavity of *Ophocephalus striatus.*

Gupta (1961) described 3 new genera (*Lytocestus*, *Bovienia* and *Djombangia*) 1 new species (*Capigentoides batrachii*) of the family caryophyllaeidae (Leuckart, 1910) from the intestine of *Heteropneustes fossilis* from U.P and Assam.

Furtado (1963) obtained a new species *Lytocestus parvulus* from the intestine of the Malayan freshwater siluroid, *Clarias batrachus*. The species belong to the family Caryophyllaeidae. Around two hundred specimens were taken from host. An overall and short explanation of this helminth had been compiled and written down.

Fischthal and Robert (1963) redescribed digenetic trematode of fish from Egypt. From *Clarias lazera*, he collected *Orientocreadium batrachoides* Tubangui, 1931 (Plagiorchioidea) which had some morphological variations. They reviewed the morphological differences and similarity of the hosts of the species of *Orientocreadium* and their related forms.

Tedla and Fermando (1969) observed that the seasonal changes in incidence and intensity of infestation of *Perca flavescens,* by eight species of larval and adult parasites. The incidence of ectoparasite, *Ergasilus confuses*, reached its peak of incidence in summer and declined in winter.

In Bangladesh *Rita rita* has been known to be infected by helminth parasites (Islam, 1970). He first studied the helminth parasites of *R. rita* from Sunamganj and identified four helminth parasites *i. e. Opisthorchis* sp., *Phyllodistomum yosufzai, Cucullanellus*

sp. and some larval nematodes. But his sample size was only eight fishes. Therefore, the parasites of this fish need to be investigated in a more detailed manner.

Datta (1971) studied on digenetic trematodes and nematodes of some fresh water fish of Dacca. He described *Camallanus adamsia* from the intestine of *Barbus saphora* (Ham.) and *Spirocamallanus olsenia* from the intestine of *Mystus vittatus* (Bloch).

Rehana *et al.* (1974, and 1979) described three new species of genus *Procamallanus, P. wallagus, P. kalriai, P. karachii* from the swim bladder of *W. attu* of lake kalri, Sind, Pakistan and also reported the infestation of *P. wallagus* from *Mastecembelus puctatus.*

Furtado and Tan (1973) have surveyed the parasite fauna of *Clarias batrachus* in the paddy fields of Sungei Besar and Sabak Bernam in Selangor. They provided some information on the seasonality of four species of parasites, namely two cestodes: *Lytocestus lativitellarium, L. parvulus* and two nematodes *Procamallanus clarias* and *P. parvulus.*

The catfish *Clarius batrachus (L.),* which is a fresh water siluroid, is usually found in the Indo-Malaysian region. From this fish, Devi (1973) collected *Lytocestus longicollis* sp. Nov. (Cestodea: Caryophyllidea). She discussed and compared the morphological descriptions of *Lytocestus longicollis* and *L. indicus.*

Srivastava and Mukharjee (1974) studied the incidence of infestation of *I. hypselobagri* metacercaria in two species of fish of the genus *Mystus; M. aor and M. seenghala.* They found the encysted metacercaria in the coelomic muscles and body cavity of these fishes.

Ahmed and Sanaullah (1976) observed the occurrence and intensity of infestation of few helminth parasites of various length groups of *Heteropneustes fossils* (Bloch) and *Clarias batrachus* (Linnaeus) in Bangladesh.

Ahmed and Sanaullah (1977) studied on the arrangement on some metazoan parasites of two specimens from six different regions in the districts of Bogra, Dhaka, Mymensing, Noakhali, Rangpur and Sylhet in Bangladesh and collected fourteen metazoan parasites.

Ashrafuddin (1977) studied on some helminth parasites from five species of commercially valuable fish of the Bay of Bengal. He described *Spirocamallanus* sp. from the intestine of *Sardinella frimbiata* and *Dussumicria acuta.*

Ahmed and Sanaullah (1977) reported *Procamallanus bengalensis, Gnathostoma spinigerum, Spirocamallanus olsenia* and a quimperid larva from *H. fossils.*
Mahajan *et al*. (1978) reported the widespread infestation by fully developed sexually adult state of *I. hypselobagri* in *Channa punctatus*, a non-siluroid fish. In a single fish usually 2-5 adult parasite with a no. of juveniles showed exceptional variation in number and size and the degree of infestation was very high.

Wabuke and Bunoti (1980) worked on the occurrence and the pathological effects that the cestode *Polyonchobothrium clarias* (Woodlamd 1925) had on their teleost host, *Clarias mossambicus* (Peters).

Shotter (1980) described the parasitology of the cat fish *Clarias anguillaris* (L.) from a river and a lake at Zaria, Kaduna State, Nigeria. The parasite fauna of *C. anguillaris* from Lake Samara and the river Galma in the northern Savannah region of Nigeria was represented by *Henneguya sp*., *Macrogyrodatylus classi* trematode species and two each of cestodes nematodes and copepods.

Mackiewicz (1982) synoptically reviewed the Caryophyllidea (Cestoda) of India, Pakistan and Nepal. He studied the biology and systematics of the approximately 18 species of caryophyllid cestodes from India, Pakistan and Nepal. He reported that the *Clarias batrachus* and *Heteropneustes fossils* were the chief hosts.

Gupta *et al.* (1983) studied on the presence of non-specific phosphomonoesterases, glucogen and pyruvic acid in *I. hypselobagri* from the air bladder and body cavity of *W. attu.*

Ahmad (1984) described *Styapalia guptai* (digenea); Agarwal and Agarwal (1984) - *Oudhia kanungoi* (digenea); Bhadauria and Dandotia (1984)- *Pleurogenoides ritai* (trematoda); Agarwall and Sharma (1989) *Nicolla fotedari* (trematoda) from *Rita rita.* In Paakistan, Khan (1985) described a new species *Phyllodistomum ritai* (trematoda) from the urinary bladder of *R. rita*.

Ahemed *et al.* (1985) investigated the original arrangement of few caryophyllid cestode parasites and their periodic fluctuation in the gut of *Clarias batrachus* and *Heteropneustes fossilis* from Dhaka, Bangladesh. They reported that the parasites showed higher abundance in summer and rainy season.

Many works have been done on the morphology of the helminth parasites of *R. rita* in India and Pakistan. Gupta and Govind (1985) described *Haplorchoides kherai* (trematoda); Gupta and Singh (1983) described *Pseudocaryophyllaeus ritai* (caryophyllaeidae) from the intestine of *R. rita* in India.

Chowdhury *et al.* (1986) studied on the intensity of infestation and abundance of *I. hypselobagri* in the catfish *Mystus vittatus, M. tengara* and *M. cavasius.*

Gupta and Jaiswal (1986) collected helminthes *Metaquimperia ophicephali* and *Paracucullanellus thapari* from the gut of the freshwater fish *Ophiocephalus marulius* and *W. attu* respectively.

Bhaduria and Dandotia (1986) described 10 new and 6 already known species, among them *Bucephalus gwaliorensis, B. attuai, Opisthorchis attuai, O. pedicellate* and *Phyllodistomum spatulaeformae* from *W. attu.*

Zaman and Leong (1987) worked on caryophyllid cestode in Malaysia and reported 37.7% prevalence of *Lytocestus parvulus* in *Clarias batrachus* with a mean intensity of 11.3. No seasonal cycles of prevalence and intensity or maturation of the cestode were detected. The abundance of the parasite decreased with increasing size of the host.

Chakravarty and Tandon (1988) worked on some histopathological effect of caryophylliasis in the cat fish *Clarias batrachus.* He also observed caryophyllidea and a few caryophyllaeid infestations in the *Clarias batrachus,* in the north-east of India.

Sinha (1988) examined *Clarias batrachus* and found infection of *Procamallanus* spp. The usual load of *Procamallanus* spp. was observed to be 2.65/ fish and there was no major difference in the load of male (1.62) and female (2.70) fish.

Venkateshappa *et al.* (1988) described a different parasitic copepod species *Ergasilus malnadensis* parasitizing *W. attu.* They again noticed the incidence and intensity of this parasite. Infestation on *W. attu* in Vanivilasa sagar reservoir, Karnataka. The incidences of infestation were 80 to 100 percent and 1 to 1,629 parasites/ fish respectively.

Chakravarty and Tandon (1989) worked on histochemical investigations of caryophyllidean cestode parasites (*Lytocestus indicus* and *Djombangia penetrans*) of *Clarias batrachus* (L.).

Mashego and Saayman (1989) observed the prevalence and intensity of some trematode and cestode parasites of African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) in Lebowa, South Africa, with taxonomic notes.

Akhtar *et al.* (1989, 1990) observed the helminth infestation in relations to seasons and length of body of *X. cancila.* 1 acanthocephalan, 3 nematode sp. encysted ascaroid larvae, *Metaquimpera bagarii a*nd *Camellanus gaboes* were found available from

X. cancila. They also found the mediocre group was more infected than the small and large size group. They discussed the incidence of helminthes parasites in this fish in relation to food items.

Gupta and Naiyer (1990) described a new nematode *Procamallanus guptai s*p. which was collected from the gut of a fresh water fish H*eteropneustes fossilis* (Bloch) from Lucknow. This worm differed from all the known species of genus *Procamallanus* except *Prcamallanus ahiri* (in the presence of esophageal gland; in the number of arrangement of caudal papillae; last pair being 'U' shaped; in the presence of phasmids near tip of male tail curved ventrally with well-developed caudal alae).
Khatun *et al.* (1992) recorded the correlation of sizes of *H. fossils* with the rate of helminth infection. They found that the first intermediate size group had the highest prevalence of infection and the second intermediate size group was associated with the highest intensity of helminthes.

Nahar (1993) reported a comparative study on the incidence of endoparasites in relation to some biological aspects of *Channa striatus* and *Channa marulius* from Dhaka, Bangladesh. She described cestode parasites *Bothriocephalus cuspidatus* from the stomach, intestine and liver of *Channa striatus* and *Channa marulius.* Digenetic trematode was *Allogomtio tremaatta* from the stomach of *Channa striatus;* nematode parasite was *Camallanus* spp. in the intestine of *Channa striatus* and *Channa marulius;* acanthocephalan parasite was *Pallisentis nagpurensis* in the stomach and intestine of *Channa striatus* and *Channa marulius.*

Nahida (1993) observed the helminth parasites and histopathology of infested organs in *N. nundus.* She found 4 trematodes *Coitocaecum orthorchis, Opegaster* sp., *Podocotyle atomen, Halipegus* sp. 2 nematodes, *Gnathostoma spinigerum, Porrocaecum* sp., 1 cestode, *Bothriocephalus* sp. and 1 acanthocephala, *Pallisentis nandai* from the host fish. She found that the female hosts were more infested than the male hosts. The occurrence and concentration was observed highest in summer and lowest in winter. The largest group and weight group showed highest prevalence and intensity.

Yasmin *et al.* (1994) worked on Identification, original distribution, seasonal variation and correlation on occurrence and concentration of infestation of helminthes in *Clarias batrachus.*

Khanum and Parveen (1997) reported on the Organal distribution and periodic occurrence of endoparasites in *Macrognathus aculeatus* (Smith) and *Mastacembelus armatus* (Day). The frequency of infestation was maximum during the rainy season in *Macrognathus aculeatus* whereas in *Mastacembelus armatus* the frequency was maximum in the winter. In case of both species, weighty infections were found in the largest groups.

Lyngdoh and Tandon (1998) studied on putative neurosecretory cells in the monozoic cestode, *Lytocestus indicus* (Caryophyllidea). In *Lytocestus indicus* putative neurosecretory cells (PNSC) are recognized on the basis of the nature of their cytoplasm. PNSC in *Lytocestus indicus* are dimensionally small. Morphologically, there are four types of PNSC: a-, uni, bi- and multipolar cells.

Eduardo *et al.* (2001) examined catfish *Clarias batrachus* and mudfish (*Ophiocephalus striatus* Bloch) from Laguna, Philippines for helminth parasites. The recovered parasites prevalence's are as follows: from the catfish- *Orientocreadum batrachoides* (16%), *Eumasenia* sp. (24%), *Oudhia* sp. (34%), *Lytocestus birmanicus* (10%), *Lytocestus lativitellarium* (48%) and *Procamallanus clarius* (46%).

Chandra and Yasmin (2003) investigated Monogenetic trematodes in air-breathing *Clarias batrachus, Heteropneustes fossils, Colisa fasciata* and *Anabus testudineus.* Three new species *Heteronchocleidus colisai, H. bengalensis* and *H. anabusi* were described, along with previously recorded *Bychowskyella tchangi, Quadricanthus kobiensis* and *Heteronchocleidus buschkiella.* All these parasites were recovered for the first time in Bangladesh.

Alam (2006) did a study where helminth parasites found in *Notopterus notopterus* were examined. In total, 4 of parasites species were recorded in this study. These were one from nematoda and three from trematoda. One species of nematoda *i.e. Spirocamallanus notopteri* and two species of trematoda *i.e. Phyllodistomum folium*

and *Singhia thapari* were found in the intestine. Whereas, *Ancylodiscoides notopterus,* an only monogenea trematoda was collected from the gills of the host. It is believed that the later species is the first report from Bangladesh.

Khanum *et al.* (2006) worked on the ectoparasitic infection in *Gudusia chapra* (Hamilton). Heavy infection (100%) was noted in the largest (14.25-17.0 cm) as well as the smallest male (6.0-8.75 cm) groups. Over-all infestation rate was lower in females (83.82%) than in males (87.72%). Intensity of infection in females and males were 2.03 and 1.62 respectively.

Parveen and Silva (2006) reported seven species of helminth parasites of *Anabas testudineus* was collected among which three species were trematodes: *Neopecoelina saharanpurensis, Ptychogonimus megastomus, Brevicreadium congeri* and four species were nematodes: *Zeylanema anabantis, Z. bidigitalis, Metaquimperia madhuai* and *Gnathostoma spinigerum.* Most of the parasites were found in the intestine. Only *G. spinigerum* was collected from the liver. The total occurrence of infestation of the parasites of *A. testudineus* was 90% and the concentration was 3.33. The prevalence was higher (100%) in male fish than in female fishes (84%). The intensity was equal in both male and female fishes (33.3). Among the all parasite groups, trematode showed the highest prevalence (90%) and intensity (2.78). However, both prevalence and intensity were observed most in the mediocre length and weight groups of fishes.

Parveen and Silva (2007) studied on 8 species of helminth parasites in *Nandus nandus* (Ham. Buch. 1822). Among them, two species were trematodes: *Coitocaecum orthorchis* and *Clinostomum piscidium;* 1 specie and 1 genera of cestodes: *Bothriocephalus cuspidatus* and *Diplopulidium* sp.; 1 specie and 2 genera of nematodes: *Gnathostoma spinigerum, Contracaecum* sp. and *Porrocaecum* sp., one species of acanthocephalan: *Pallisentis nandai* was observed in the gut of the host. The complete prevalence and intensity of infestation was 55% and 3.72 respectively. The prevalence was higher (65.21%) in female than in male (41.17%) fishes. Similarly, the intensity in the male and female fishes were 4.00 and 3.6 respectively. The highest number of host fish was infected by nematode parasites (45%) with the highest mean intensity 2.22. Among the parasites, *Porrocaecum* sp. had the highest prevalence

(22.5%) and *Clinostomum piscidium* had the lowest prevalence. Fishes of intermediate length but highest weight group had the highest prevalence of infection.

Khanum *et al.* (2008) reported the endohelminth infestation in *Channa punctatus* (Bloch, 1794). They found 46.7%were infected with four species of endohelminths such as *Ancistrocephalus microcephalus, Haplonema immulatum, Camallanus anabantis* and *Pallisentis ophiocephali.* The infestation was lower in female (35.2%) than male (63.95). Among the identified endohelminths, highest (33.3%) and lowest (3.3%) prevalence showed by *P. ophiocephali* and *A. microcephalus,* respectively.

Khanum and Yesmin (2010) worked on the parasitic infection in *Clarias batrachus* (Linnaeus) and *Clarias gariepinus* (Burchell). They found 16 species of parasites (Ten cestodes: *Djombangia penetrans, Pseudocaryophyllaeus indica, Capingentoides batrachii, Lytocestus parvulus, Lytocestus indicus, Marsipometra confusa, Stocksia pujehuni, Caryophyllaeus laticeps, Bothriocephalus scorpii and Bothriocephalus salvelini;* three trematodes: *Lissorchis fairporti, Holorchis lengendrei and Allorcreadium isoporum and* three nematodes: *Spirocamallanus olsenia, Procamallanus bengalensis,* larva of *Paraquimperia tenerrima) in C. batrachus* while only four species found in *C. gariepinus* (two cestodes: *Djombangia penetrans, Lytocestus parvulus;* one trematode- *Allocreadium isoporum* and one nematode- *Procamallanus bengalensis).* The prevalence was 80.60% in *C. batrachus* and mean intensity was 13.74 (SD ± 12.67) while in *C. gariepinus,* prevalence was 37.20% and intensity was 1.60 (SD ± 0.958).

Khanum *et al.* (2011) studied the periodic occurrence, concentration and distribution of helminth parasites in different organs of host, *Macrognathus aculeatus.* They detected six species of helminthes (two trematodes- *Clinostomum piscidum, Rhynchooharynx paradoxa;* one cestode-*Marsipometra parva, three* nematodes- Pseudoproleptus vestibules, *Cucullanus cirratus* and *Porrocaecum trichiuri* L_3 larva). Prevalence and intensity of parasites were observed a bit lower in male fish than in female. The intensity of parasites were found to be plentiful in monsoon (75%) followed by summer (62.5%) and winter (31.81%). The bigger fishes were greatly affected (71.01%) than medium (53.33%) and smaller (52.17%) fishes.

Farhana and Khanum (2013) reported the dissemination of helminth parasites in various body parts of *Mystus aor* (Hamilton) and *Mystus bleekeri* (Day). To investigate the helminth infestation and their organal distribution, a total of 1011 *Mystus aor* and 1039 *Mystus bleekeri* were examined during January 2004 to December 2005. In *M. aor*, the overall prevalence was 85.95% with a mean intensity of 46.26± 12.0, whereas in *M. bleekeri*, 72.95% prevalence with mean intensity of 56.49± 12.29 was recorded. Out of 22 species of helminth parasites from *M. aor*, 10 sp. of trematodes, two sp. of cestodes, six sp. of nematodes and four sp. of acanthocephalan. From *M. bleekeri*, out of 18 species of helminth parasites, eight sp. of trematodes, one sp. of cestode, five sp. of nematodes and four sp. of acanthocephala were recovered. In *M. aor* and *M. bleekeri*, two trematodes *Masenia collate and Isoparorchis hypselobagri* were found most prevalent and most numerically dominant. *Macrolecithus gotoi* was found only in *M. aor*. *Procamallanus mysti, Pallisentis gaboes* and *Acanthosentis datti* were more prevalent. Majority of the parasites harbored the intestine and some harbored the stomach and other visceral organs. Some larval forms of nematode and acanthocephalan were found to infest the coelomic cavity and mesenteries of the two fishes. The juvenile or immature trematode *Isoparorchis hypselobagri* was recovered from body muscles, swim bladder and visceral organs of the fishes causing massive tissue damages in *M. aor*.

Histopathological aspects

Among the workers who have undertaken histological research on tissues of various freshwater fishes are: Hunter (1928, 1930), Chauhan and Ramakrishnan (1958), Bauer (1959), William (1960), Bullock (1963), Kennedy and Walker (1969), Mackiewicz (1972), Esch and Huffines (1973), Hine and Kennedy (1974), Jain *et al.* (1976), Ahmed

and Sanaullah (1979), Wabuke and Bunoti (1980), Mitchell *et al.* (1982), Khanum (1994), Khanum and Farhana (2002) etc.

Hunter and Hunter (1942) theorized that the black pigment of the interfacicular connective tissue of the host was medicated by an enzyme reaction, which caused mechanical obstruction due to the occurrence of parasite in clusters. Roberts *et. al.*

(1986) pointed out that the internal organ of infected fish show only mild histopathological changes which may be the result of background pathology.

Helminths in fishes are also recognized as causing serious effect on their hosts (Dogiel, 1964; Sindermann, 1970; Ribelin and Migaki, 1975). Ozaki (1926) first described changes in the stomach of a fish host brought on by a trematode *Genarchopsis* sp. Woodland (1935) mentioned the condition of the intestine due to the presence of a cestode *Gangesia* sp. Changes brought on by nematodes have been noted by Yeh, 1960.

Very few studies have been done on parasitization, host-parasite relationship and histpathology of *I. hypselobagri.* Siddique and Nizami (1978) reported incidence of this trematode from *W. attu*. Deveraj and Ranganathan (1971) studied the incidence of this trematode and its destructive effects on air bladder of *W. attu*, viscera and body musculature of *Callichrous bimaculatus* and ovary of *Mystus aor*.

Ahmed and Sanaullah (1979) analysed the relative histopathology as associated to modes of connection and scolex structure, microscopic anatomy, host reaction and consequences of the three caryophyllid cestodes: *Djombangia penetrans*, *Lytocestus indicus* and *L. parvulus.*

Khanum (1994) observed severe pathogenic lesions done by juvenile *I. hypselobagri* on the skin surface, body musculature, liver, intestine, kidney and other visceral organs in two species of *Ompok*.

Khanum and Farhana (2002 b) studied the prevalence and intensity of the trematode *Isoparorchis hypselobagri* (Billet) in *Wallago attu* (Bloch and Schneider) and observed the host's histopathological impacts due to the parasite. In this experiment, encysted as well as free forms were observed in the host's body parts resulting in tissue damage and

in severe cases perforations in the liver. The larval form of *I. hypselobagri* interrupted the structural unity of the body muscles and visceral organ of the host.

Naser and Mustafa (2006) studied the histoanatomical and histonumerical analysis of the digestive system of *Channa punctatus*. The digestive tract was short in length and

the digestive system includes large mouth with sharp teeth, highly distensible esophagus, a large stomach, a number of pyloric caecae and a short intestinal tract. The mucosal layer of the esophagus is highly folded, the stomach was distinct with strong and branched villi and the intestine was short with long villi.

Yesmin and Khanum (2013) investiated the Histo-pathological effects due to helminth infection in *Clarias batrachus* (Linnaeus) and *Clarias gariepinus* (Burchell). Two catfish, *Clarias batrachus* (1000) and *Clarias gariepinus* (500) were examined throughout two year time period. Total sixteen parasites were recovered from these two fishes. In the present observation, the liver, stomach and intestine were found to be infected by numerous nematode, cestode and trematode parasites. Serious pathogenicity was observed due to infection of the cestodes in *C. batrachus*. Among the recovered sixteen parasites, the maximum damage were caused by *Djombangia penetrans* followed by *Lytocestus parvulus, L. indicus, Capingentoides batrachii* caused complete penetration through the stomach and the scolex was deeply buried up to the serosa layers caused shallow ulcers and lesions. While in *C. gariepinus*, no remarkable histopathological damages observed caused by the inhabiting parasites. Histozoic helminthes, particularly migrating forms causes greater damage. In some cases, produced most serious reactions: leukocytosis, fibrosis, hemorrhage, hyperemia and necrosis. The caryophyllaeid cestodes inflict by their scolex, as they anchor to the wall of the stomach and intestine and causes shallow ulcers and lesions. Due to severe infection of these species, intestine becomes porous through the epithelial layer and ultimately become sieve-like. The muscularis mucosa were fully disrupted and damaged by the parasites like, *Djombangia penetrans*, *Capingenoides batrachii*, *Pseudocaryophylaeus indicus*, *Procamallanus bengalensis* and *Spiracamallanus olsenia*. All these species generally capable of local destruction mainly through cellular damage and depletion.

Biochemical composition of (fresh) fish:

Johnstone (1918) analyzed the amount of fat in Halibut and the ranged from 0.5%-9.6% where the protein content remained constant at close to 18%.

Stansby (1954) found the micronutrient content of the edible flesh of certain freshwater fishes and those were 76.8% moisture, 1.2% ash, 5% fat and 19% protein.

Jafri (1968a, 1968b and 1969) published a series of works on the seasonal variations in the biochemical compositions of major carp *Cirrhina mrigala*, catfish *Mystus seenghala*, freshwater murrel *Ophiocephalus punctatus* and catfish *Wallagonia attu*. He indicated that the nutritive value of *W. attu* is greater than that of *M. seenghala*. He determined the changes in fat, moisture, protein and ash contents. The greatest amount of fat in the musculature coincided with the period of peak ripeness. The protein cycle in different tissues exhibited a close connection with maturing and breeding. Protein value in every tissue was usually low in the winter.

People of all ages from children over a year to older persons can enjoy fish because its protein is highly digestible (Nittleton 1985). The protein from fish source would of utmost importance in supplying the nutritional need for the under nourished children as well as pregnant and lactating woman. Thus fish protein is the best animal protein and very much essential for human body development, but parasitic infestation interferes with the protein contents of fish body. Fish proteins contain all the essential amino acids in required amount for human ingestion. Aside from the main components such as moisture, protein, fat and ash, fish contains many other important micro nutrients (Calcium, phosphorus, iron, vitamins, etc.). It is rich in essential dietary requirements constituted of protein (6-28%), moisture (28-90%), oil (0.2-64%), ash (0.4-1.5%), carbohydrate 0.6% (maximum), vitamins: A, B, C, D and E. Fishes are also good sources of riboflavin, iron, calcium, phosphorus and magnesium (Banu *et al*. 1991).

Roopma (2013) analyzed the impact on the adjacent, biochemical and microbial outline of the musculature of a silurid cat fish (*Wallago attu*) when it was stored in a very cold environment. The fish muscle was in a frozen storage for one month and the investigation was done with in a 10 days duration. It was seen that adjacent constituents viz. protein, lipid, moisture and ash content fell considerably ($P<0.05$) due to the increase of time for which the specimens were frozen. The specimens that were not

frozen showed the greatest result among them, i.e. 15.45±0.2% for protein, 4.02±0.04% for lipid, 81.66±0.03 for moisture and 1.48±0.1% for ash while the smallest results were seen at the end of a month during which the specimens were frozen i.e. 10.14±0.015%, 2.36±0.03%, 74±0.05% and 1.33±0.02% for protein, lipid, moisture and ash

respectively. Hence, taking into account the significance from consumer's perspective, these analyses the impact caused to the frozen fish due to the huge loss. Still, it could be understood that in order to maintain its taste and nutrition, the fish can be kept in frozen conditions when preservation

Zaman and Khanum (2013) worked on proximate study of *Mystus aor* (Hamilton) and *Mysus bleekeri* (Day) in relation to parasitic infestation. A total 1011 *M. aor* and 1039 *M. bleekeri* were examined during January 2004 to December 2005. The results of the biochemical analysis revealed that protein and carbohydrate contents were found higher in *M. bleekeri* than in *M. aor*, while lipid content was much higher in *M. aor*. In uninfected *M. aor*, the percentage of moisture and lipid (68.54±1.40 g/100g and 5.70±0.45 g/100g) observed higher than uninfected *M. bleekeri* (67.11±1.59 g/100g and 4.49±0.33 g/100g) while the values of protein and carbohydrate contents were higher in uninfected *M. bleekeri* than uninfected M. aor. In infected M. aor and *M. bleekeri*, the percentage of moisture (72.38±1.5 g/100g and 72.85±1.52 g/100g) found higher than uninfected one. The percentage of protein, lipids and carbohydrate were higher in uninfected fishes than in the infected fishes. Moisture content in both the catfishes found higher during hot and wet seasons and lower in dry season, while the value of carbohydrate found higher in dry season and comparatively lower in hot and wet seasons.

Parasites infestation in relation to climatic factors (Temperature, Rainfall and Humidity)

The ecological issues comprising of climate, season and rainfall plays a vital role in the growth of helminthes parasites. In our atmosphere the increasing greenhouse gases are causing our climate to change continually. In the next few years, worldwide usual temperatures will increase, precipitation patterns will alter, natural disasters may get more intense, sea levels will rise and many other environmental changes will occur (IPCC, 2007). Agriculture will be affected with the impact on food security. Aquaculture will also get affected by the increasing heat.

The alteration in climate can openly hamper fish farming along many routes. Fish breeding, development and movement patterns are affected by temperature, rainfall and hydrology (Ficke *et al.* 2007). Alterations in these factors will modify the number and

availability of certain species. Salt water interruption due to the increase in sea level can impose a threat to freshwater fisheries and simultaneously produce occasions for capturing and farming high-value brackish or marine species (World Fish Center 2007). Variations in rainfall will affect seasonal flooding and may increase production in some inland fisheries. However, the drier seasons may loom stocks of both wild and cultured fish.

Given the situation of extended periods of time and climate variations, increase in sea-level and water temperatures can have direct consequences on the fish parasitic arrangement within a certain territory. Only a small number of freshwater territories are located within perfect environments and anthropogenic species summary connected to fisheries along with consistent immigration events of neozoons change the fixed fish and parasite fauna. Many marine locations have had intense burden of fishing over the last 100 years. Anthropogenic alterations have largely changed the fish species configuration, particularly of larger predators at higher trophic levels (Hutchings and Baum 2005; Baum and Worm 2009 which has quantifiable impacts, even on the traits of life history, considerably changing the maturation size and age (Sharpe and Hendry 2009). Accordingly, the number of fish parasite that are related to their changing host numbers may also vary with a shift in environmental situations. A final account of the situations under which parasites can be used as indicators of environmental effect, is still difficult to achieve (Vidal-Marti ‘nez *et al.* 2010).

The marine surroundings can be investigated either directly by continuously checking the water quality compared with its guidelines or indirectly by the use of bio indicators (Palm and Reuckert 2009), like fish parasites (Galli *et al.* 2001). These organisms respond on precise environmental situations or change, resulting in a diverse range of uses (bioindication for water quality, MacKenzie *et al*. 1995; environmental stress, Landsberg *et al.* 1998; pollution, Khan and Thulin 1991; Yeomans *et al.*1997). Vidal-Marti ‘nez *et al.* (2010) normally differentiated between accumulation or effect bio indicators, where organisms skillfully take upsubstances in the previous or are used to notice effect on surroundings in the latter. This is done during recording a certain variation in their physiology, chemical constituent, behaviour or number. Additionally, other parasite metrics like change in indices or species richness can be a source of data, showing a probable impact on particular environmental circumstances on the fish

parasite community. The existence of parasites in the surroundings frequently becomes obvious after a huge infestation, resulting in symptoms or leading to mortality of the infested hosts. Situations like these can be joined with biotic or antibiotic alterations in the environmental indicators (Meoller 1987). Information of the biology of the parasite and its host(s), the host-parasite relationship and the surroundings can assist to notice change in the environment.

(a) Temperature

Parasites that infect fish and have a disastrous impact on fish reproduction, grow four times faster at raised temperatures – providing a number of the first indication that global warming affects the interactions between parasites and their hosts.

The analysis from the University of Leicester showed that global warming had the possibility to alter the balance between parasite and host – with possibly severe inferences for fish populations. The investigators from the University of Leicester's Department of Biology also detected change in behavior in infected fish – signifying parasites may influence host behavior to make them search for higher temperatures. They also learnt that while parasites grew faster in higher temperatures, the host's growth rate reduced.

The experts detected that parasitic worms infecting stickleback fish grew four times faster in experimentally infected sticklebacks raised at 20°C than at 15°C. However, the fish grew slowly at the higher temperature, suggesting that fish parasites survive at increased temperatures considerably better than the fish they infect. In a follow up study, the authors also showed that fish infected with the largest worms showed a preference for warmer water, suggesting that these parasites also influence the behavior of host fish in ways that help the parasites and maximize their growth rates. The results show some of the early sign that raising environmental temperatures can cause a change in the delicate balance that is present between co-evolved hosts and parasites, increasing the speed with which parasites complete their life cycles that could cause an increase in the overall level of parasitism in natural animal populations (Macnab and Barber, 2011).

(b) Rainfall

Being a low-lying country, Bangladesh is at high risk and in high danger because the disasters that come with climate change. Bangladesh is facing certain straightforward and major changes in its climate and pattern of weather. Nowadays, unpredictable rainfall has become very common in Bangladesh. Climate change has brought erratic

rainfalls in several areas around the globe. This rate is rising abruptly. Long term unmitigated climate change will "likely" surpass the capability of people and the natural world to adjust (IPCC 2007).

Some authors say that a high abundance of parasites is sustained in tropical locations all through the year (Coley and Aide 1991; Martin *et. al*. 2004), during which others say that these parasites could exhibit major temporal changes because of the alterations in rainfall (Steinauer and Font 2003). Latest analyses on fish infections due to by digenean parasites (Jiménez-García and Vidal-Martínez 2005) show the necessity of seasonality in infection parameters of parasites in tropical environments, signifying a connection between seasonal factors such as rainfall or temperature, the presence of new host cohorts and maximum variability of the infection parameters.

(c) Humidity

Humidity is the volume of water vapor in the air. Water vapor is the gaseous state of water and is not visible. Humidity shows the probability of precipitation, dew or fog. Higher humidity decreases the efficacy of sweating and cooling the body by decreasing the rate of evaporation of moisture from the skin. This consequence is calculated in a heat index table or humidex (Wikipedia, the free encyclopedia).

Humidity is one of the essential abiotic factors that says a lot regarding any habitat and is a determinant of which animals and plants can flourish in a particular location. The human body dispels heat through perspiration and evaporation. Heat convection to the surrounding air and thermal radiation are the main ways heat is transferred out from the body. Under circumstances of high humidity, the rate of evaporation of sweat from the skin is reduced. Moreover, if the atmosphere is as warm as or warmer than the skin during times of high humidity, blood brought to the body surface cannot dissipate heat

by conduction to the air and a condition called hyperthermia occurs. With so much blood going to the external surface of the body, a lower amount goes to the active muscles, the brain and other internal organs. Physical strength is lowered and fatigue results sooner than it would otherwise. Alertness and mental capacity also may be changed, causing heat stroke or hyperthermia (Hogan 2010).

DNA barcoding

Özcan and Bozdoğan (2020) used DNA barcoding for the molecular identification of the parasite *Neoechinorhynchus rutili* diagnosed in fish (*Capoeta barroisi*) caught in Menzelet Dam Lake, Turkey. Parasite specimens were collected from the guts of fish which were caught in between January to June of 2013. These obtained parasites were kept in 70% alcohol. By the use of staining processes and based on morphology, 120 *N. rutili* had been identified. DNA extraction of *N. rutili* was performed by using certain tissue sets for parasites. Particular primers were used for molecular identification of *N. rutili* by means of polymerase chain reaction (PCR). After that it was probable to authenticate that all the parasites had *N. rutili* molecules. Finally, by the use of numerous processes, it had been fruitfully identified and proved that there are *N. rutili* parasites present in the fish which had been caught in the Menzelet Dam Lake. The method of identification of *N. rutili* by the use of morphology and staining takes a lot of time. Still, PCR was magnificently completed in a small amount of time to achieve the exact same results. The success of this experiment might help to create even more original and widespread tasks intended to achieve effective molecular identification of parasitic agents present in fish.

Dabara and Takiso (2020) performed the experiments on the the molecular analysis of the parasite *Ligula intestinalis* found in *Alburnus adanensis*, from Menzelet Dam Lake in Kahramanmaraş. For this reason, parasite specimens were collected from the gut of 60 of the hosts which were caught in a certain period in 2018. After collecting the parasites, they were kept in 70% alcohol. By morphological and staining methods, 27 *Ligula intestinalis* were identified. The DNA extraction of *Ligula intestinalis* was done by using a specific tissue kit. In the molecular identification of Ligula intestinalis by PCR method, exact primers were used. Consequently, all the parasites were affirmed molecularly to be *Ligula intestinalis*. The method of identification of *Ligula intestinalis*

by the use of morphology and staining takes a lot of time. Still, PCR was splendidly completed in a small amount of time to achieve the exact same the achievement of this experiment shall pave the way for more unique and widespread investigation on molecular identification of parasites in fish.

Siddall *et al*. (2012) Nematode parasites were encountered in kosher-certified fish meat and roe. So to find out the parasitic nematodes' identities, molecular procedures were done in the form of DNA barcoding because of the impaired samples. Possibly, this is the first use of this method to a clearly traditional apprehension as divergent to one of health or monetary importance. Outcomes, put together on both cytochrome *c* oxidase subunits I and II, recommended that the parasite species collected from the fish products are anisakis species that does not live in the intestinal lumen of the fish hosts studied. Nevertheless, the achievement of DNA barcoding in defining at any rate the upper taxonomic characteristics of the parasites, few inadequacies of the DNA barcoding channel as it concerns to nematode parasites were met; particularly, the scarcity of information that exists for the DNA barcoding locus, even for very common nematode taxa.

Reier *et al.* (2020) Acanthocephalans are obligate parasites of vertebrates, mostly of fish. There is little data regarding the variety of Acanthocephalan fish-parasites in Austria. In Austrian water bodies seven known and one unknown species were documented. Acanthocephalans are still difficult to identify because of their scant morphological characteristics and large intraspecific disparities. DNA barcoding is a powerful weapon for taxonomic task at the species level. In this investigation, it has been provided that new DNA barcoding data for three species of Acanthocephala (*Pomphorhynchus monticelli*, 1905, *Echinorhynchus zoega* in Müller, 1776 and *Acanthocephalus koelreuter* 1771) collected from various species of fish in Austria. It also delivers a significant contribution to the taxonomy of acanthocephalan parasites and their dispersal in Austrian fish. Nonetheless, the taxonomic assignment of one species should stay open. Clues have been found for cryptic species within *Echinorhynchus cinctulus* Porta 1905. These type of studies highlights the challenges in handling dependable DNA barcodes to identify acanthocephalan parasites molecularly.

CHAPTER-3

MATERIALS AND METHODS

MATERIALS AND METHODS

The two host species were autopsied for collection of helminth and other parasites, for taxonomic identification of the parasites, histopathological studies, biochemical analysis, food contents, monthly and seasonal infestation of parasites, their organal distribution etc.

Sample Collection and Description

The experiment was conducted from January 2017 to December 2018 at the Parasitology laboratory of the Department of Zoology, University of Dhaka. A total of 321 *Polynemus paradiseus* and 321 *Xenentodon cancila* were autopsied and examined during January 2017 to December 2018, from Swarighat under Dhaka district of Bangladesh.

Collection Methods

After collection, the fishes were kept in an icebox with ice and carried to the Parasitology laboratory, Department of Zoology, University of Dhaka for the present observation. In the laboratory they were examined within 2-3 hours. On arrival at the laboratory, the fishes were given serial numbers and then the total length and weight were measured. The sexes of each fish were identified according to Haq (1977). The genital pore, the shape of the body, abdomen of the fishes and the body coloration of both the sexes were examined for finding a practical way of discerning the sexes. The area of genital pore was observed to be quite helpful for identification of the sexes (Hossain and Islam 1983).

Sampling technique, measurement of length and weight, sex differentiation

Sample Description

Identification of host fishes: Identification of *Polynemus paradiseus* (Linnaeus, 1758) and *Xenentodon cancila* (Hamilton, 1822) were done following Day (1878), Lagler (1956), Shafi and Kuddus (1982) and Rahman (1989).

Scientific name: ***Xenentodon cancila***

Common name: Garfish

Local name: Kaikka, Kakila

The length groups of Xenentodon cancila ranging from 20 cm. to 40 cm. were divided into 4 groups: viz.

(a) 20-25cm.

(b) 25.1-30cm.

(c) 30.1-35cm.

(d) 35.1-40cm.

Scientific name: ***Polynemus paradiseus***

Common name: Thread fin fish

Local name: Tapasi, Muni, Rishi

The length groups of *Polynemus paradiseus* ranging from 7 cm. to 15 cm. were divided into 4 groups: viz.

(a) 7-9 cm.

(b) 9.1-11cm.

(c) 11.1-13cm.

d) 13.1-15cm.

Examination of the fish and collection of parasites

At the laboratory, the skin, fins, body surface, tail etc. of fish were observed very finely to see the pathological condition such as ulcers, scars, cysts and injuries. The fishes were opened by an incision through midventrally and the entire intestinal tract together with digestive gland and urogenital system were removed. The different parts of the alimentary canal viz. esophagus, stomach, intestine and other internal organs like liver, kidney etc. were kept in normal saline solution (0.8% Nacl solution) in different Petri dishes. The separated parts of stomach and intestine were split opened carefully by a longitudinal incision throughout their length and shaken to dislodge the parasites, and then the epithelial layers of stomach and intestine were scrapped to remove any parasite that might have remained attached to the mucus layer. The liver and kidneys were

stretched to dislocate the parasites. The collected parasites were then washed in fresh saline solution to clean them and to free of any debris.

Isolation, fixation, staining and preservation of helminth parasites

The helminth parasites were detected by both macro and microscopically into different groups: Trematoda, Cestoda, Nematoda and Acanthocephala. The parasites belonging to the major groups were separated and counted. In many cases some larval and juvenile stages were also observed.

Once, the genus and species of each group were identified and familiarized, the parasites were easily separated and counted directly. For microscopic examination and identification, the helminths were fixed and preserved in different suitable preservatives for each group and kept in separate vial.

The trematodes and cestodes were fixed in acetic-formalin-alcohol (AFA), stained in borax-carmine or Semichon's aceto -carmine; cleared in lactophenol and then mounted in Canada balsam (Cable 1963).

The nematodes and acanthocephalan were fixed in glycerinalcohol, stained in borax carmine, cleared in lactophenol followed by permanent mount on Canada balsam (Cable 1963).

Taxonomic identification of the parasites

For taxonomic classification of the helminth parasites, Yamaguti (1958, 1959 and 1961) and other relevant reference articles were consulted.

Micromeasurements

The measurement of parasites and their different organs was done by using a calibrated eyepiece scale (micrometer). The eyepiece micrometer scale required calibration from a measured scale engraved on a glass slide (stage graticule). A suitable line scale for the eyepiece micrometer is one that is divided into 50 divisions. A suitable scale for the stage graticule is one that measures 2 mm in length with each large division measuring

0.1mm (100µm) and each small division measuring 0.01 mm (10µm). For parasitology, the scale should be calibrated for the 40X objective (Cheesbrough 1987).

The method is as follows-

1. Adjust the field until the 0 line of the eyepiece scale aligns exactly with the 0 line of the calibration scale.
2. Look along the scales and note where a division of the eyepiece scale aligns exactly with a division of the calibration scale.
3. Measure the distance between the 0 point and where the alignment occurs.
4. Count the number of divisions of the eyepiece scale covered between the 0 point and where the alignment occurs.
5. Calculate the measurement of 1 of the divisions of the eyepiece scale, in µm e.g. Distance measured=0.2 mm

   Number of division=27

   1 division measured: $= \frac{0.2}{27}$=0.0074 mm

   To convert mm to µm: 0.0074x1000=7.4 µm

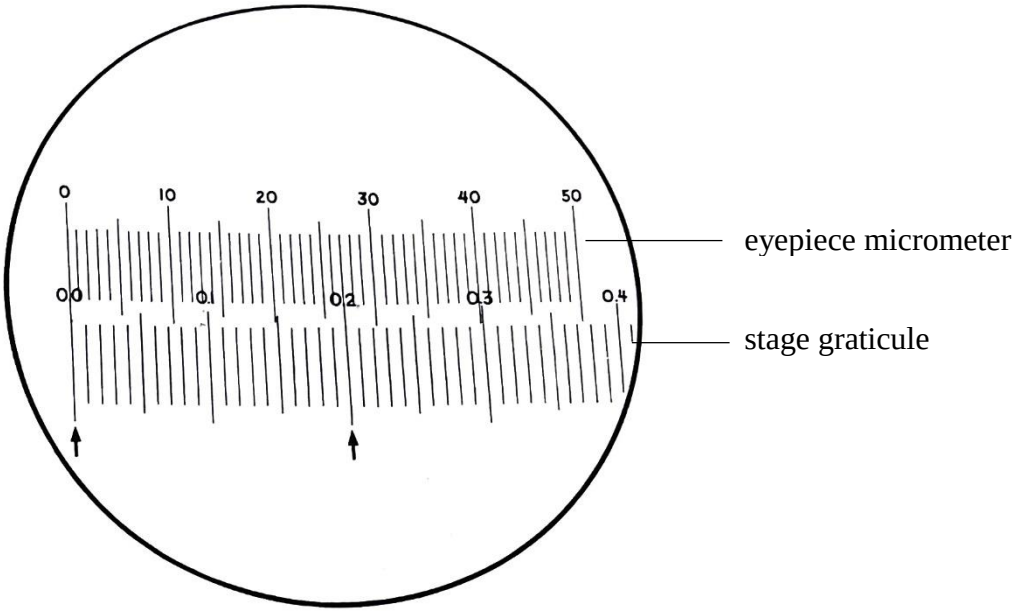

Fig.-2. Calibrating an eyepiece micrometer. Top scale is that of the eyepiece micrometer. Lower scale is that of the stage graticule (Cheesbrough 1987).

Collection of data about climatic factors (Temperature, Rainfall, Humidity)

The information about climatic factors i.e. temperature, rainfall and humidity were collected from "Bangladesh Meteorological Department (BMD)", Meteorological complex, Agargaon, Dhaka-1207.

Histopathological examination

Tissue collection

The affected parts and organs of the fish, e.g. skin, liver, muscles, kidney, swim bladder and alimentary canal were separated and treated according to the methods instructed by Drury and Wallington (1967) for histological studies. After detection, the affected tissues were carefully fixed by a gradual addition of 10% Buffered neutral formalin solution.

Technique for histological study

The methods of Gray (1964) and Humason (1979) were followed for the preparation of the permanent histological slides. For preparation of histological slides, the tissue materials were kept in 10% Buffered neutral formalin solution for 24-48 hr for fixation, dehydrated in ascending grades of ethanol (50%, 70%, 10% and 100%), impregnated and embedded in paraffin and sectioned at 5µ. Sections were mounted on slides, deparafined by low grading and stained with haematoxylin and counter stained with eosin, dehydrated and finally mounted in Canada balsam.

Analysis of collected food items from the stomach of fish

To establish the food habits of *P. paradiseus* and *X. cancila*, the stomach contents were analyzed by occurrence method (Hynes 1950). Stomach contents were examined and the individual food item was sorted out and identified with the aid of a dissecting microscope. The number of stomachs in which each item occurred was recorded and expressed as a percentage of the total number of stomachs examined (Forbes 1888). Stomachs with food were noted and the food items were grouped into 6 categories, as given below, according to the dominance of the particular group taken in by the fish as food item (Mellanby 1963; Cannon 1973; Hyslop 1980):

Category 1: small fishes, whole or remains of scales, bones, skin, flesh etc.

Category 2: Crustaceans e.g. Copepods, crayfishes, ostracod, crabs etc.

Category 3: Aquatic insects e.g. fleas, beetles, eggs, pupae, wings, legs of insects etc.

Category 4: Mollusca e.g. broken or empty shells, mantles of gastropods, pelecypods etc.

Category 5: Annelids e.g. leeches, oligochaetes, polychaetes etc.

Preparation of samples for the biochemical analysis

The weighted samples of fishes were thoroughly washed with saline water (0.75%) and dried by soaking them with filter papers. The dried and cleaned fishes were treated separately as required by the specific methods of analysis for different nutrient content of *P. paradiseus* and *X. cancila*. For the determination of each type of nutrient, every analysis was repeated 3-5 times and the mean value was recorded.

Proximate analysis of tissue sample

The washed materials were soaked with blotting paper followed by filter paper at room temperature to remove the surface water. These were immediately kept in desiccators to avoid further evaporation of moisture from the materials. The samples become ready for the determination of their proximate composition such as the moisture content, protein and fat. These determinations were made according to the method described by Gopalan 1971.

Methods for analytical determination of nutrient contents

Determination of moisture content

The fish sample (2-5gm) was taken in a constant weight crucible. It was then dried at 100-105 ^{0} C temperature in an oven for 4 hours and cooled in a desiccators and weight again. Heating, cooling and weighting were continued until a constant weight was obtained.

Calculation

Initial weight = Sample weight + crucible weight

(Before heating

Final weight = Sample weight + crucible weight

(After heating)

$$\text{Percentage of moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \text{ X } 100$$

Determination of protein content

The protein content of fish flesh may be obtained by estimating the nitrogen content of the material and multiplying the nitrogen value by 6.25. This was referred to as crude protein content, since the non-protein nitrogen (NPN) present in the material was taken into consideration in the present investigation. The estimation of nitrogen was made by modified Kjeldhl method (Gopalan, 1971), which depends on the fact that when organic nitrogen digested with concentrated sulphuric acid in the presence of a catalyst was converted into ammonium sulphate. Ammonia liberated by making the solution alkaline was distilled into a known volume of standard acid, which was then back titrated.

Reagent preparation

1) Digestion mixture: Potassium sulphate and Copper sulphate in a ratio of 98 g: 2 g, were powdered with, mortar pestle and mixed well.

2) Sulphuric acid solutions (0.1N): concentrated sulphuric acid (2.78 ml) was added in distilled water and the volume was made up to 1000 ml. The solution was standardized by standard sodium carbonate (0.1 N) solution.

3) Sodium hydroxide solution (0.1N): Sodium hydroxide (4gm) was dissolved in distilled water and the volume was made up to 1000 ml. it was standard by the standard sulphuric acid (0.1 N) solution.

4) Sodium carbonate solution (0.1N): Anhydrous sodium carbonate (5.3 gm) was dissolved in distilled water and the volume was made up to 1000 ml.

5) Sodium hydroxide (40%): Sodium hydroxide (40%) was dissolved in distilled water and the volume was made up to 100 ml.

6) Methyl red indicator: Methyl red indicator (0.1g) was dissolved in 60 ml alcohol and the volume was made up to 100 ml with distilled water.

Procedure: According to principle, Kjeldahl method consists of following steps:

1. Digestion of sample
2. Distillation
3. Titration.

1. Digestion

The sample (0.5-0.2gm) was taken in weighting paper and measured accurately. This sample was poured in a 500ml clean and oven dried Kjeldahl flask, to which 5gm of digestion mixture and 25 ml of pure concentrated sulphuric acid were added. To avoid frothing and bumping, a glass rod was placed inside the flask. A blank flask was carried with all reagents except sample material for the comparison. These flask were then heated in a Kjeldahl digestion chamber initially at low temperature (20^0 C) until the mixture no longer froths and then temperature was increased to 60 ^{0} C and heating was continued until the solution became colorless. At the end of digestion period the flasks were cooled and diluted with 100 ml distilled water. A small piece of litmus paper was placed in the solution and the reaction was found to be acidic.

2. Distillation

The distilling set of Kjeldahl apparatus was thoroughly washed with distilled water before starting the distillation experiment. 25 ml of 0.1 N sulphuric acids was taken into the receiving 250 ml conical flask. In a measuring cylinder 75 ml of 40% Sodium hydroxide was taken and it was carefully poured down the side of the Kjeldahl flask. The litmus paper appeared blue indicated the solution became alkaline. The mouth of the flask was closed with a stopper containing connective tube, which was ultimately connected to the ammonia receiving flask containing 0.1 N sulphuric acids. The mixture was boiled at such a rate that water and ammonia distilled over at a steady moderate rate. The heating was not too slow so that the sulphuric acid solution might be sucked into the Kjeldahl flask and not too fast so that the distilling ammonia did not escape the sulphuric acid without absorption.

3. Titration

Ammonia was absorbed in a receiving flask which contained 0.1 N sodium hydroxide and 3 drops of methyl red was used as indicator. Similarly, a blank reagent was distilled and titrated.

Calculation

The calculation of the protein content of the sample on the percentage basis was given by the following formula:

$$\text{Percentage of protein} = \frac{(c-b)\times 14d \times 6.25 \times 100}{a \times 100}$$

Here,

a= Sample weight (g)

b= Volume of sodium hydroxide required for the back titration and to neutralize 25 ml of 0.1 N $H_2SO_{4\,r}$

c= Volume of sodium hydroxide required for the back titration and to neutralize 25 ml of 0.1 N H_2SO_4

d= Normality of sodium hydroxide used for titration, conversion factor of nitrogen to protein is 6.25; atomic weight of nitrogen is 14.

Determination of fat content

Reagents of fat determination are Chloroform: Methanol solution (Chloroform was mixed with Methanol in the ratio of 2:1) and Sodium Chloride solution (0.58%) (Sodium Chloride (0.58%) was dissolved in distilled water and the final volume was made up to 100 ml).

Reagent preparation

1) Chloroform Methanol solution: Chloroform was mixed with Methanol in the ratio of 2:1.
2) Sodium chloride solution (0.58%): Sodium chloride (0.58g) was dissolved in distilled water and the final volume was made up to 100 ml.

Procedure

Total lipids were extracted by modification of the methods described by Folch *et al*. (1957). The moisture free sample was taken in a conical flask and to it 100 ml of Chloroform: Methanol solution (2:1) was added. The sample was allowed to stand for overnight and was filtered. The filtrate was taken in separating funnel and to it 0.58% Sodium Chloride solution (20ml) was added. The separating funnel was vigorously shacked for proper mixing and allowed to stand for 4-6 hours. The lower phase was then collected and washed with sodium chloride solution repeatedly till the lower phase was clear. Finally, the lower phase was collected in a dry weighted conical flask. The fat was then estimated gravimetrically.

Calculation

$$\text{Percentage of fat} = \frac{\text{weight of extract}}{\text{Sample weight}} \text{ X } 100$$

Calculation of Calorie content

The calorie content of the flesh material was calculated by multiplying by protein and fat by 4 and 9 respectively

Statistical techniques for analysis of data

In the present study, some terms have been expressed as required concepts related to the number of the hosts in sample infected with a particular species of parasite groups and to the number of individuals of a particular species in each host in a sample (Margolis, *et al*. 1982a and b). Statistical calculation of data of the present observation was done on a personal computer. The SPSS program was utilized for statistical analyses which were obtained by the following formulas-

Prevalence: Percentage of infected host

$$\text{Prevalence} = \frac{\text{Number of infected hosts}}{\text{Total number of hosts examined}} \times 100$$

Intensity: Average number of parasites in each infected host

$$\text{Intensity} = \frac{\text{Total no. of parasites}}{\text{No. of infected hosts examined}}$$

Standard deviation: dispersion of probability about the highest point.

$$S = \sqrt{\frac{\sum(x - \bar{x})}{n}}$$

Where, S = Standard deviation

x = the individual value

$\bar{x}$ = the arithmetic mean

n = number of observation

Chi-square x^2 test

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Where, O = Observed number

E = Expected number

$\sum$ = Summation

The simple significance student"t-test"

Student"t-test" (Equality of means) has been done for the comparison of the results and data of prevalence and intensity of the two species of fishes examined in two successive years (2017 and 2018).

Significant correlation

To find out the correlation (if any) of the prevalence and intensity of parasites with the size, season, sexes and food of the hosts. Correlation-coefficient tests were calculated by Linea regression and multiple regression methods.

Analysis of variance (ANOVA-one-way)

The seasonal data were analyzed by an Analysis of variance (ANOVA-one-way) for unequal samples, to find out whether the difference observed in the prevalence of infection and intensity in seasons of fishes during the study period are significant or not. (Bailey 1959; Winifred 1972).

Microphotographs

The photomicrographs of the parasites and the histological section were taken by a camera (Sony, DSC-H10, 8.1 mega pixels) after fixing with compound microscope in the laboratory of Department of Zoology, University of Dhaka.

Seasons

Summer= March to June

Rainy= July to October

Winter= November to February

MOLECULAR STUDIES

Sampling and morphological analysis

After collecting the parasites from the host fish they were identified using the methods of Bauer (1987) and Ekingen (1983). Once morphological analysis has been done all of the collected parasites samples were subjected to molecular study by using mitochondrial cytochrome c oxidase I (COI) gene for molecular identification. DNA barcoding which is an experimental procedure can be performed through following steps-

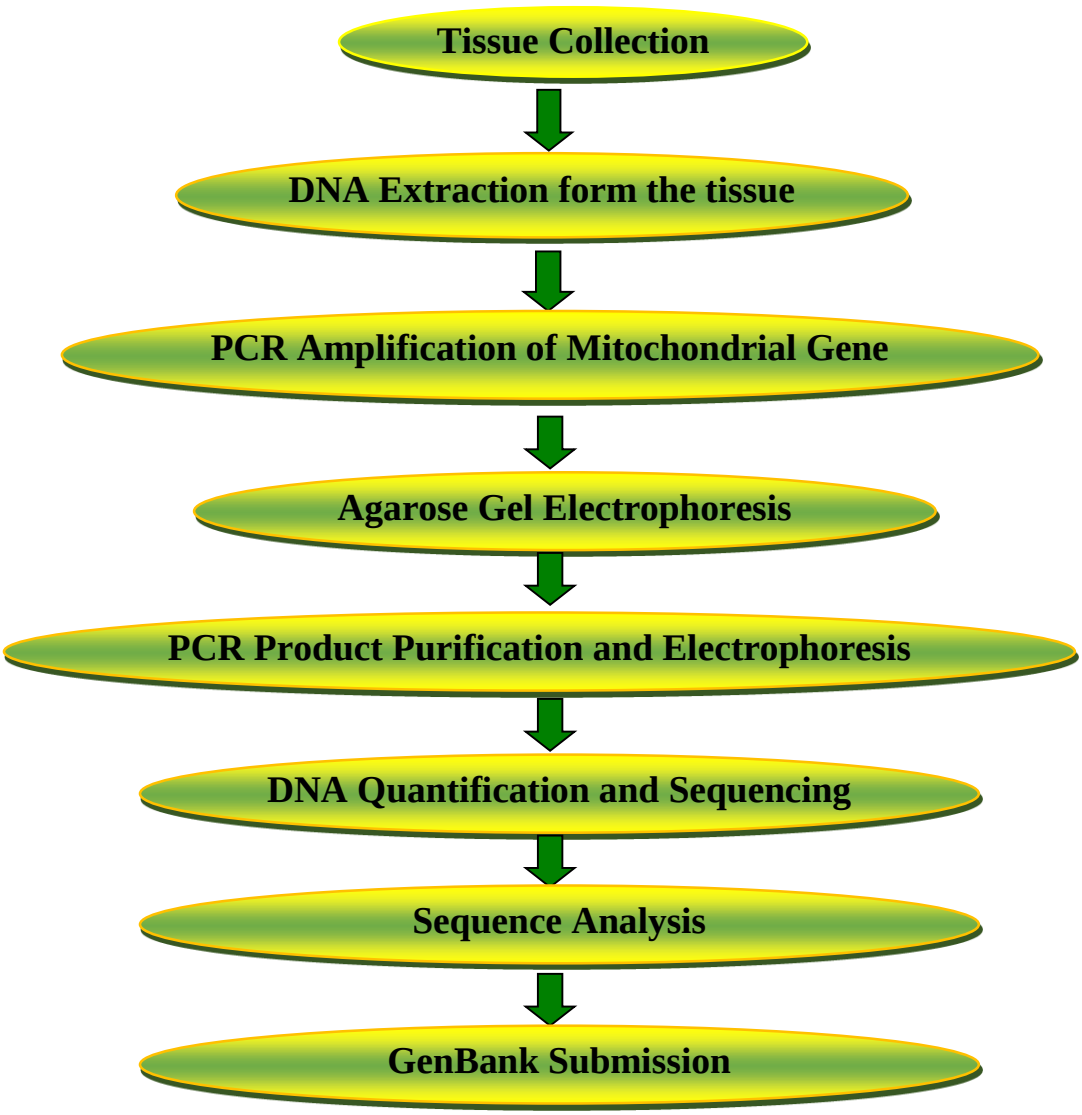

Fig. 3: Flow diagram showing experimental procedure of the study

Tissue Collection

Each one of the parasite samples was cut into pieces of 0.2 gr using sterile scissors and then placed into 1.5 ml micro-centrifuge (MC) tube

DNA extraction

1. The parasite pieces were washed repeatedly after adding 2 ml of sterile distilled water, were centrifuged at 5000 rpm for 10 minutes.
2. Following centrifugation, the water remaining on top of the Eppendorf tube was discarded and 20 µl of proteinase K and 400 µl of lysine buffer were added to the pellet remaining at the bottom of the Eppendorf tube, and all were mixed.
3. These samples were incubated overnight at 56°C in a shaking water bath, mixed for 15 seconds in a vortex, and 10 µl of RNase A was added and mixed in the vortex.
4. Then 430µl of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and mixed thoroughly in an up-and-down fashion for 5 minutes. The mixture was centrifuged at 12000 rpm for 5 minutes.
5. Then supernatant (about 200 µl) was transferred to a new sterilized MC tube and chilled absolute ethanol (100%) was added to become 1ml.
6. To break down certain bonding between DNA and other salts, 20 µl 7.5M ammonium acetate was added and centrifuged at 12000 rpm for 5 minutes. After this step DNA precipitated as pellet and the supernatant was discarded.
7. Then 70% chilled alcohol was added at the volume of 1ml to wash the DNA and centrifuged at 12000 rpm for 5 minutes. Pellet was retained discarding the supernatant and kept for air dry for 30 minutes under laminar flow.
8. Finally, the pellet was suspended in 50 µl TE (Tris Edditate) buffer and stored at -20°C for long preservation.

Agarose gel electrophoresis (AGE) and visualization of DNA bands

AGE is a standard method to separate and identify DNA fragments. After DNA extraction, the extracted DNA products were examined by this method. 1% agarose gel was used to separate, identify and purify DNA fragments.

Gel (1.5%) Preparation

1. 150 ml 1X TBE buffer was taken in a conical flask

2. 5gm Agarose powder was weighted and mixed with the 1 X TBE buffer

3. The mixture was then put into the oven for 60 seconds for melting

4. After that the conical flask was put out from the oven and kept it for sometimes for being cool

5. 15 µl ethidium bromide was added and poured the mixture on the gel case.

6. The comb was set at negative side of the plate and left the gel for being solidify

7. After being gel, the comb and rubber pad were removed from the gel plate

8. Then the gel plate was set into the gel tank, the comb side must at the negative side of the gel tank.

9. After the gel solidified, the comb was removed carefully and it was placed horizontally into electrophoresis chamber containing 1XTBE buffer.

Sample loading and gel running

1. Agarose Gel Electrophoresis was carried out on a horizontal slab gel apparatus

2. 50 bp ladder was loaded into the first well of the gel through pipetting

3. The extracted DNA and ethidium bromide was mixed in a ratio of 5:1

4. The DNA was loaded into the well

5. The electrophoresis was carried out at 120 volts for about 40 minutes

6. After electrophoresis, the gel was placed on ultraviolet (UV) transilluminator to visualize the DNA; the photograph was taken immediately in GEL-DOC (BioRAD).

PCR amplification of mitochondrial COI gene

Amplification of target DNA sequences is accomplished by polymerase chain reaction usually referred to as PCR. PCR is a common molecular technique which used to amplify a specific region of DNA strand, generating thousands to millions of copies of a particular DNA sequence. For PCR amplification we used short fragment of DNA called primer to define a 432 bp of region of COI gene to be copied.

Reagents required for PCR are as follows

1. 10 X PCR buffer
2. 25 mM $Mgcl_2$
3. 10 m M dNTP (Dinucleotide phosphate)
4. Forward primer
5. Reverse primer
6. Taq DNA polymerase
7. Template DNA
8. Sterilized distill water

The PCR mixture was prepared with a total volume of 25µl containing 23 µl Master Mix and 2 µl of DNA sample and mixed and spun for 30 s for homogenization of the mixture.

Primers used in Polymerase Chain Reaction

From the extracted DNA, approximately 432 bp nucleotide was amplified from the 5' region of the COI gene by PCR (Polymerase Chain Reaction) using one pair of primers.
Forward primer (COI P1-F: TTTTTTGGGCATCCTGAGGTTTAT)
Reverse primer (COI P2-R: TAAAGAAAGAACATAATGAAAATG)
(Near et al. 1998; Král'ová-Hromadová et al. 2003; Goméz et al. 2002

Preparation of master mix

Master Mix is the mixture of all the components for PCR except the template DNA. For 23 µl master mix reagents are added in the following amount:

Table-1: **Composition of the Master Mix.**

Reagents	**Volume (µl)**
Maximo premix ($MgCl_2$, dNTPs, Taq polymerase and reaction buffer)	12.5
Forward primer	1
Reverse primwe	1
Nuclease free water	8.5

Thermal cycling profile used in PCR

The PCR procedure involves three steps, each repeated many times; these steps are:

1. Denaturation phase
2. Annealing phase
3. Elongation phase

In PCR amplification, the pre-denaturation stage for 4 minutes at 94ºC was followed by a total of 35 PCR cycles, denaturation at 94ºC for 40 seconds, annealing at 50ºC for 40 seconds, elongation 72ºC for 1 minute and final elongation at 72ºC for 10 minutes.35 cycles of these steps were performed in the Prime Thermal Cycler.
Thermal cycling profile programmed for amplification of COI gene:

Table-2: The thermal cycling profile that was programmed to amplify the COI gene by polymerase chain reaction for 35 cycles is as follows

Name of stages in PCR	**Temperature**	**Time**	**No. of cycles**
Initial denaturation	94°C	4 minutes	1(first)
Denaturation	94°C	40 seconds	35
Annealing	50°C	40 seconds	35
Elongation	72°C	1 minute	35
Final elongation	72°C	10 minutes	1(last)
Holding stage	22°C		

A total of 5 µl was collected from the DNA products amplified in PCR, mixed with 1 µl of g, and placed in previously prepared wells over 1.5% agarose gels with the last well saved for the DNA marker. After the gel was subjected to electrophoresis using Tris/Borate/EDTA buffer in 1.5% agarose gel at 80 V for 1.5 hours, it was stained for 30 minutes with ethidium bromide. It was photographed with apolaroid camera system in a dark room using an ultraviolet trans-illuminator looking at the specific DNA bandwidth for *P. ophiocephali*

PCR products were then visualized under UV transilluminator on 1% agarose gel containing ethidium bromide staining (10mg/ml)
Heated lid was 104°C. The holding stage did not exceed 10 minutes.

Purification of PCR product and gel electrophoresis

DNA cleanup and Concentration Protocal Steps

Purification step was performed in order to isolate DNA from other macromolecules. The steps are given below:

1. Dilute sample with DNA cleanup Binding Buffer with a ratio of 2:1 and mix well by pipetting up and down or flicking the tube.
2. Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.
3. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.
4. Repeat step 3.
5. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute.
6. Add≥6 µl of DNA Elution Buffer to the centre of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA

Oligonucleotide cleanup protocol steps

1. 100 µl DNA cleanup binding buffer was added to the 50 µl of sample.
2. 300 µl was added and mixed well by pipetting
3. Then insert the column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.
4. Re-insert column into collection tube. Add 500 µl DNA Wash Buffer and spin for 1 minute. Discard flow-through.
5. Repeat step 4. This second wash step is optional, but recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K). Please note that if carrying out a second wash step, additional DNA Wash Buffer may be required.
6. Transfer column to a clean1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute.
7. Add≥ 6 µl of DNA Elution Buffer to the centre of the matrix. Wait for 1 minute, then spin for 1 minute to elute the DNA. Typical elution volumes are 6-20 µl. Nuclease free

water (PH 7-8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated.

8. After this step the PCR product becomes purified and ready for being sent for sequencing

9. The purified PCR products were loaded in the 1% (w/v) agarose gel and electrophoresed at 100 v for 40 minutes. The purified PCR products were observed under gel documenter (EZEE clearview UV Transilluminator) and photograph was taken immediately in GEL-DOC.

Analysis for molecular confirmation study

DNA quantification and sequencing

After purification, the purity and yield of the purified DNA was performed using Nanodrop spectrophotometer. Then the purified PCR products were sent to the Molecular laboratory of NIB for sequencing. DNA was sequenced bidirectional by using Commercial Sanger Sequencer and the results were received by Email.

Sequences analysis

The raw sequences were viewed by CHROMAS software. The unwanted and noise bases were deleted to obtain a better quality sequence. Then the sequences were transferred to FASTA file format.

Identification of samples and finding homology by BLAST search

Basic Local Alignment Search Tool (BLAST) is an algorithm that used to compare primary biological information, such as the amino-acid sequences of proteins or the nucleotides of DNA sequences. BLAST was also used for finding homology. The sequence was identified from the pre-existing data of the National Center for Biodiversity Information database (NCBI) to determine the highest homology. Nucleotide Basic Local Alignment Search Tool (BLAST) was used for searching similar sequence. Besides this was also compared with morphological features following Ahmed *et al.*2007, Encyclopedia of Flora and Fauna of Bangladesh, Volume-17.

GenBank submission

After identification of all the best match sequences, they were submitted to the GenBank through individual submission process for accession number.

Sequence alignment and genetic variation analysis

Sequence alignment was done in order to find the genetic variation among the individuals of the same species, to find the interspecies and intraspecies distance and to construct a phylogenetic tree. Multiple Sequence Comparison by Log-Expectation (MUSCLE) tool was used from the software Molecular Evolutionary Genetic Analysis (MEGA) 11. FASTA file (fas) containing multiple sequences were used to identify genetic variation among different individual specimens that belong to the same species. To identify the position of nucleotide variation, the sequence used in this study was aligned first with at least 3 different sequences of the same species using Mega11 software.

Phylogenetic tree construction in MEGA

After analyzing the sequences by using BLAST and FASTA tools and defining the best match homology, a phylogenetic tree was constructed. Neighbor joining trees of K2P distance was created to deliver a graphical illustration of the modelling of divergence between species (Saitou and Nei 1987). In the chosen group of parasites were performed in MEGA 11. All the data including taxonomic characteristics and GenBank accession number were tagged with the voucher specimens which were preserved at the Lab of National Institute of Biotechnology.

Statistical analysis

Necessary Statistical Analysis was performed by using Microsoft Excel 2013 software package. The output files of K2P distances were saved using this software.

CHAPTER 4.

DESCRIPTION OF HOST FISHES

Taxonomical Identification of Host Fish

Host: *Xenentodon cancila* (Hamilton, 1822)

Classification

Phylum: Chordata

Class: Osteichthyes

Order: Beloniformes

Family: Belonidae

Genus: *Xenentodon*

Species: *Xenentodon cancila* (Hamilton, 1822)

Synonyms

Esox cancila Hamilton, 1822, *Fishes of the Ganges*, p. 213;

Belone graii Sykes, 1841 Trans. *Zool. Soc. Lond*., p. 367;

Belone cancila Day, 1878, *Fishes of India*, p. 511;

Xenentodon cancila Shaw and Shebbeare, 1937, *Fishes of Northern Bengal*, p.108.

English names: Freshwater Garfish, Needle Fish, Silver Needle Fish

Local names: Kankila, Kaikya, Kakila

Taxonomic formula: D 15-16; P1 10-11; P2 6; A 17-18

Description

The body is very long and somewhat compressed. The eyes are tiny. The cheeks are elongated. A deep longitudinal furrow with the upper surface of the head. Both the jaws are produced into very long beaks, the lower being a little longer than the upper. A single row of sharp, needle-like and unequal teeth are there on each beak. Paired, slit-like nostrils at the anterior superior angle of the eyes are present. No spines are present in the fins. Dorsal fin is inserted opposite the origin of the anal fin in the posterior region of the body. Pectoral fins are small and inserted high up on the sides. Pelvic fins are abdominal. Caudal fin is truncate. Lateral line is present near the lower profile. It has small cycloid and deciduous scales. The color code is greenish above, flanks greenish-silvery and whitish below. A silvery lateral band with a dark margin runs along the side; a series of 4-5 blotches (not present in the young) on the sides between the pectoral and anal fins. Dorsal and anal fins have dark edges. Adult males and females differ morphologically. Mature males are easily identified by the presence of a red crest on the top of their heads. Males are slimmer than the females.

Habit and habitat

They are predators, feed on live beings and hostile. Their food are comprised of live fish, tadpoles, shrimp, crickets and other insects. They are usually found in rivers, ponds, canals, beels and flooded fields. They are frequently found in slow-flowing pools in rivers with rock or sand substrates. They are oviparous, lays eggs on the underside of leaves.

Distribution

Sri Lanka, India, Bangladesh, Myanmar, Thailand and Malaysia.

Economic Importance

An elegant surface-living freshwater pipe fish attaining an overall length of about 40 cm, it is of minor commercial significance. The major part of the catch is consumed locally and a small part is exported to the Middle East, Europe and America to meet the demand of Bangladeshi wage earners. It is also a potential aquarium fish.

Ecological role

It is a predator in the upper layer of the ecosystem.

Status and conservation

Obstruction of migratory routs from rivers to adjoining low-lying lands through construction of flood control, drainage and irrigation (FCDI) structures, compartmentalization of floodplain areas through unplanned construction of road networks, encroachment on floodplains for urbanization, agricultural farming, etc. have posed to be the potential threats. Establishment of sanctuaries and ban on fishing at strategic location, along with judicious enforcement of the Fish Conservation Acts are needed to conserve the Kakila population.

Remarks

Kakila is a beautiful pelagic freshwater fish having a nice taste. ‘Fried Kakila’ and ‘Kakila dopiaji’ are considered to be a delicacy in the diet of the Bangladeshi people.

Host: *Polynemus paradiseus* (Linnaeus, 1758)

Classification

Phylum: Chordata

Class: Osteichthyes

Order: Polynemiformes

Family: Polynemidae

Genus: *Polynemus*

Species: *P. paradiseus* (Linnaeus, 1758)

Synonyms

Polynemus aureus Hamilton, 1822, *Fishes of the Ganges*, p. 232;

Polynemus toposui Hamilton, 1822, *Fishes of the Ganges*, p. 381;

Polynemus longifilis Cuvier, 1829, *Hist. Nat.Poiss.* p. 365;

English names: Paradise Threadfin

Local names: Taposhi, Tapsi, Bairagi, Muni, Rishi

Taxonomic formula: D_1 VII; D_2 I/15-17; P_1 16-17+7; P_2 I/5; A II/12

Description

Head small with a projecting snout. Mouth large, cleft lateral. Preopercle serrated, having a produced and rounded angle. Villiform teeth in several rows on both jaws. Gillrakers fine, closely set, about 20 in the lower part of the first arch. Eyes with an adipose lid. Pectoral fins inserted high, upper part of its base at mid-depth of the body; 7-8 very long pectoral filaments about twice as long as the body. Lateral line continuous up to the caudal. Anterior portion of the lateral line forming a curve above the pectoral fin. Caudal peduncle elongate. Scales ctenoid along the lateral line, 5.5 rows between the lateral line and origin of the first dorsal. Fine scales over the vertical fins and somewhat. Pelvics thoracic. Caudal deeply lobed, upper lobe longer. Oblong and somewhat compressed fish with two dorsal fins. Body golden in colour, sometimes greenish back with a silvery belly. Fins greyish (Talwar and Jhingran 1991)

Habit and habitat

Predator; hunts macro-fauna. Feeds on benthic organisms, small fin fishes and shrimps. Inhabits shallow, sandy inshore areas; enters freshwaters during the breeding season. Seas, bays, gulfs and rivers. Abundant in the Meghna River near Chandpur and Hatiya.

Distribution

Arabian Sea, Bay of Bengal, Indian Ocean, Indonesian seas. Mekong River, South China Sea and Pacific Ocean. In Bangladesh, the species is distributed in the tidal rivers, estuaries and the Bay of Bengal.

Economic Importance

Threadfins contribute significantly to marine fish production in Bangladesh. In the Hoogly estuary in West Bengal, the species is next in importance to Hilsa fishery. Fishing is carried out in the estuaries with seine nets and lines. The peak fishing season is May-June. Bait fishing with live prawn and lure casting with minnows are other methods of fishing. The fish is a good source of cheap protein and iodine. The species is also sold after drying. It compensates iodine deficiency and protects people from goiter. *P. paradiseus* is a recent entry in the aquarium trade in UK and Thailand.

Ecological role

Takes part in the aquatic food chain and consume benthic organisms and small fishes and shrimps.

Status and conservation

Not considered as threatened in the Red List of IUCN Bangladesh (2000). The fish is harvested commercially without considering its stock, size and maturity. The catch of the species has declined in recent years because of overfishing. Since the fish contributes significantly to fish production, it is highly essential to conserve its stock.

Remarks

Maximum size recorded is 23 cm in total length (Rahman 2005). In allusion to the sparse chin whiskers worn by the Brahmin priests, the fish is called Muni, Rishi or Taposhi by the fishermen.

CHAPTER 5

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Identification and Description of the Helminth Parasites

During the study period for data collection, six hundred and forty two host specimens, belonging to two species, viz., *X. cancila* and *P. paradiseus* were examined. From these 642 fish specimens, 390 helminth parasites were collected. Altogether seventeen helminth species representing four major helminth groups, viz., Trematoda, Cestoda, Nematoda and Acanthocephala were recorded from the two hosts' collection.

List of helminth parasites collected from the host fishes

Host: *Xenentodon cancila*

Trematoda

Bolbocephalus sp. Dubois, 1934
Isoparorchis hypselobagri Billet, 1898

Nematoda

Gnathostoma spinigerum (L_3 larva) Owen, 1836
Metaquimperia bagarii Karve, 1941
Camallanus ophiocephali Pearse, 1933
Porrecaecum trichuri. Railliet et Henry, 1912

Acanthocephala

Neoechinorhynchus prolixum Van Cleave et Timons, 1952
Acanthocentis nigeriensis Dollfus et Golvan, 1956
Pallisentis ophiocephali Thapar, 1930

Host 2: *Polynemus paradiseus*

Trematoda

Hypohepaticola callionymi Yamaguti, 1934

Prosogonotrema bilabiatum Perez Vigueras, 1940

Thaparotrema vittalani Dayal et Gupta, 1954

Uterovesiculurus hamati Yamaguti, 1934

Cestode

Nybelinia lingualis Cuvier, 1817

Parachristianella trygonis Dollfus 1946

Nematoda

Dujardinascaris sp. (L_4 larva) Baylis, 1947

Metaquimperia bagarii Karve, 1941

Acanthocephala

Neorhadinorhynchus aspinosum Fukui et Morisita, 1937

Pallisentis ophiocephali Thapar, 1930

Taxonomical Identification, brief description and illustration of the parasites

Bolbocephalus sp. Dubois, 1934

Description of the parasite

Phylum: Platyhelminthes

Class: Trematoda

Order: Digenea Van Beneden, 1858

Family: Strigeidae Railliet, 1919

Genus: *Bolbocephalus* Dubois, 1934

Species**:** *Bolbocephalus sp.* Dubois, 1934

This species was collected from the host's intestine of *Xenentodon cancia*. Three hundred twenty-one specimens were examined of which 14 specimens' harboured 16 individuals of *Bolbocephalus sp*.

Description: (Plate-1, Photograph-1)

Forebody bulbiform, penetrable into intestinal wall of host, with cup-shaped thickening at base. Hindbody conical or ovoid, well constricted off from cupule. No oral sucker. Digestive tract atrophied. Pharynx poorly developed. Acetabulum present in front of tribocytic organ which is transversely elongated in front of a two-lipped ventral lobe. Anterior testis asymmetrically developed, lateral; posterior testis larger, bilobed, curved in form of a horse-shoe, excavated ventrally. Vesicula seminalis winding ventral and posterior to hind testis; hermaphroditic duct short, opening at tip of muscular genital cone. Bursa copulatrix occupied for most part by genital cone, may be evaginable through wide terminal pore. Overy opposite anterior testis. Uterus coiled in median field ventral to overy and testis; eggs large, numerous. Vitellaria confined to equatorial cupule. Anterior extremity: 0.39-0.40 mm; seminal vesicle: 0.36-0.52 mm; eggs: 0.018-0.029 mm by 0.013-0.021 mm; number of vitelline glands on the left: 14-18 mm, right: 12-16 mm.

Remarks

In Bangladesh, metacercaria of *Bolbocephalus sp.* was first recorded by Sharmin *et al.* (2003) from the intestine of *Xenentodon cancila* of Chandpur. But this is the first description of *Bolbocephalus sp.* from *X. cancila* in Bangladesh. And having similarity of the the adult worm. This genus is preoccupied and as a synonym of *Bolbocephaloides* (Strand 1935)

Isoparorchis hypselobagri Billet, 1898

Order: Digenea Van Beneden, 1858

Family: Isoparorchiidae Poche, 1926

Genus: *Isoparorchis* Southwell, 1913

Species**:** *Isoparorchis hypselobagri* Billet, 1898

Twenty-one specimens were collected from the intestine and body cavity of *Xenentodon cancila*. Three hundred Twenty-one specimens were examined of which 13 specimens' harboured 15 individuals of *Isoparorchis hypselobagri*.

Description: (Plate-1, Photograph-2)

Body aspinose, thick and elongated, anterior end being more attenuated than posterior end. Body1.7-1.94 mm long, 0.84-0.8 mm wide. Anterior sucker, sub-terminal, spherical 0.2-0.24 mm. Ventral sucker much larger than oral sucker, spherical, 0.31-0.43 mm long, 0.34-0.40 mm wide, at 0.5-0.53 mm from anterior extremity. Pre-pharynx, oval, well developed. Oesophagus short, tubular, 0.04-0.08 mm long, 0.08-0.11 mm wide. Oesophagus ran into intestinal caeca. Intestinal caeca, broad, and appeared yellow or brown with the contained food matters. It ran from oesophagus up to posterior end of body. Testes two, rounded, anterior testes were larger than posterior one. Vesicula seminalis was continued into a short ejaculatory but enclosed in the so-called "Sinus sac" of Manter (1936). Genital pore was median and present just below the intestinal bifurcation. Ovary was present on the right side in oval shape structure in the hind region of the body in front of excretory bladder. A small oval shaped Receptaculum seminis was present. Vitellaria were in the incipient stage of development were represented by dark staining cells in front excretory bladder. Excretory bladder was cylindrical shaped between intestinal caeca and leads to the outside by a terminal excretory pore.

Remarks

The present specimens closely resembled with *I. hypselobagri* (Billet 1898) found in *Mystus vittatus*, *Ompok bimaculatus* of Bangladesh (Khanum 1994).

Prosogonotrema bilabiatum Perez vigueras 1940

Order: Digenea Van Beneden, 1858

Family: Prosogonotrematidae Perez Vigueras, 1940

Genus: *Prosogonotrema* Perez vigueras 1940

Species: *Prosogonotrema bilabiatum* Perez vigueras 1940

This parasite was taken from the stomach and intestine of *Polynemus paradiseus*. Three hundred twenty-one specimens were examined of which 37 specimens' harboured 38 individuals of *Prosogonotrema bilabiatum*.

Description: (Plate-2, Photograph-3)

The description of the parasite was based on the measurements (in mm) of thirty-eight specimens. Body 4.6-6.2 mm long, 1.3-1.5 mm wide. Acetabulum 749-1085 by 805-1115. Oral sucker 309-425 by 405-514 spherical, subterminal; pre-oral lobe 70-105 wide. Pharynx 185-215 by 211-285, sub-globular, over lapped by oral sucker. Esophagus is enlarged. Caeca arises from the surface of esophageal swelling, wide, reaching posterior portion of body. Testes 274.8-3.01 by 3.00-3.45 about midway between suckers. Ovary 290-364 by 274-400, globuler and pre-acetabular. Vitellaria seven long thin winding tubules, intercaecal, and uterine seminal receptacle.

Remarks

Four species of Prosogonotrema have been explained, viz. *P. bilabiatum* redescribed by Manter (1969), *P. clupeae* Yamaguti (1952), *P. carangi* Velasquez, 1961 and *P. subeequilatum* Pritchard (1963). *P. bilabiatum* is different from all the other species of *Prosogonotrema* in smaller sucker ratio. Its vitelline tubules never reached testicular area and its acetabulum is location more posteriorly. It also varies in the pre-acetabular location of ovary. In 2008, Latifa *et al.* found the infection of *Prosogonotrema bilabiatum* in the same host. The present specimens are absolutely similar in all morphological details.

Uterovesiculurus hamati Yamaguti 1934

Order: Digenea Van Beneden, 1858

Family: Hemiuridae Luhe, 1901

Genus: *Uterovesiculurus* Skrjabin et Guschanskaja, 1954

Species: *Uterovesiculurus hamati* Yamaguti 1934

The parasite was collected from the stomach and intestine of the fish. Out of 158 infected specimens of *Polynemus paradiseus* 32 specimens harbored 35 individuals of *Uterovesiculurus hamati.*

Description: (Plate-2, Photograph-4)

The description was based on the measurement (in mm) of thirty-five specimens. Total body length 6.70 mm long. Oral sucker terminal, sub-spherical, 0.35mm long, 0.41 mm wide. Caeca is extended up to posterior end of the body. Ventral sucker is sub-spherical in middle-third of the body, larger than oral sucker, 1.06 mm long, 0.80 wide. Testes spherical, sub-equal, diagonally tandem and well separated from each other. Right testes 0.37 mm and left testes slightly smaller, 0.31 mm long 0.41 mm in diameter. Ovary post-tentacular, 0.39 mm long and 0.46 mm wide. Vitellaria consisting of seven tubular lobes, with four on right side and three on left side. Uterus coiled, ascending anteriorly, distal end abruptly inflated forming a saccular uterine swelling. Eggs 0.010-0.015 mm x 0.005-0.009 mm in size.

Remarks

U. hamati was first described from marine fish. The present specimen differs from *U. indica, U. trichiurusi, U. puriensis*. In *U. indica* vesicula seminalis extends to the anterior margin of ventral sucker. But, in this species vesicular seminalis extend up to the anteriormargin of the ovary. The present specimens are absolutely similar in all morphological details with *Prosogonotrema bilabiatum* found in the same host (Latifa *et al.* 2008).

Thaparotrema vittalani Gupta 1954

Order: Digenea Van Beneden, 1858

Family: Opisthorchiidae Braun, 1901

Genus: *Thaparotrema* Dayal et Gupta, 1954

Species: *Thaparotrema vittalani* Dayal et Gupta 1954

The parasite was collected from the stomach and intestine of *Polynemus paradiseus*. Three Hundred Twenty-one specimens were examined of which 24 specimens' harbored 27 individuals of *T. vittalani.*

Description: (Plate-3, Photograph-5)

Long, dorsoventrally flattened, with narrower anterior and broader posterior region, the body covered with tiny spines, length of the worm varies from 2.32-4.00 mm and the breadth is from 0.46-0.77 mm. in the region of the ovary. The oral sucker sub-terminal and measured 0.21 mm and ventral sucker 0.14 mm by 0.16 mm. Genital pore median. Testes with irregular margins, tandem, inter-caecal in position in the posterior fourth of the body, measure 0.17 mm by 0.29 mm in size. The ovary 0.13 mm by 0.14 mm. The uterus arises from posterior side of the ootype and extends anteriorly forming intercaecal transverse coils between the ovary and the ventral sucker. The eggs are oval, measuring 0.07-0.1 mm by 0.04-0.06 mm in size.

Remarks

The new form as appear from the description belonging to the family opisthorchiidae (Braun 1901). It shows relationships to the sub-families Metorchiinae Luhe (1990). Ratzininae Dolfus 1929 in the extension of vitelline glands to ventral sucker but differ from Metorchiinae in having uterine coils posterior to ventral sucker but differ from Ratziinae in the absence of a rudimentary cirrus pouch. It was, therefore, placed in the subfamily Opisthorchinae Loose, 1899. It closely resembles the genus Opisthorchis Blanchard 1895 in the general topography of organs, but differs from it and other known genera of the subfamily Opisthorchiinae in the extension of vitelline glands anterior to ventral sucker and up to hinder end of the anterior testes. In 2008, Latifa *et al.* found the infection of *Prosogonotrema bilabiatum* in the same host. The present specimens are absolutely similar in all morphological details.

Hypohepaticola callionymi Yamaguti 1934

Order: Digenea Van Beneden, 1858

Family: Hemiuridae, Luhe, 1901

Genus: *Hypohepaticola* Yamaguti, 1934

Species: *Hypohepaticola callionymi* Yamaguti 1934

The parasite was collected from the stomach and intestine of *Polynemus paradiseus*. Three Hundred Twenty-one specimens were examined. Out of 158 infected specimens of *Polynemus paradiseus* 15 specimens' harbored 17 individuals of *Hypohepaticola callionymi.*

Description: (Plate-3, Photograph-6)

Body small, fusiform and measuring 1.78-2.30 mm in length and 0.81-1.02 mm wide. The oral sucker is oval and measures 0.12-0.15 x 0.15-0.18 mm. Acetabulum larger than the oral sucker and measures 0.23-0.31 x 0.24-0.32mm in size. Testes round and equatorial, and equal in size, measures 0.18-0.21 x 0.17-0.20 mm. Ovary more or less oval in shape, post acetabular in position and larger than the testes. Measured 0.22- 0.34 x 0.21-0.28mm. Eggs are small, numerous, elliptical in shape and measures 0.024-0.038 x 0.012-0.026 mm.

Remarks

This species was originally found under the connective tissue membranes of the liver. It has been recorded by Yamaguti (1942) from the stomach of *Callionymus valenciennesi* and from the intestine of *Monacanthus cirrhifer*, Yamaguti states that" the proper location of the worm may be stomach". Gibson and Bray (1979) gave a concept of *Hypohepaticola.* They viewed the genus as morphologically similar to the Lecithochiriinae, differing fundamentally according to the originally description, only in the apparent absence of an ecsoma and the presence of filamented eggs. The present specimens are absolutely similar with *Hypohepaticola callionymi* in all morphological details which was also observed by Latifa *et al.*2008 from the same host fish.

Nybelinia lingualis Cuvier 1817

Phylum: Platyhelminthes

Class: Cestoda

Order: Trypanorhyncha Diesing, 1863

Family: Tentaculariidae Poche, 1926

Genus: *Nybelinia* Poche, 1926

Species: *Nybelinia lingualis* Cuvier 1817

The parasite was collected from the intestine and liver of *Polynemus paradiseus*. Three Hundred Twenty-one specimens were examined. Out of 158 infected specimens of *Polynemus paradiseus* 10 specimens' harbored 10 individuals of *Nybelinia lingualis*

Description: (Plate-4, Photograph-1)

The description of the parasite is based on the measurements (in mm) of ten specimens. The worms up to 1.18 mm in length and 0.97 mm in breadth. The scolex measures 0.60-0.82 mm. Each of the four bothridia measures from 798 to 898 µ long and their posterior extremities lie over the centre of the proboscies sac. The proboscis short. Pars bulbosa overlapping pars bothridialis. Vellum well developed. Testes numerous. Cirrus pouch muscular and long. Seminal vesicle present. Genital pores ventromarginal. Ovary "X" shaped in cross section. Vitellaria encircling testes. Uterus occupying most of central testicular zone. Eggs sub-globular and small.

Remarks

The species *N. lingualis* was discovered by Cuvier 1817 from teleost fishes. Dollfus and Baer (1936) also described this parasite from the fish. In India Radhakrisnan and Nair (1981) described this species from *Diodon histrix*. In Bangladesh, *N. lingualis* was first recorded by Latifa *et al.* (2008) from Threadfin fish. Present specimen resembles *N. lingualis* in shape size, position of genital pore. Ovary testes and other body structures. However, due to major points of resemblance, the present specimen is placed under the species *N. lingualis* Cuvier 1817.

Parachristianella trygonis Dollfus

Order: Trypanorhyncha Diesing, 1863

Family: Eutetrarhynchidae Guiart, 1927

Genus: *Parachristianella* Dollfus, 1946

Species**:** *Parachristianella trygonis* Dollfus 1946

The parasite was collected from the intestine and liver of *Polynemus paradiseus*. Three Hundred Twenty-one specimens were examined of which 6 specimens' harboured 7 individuals of *Parachristianella trygonis*.

Description: (Plate-4, Photograph-2)

Two bothridia, proboscis with hetermorphic aramature, each obliquely ascending row of metabasal hooks, starting from middle of internal face as a large triangular hook with recovered point, terminating in small hooks on external face. Plerocercoid is very complex in structure. Body divided into two regions. The total body length 2.21-2.41 mm, fore body measure 1-1.04 x 0.53-0.6 mm and hind body in 1.21-1.37 x 0.01-1.04 mm. The alimentary tract located within the fore body. Proboscis well developed and consists of hooks. Indistinct demarcation line of proglottids noticed, distinct neck region present.

Remarks

In 2008, Latifa *et al*. found the infection of *Parachristianella trygonis* in the same host. The present specimens are absolutely similar with all morphological details of *Parachristianella trygonis.*

Gnathostoma spinigerum Owen, 1836

Phylum: Platyhelminthes

Class: Nematoda

Order: Spiruridea Diesing, 1861

Family: Gnathostomatidae Lane, 1923

Genus: *Gnathostoma* Owen, 1836

Species**:** *Gnathostoma spinigerum* Owen, 1836

The parasite was collected from the intestine of the fish. Out of 192 infected specimens of *X. cancila* 16 specimens' harbored 18 individuals of *G. spinigerum.*

Description: (Plate-5, Photograph-1)

Twelve specimens of 3rd stage larvae (Cheng 1964) of this type were collected from the intestine and stomach and six encysted 3rd stage larvae from the body cavity and swim bladder. Head bulb prominent, containing four rows of hooks. Esophagus long, four elongate cervical glands present. Valva is at the posterior portion of the body. Tail blunt and short. Body length: 2.53-3.07 mm; body width: 0.25-0.44 mm; head bulb length and breadth: 0.15-0.17 mm by 0.08-0.17 mm; esophagus length: 0.94-1.51 mm.

Remarks

The present larval form resembles *G. spinigerum* for the armed body and head bulb, position of vulva, cuticular spines and cervical glands. Therefore, the present form is identified as *G. spinigerum*. It was recorded for the first time in the intestine of *X. cancila* from Chandpur (Sharmin *et al*. 2003).

Dujardinascaris sp. Baylis 1947

Phylum: Platyhelminthes

Class: Nematoda

Order: Ascarididea Henry, 1915

Family: Heterocheilidae Railliet et Henry, 1915

Genus: *Dujardinascaris* Baylis, 1947

Species: *Dujardinascaris sp*. Baylis 1947

The parasite was collected from the stomach, intestine and liver of *Polynemus paradiseus*. Three Hundred Twenty-one specimens were examined of which 8 specimens' harboured 9 individuals of *Dujardinascaris sp.*

Description: (Plate-5, Photograph-2)

The description of the parasite was based on the measurement (in mm) of ten specimens. It was a fourth stage larva of *Dujardinascaris.* The length 2.06-3.21 mm.

Thickness was 0.15-0.17 mm. A pointed tooth present at the mouth. The esophagus including bulb measures 0.53-0.61 mm. The esophagus bulb is 0.12-0.14 mm in size. Anteriorly directed intestinal caecum is 0.23-0.26 mm long in male and 0.21-0.25 mm in female. Tail slender with pointed cuticular ring shaped striation.

Remarks

Baylis and Daubney (1922) was the first to study the larval forms of *Dujardinascaris sp*. having rounded anterior end, oval globular ventriculus and large caecum in the mesenteries of a fish of *Lates spp*. from Africa. The present author also received fourth stage larval forms of *Dujardinascaris* from estuarine fish *P. paradiseus*. It was recorded

for the first time in the stomach, intestine and liver of *Polynemus paradiseus* from Chandpur (Latifa *et al.* 2008).

Metaquimperia bagarii Karve, 1941

Order: Ascarididea Henry, 1915

Family: Quimperiidae Baylis, 1930

Genus: *Metaquimperia* Karve, 1941

Species: *Metaquimperia bagarii* Karve, 1941

The parasite was collected from the intestine, rectum and liver of *X. cancila*. Out of 192 infected specimens of *X. cancila* 14 specimens' harbored 16 individuals of *M. bagarii.* Three Hundred Twenty-one specimens of *P. paradiseus* were examined of which 10 specimens' harboured 11 individuals of *M. bagarii*. The parasite was collected from the stomach, intestine and liver of *P. paradiseus*.

Description: (Plate-6, Photograph-5)

Male. Body 6.4-7.0 long, 0.13-0.15 wide; lips three, one dorsal, two subventral; cephalic papillae one pair; teeth 3, 1 dorsal, 2 sub-ventral; lateral alae starting from head, broadening at level of or a little behind cervical papillae, then narrowing behind oesophagus and ending in front of cloacal opening; oesophagus divided into two parts, anterior muscular 0.22-0.26 long, posterior glandular 0.39-0.4; cervical papillae 0.3-0.45 from anterior end; excretory pore not discernible; tail 0.16-0.19 long, with fairly

well developed caudal alae, and stout spike ending in small spine; preanal sucker present; spicules alate, sickle-shaped, equal, 0.08-0.095 long; gubernaculum present; caudal papillae 13 pairs, 4 preanal, 1 adanal, 8 postanal; two median papillae, one in front of cloacal opening, other at base of caudal spike.

Female. Body 6.7-7.05 long, 0.15 wide; head and cervical alae as in male; oesophagus, anterior muscular 0.23-0.25 long, posterior glandular 0.38-0.41; cervical papillae 0.41-0.5 from anterior end; tail 0.24-0.27 long, with small spine, and pair of small papillae just in front of tip; vulva post-equatorial.

Remarks

The species described by Karve (1941) from *Bagarius bagarius* from Pune, Maharashtra, and separated from the same locality (Intestine and rectum), by having semi-globular lips, a comparatively slender oesophagus, two median papillae in male, smaller spicules, and a gubernaculum with coiled chitinous band attached to it. Khanum *et al*. 1989 recorded *M. bagarii* in the intestine of *X. cancila*. But it was observed first time in the the stomach, intestine and liver of *P. paradiseus*. The species collected from the both host fishes resemble *Metaquimperia bagarii* Karve, 1941 in all morphological details.

Camallanus ophiocephali Pearse, 1933

Order: Spiruridea Diesing, 1861

Family: Camallanidae Railliet el Henry, 1915

Genus: *Camallanus* Railliet et Henry, 1915

Species: *Camallanus ophiocephali* Pearse, 1933

The parasite was collected from the intestine and body cavity of *X. cancila*. Out of 192 infected specimens of *X. cancila* 11 specimens' harbored 13 individuals of *C. ophiocephali.*

Description: (Plate-6, Photograph-4)

Body slender, filiform and blood red in live. The buccal capsule consists of two valves. The buccal valves are situated at the junction of the valves with the esophagus. The esophagus is divided into two portions, the anterior musculature and posterior glandular

part. Reduced tridents were also observed. All female specimens were recovered and agrees well with the description of Yamaguti (1961). The female measures 7.80 mm in length, the maximum breadth being 0.238 mm. The tail is conical. Vulva about middle of body; uteri opposed; posterior overy lacking in female the chitinous ring measures 0.116 mm in length and 0.108 mm in breadth. The muscular esophagus is 0.680 mm and glandular esophagus measures 0.843 mm in length.

Remarks

This species was also collected and recorded by Sharmin *et al*. (2003) from the same host, *Xenentodon cancila*. The two collections of specimens are absolutely similar in all details.

Porrocaecum trichuri Railliet et Henry, 1912

The parasite was collected from the intestine, rectum, body cavity and liver of the fish. Out of 192 infected specimens of *X. cancila* 13 specimens' harbored 15 individuals of *Porrocaecum trichuri.*

Order: Ascaridídea Henry, 1915
Family: Heterocheilidae Railliet et Henry, 1915
Genus: *Porrocaecum* Railliet el Henry, 1912
Species: *Porrocaecum trichuri* Railliet el Henry, 1912

Description: (Plate-5, Photograph-3)

Body long, cylindrical, tapering towards both extremities. Mouth surrounded by three lips with dentingerus ridges, dorsal one bearing two large papillae, two subventral each bearing two small papillae. Interlabia present, intestinal caecum well developed. Cuticle is thick and prominent cuticular striations are present. Vulva situated in the anterior third to middle of the body. Esophagus oblong with ventriculus. Nerve ring present and excretory pore to posterior end of the body. Tail bluntly conical. Body length: 13.3-15.2 mm; breadth: 0.37-0.56 mm; length of esophagus: 1.85-2.26 mm and tail length: 0.17-0.03 mm.

Remarks

This specimen showed resemblance with *Porrocaecum trichuri* found in *Polydactylus indicus* (Arthur & Ahmed, 2002). This species was also collected and recorded by Sharmin *et al*. (2003) from the same host, *Xenentodon cancila*. The two collections of specimens are absolutely similar in all details.

Neorhadinorhynchus aspinosum Fukui et Morisita, 1937

Phylum: Platyhelminthes

Class: Acanthocephala

Order: Echinorhyncidea Southwell et Macfie, 1925

Family: Echinorhynchidae Cobbold, 1879, emend.

Genus: *Neorhadinorhynchus* Yamaguti, 1939

Species: *Neorhadinorhynchus aspinosum* Fukui et Morisita, 1937

The parasite was collected from the intestine of *P. paradiseus*. Out of 158 infected specimens of *P. paradiseus* 10 specimens harbored 11 individuals of *N. aspinosum.*

Description: (Plate-8, Photograph-4)

The parasite was collected from the stomach and intestine of the fish.

The description of the parasite was based on the measurement of three species ten species. Body elongated, cylindrical, spinose with tapering extremities. Anterior trunk spines arranged in two groups of which anterior spines encircles the body. Proboscis elongated, cylindrical with 12-14 longitudinal rows of 24-26 hook per row. Neck short, lemnisci clavi form lying close to proboscis. Genital pore terminal. Male: Body 7.85 mm longy, 0.60 mm wide. Proboscis 1.60 mm long, 0.22 mm wide. Proboscis hook from anterior region to base 0.035- 0.042 mm long field.

Female: Body 6.8-6.95 mm long, 0.50- 0.58 mm wide. Anterior trunk spines 6-8 circles of 4-5 spines in one and 3-4 spines in second field extending posteriorly. Proboscis receptacle 1.85 mm to 2.00 mm long. Uterine bell elongated tubular vaginal muscular with genital sphincter. Egg elongated three shelled, with polar prolongation of middle shell.

Remarks

The species was also collected and recorded by by Latifa *et al.* (2008) from the same host, *P. paradiseus*. The two collections of specimens are absolutely similar in all details.

Neoechinorhynchus prolixum Van Cieave et Timons, 1952

Order: Neoechinorhyncidea Southwell et Macfie, 1925

Family: Neoechinorhynchidae Van Cleave, 1919

Genus: *Neoechinorhynchus* Hamann, 1892

Species: *Neoechinorhynchus prolixum* Van Cleave et Timons, 1952

This species was recovered from the intestine and rectum of the host, *Xenentodon cancila*. Three hundred Twenty-one specimens were examined of which 35 specimens' harboured 39 individuals of *Neoechinorhynchus prolixum*

Description: (Plate-7, Photograph-1)

Male: Body stout, aspinose rounded anteriorly, in all the specimens' proboscis and associated structures were very clear but inside the body. Posterior region relatively narrow with flat posterior end. Body length 3.45–4.84, width 0.75–0.79, with greater width behind proboscis. Proboscis globular 0.56–0.57 × 0.24–0.26 in size with three circles of hooks six in each row. Anterior circle has large hooks, middle and posterior rows of hooks become progressively smaller. Proboscis receptacle muscular double-walled small as compared to body size. Testes in posterior half of body, large, 0.80–1.0 in length, 0.39–0.45. Cement gland swollen anteriorly and elongated posteriorly. Saeftigen's pouch also rounded anteriorly. Two bursal glands are present anterior to bursa. Bursa well developed with three bursal ray.

Remarks

The species was recorded for the first time in the intestine of *X. cancila* and resemble *Neoechinorhynchus prolixum* Van Cleave et Timons 1952 in all morphological details.

Acanthosentis nigeriensis Dollfus et Golvan, 1956

Order: Neoechinorhyncidea Southwell et Macfie, 1925

Family: Quadrigyridae Van Cleave, 1920

Genus: *Acanthosentis* Verma et Datta, 1929

Species: *Acanthosentis nigeriensis* Dollfus et Golvan, 1956

The parasite was collected from intestine, rectum and body cavity of the fish. Out of 192 infected specimens of *X. cancila* 32 specimens harbored 36 individuals of *A. nigariensis*

Description: (Plate-7, Photograph-2)

The ovoid shape of male and elongate shape of females. The arrangement of anterior proboscis hooks at alternative levels and not in a straight circle. Trunk is small, cylindrical, tapering posteriorly, 0.96 × 0.26 mm, with 8 prominent giant hypodermal nuclei: 6 dorsal and 2 ventrals. Rosethorn-shaped cuticular spines, 7-10 long, with discoid base cover entire trunk in 25 circles, with 20-22 spines per complete circle anteriorly. Circles of spines more widely spaced and with slightly fewer spines posteriorly, incomplete dorsally. Posteriormost spines directed anteriorly, never reaching posterior tip of trunk. Proboscis cylindrical, plump in middle, longer than wide, 100 × 75. Anterior, middle and posterior hooks 50, 40 and 25 long, respectively. Proboscis hooks slender, with simple, short roots. Roots of anterior hooks narrow posteriorly; those of middle and posterior hooks truncate posteriorly. Two lateral proboscis hooks in first and middle circle displaced posteriorly, but do not differ in size from 2 dorsal and 2 ventral hooks of each circle. Proboscis receptacle with cerebral ganglion at its base. Lemnisci ribbon-shaped, long, subequal, multibranched, reaching anterior testis. Reproductive system in posterior half of trunk. Testes ovoid, overlapping; anterior testis larger than posterior testis.

Remarks

The species was recorded for the first time in the intestine, rectum and body cavity of *Xenentodon cancila* and resemble *Acanthosentis nigeriensis* Dollfus et Golvan, 1956 in all morphological details. Golvan in 1959 divided the genus *Acanthogyrus* into two subgenera: *Acanthogyrus* and *Acanthosentis* based on the number of proboscis hooks

18 (3 circles of 6 hooks each) in *Acanthosentis* and 24 (3 circles of 8 hooks each) in *Acanthogyrus*.

Pallisentis ophiocephali (Thapar, 1930)

Order: Neoechinorhyncidea Southwell et Macfie, 1925

Family: Quadrigyridae Van Cleave, 1920

Genus: *Pallisentis* Van Cleave, 1928

Species: *Pallisentis ophiocephali* Thapar, 1930

The parasite was collected from the intestine and rectum of *X. cancila*. Out of 192 infected specimens of *X. cancila* 44 specimens' harbored 50 individuals of *P. ophiocephali*. Three Hundred Twenty-one specimens of *P. paradiseus* were examined of which 6 specimens' harboured 7 individuals of *P. ophiocephali*. The parasite was collected from the intestine of the host.

Description: (Plate-8, Photograph-3)

The proboscis hooks are arranged in four circles and the size length of the hooks diminished gradually from 1st to 4th circle. Vulva placed posterioventrally. A long tube opens into the vagina and continued into the basal portion of the funnel shaped uterine bell. Body length: 7.01-8.39 mm; body breadth: 0.36-0.58 mm; proboscis length and breadth: 0.48-0.54 mm by 0.13-0.15 mm; hooks length: 1st circle: 0.08-0.07 mm, 2nd circle: 0.081-0.084 mm, 3rd circle: 0.064-0.07 mm, 4th circle: 0.034-0.04 mm.

Remarks

The present specimens were collected from both the host fish *X. cancila* and *P. paradiseus.* Bashirullah 1973 and Khanum *et al*. 1989 collected and recorded *Pallisentis ophiocephali* in the intestine of *X. cancila*. But it was observed first time in the the intestine and rectum of *P. paradiseus*. The two collections of specimens are absolutely similar in all details.

The communities of parasites in *Xenentodon cancila* and *Polynemus paradiseus*

The present investigation was done on two species of fish, *Polynemus paradiseus* and *Xenentodon cancila*. Out of 321 *P. paradiseus*, 158 were infected (prevalence-49%) and out of 321 *X. cancila*, 192 were infected (prevalence-60%) with different helminth parasites. In the present study, ten species of parasites from different helminth groups were collected and identified from *P. paradiseus*, while nine species of parasites were collected from *X. Cancila*. The mean intensity of parasites at per infected *P. paradiseus* was 1.09 and in X. cancila was 1.14 (Table-3).

Prevalence of infestation in male *P. paradiseus* and *X. cancila* were 46.62% and 47.75% while, in female *P. paradiseus* and *X. cancila* were 51.45% and 74.83% respectively. Intensity of parasites in male *P. paradiseus* and *X. cancila* were 1.10 and 1.11. On the other hand, Intensity of parasites in female *P. paradiseus* and *X. cancila* were 1.08 and 1.16 (Table- 3).

Table-3: **Diversity of endo-parasites in *X. cancila* and *P. paradiseus***

Factors	***Xenentodon cancila***	***Polynemus paradiseus***
Name of fish examined	321	321
Name of fish infected	192	158
Prevalence of infestation	59.81%	49.22%
Total number of parasites Collected from host fish	218	172
Total number of parasite species Collected from host fishes	12	10
Mean intensity per infected fish	1.14	1.09
Prevalence of infestation in Male	47.75%	46.62%
Prevalence of infestation in female	74.83%	51.45%
Mean intensity of parasites in Male	1.11	1.10
Mean intensity of parasites in Female	1.16	1.08

During Jan'17-Dec'17, a total of 161 *P. paradiseus* were examined and among them 85 were infected, the prevalence of infestation was 52.80% and the mean intensity of

the parasites was 1.08. In the next year (Jan'18-Dec'18), 160 numbers of host fishes were examined and 73 hosts were infected, the prevalence and the mean intensity of the parasites was 45.63 % and 1.10.

In case of *X. cancila*, (Jan'17-Dec'17), a total of 160 fishes were examined and among them 91 were infected, the prevalence of infestation was 56.88% and the mean intensity of the parasites was 1.17 . In the next year (Jan'18-Dec'18), 161 numbers of host fishes were examined and 101 hosts were infected, the prevalence and the mean intensity of parasite were observed 62.73 % and 1.11

In *P. paradiseus* among the ten endo-parasites species, four belong to Trematoda (*Prosogonotrema bilabiatum, Uterovesiculurus hamali, Thaparotrema vittalani, Hypohepaticola callionymi*,); two belong to Nematoda (*Dujardinascaris* sp., *Metaquimperia bagarii*); two belong to Cestoda (*Nybelinia lingualis, Parachristianella trygonis*) and two belong to Acanthocephala (*Neorhadinorhynchus aspinosum, Pallisentis ophiocephali* (Table- 4).

Table-4: List of the parasites collected from *Xenentodon cancila* and *Polynemus paradiseus* during the study period (Jan'17-Dec'18)

Group	***X. cancila***	***P. paradiseus***
Trematoda	*Bolbocephalus sp*	*Prosogonotrema bilabiatum*
	Isoparorchis hypselobagri	*Uterovesiculurus hamati*
		Thaparotrema vittalani
		Hypohepaticola callionymi
Cestoda		*Nybelinia lingualis*
		Parachristianella trygonis
Nematoda	*Metaquimperia bagarii*	*Dujardinascaris sp.* (L_4 larva)
	Gnathostoma spinigerum (L_3 larva)	*Metaquimperia bagarii*
	Camallanus ophiocephali	
	Porrocaecum trichuri	
Acanthocephala	*Neoechinorhynchus prolixum*	*Neorhadinorhynchus aspinosum*
	Acanthosentis nigeriensis	*Pallisentis ophiocephali*
	Pallisentis ophiocephali	

In *X. cancila*, nine species of endo-parasites were collected; among them two were from Trematoda (*Bolbocephalus sp*, *Isoparorchis hypselobagri* sp.) four from Nematoda (*M. bagarii*, *Gnathostoma spinigerum*, *Camallanus ophicephali*, *Porrocaecum sp.*) and three from Acanthocephala (*Neoechinorhynchus prolixum*, *Acanthosentis nigeriensis* and *Pallisentis ophiocephali*) (Table- 4).

Table-5: Analysis of endo-parasites in *Xenentodon cancila* and *Polynemus paradiseus*

Host fish	**2017**	**2018**	**P-value**
X. cancila			
Prevalence (%)	56.88%	62.73%	0.7039
Intensity of parasites	1.17	1.11	
P. paradiseus			
Prevalence (%)	52.80%	45.63%	0.2851
Intensity of parasites	1.08	1.10	

Table-5 shows the result of "two samples propotion test". In case of "propotion test", the overall proportion of infected *P. paradiseus* does not differs significantly at 5% level of significance between two periods 2017 and 2018. Similar trend was shown in case of *X. cancila*.

Percentage of helminth parasites in *X. cancila* and *P. paradiseus*

In the present investigation, trematodes and nematodes were found to be dominant among the parasites (4 trematodes and 2 nematodes) in *P. paradise;* nematodes (4) and acanthocephalan (3) were found to be dominant in *X. cancila*, (Table-6). The trematodes were 68.02% and nematodes were 11.63% of the total number of parasites recovered from *P. paradiseus*. On the other hand, the nematodes were 28.44% and acanthocephalans were 57.33 % of the total number of parasites recovered from *X. cancila* (Table-6).

Table-6: Prevalence and intensity of parasites in *P. paradiseus* and *X. cancila*

Parasite groups	Parasite species	*P. paradiseus*		*X. cancila*	
		No. of hosts infected	Prevalence of infestation (%) & Mean Intensity	No. of hosts infected	Prevalence of infestation (%) & Mean Intensity
Trematoda	*Bolbocephalus sp.*			14	7.29%, 1.14
	Isoparorchis hypselobagri			13	6.77%, 1.15
	Prosogonotrema bilabiatum	37	23.41%, 1.03	-	-
	Uterovesiculurus hamati	32	20.25%, 1.09	-	-
	Thaparotrema vittalani	24	15.18%, 1.13	-	-
	Hypohepaticola callionymi	15	10%, 1.13	-	-
Cestoda	*Nybelinia lingualis*	10	6.32%, 1	-	-
	Parachristianella trygonis	06	4%, 1.17		
Nematoda	*Dujardinascaris sp.*	8	5.06%, 1.13	-	-
	Metaquimperia bagarii	10	6.32%, 1.1	14	7.29%, 1.14
	Gnathostoma spinigerum	-	-	16	8.33%, 1.13
	Camallanus ophiocephali	-	-	11	5.72%, 1.18
	Porrocaecum trichuri	-	-	13	6.77%, 1.15
Acanthocephala	*Neorhadinorhynchus aspinosum*	10	6.32%, 1.1	-	-
	Neoechinorhynchus. prolixum	-	-	35	18.23%, 1.12
	Acanthosentis nigeriensis	-	-	32	16.67%, 1.13
	Pallisentis ophiocephali	6	4%, 1.17	44	22.92%, 1.14

Bolbocephalus sp. was the most dominant among the trematodes recovered from *X. cancila* and the prevalence was 7.29% with mean intensity 1.14 (Table-6). The occurrence of this trematode was recorded 7.33% of the total number of parasites collected and 51.61% of the trematode fauna observed (Table-7).

Isoparorchis hypselobagri was recovered from *X. cancila* and the prevalence was 6.77% with mean intensity 1.15 (Table-6). The occurrence of the trematode was recorded 6.88% of the total number of parasites collected and 48.39 % of the trematode fauna observed (Table-7).

The four trematodes found in *P. paradiseus* were *P. bilabiatum*, *U. hamati*, *T. vittalani*, and *H. callionymi* showed the prevalence of 23.41%, 20.25%, 15.18% and 10% with mean intensity 1.03, 1.09, 1.13 and 1.13 (Table-6). They were comprised 22.10%, 20.35%, 15.70%, 9.88% of the total parasites (Table-7) and 32.47%, 29.91%, 23.08%, 14.52% of the trematode group.

The cestodes *Nybelina lingualis* and *Parachristianella trygonis* were found in *P. paradiseus* which were 5.81% and 4.07% of the total parasites (Table-7). The prevalence was recorded 6.32% and 4% with mean intensity 1 And 1.17 (Table-6).
No cestodes were found in *X. cancila.*

In *P. paradiseus* two nematodes, *Dujardinascaris* sp and *M. bagarii* were found. The prevalence and mean intensity were found to be 5.06%, 6.32 and 1.13, 1.1 respectively. These parasites comprised 5.23% and 6.39% of the total parasite collected and 45%, 55% of the nematode obtained (Table-7).

A total of four species of nematodes were collected from *X. cancila.* They were *M. bagarii*, *Gnathostoma spinigerum* (third stage larva), *Camallanus ophiocephali* and *Porrocaecum sp.* with prevalence 7.29%, 8.33%, 5.72% and 6.77% and mean intensity 1.14, 1.13, 1.18 and 1.15. (Table-6) These parasites composed 7.34%, 8.26%, 5.96% and 6.88% of the total parasites while 25.80%, 29.03%, 20.96% and 24.19% of the total number of nematodes fauna were observed (Table-7).

Table-7: Percentage of parasites in *P. paradiseus* and *X. cancila*

Parasites	*P. paradiseus*			*X. cancila*		
	No. of parasites collected	**Occurrence in Group (%)**	**% in total**	**No. of parasites collected**	**Occurrence in Group (%)**	**% in total**
Bolbocephalus sp.				16	51.61	7.33
I. hypselobagri				15	48.39	6.88
P. bilabiatum	38	32.47	22.10			
U. hamati	35	29.91	20.35			
T. vittalani	27	23.08	15.70			
H. callionymi	17	14.52	9.88			
Total	**117**	**100**	**68.02**	**31**	**100**	**14.21**
N. lingualis	10	58.82	5.81			
P. trigonis	7	41.17	4.07			
Total	**17**	**100**	**9.88**			
Dujardinascaris sp.	9	45	5.23			
M. bagarii	11	55	6.39	16	25.80	7.34
G. spinigerum	-	-		18	29.03	8.26
C. ophiocephali	-	-		13	20.96	5.96
Porrocaecum trichuri	-	-		15	24.19	6.88
Total	**20**	**100**	**11.63**	**62**	**100**	**28.44**
N. aspinosum	11	61.11	6.40			
N. prolixum				39	31.2	17.89
A. nigariensis				36	28.8	16.51
P. Ophiocephali	7	38.88	4.07	50	40	22.93
Total	**18**	**100**	**10.47**	**125**	**100**	**57.33**

In *P. paradiseus* only two species of acanthocephalans were observed that are *N. aspinosum, P. ophiocephali*. They showed the prevalence 6.32% and 4% with mean intensity 1.1 and 1.17 (Table-6). They comprised 6.40% and 4.07% of the total parasites and 61.11% and 38.88 %of the parasitic groups (Table-7).

A total of three species of acanthocephala were collected from *X. cancila*. These were *N. prolixum, A. nigariensis* and *P. ophiocephali* with prevalence 18.23%, 16.67% and 22.92% and mean intensity 1.12, 1.13 and 1.14. These parasites composed 17.89%, 16.51% and 22.93% of the total parasites while, 31.2%, 28.8% and 40% of the total number of acanthocephalan fauna were observed (Table-7).

Organal distribution of different parasites in *X. cancila* and *P. paradiseus*

In *P. paradiseus* and *X. cancila*, the parasitic fauna was observed to occupy the oesophagus, stomach, anterior and posterior intestine, body cavity, liver and the larval forms were found to be attached to the anterior intestine, liver and fat bodies.

In *P. paradiseus*, the parasites *Prosogonotrema bilabiatum*, *Uterovesiculurus hamati*, *Thaparotrema vittalani*, *Hypohepaticola callionymi*, *Dujardinascaris sp.* and *Metaquimperia bagarii* were found to be located in stomach, anterior and posterior intestine. *Dujardinascaris sp*. and *M. bagarii* also found in the body cavity. The cestode parasites *Nybelinia lingualis* and *Parachristianella trygonis* in *P. paradiseus* were collected mainly from anterior and posterior intestine. Similarly, two acanthocephalan parasites *Neoechinorhynchus aspinosum* and *Pallisentis ophiocephali* was collected from anterior and posterior intestine of the same host (Table-8).

In *X. cancila*, trematodes *Bolbocephalus sp., Isoparorchis hypselobagri;* nematodes *Metaquimperia bagarii*, *Gnathostoma spinigerum*, *Camallanus ophiocephali* and *Porrocaecum sp* third stage larva were mostly found in anterior intestine, posterior intestine and body cavity. Rare and few were found in liver. All the acanthocephalan parasites in *X. cancila* are *N. prolixum, A. nigeriensis* and *P. ophicepali* were collected from anterior and posterior intestine (Table-8).

Table-8: Organal distribution of helminth parasites in *P. paradiseus* and *X. cancila*

Host	Name of the parasites	Stomach	Anterior intestine	Posterior intestine	Rectum	Body cavity	Liver	Total
P. paradiseus	*P. bilabiatum*	6	16	16	-	-	-	38
	U. hamali	5	19	11	-	-	-	35
	T. vittalani	3	13	11	-	-	-	27
	H. callionymi	1	9	7	-	-	-	17
	N. lingualis	-	04	2	-	-	4	10
	P. trygonis	-	3	2	-	-	2	07
	Dujardinascaris sp.	2	3	2	-	-	2	09
	M. bagarii	3	2	3	-	-	3	11
	N. aspinosum		6	5	-	-		11
	P. ophiocephalus	-	4	3	-	-	-	07
	Total	**20**	**79**	**62**	**-**	**-**	**11**	**172**
X. cancila	*Bolbocephalus ssp.*		08	08	-	-		16
	Isoparorchis hypselobagri	-	08	06	-	-	1	15
	G.spinigerum	-	10	8	-	-	-	18
	C. ophiocephali		04	05		04	-	13
	M. bagarii		7	6	1	-	2	16
	Porrocaecum sp.		6	5	2	1	1	15
	N. prolixum		18	17	4		-	39
	A. nigeriensis		15	16	2	3		36
	P. ophiocephali		27	22	1			50
	Total		**103**	**93**	**10**	**8**	**4**	**218**

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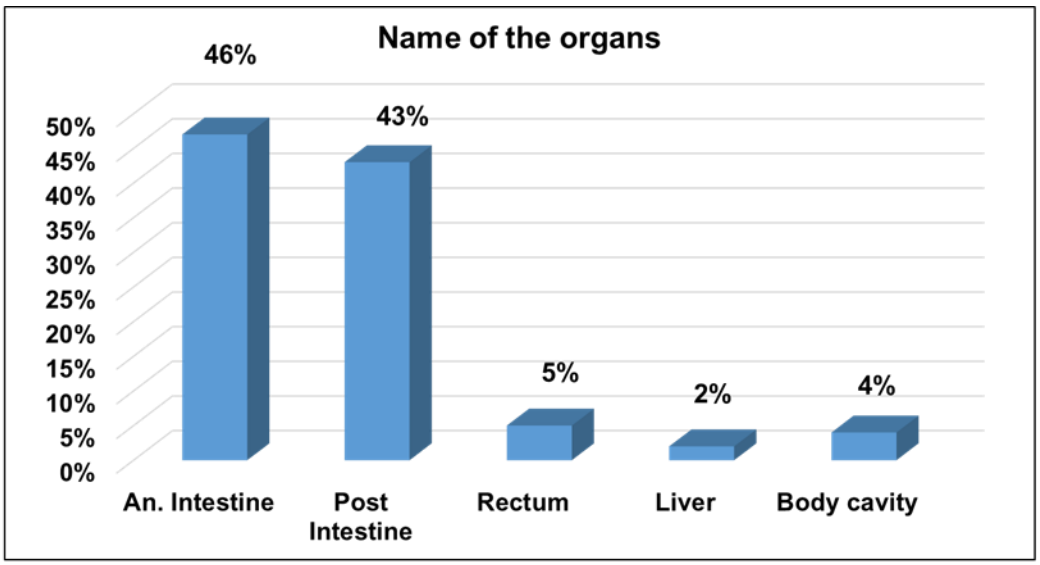
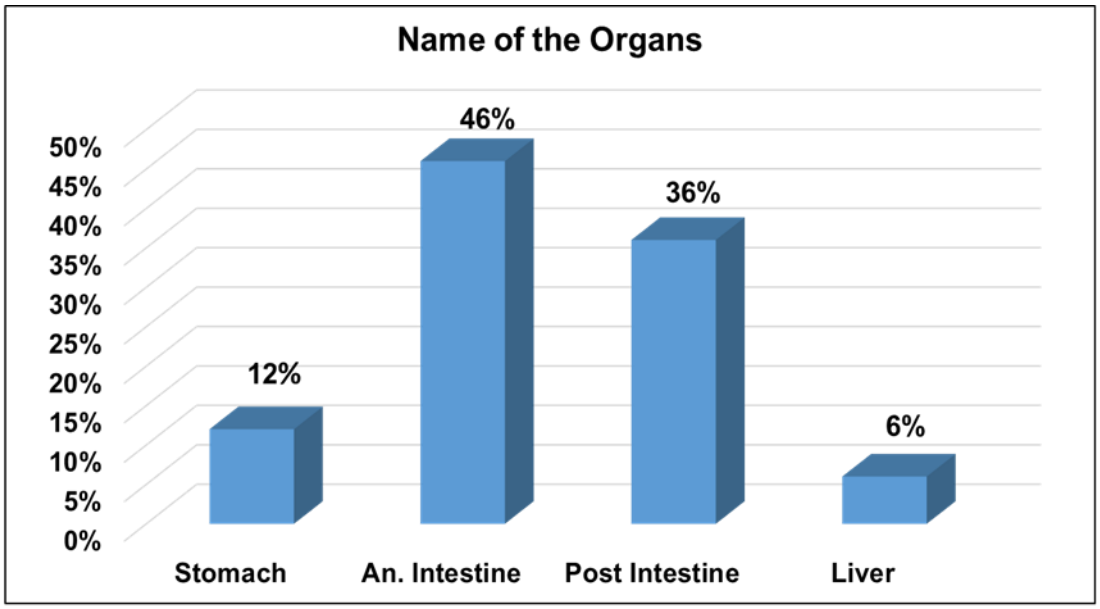
Percentage of helminth parasites found in the various organs of *Xenentodon cancila* and *Polynemus paradiseus*

In *X. cancila*, the percentage of parasites present in different organs was: anterior intestine-46%, posterior intestine-42%, rectum-5%, body cavity-4%, liver-2% (Fig-3).



(January 2017-December 2018)

Fig. 3. Percentage of total helminth parasites found in various organs of *Xenentodon cancila*



(January 2017-December 2018)

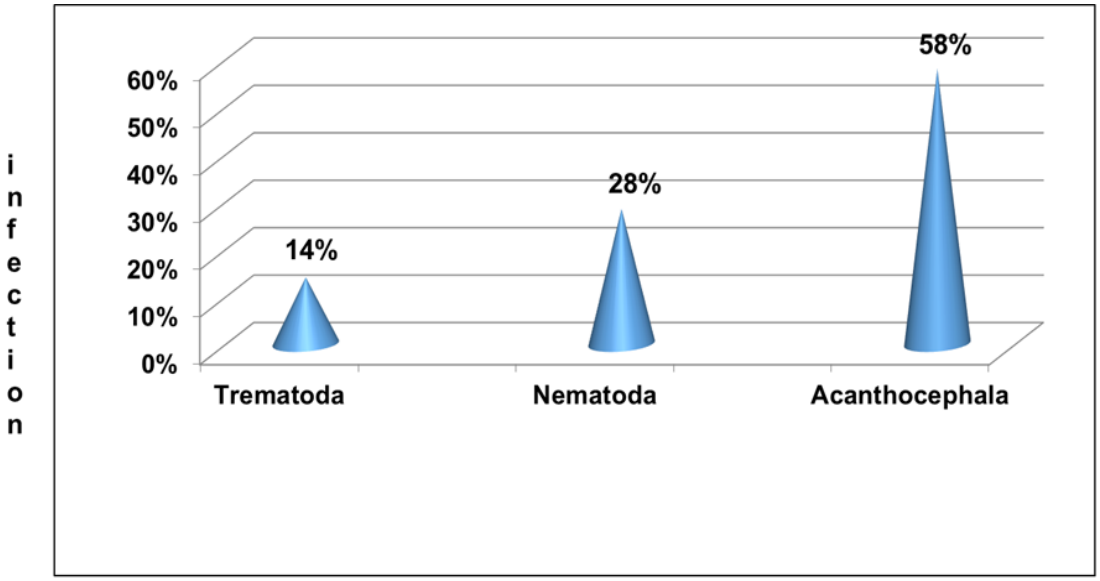
Fig. 4. Percentage of total helminth parasites found in various organs of *Polynemus paradiseus*

In *P. paradiseus*, the percentage of parasites present in different organs was: stomach-12%, anterior intestine-46%, posterior intestine-36%, liver-6% (Fig-4).

Interpretation: In the present investigation, a comparative and detailed analysis of prevalence and intensity of helminthes parasites, their organal distribution in *P. paradiseus* and *X. cancila* have been discussed. *P. bilabiatum* was the largest and most striking fish trematode recorded. Most of the parasites were found to infect more organs in both host fishes (Table-8).

Percentage of different parasite groups in *X. cancila* and *P. paradiseus*

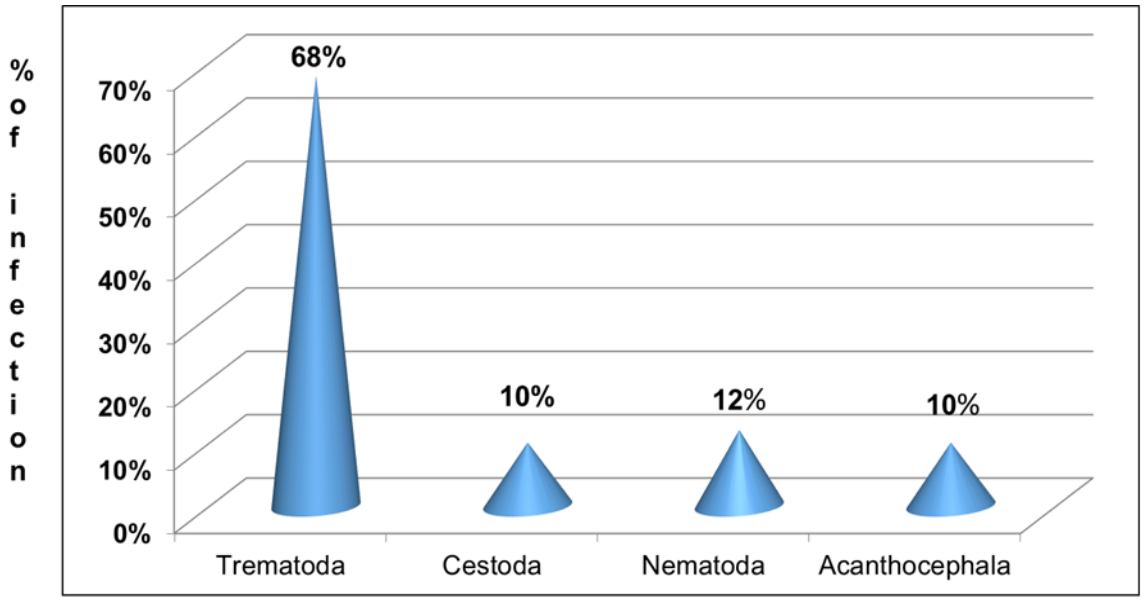
Among the different classes of parasites, the acanthocephala showed the highest infestation 58% in *X. cancila*. The second highest infestation rate was 28.44% for Nematoda and Trematoda showed the last position (14.21%) (Fig-5).



(January 2017-December 2018)

Fig. 5. Infestation of different helminth parasite groups in *X. Cancila*

The trematode showed the highest infestation 68%, the cestode parasites 10%, nematodes 12% and acanthocephalan 10% in *P. paradiseus* (Fig-6).



(January 2017-December 2018)

Fig. 6. Infestation of different helminth parasite groups in *P. paradiseus*

Infestation of parasites in different months and seasons

Infestation of endo-parasites in *Xenentodon cancila* and *Polynemus paradiseus* in relation to different months and seasons

In the present investigation, the prevalence and intensity of parasites have been described according to different months and seasons. The overall recorded infestation of parasites in both the host fishes were statistically analyzed to determine the seasonal variation during January 2017 to December 2018.

Regarding the yearly incidence, in *P. paradiseus*, the prevalence was 52.16% in 2017 and 45.81% in 2018 (Table-9, 10). In *X. cancila*, the prevalence was comparatively lower 56.65% in 2017 than in 2018 (63.10 %) (Table-11, 12).

In *P. paradiseus* (Jan' 17-Dec'17), the prevalence of infestation was highest (78.57%) in July'17 and the highest mean intensity (1.33) of parasites was observed in October'17. The lowest prevalence (33.33%) was found in the month of March'17 and the lowest intensity of infestation (1) was observed in several different months of the year (Table-9).

In 2018, the highest prevalence (66.67%) was found in July'18 and highest intensity (1.33) of parasites was found in March'18. The intensity of parasites was found lowest (1) in several different months of the year. The lowest prevalence (23.08%) was observed in January'18 (Table-10).

Table-9: Monthly prevalence and intensity of helminthes in *P. paradiseus* (Jan'17-Dec'17)

Month	No. of fish examined	No. of fish infected	Prevalence of infestation (%)	No. of worms collected	Mean Intensity of parasites
January	12	5	41.67	5	1
February	12	5	41.67	6	1.2
March	12	4	33.33	5	1.25
April	13	7	53.85	7	1
May	14	9	64.29	11	1.22
June	14	9	64.29	9	1
July	14	11	78.57	11	1
August	16	10	62.5	10	1
September	14	7	50	8	1.14
October	14	6	42.86	8	1.33
November	12	6	50	6	1
December	14	6	42.86	6	1
Overall	**161**	**85**	**52.16**	**92**	**1.10**

Table-10: Monthly prevalence and intensity of helminthes in *P. paradiseus* (Jan'18-Dec'18)

Month	No. of fish examined	No. of fish infected	Prevalence of infestation (%)	No. of worms collected	MIntensity of parasites
January	13	3	23.08	3	1
February	14	4	28.57	4	1
March	14	6	42.86	8	1.33
April	12	7	58.33	9	1.29
May	15	8	53.33	8	1
June	14	8	57.14	9	1.13
July	12	8	66.67	9	1. 13
August	13	7	53.85	8	1.14
September	13	6	46.15	6	1
October	14	6	42.86	6	1
November	13	6	46.15	6	1
December	13	4	30.77	4	1
Overall	**160**	**73**	**45.81**	**80**	**1.09**
Total Jan'17-Dec'18	**321**	**158**		**172**	

Table-11: Monthly prevalence and intensity of helminthes in *X. cancila* (Jan'17-Dec'17)

Month	No. of fish examined	No. of fish infected	Prevalence of infestation (%)	No. of worms collected	Intensity of Parasites
January	15	9	60	10	1.11
February	14	9	64.29	10	1.11
March	12	5	41.67	7	1.14
April	13	8	61.54	9	1.13
May	13	6	46.15	7	1. 17
June	13	8	61.54	9	1.13
July	15	9	60	10	1.11
August	13	8	61.54	9	1.13
September	13	5	38.46	8	1. 6
October	13	7	53.85	8	1. 14
November	13	8	61.54	9	1.13
December	13	9	69.23	10	1.11
Overall	**160**	**91**	**56.65**	**106**	**1.19**

In *X. cancila*, during Jan'17-Dec'17, the maximum prevalence (69.23%) was observed in the month of December'17 and intensity (1.6) was observed in September'17. The lowest prevalence (38.46%) of infestation was observed in September'17 and the intensity (1.11) was observed in January'17, February'17, July'17 and December'17 (Table-11).

Table-12: Monthly prevalence and intensity of helminths in *Xenentodon cancila* (Jan'18-Dec'18)

Month	No. of fish examined	No. of fish infected	Prevalence of infestation (%)	No. of worms collected	Mean Intensity of parasites
January	13	9	69.23	10	1.11
February	13	9	69.23	10	1.11
March	13	7	53.85	8	1. 14
April	12	8	66.67	8	1
May	15	10	66.67	11	1.1
June	16	8	50	8	1
July	13	8	61.54	9	1.13
August	14	7	50	8	1.14
September	13	8	61.54	9	1.13
October	12	8	66.67	8	1
November	12	9	75	11	1.22
December	13	9	69.23	10	1. 11
Overall	**161**	**101**	**63.10**	**112**	**1.11**
Total Jan'17-Dec'18	**321**	**192**		**218**	

In 2018, the highest prevalence (75%) and highest intensity (1.22) were found in November'18 while the lowest intensity (1) was found in June'18 and October'18. The lowest prevalence 50% was observed in June'18 and August'18.

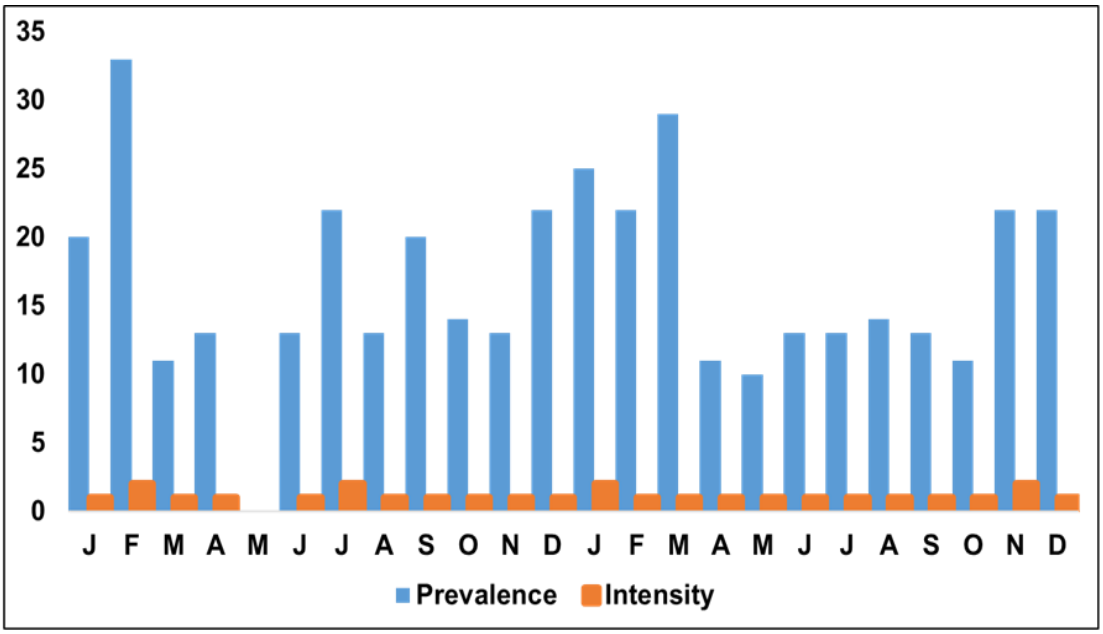
Table-13: Infestation by helminth parasites over the periods 2017 and 2018

Host fish	2017	2018	P-value (using proportion test)
P. paradiseus			0.198
Infected fish (%)	52.80	45.63	
N	161	160	
X. cancila			0.285
Infected fish (%)	56.88	62.73	
N	160	161	

Table-13 shows the result of two sample binomial proportion test. The overall proportion of infected *P. paradiseus* does not differ significantly at 5% level of significance between two periods 2017 and 2018. Similar trend was shown in case of *X. cancila*.

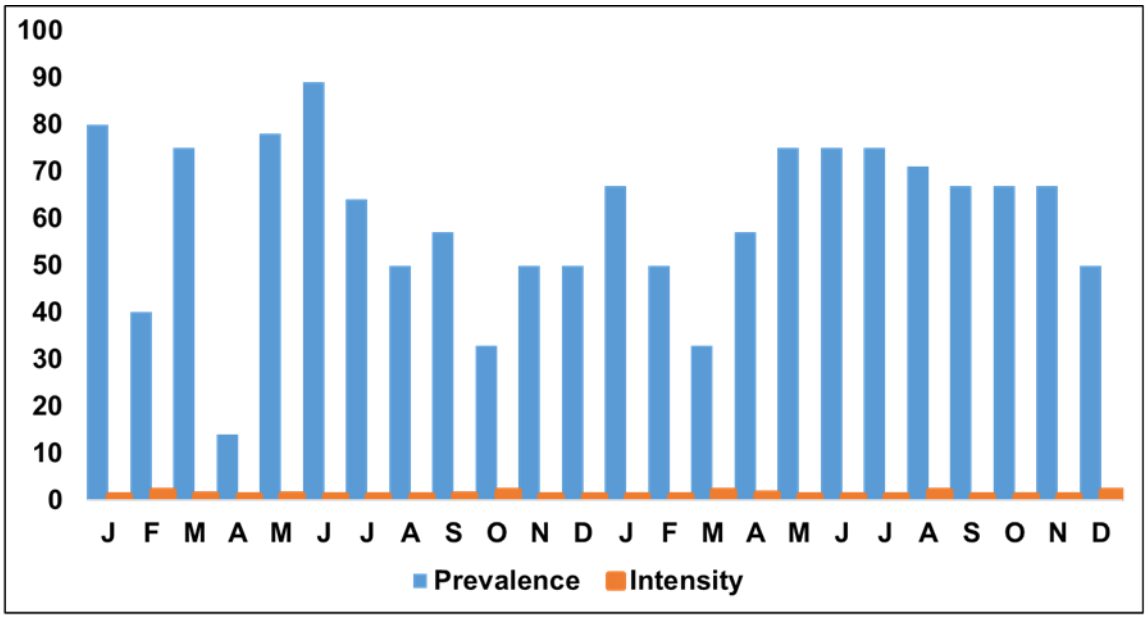
In *X. cancila*, the maximum prevalence of trematodes was shown to be the highest in February of 2017 (33.33%) and in March of 2018 (29%) during winter and summer seasons while the minimum prevalence was 11% in March'17 and 10% in May'18 during summer seasons. The highest intensity was observed (2) in February'17, July'17, January'18 and November'18 during winter and rainy seasons whereas the lowest intensity 1 was observed in several different months of both the years (Fig.- 7).

In *P. paradiseus*, the prevalence of trematodes was shown to be the highest in June of 2017 (88.89 %) during summer season and in May, June and July of 2018 (75%) during summer and monsoon. The lowest prevalence of infestation was recorded in April'17(14.29%) and March'18 (33.33%) during summer season. However, the highest intensity (2) was observed in February, October of 2017 and March, August, December of 2018 while the lowest intensity (1) was observed in March, April, July, November, December of 2017 and January, June, August, September, October'18 and November of 2018. Both highest and lowest intensity was observed in all seasons of both the years of (Fig.- 8).



(January 2017-December 2018)

Fig.7. Monthly Prevalence and intensity of Trematode parasites in *X. cancila*



(January 2017-December 2018)

Fig. 8. Monthly Prevalence and intensity of Trematode parasites in *P. paradiseus*

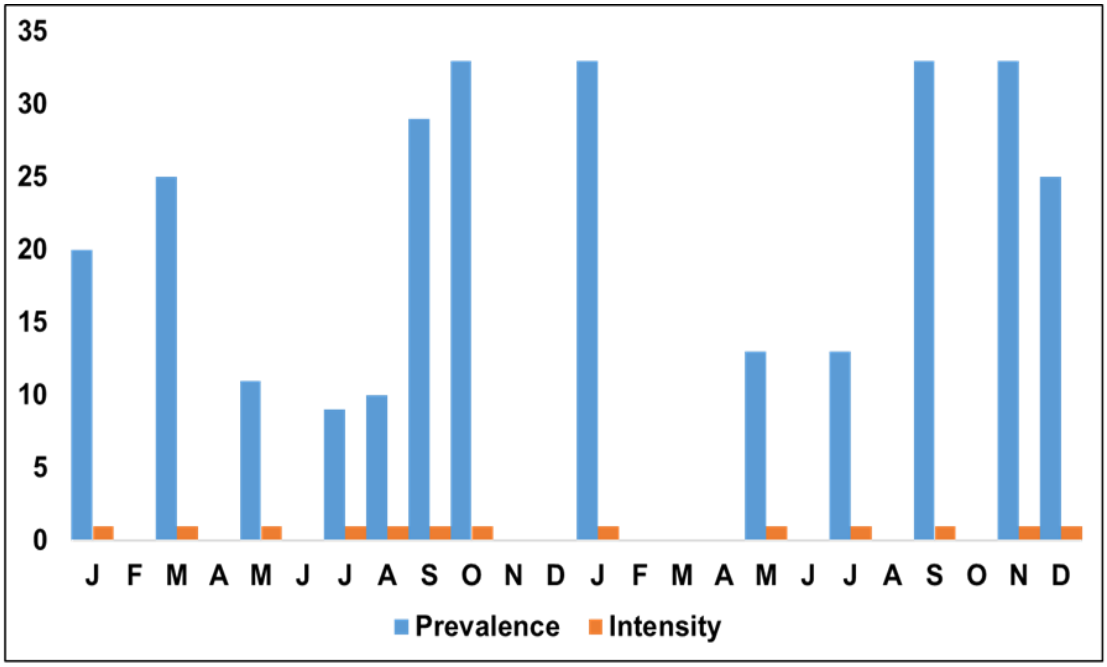
In *P. paradiseus*, the prevalence of cestodes was shown to be the highest (33.33%) in October of 2017 during rainy and in January, September and November of 2018 during winter and rainy seasons. The lowest prevalence of infestation was recorded in July of 2017 (9.09%) and May and July of 2018 (33.33%) in summer and rainy seasons. The Cestodes was not found in February, April, June, November, December of 2017 and in February, March, April, June, August, October of 2018. The intensity was recorded (1) for rest of the months of both the years Fig.-9.

In *X. cancila*, Cestode was not found at all.

In *X. cancila*, the maximum prevalence of nematodes was shown to be the highest in September of 2017 (60%) and in May of 2018 (60%) during rainy and summer seasons while the prevalence was shown to be lowest in December of 2017 (11.11%) and in April, November, December of 2018 (11.11%) during summer and winter seasons. The highest intensity was observed (2) in April, June of 2017 and February of 2018 during summer and winter seasons whereas the lowest intensity 1 was observed in several different months of both the years (Fig.-10).

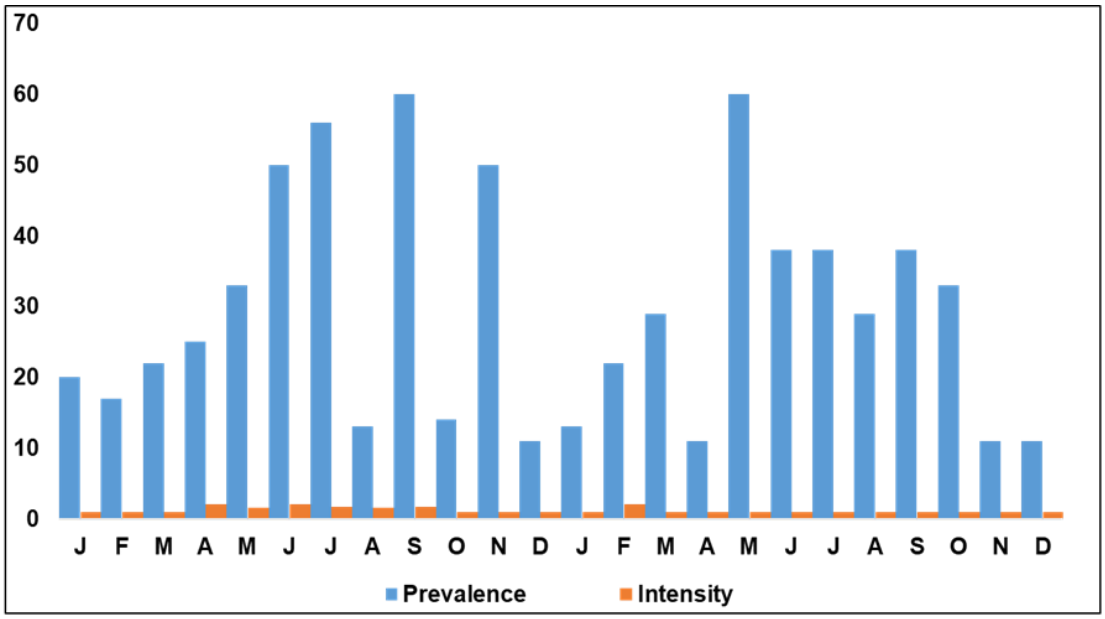
In *P. paradiseus*, the prevalence of nematode was shown to be the highest in February of 2017 (40 %) during winter and in March of 2018 (33.33%) during summer. The Cestodes was absent in January, March, April, June, September of 2017 and in January, February, August, September, October, November, December of 2018. The mean intensity was recorded (1) for rest of the months of both the years (Fig.-11).

In *X. cancila*, the maximum prevalence of Acanthocephala was noted as 75 % in August'17 during rainy and 79% in April'18 during summer season while the minimum prevalence was 20% in September'17 during rainy and 30% in May'18 during summer season. The highest mean intensity were observed (2.33) in March'17 during summer and (1.75) in December'18, during winter season whereas the lowest intensity 1 was observed in several different months of both the years (Fig.-12).



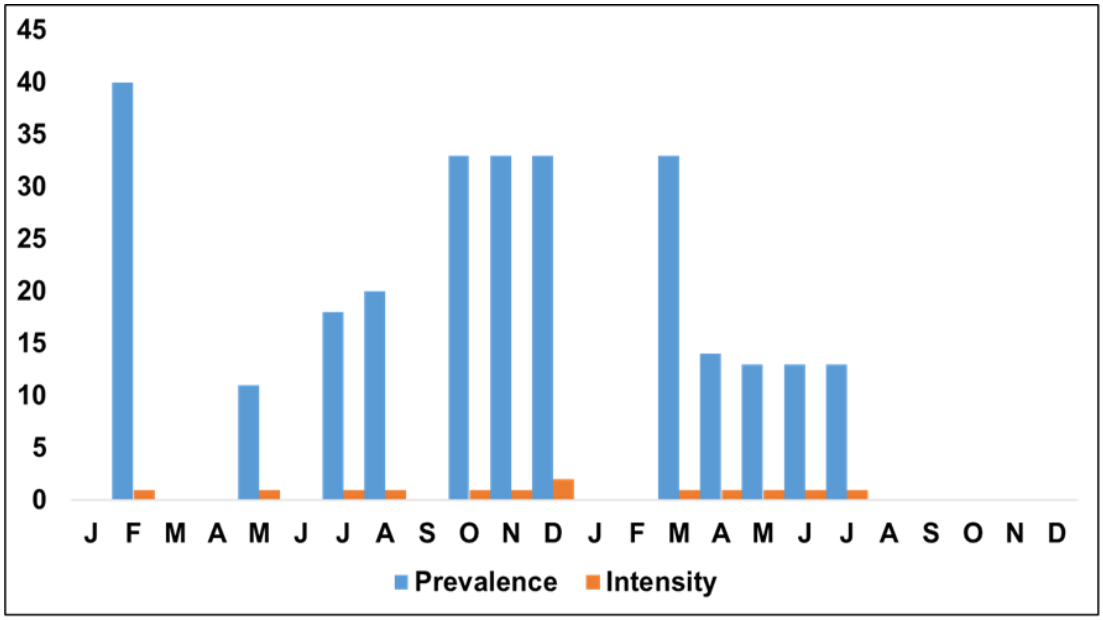
January 2017-December 2018

Fig. 9. Monthly Prevalence and intensity of Cestode parasites in *P. paradiseus*



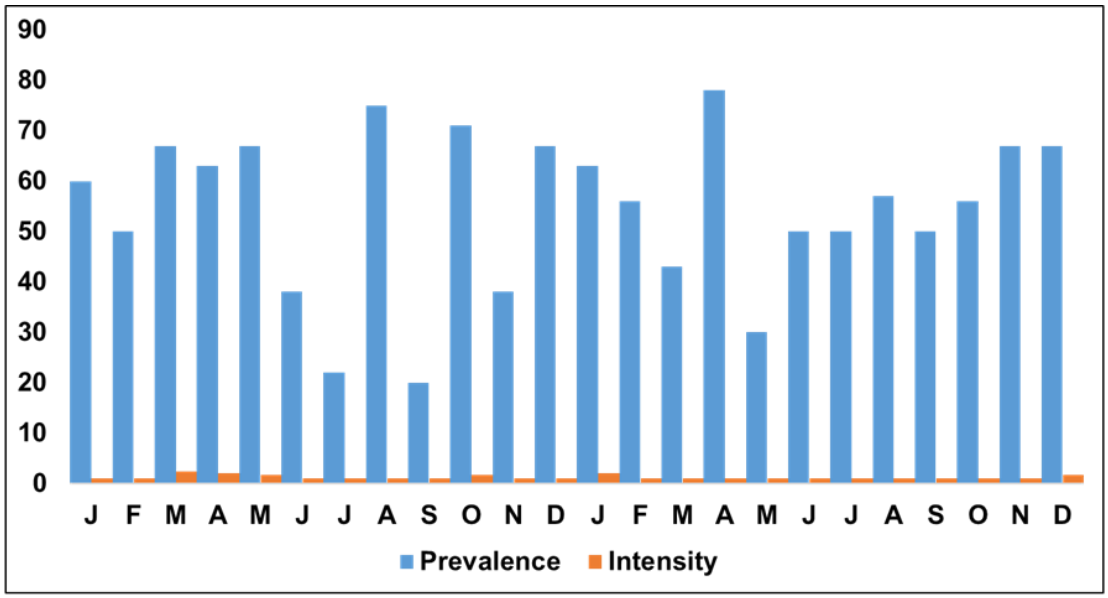
January 2017-December 2018

Fig. 10. Monthly Prevalence and intensity of nematode parasites in *Xenentodon cancila*



January 2017-December 2018

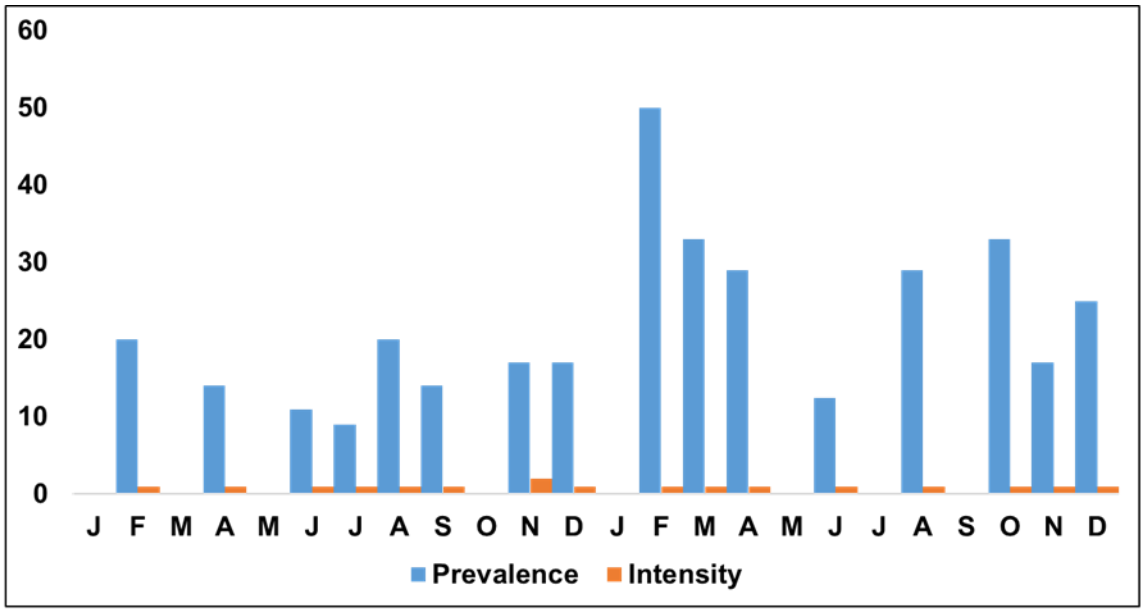
Fig. 11. Monthly Prevalence and intensity of nematode parasites in *Polynemus paradiseus*



January 2017-December 2018

Fig. 12. Monthly Prevalence and intensity of acanthocephalan parasites in *X. cancila*

In *P. paradiseus*, the prevalence of Acanthocephala was shown to be the highest in February and August of 2017 (20 %) during winter and rainy seasons and in February of 2018 (50%) during winter. The Acanthocephala was not found at all in January, March, May and October of 2017 and in January, May, July and September of 2018. The highest intensity was recorded 2 in November of 2017 whereas it was observed 1 in remaining months of 2018 (Fig. -13).



(January 2017-December 2018)

Fig. 13. Monthly Prevalence and intensity of acanthocephalan parasites in *P. paradiseus*

The maximum prevalence of *P. bilabiatum* in *P. paradiseus* was observed in rainy and summer seasons (36% in July'17; 38% in May'18 and June'18) while the minimum prevalence was recorded during summer and rainy seasons (14% in April'17, September'17 and August'18). *P. bilabiatum* in *P. paradiseus* showed the highest intensity during rainy and summer seasons (2 in September'17, October'17 and March'18) while the lowest intensity was observed (1) in rest of the months of the year 2017. No *P. bilabiatum* was found from August to December of 2018) (Fig.-14, 15).

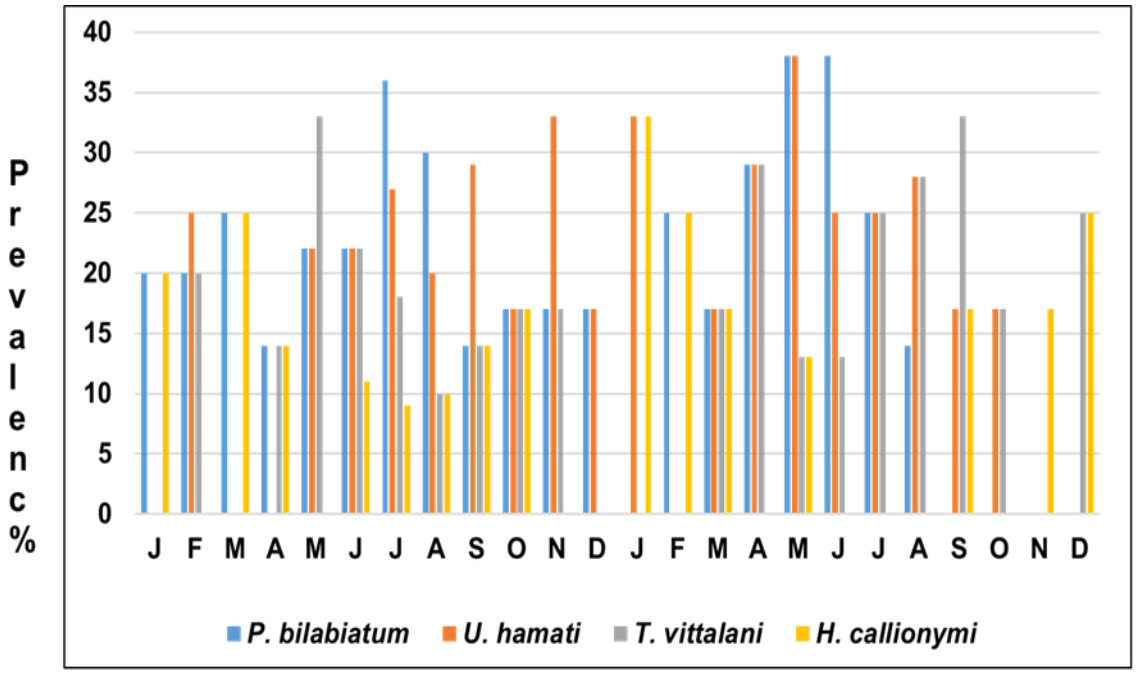


Fig. 14. Monthly prevalence of trematode parasites in *Polynemus paradiseus* (January 2017-December 2018)

Uterovesiculurus hamati showed the maximum prevalence during winter and summer seasons (33%, November'17 and 37.5% in May'18) in *P. paradiseus*. No *U. hamati* was found in January'17, March'17, April'17, February'18, November'18 and December'18. *U. hamati* showed the highest intensity (2 in May'17, October'17 and 1.5 in April, 18) during summer and rainy seasons while the lowest intensity (1) was observed in all the seasons of the study period (Fig.-14, 15).

The highest prevalence of *T. vittalani* in *P. paradiseus* was observed during summer season (33% in May'17) and rainy season (33% in September'18) and lowest prevalence was observed during rainy season (10% in August'17). No *T. vittalani* was found in January, March, August, December of 2017 and January, February and November of 2018. This trematode showed the highest intensity (1.5, January'18) during winter while the lowest intensity (1) was observed in all the seasons of both the years (Fig-14, 15).

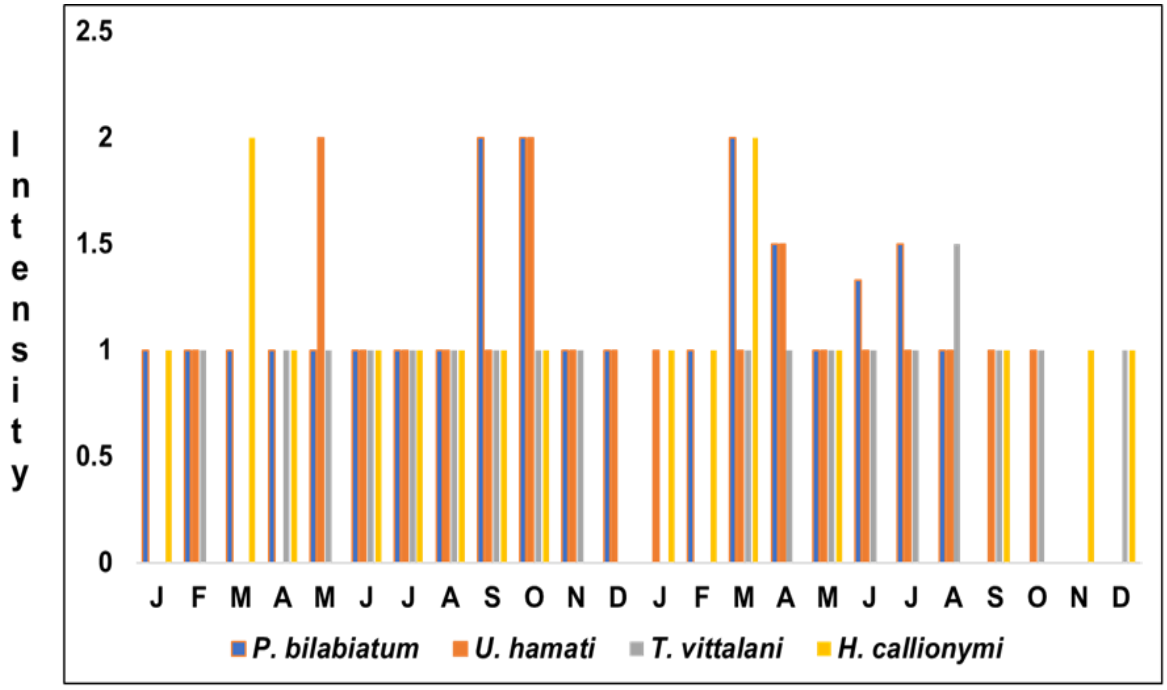


Fig. 15. Monthly intensity of trematode parasites in *Polynemus paradiseus* (January 2017-December 2018)

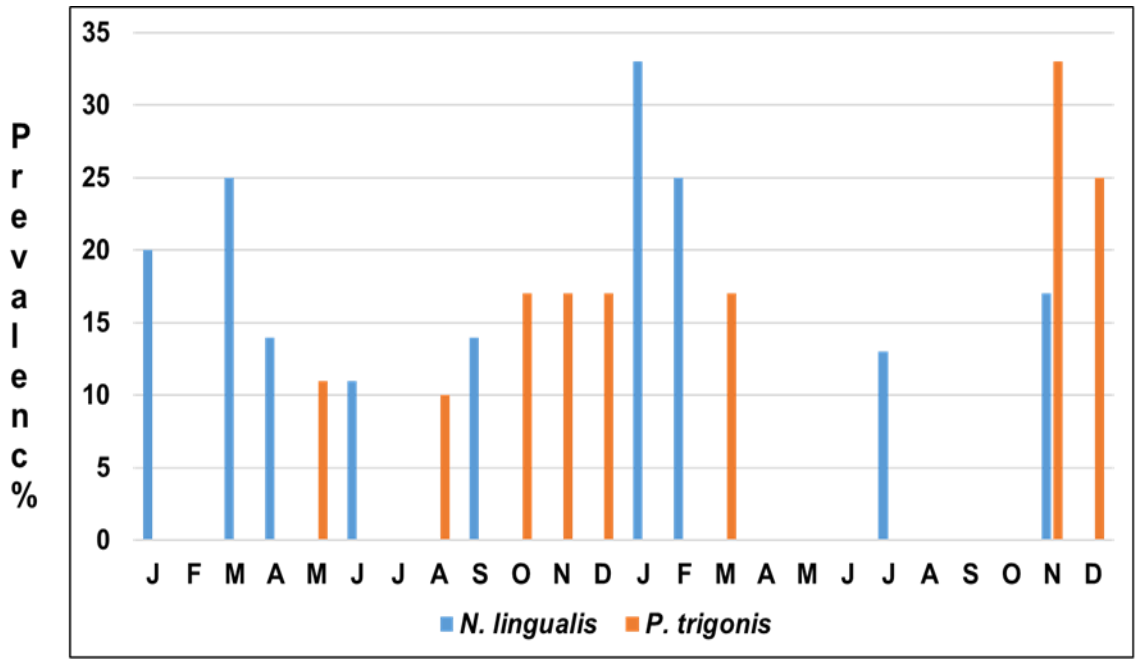


Fig. 16. Monthly prevalence of cestode parasites in *Polynemus paradiseus* (January 2017-December 2018)

H. callionymi showed the maximum prevalence during summer and winter seasons (25% in March'17 and 33.33% in January'18) and lowest prevalence during rainy and summer seasons (9% in July'17 and 12.5% in May'18) for both the years. It also showed the highest intensity (2 in March'17 and March'18) and lowest intensity (1) in all the seasons of both the year. No *H. callionymi* was found in February'17, May'17, November'17, December'17, April'18, May'18, June'18, July'18, August'18 and October'18) (Fig.-14, 15).

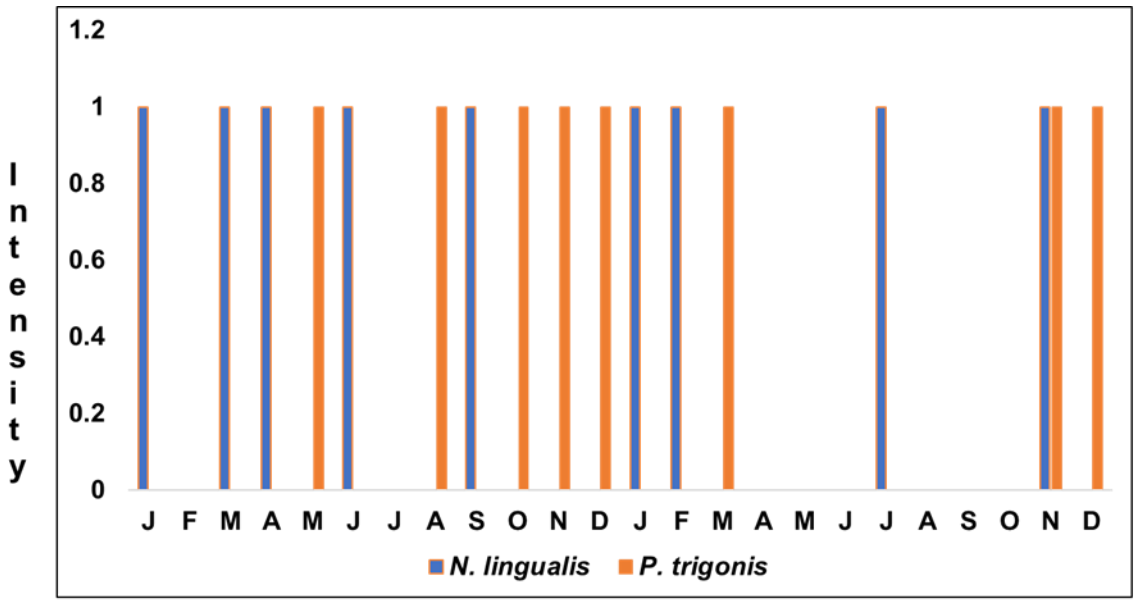
The cestode, *Nybelinia lingualis* showed the highest prevalence of infestation in *P. paradiseus* during summer and winter (25% in March'17 and 33.33% in January'18) and lowest prevalence (11% in June'17 and 12.5% in July'18) during summer and rainy seasons (Fig.-16).

The cestode *P. trygonis* was shown to be the highest during rainy and winter seasons (17% in October'17, November'17, December'17 and 33.33% in November'18) and lowest prevalence of infestation in *P. paradiseus* during rainy (10% in August'17) and summer seasons (17% in March'18) (Fig.-16).

In *P. paradiseus*, the cestodes were absent in most of the months but they were found abundantly in a few particular months. The intensity of cestodes was observed constant (1) in the months they present. (Fig. 17).

In X. cancila, cestode parasites were totally absent.

The nematode species *Dujardinascaris sp.* showed maximum prevalence of infestation during winter and monsoon (20% in January'17, February'17 and 33.33% in September'18) and minimum prevalence in rainy seasons (10% in August'17 and 14% in August'18). The intensity of *Dujardinascaris sp.* was observed highest in winter (2, February'17). The nematodes were absent in most of the months but they were found abundantly in a few particular months (Fig.-18, 19).

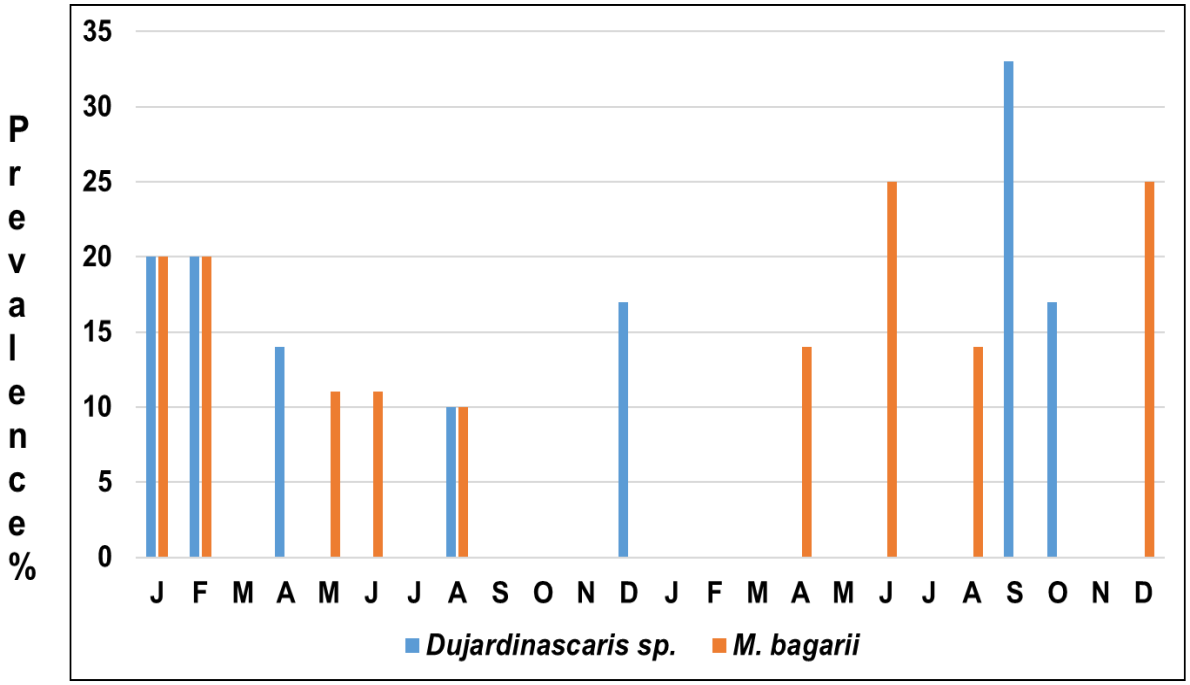


(January 2017-December 2018)

Fig. 17. Monthly intensity of cestode parasites in *Polynemus paradiseus*

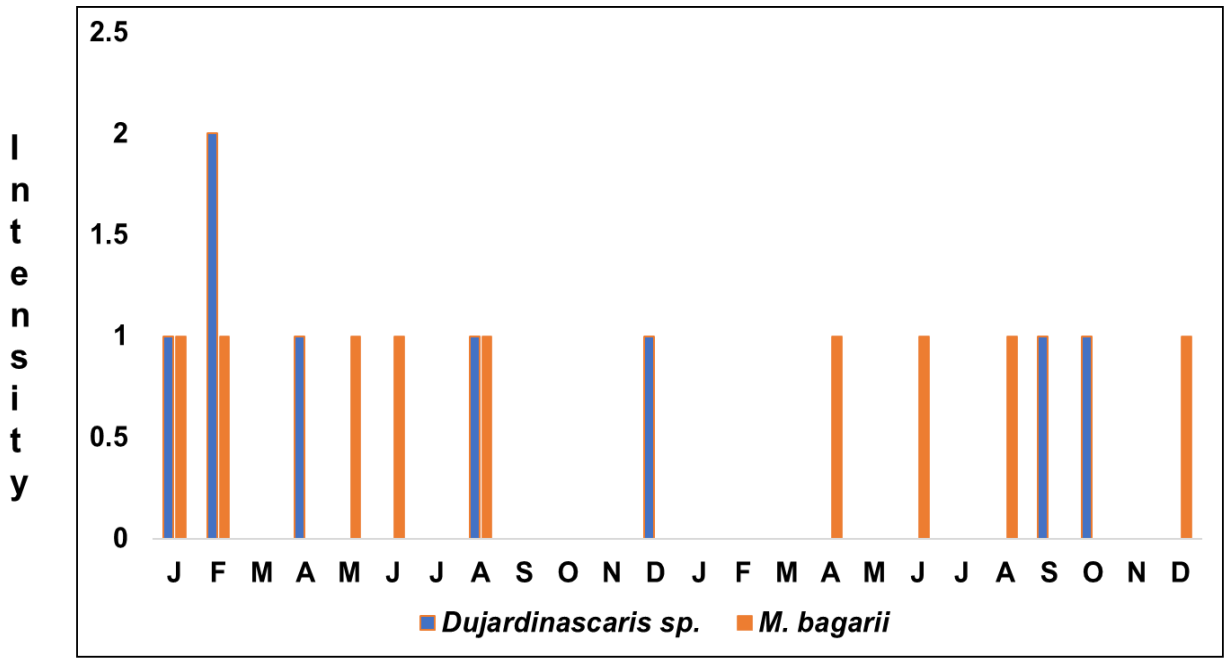
Metaquimperia bagarii showed highest prevalence during winter and summer (20% in January'17, February and 25% in June'18 and December'18) while in *P. paradiseus* the lowest prevalence of the nematode was observed in all three seasons (10% in August'17 and 14% in April'18 and August'18).The intensity of *M. bagarii* was observed constant (1) in the months when it was present. In *P. paradiseus*, the *M. bagarii* was absent in most of the months but was found abundantly in a few particular months (Fig.-18, 19).

N. aspinosum, showed the highest prevalence (33.33%) of infestation in *P. paradiseus* during winter and monsoon (December'17 and October'18). The lowest prevalence was observed (14% in April'17, Sept'17and 12.5% in July'18) during summer and rainy seasons. The intensity of *N. aspinosum* was observed same (1) in all months when it was present. In *P. paradiseus*, the *N. aspinosum* was absent in most of the months but only found abundantly in a few particular months (Fig.-20, 21).



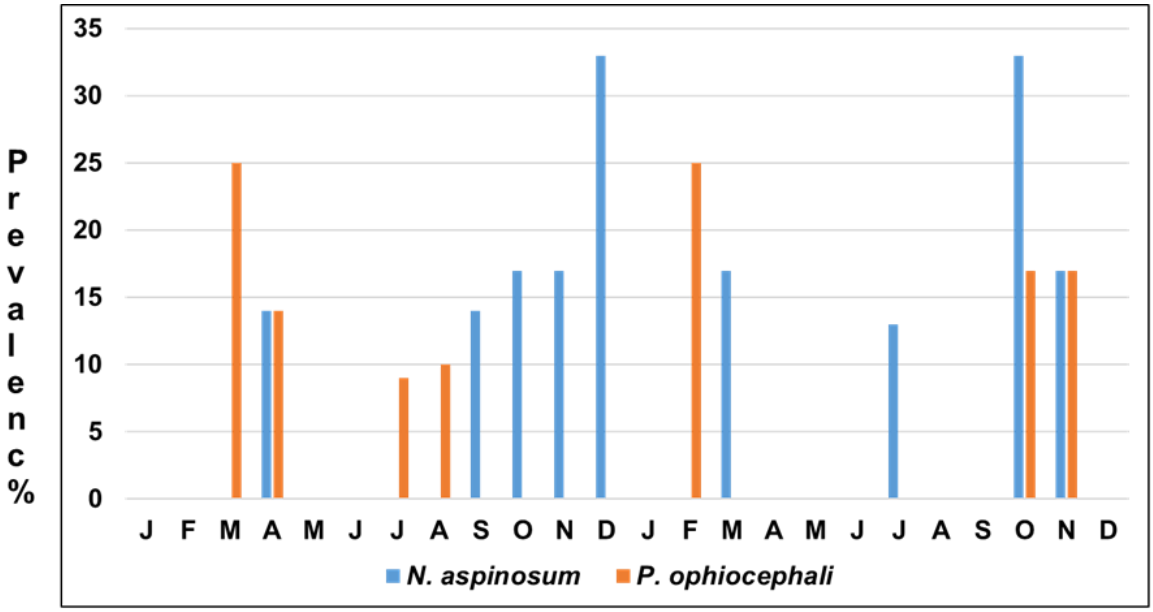
(January 2017-December 2018)

Fig. 18. Monthly prevalence of nematode parasites in *Polynemus paradiseus*



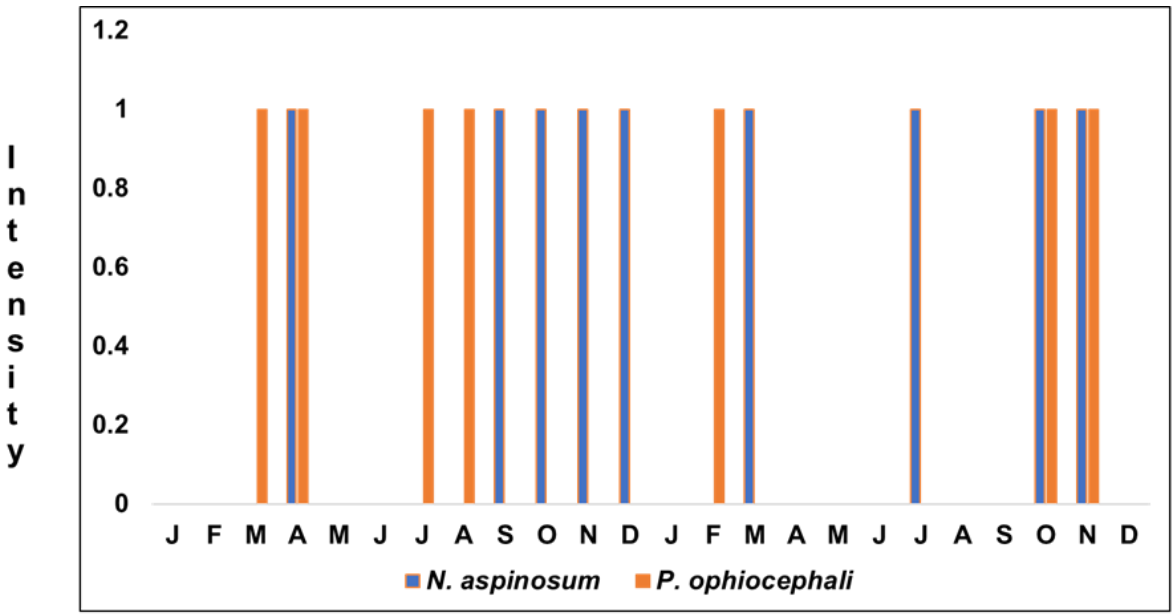
(January 2017-December 2018)

Fig. 19 . Monthly intensity of nematode parasites in *Polynemus paradiseus*



(January 2017-December 2018)

Fig. 20. Monthly Prevalence of acanthocephalans in *Polynemus paradiseus*



(January 2017-December 2018)

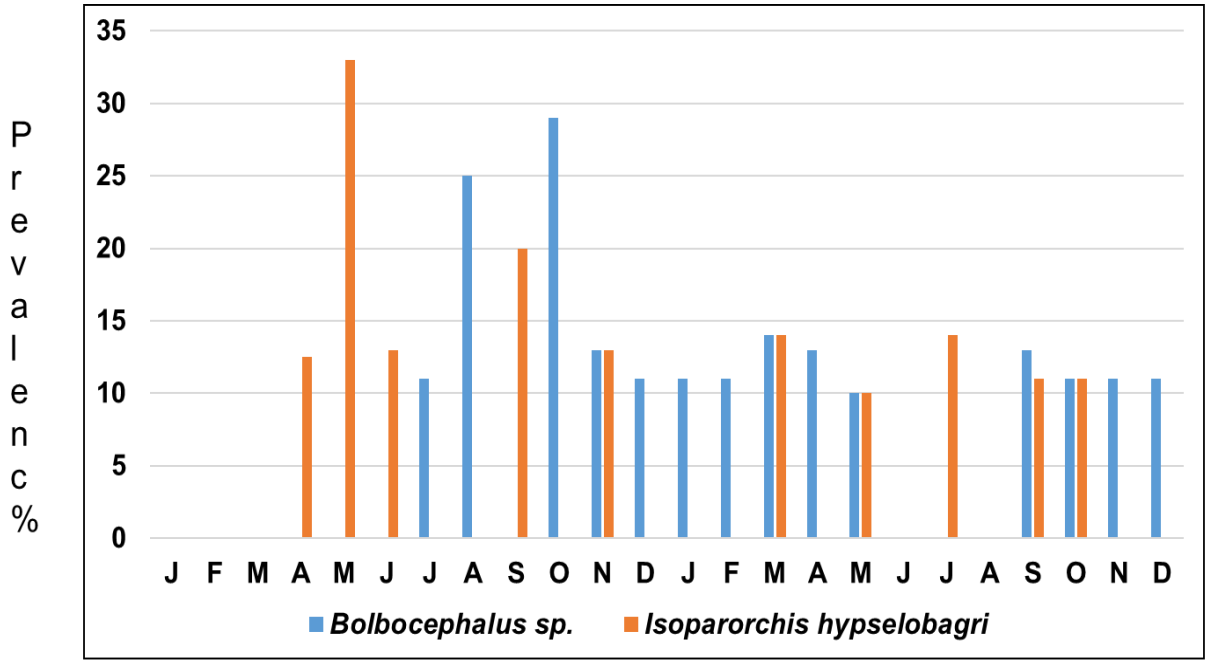
Fig. 21. Monthly intensity of of acanthocephalan parasites in *P. paradiseus*

The maximum prevalence of *Pallisentis ophiocephali* was observed in summer and winter (25% in March'17 and February'18) in *P. paradiseus* and the lowest prevalence was also found in summer and winter (9 % in July'17 and 16.67% in Oct'18 and November"18). The intensity of *P. ophiocephali* was observed same (1) in all months it present. In *P. paradiseus,* the *P. ophiocephali* was absent in most of the months but was found abundantly in a few particular months (Fig.-20, 21).

Bolbocephalus sp showed the maximum prevalence in *X. cancila* which was 29% in Oct'17 and 14% in March'18 during rainy and summer seasons of the study period. The lowest prevalence was found during summer, rainy and winter seasons (11.11% in July17, December17 and10% in May'18). The intensity of *Bolbocephalus sp* was observed (1) throughout all the seasons (Fig -22, 23).

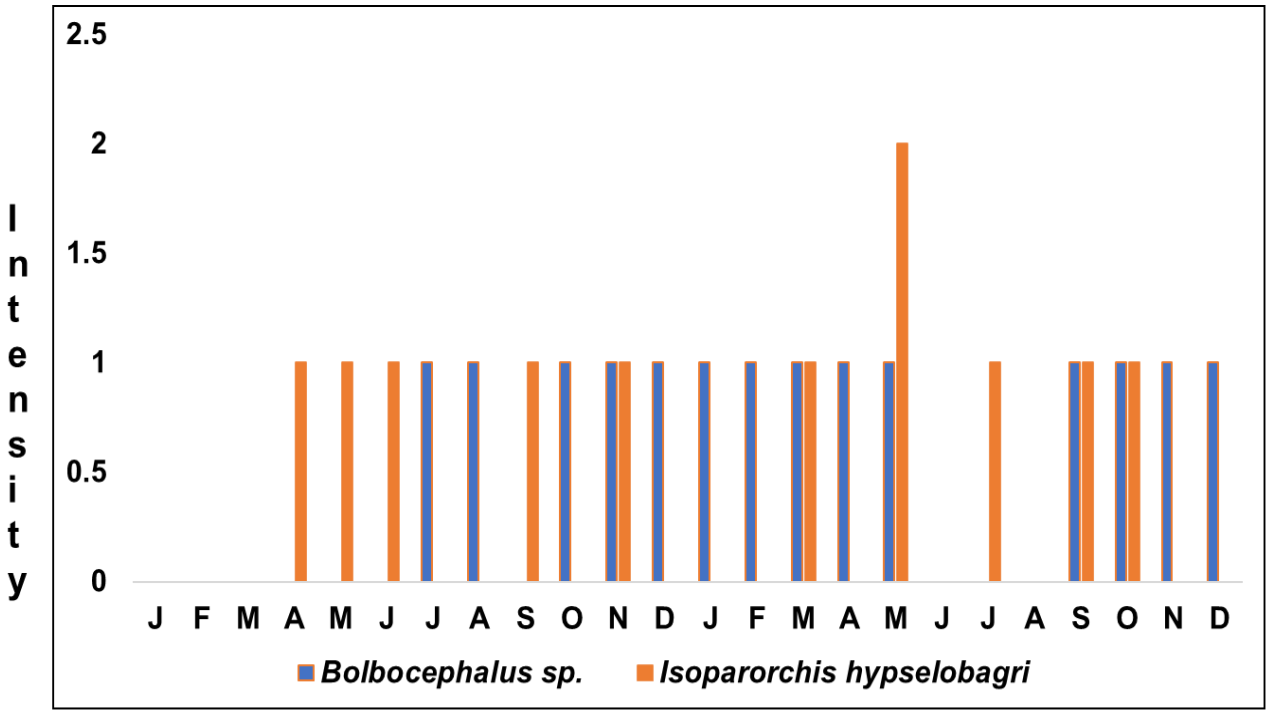
Isoparorchis hypselobagri showed the maximum prevalence in *X. cancila* during summer and rainy seasons (33.33% in May'17 and 14 % in March'18 and July'18). The lowest prevalence was found during summer and winter seasons (12.5% in April'17, June'17, November'17 and 10% in May'18). The highest mean intensity was observed (2 in May'18) during summer season of 2018 whereas, the lowest intensity (1) was observed in rainy, winter and summer seasons of both the years (Fig -22, 23).

In *X. cancila*, the maximum prevalence of *M. bagarii* was shown to be the highest in April, June and August of 2017 (12.5%) and in May of 2018 (28%) during summer and rainy seasons while the prevalence was shown to be lowest in February, July and December of 2017 (11.11%) during winter and rainy seasons and in May of 2018 (10%) during summer. The highest intensity was observed (2) in July of 2017 during monsoon whereas the lowest intensity 1 was observed in several different months of both the years. No *M. bagarii* was found in January, March May, September, October and November of 2017 and January, March, April and November of 2018 (Fig.-24, 25).



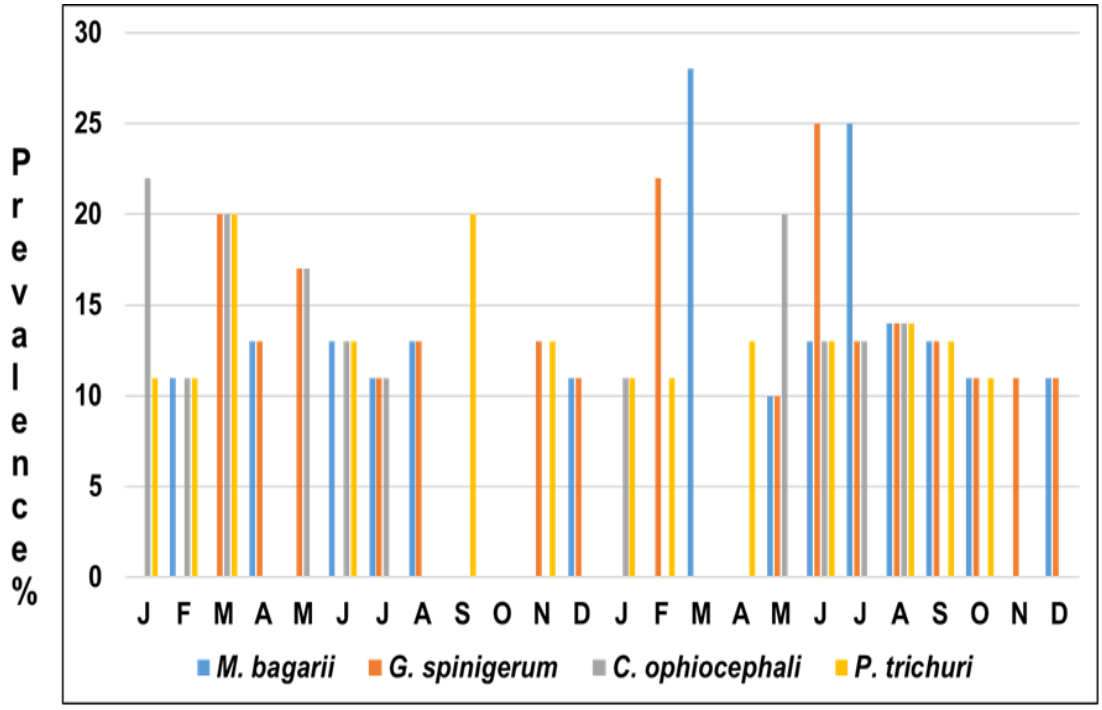
(January 2017-December 2018)

Fig. 22. Monthly Prevalence of trematode parasites in *Xenentodon cancila*



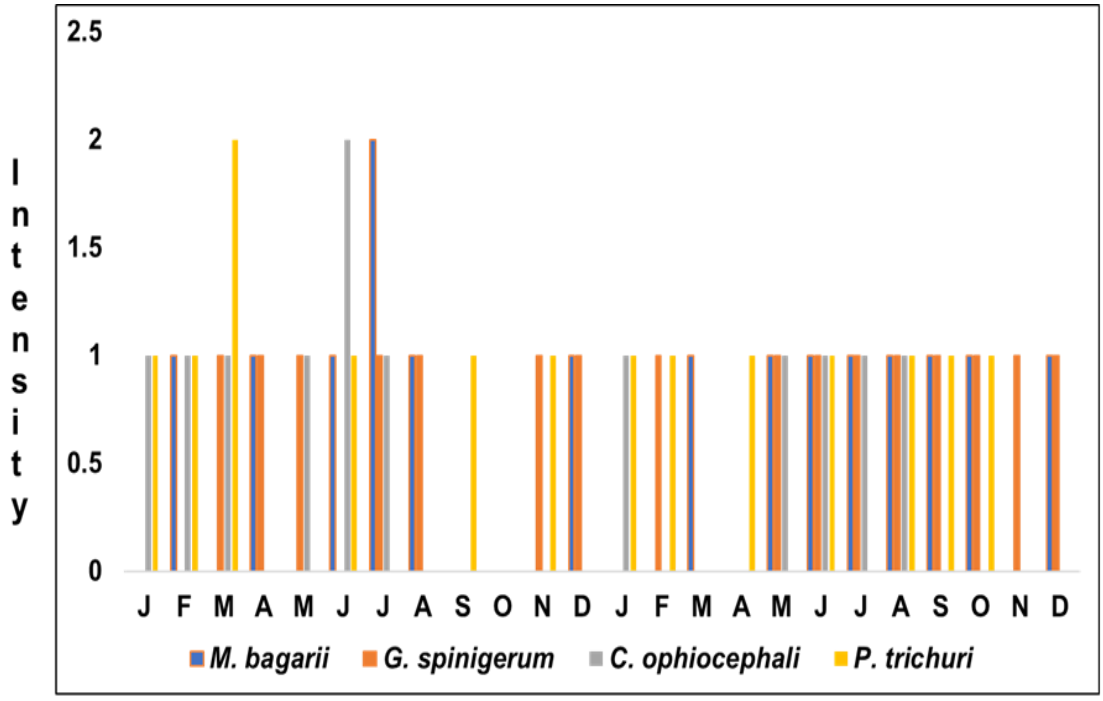
(January 2017-December 2018)

Fig. 23. Monthly intensity of trematode parasites in *Xenentodon cancila*



(January 2017-December 2018)

Fig. 24. Monthly Prevalence of Nematodes in *Xenentodon cancila*



(January 2017-December 2018)

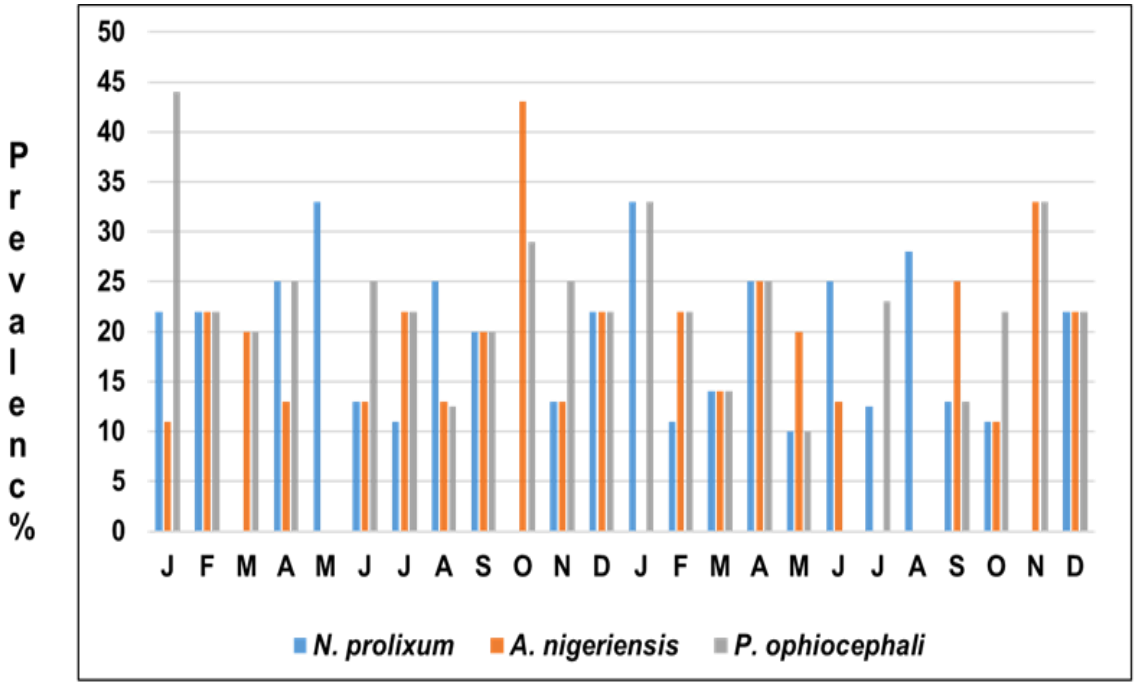
Fig. 25. Monthly intensity of Nematode parasites in *X. cancila*

Maximum prevalence of *Gnathostoma spinigerum* in *X. cancila* was found 20% in March of 2017 and 25% in June of 2018 during summer season. The lowest prevalence was found during summer, rainy and winter seasons (11.11% in July'17, December'17 and 10% in May'18). The intensity (1) remained constant during all seasons. No *G. spinigerum* was found in January, February, June, September, October of 2017 and in March, April of 2018 (Fig.-24, 25).

The highest prevalence of *Camallanus ophiocephali* in *X. cancila* was found 22.22% in January'17 during winter and 20% in May'18 during summer. The lowest prevalence (11.11%) was found during winter and rainy seasons (February and July of 2017 and January of 2018). The highest intensity (2) was observed in June'2017 during summer season whereas the lowest intensity was (1) observed in rest of the months of both the years. *C. ophiocephali* was absent for thirteen months of the study periods (Fig.-24, 25).

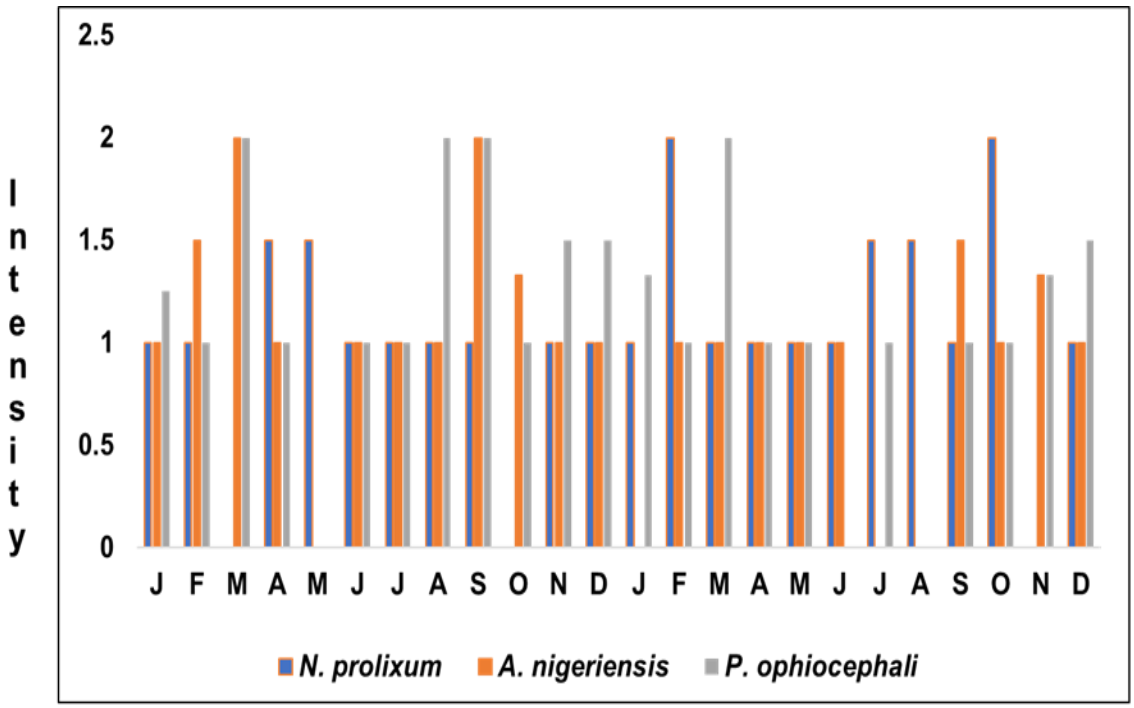
Along with *Porrecaecum trichuri*, month March'17 (20%), September'17(20%) and August'18 (14%) showed highest prevalence of infestation in *X. cancila* during summer and monsoon. In 2017, the lowest prevalence (11.11%) was found in January, February and in 2018 the lowest prevalence (11.11%) was found in January, February, October during winter and rainy seasons. The maximum intensity (2) occurred in March'17 whereas the minimum (1) intensity of *P. trichuri*, was observed in most of the months of both the years (Fig.-24, 25).

Maximum prevalence of *N. prolixum* in *X. cancila* was found 33.33 % in May'17 during summer season and January'18 during winter season. The lowest prevalence was found during rainy season, in July'17 (11.11%) and during summer season, in May'18 (10%). The highest intensity (1.5 in April'17, May'17) was observed during summer and (2 in February'18 and October'18) was observed during winter and rainy seasons whereas the lowest intensity (1) remained constant during all seasons of both the years (Fig -26, 27).



(January 2017-December 2018)

Fig. 26. Monthly Prevalence of acanthocephalan parasites in *X. cancila*



(January 2017-December 2018)

Fig. 27. Monthly intensity of Acanthocephalans in *Xenentodon cancila*

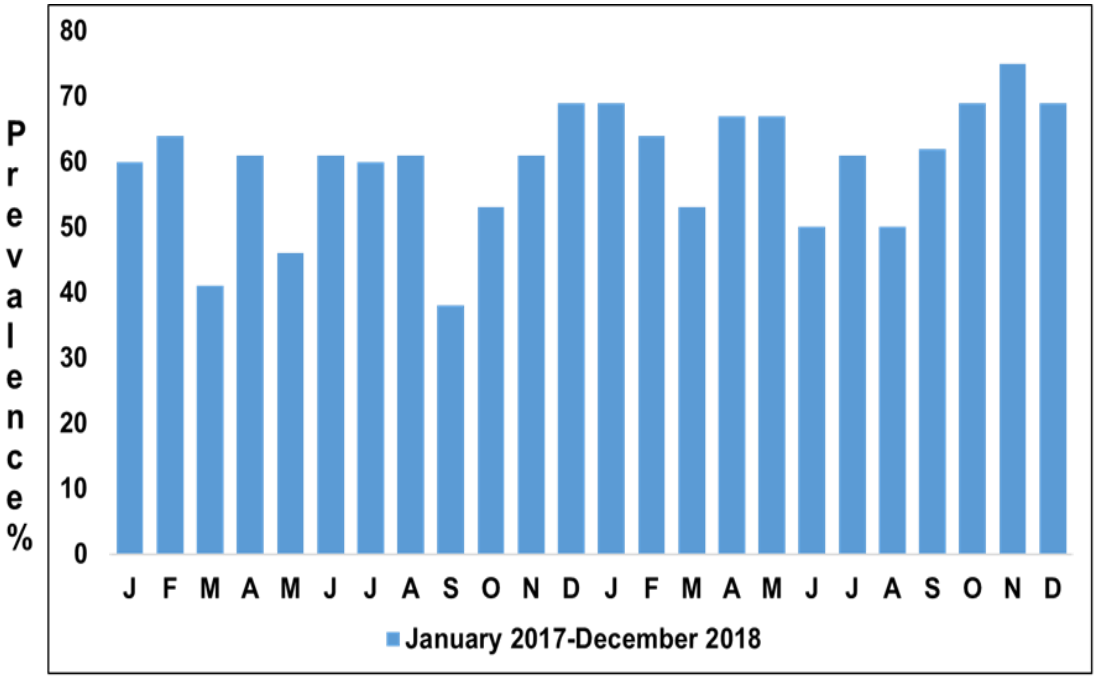
Maximum prevalence of *A. nigeriensis* in *X. cancila* was found 42.85% in Oct'17 and 33.33% in Nov'18 during rainy and winter season. The lowest prevalence was found (11.11%) during winter and rainy seasons (in January '17 and Oct'18) (Fig 26).

The highest intensity of *A. nigeriensis* were observed (2 , March'17 and 1.5, Sep '18) during summer and rainy seasons whereas, the lowest intensity was (1) observed in summer, rainy and winter seasons respectively (Fig -26, 27).

In *P. ophiocephali*, month January'17 (44.44 %) and January'18 (33.33%), November'18 (33.33%) showed highest prevalence of infestation in *X. cancila* during winter season. The lowest prevalence was found in August'17 (12.5%) and May'18(10%) during monsoon and summer seasons. The maximum intensity (2 in March'17, August'17, September'17 and March'18) during summer and rainy seasons and minimum intensity (1 in February'17, April'17, July'17, July'17, Feb'18, April'18, May'18, July'18, sept'18 and Oct'18) was observed in all over the seasons, Fig (26, 27).

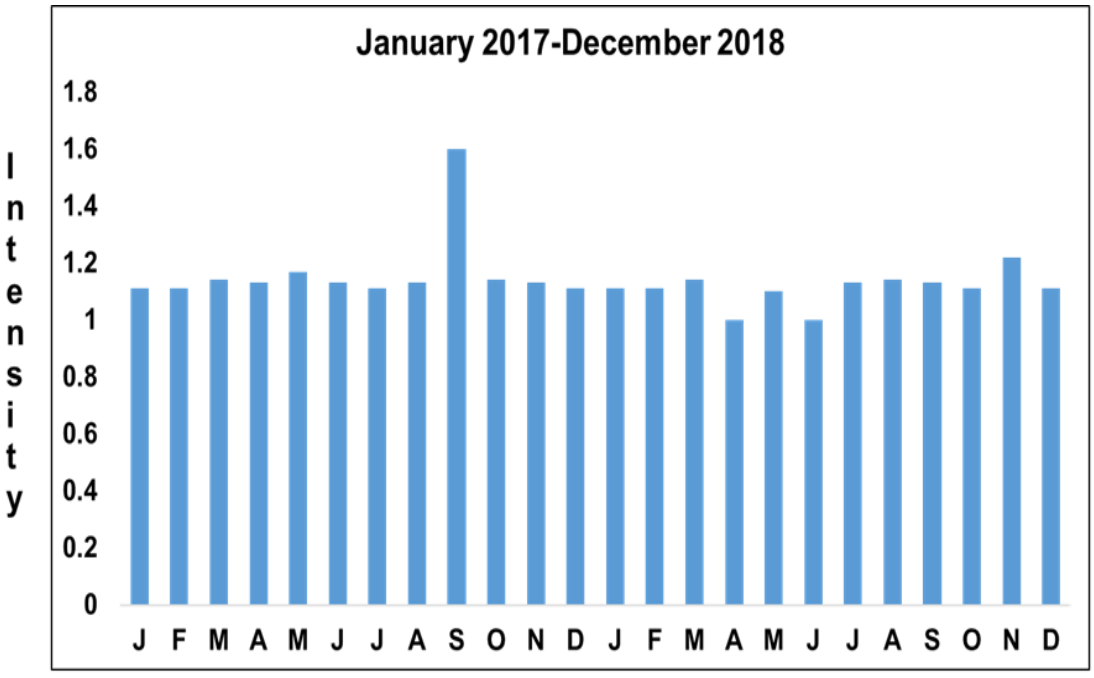
In *Xenentodon cancila*

In 2017, the maximum prevalence (69.23%) was observed in the month of December'17 and lowest prevalence (38.46%) of infestation was in September'17. In 2018, the highest prevalence (75%) was found in November'18 whereas the lowest prevalence (50%) was found in both June'18 and August'18. In 2017, the highest intensity (1.6) of infestation was observed in the month of September'17 and lowest intensity (1.11) was observed in the months of January'17, February'17, July'17 and December'17. In 2018, the highest intensity (1.22) of infestation was found in November'18 while the intensity of parasites was lowest (1) observed in the month of April'18 and June'18 (Fig.-28, 29).



(January 2017-December 2018)

Fig. 28. Monthly prevalence of parasites in *Xenentodon cancila*



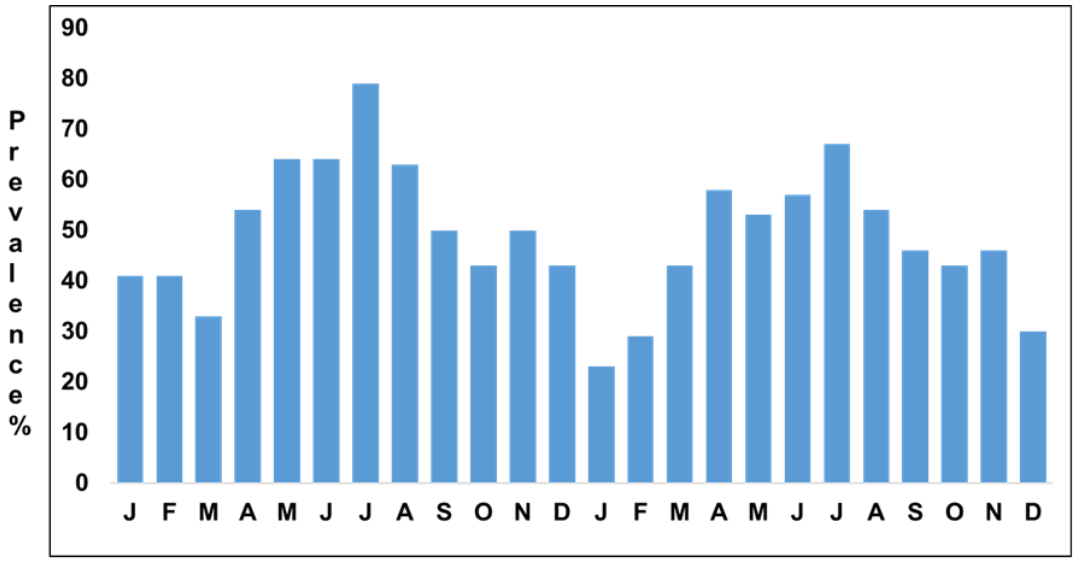
(January 2017-December 2018)

Fig. 29. Monthly intensity of parasites in *Xenentodon cancila*

In *Polynemus parasiseus*

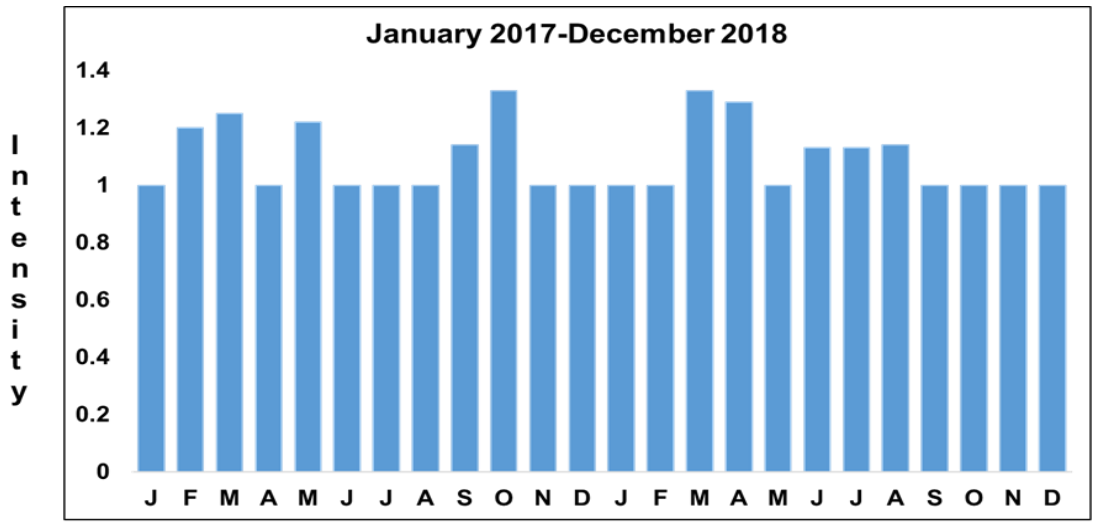
In 2017, the prevalence of infestation was highest (78.57%) in July'17 and lowest prevalence (33.33%) was found in the month of March'17.The highest prevalence (66.67%) found in July'18 and the lowest prevalence (23.08%) observed in January'18 (Fig-30).

The highest intensity of parasites (1.33) was observed in October'17 and March'18 .The lowest intensity of parasites (1) was observed in several different months of both these years (Fig. 31)



(January 2017-December 2018)

Fig. 30. Monthly prevalence of parasites in *Polynemus paradiseus*



(January 2017-December 2018)

Fig. 31. Monthly intensity of parasites in *Polynemus paradiseus*

In *Xenentodon cancila*

In 2017, the prevalence of infestation was highest (64%) during winter and lowest prevalence (53%) was found during summer season. On the other hand, in 2018, the highest prevalence (71%) was found during the winter and the lowest prevalence (59 %) was observed during summer season (Fig-32).

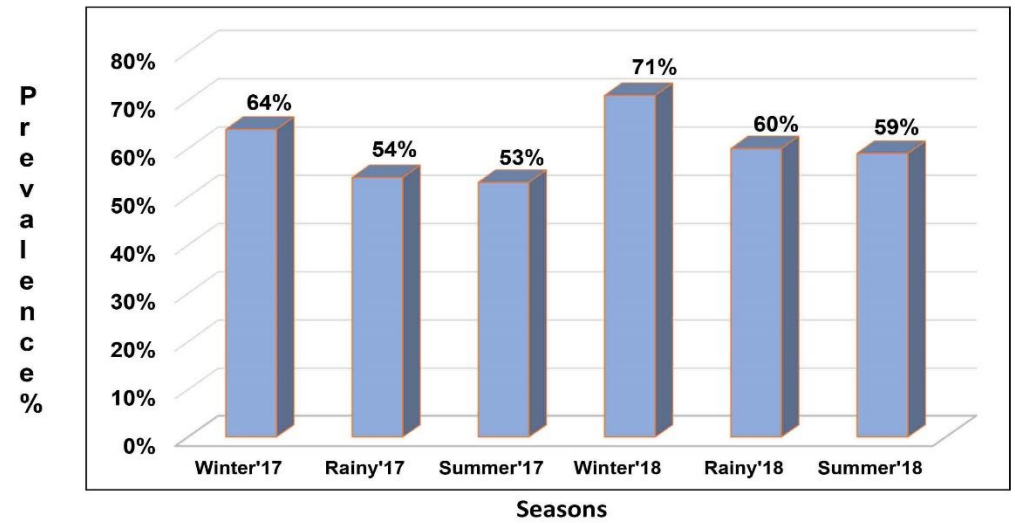


Fig. 32. Seasonal prevalence of parasites in *X. cancila* during the study period (January 2017-December 2018)

The intensity was highest (1.21) during rainy season and lowest (1.11) was observed during winter season of 2017 while in 2018, the highest intensity (1.14) was found during winter and the lowest intensity (1.06) observed during the summer season (Fig -33).

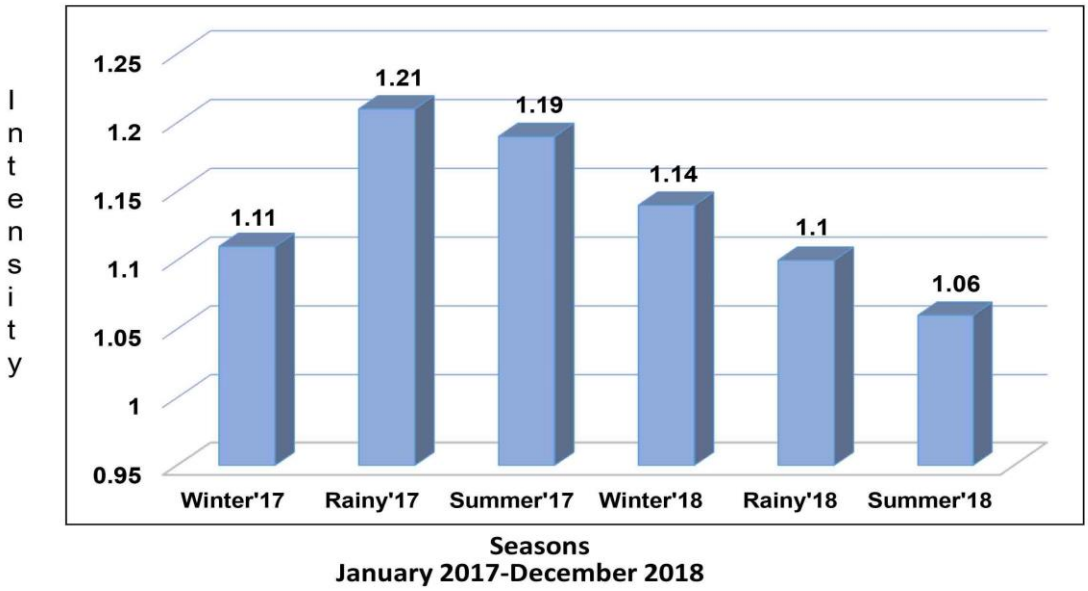


Fig. 33. Seasonal intensity of parasites in *X. cancila* during the study period January 2017 to December 2018

In *Polynemus parasiseus*

In case of *P. pardiseus*, in 2017, the prevalence of infestation was highest (58%) during rainy'17 and lowest prevalence (44%) was found during winter'17. On the other, in 2018, the highest prevalence (53%) was observed during summer and the lowest prevalence (32%) was observed during winter season (Fig-34).

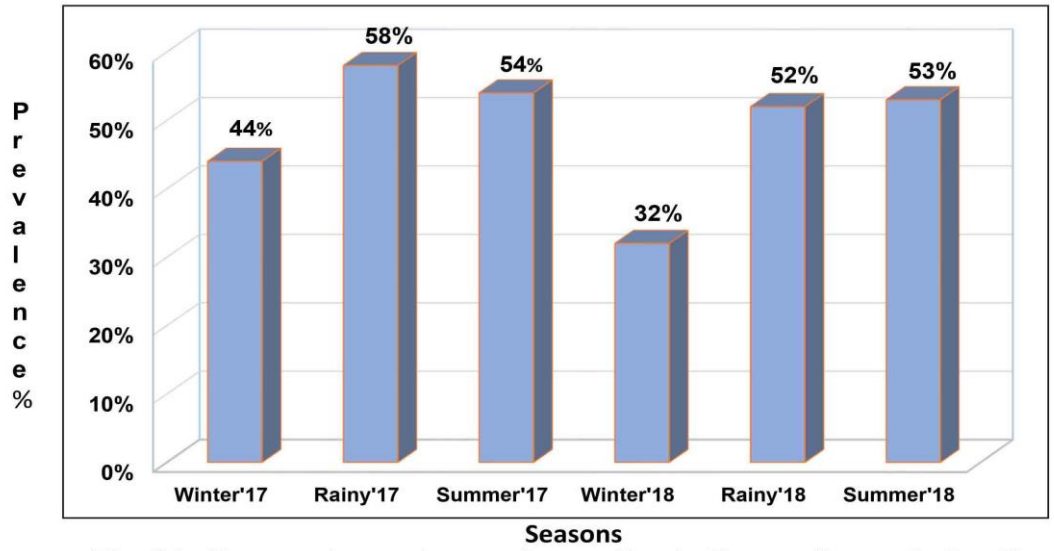


Fig. 34. Seasonal prevalence of parasites in *P. paradiseus* during the study period of January 2017 to December 2018

The intensity was observed highest (1.12) during summer and rainy seasons and lowest (1.05) during winter season of 2017 while in 2018, the highest intensity (1.19) was found during summer and the lowest intensity (1) during the winter season (Fig-35).

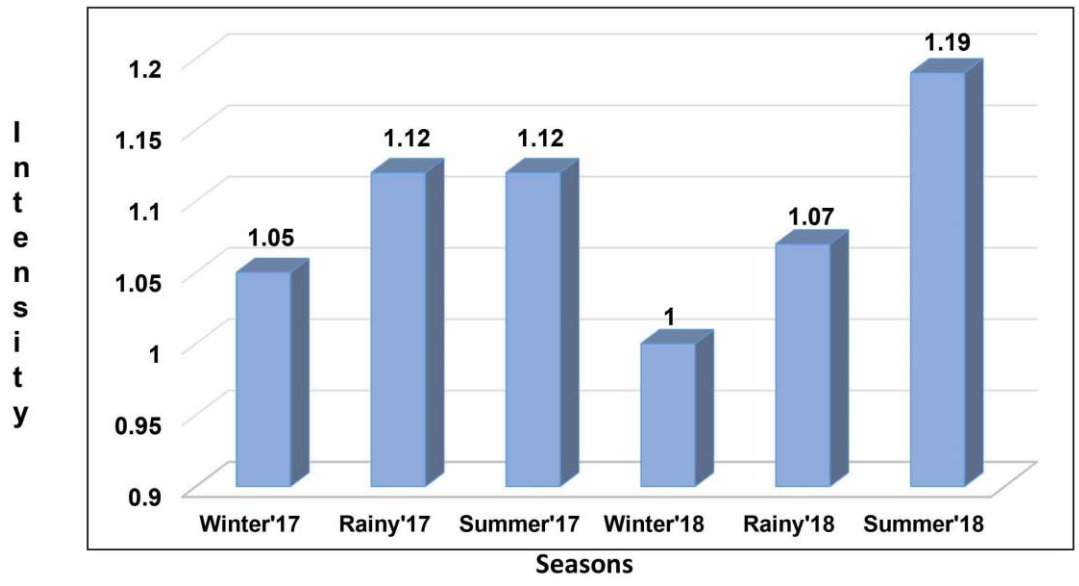


Fig. 35. Seasonal intensity of parasites in *P. paradiseus* during the study period (January 2017-December 2018)

Table-14: **Seasonal association of the infected fishes by helminth parasites**

Seasons	***Polynemus paradiseus***			***Xenentodon cancila***		
	No. of infected fish	**No. of non-Infected fish**	**P-value (using chi Square test)**	**No. of infected fish**	**No. of non-Infected fish**	**P-value (using chi Square test)**
2017						
Summer	29	24		27	24	
Rainy	34	24	0.298	29	25	0.457
Winter	22	28		35	20	
Total	**85**	**76**		**91**	**69**	
2018						
Summer	29	26		34	24	
Rainy	27	25	0.053	32	21	0.433
Winter	17	36		35	15	
Total	**73**	**87**		**101**	**60**	

Table-14 shows the "chi square test" providing the association of the number of infected and non-infected fish with different seasons. In *P. paradiseus* there was no statistically significance of being infected by parasites over the seasons during 2017 but there was an association to be infected by parasites over the seasons during 2018 at 10% level of significance. In case of *X. cancila,* there was no association of being infected by the parasites with the season's durind 2017 and 2018.

Table-15: Statistical analysis of infected fishes over seasons

Host fish	Season	No. of infected fish	No. of non-infected fish	P-value (using chi Square test)
P. paradiseus	Summer	58	50	0.019
	Rainy	61	49	
	Winter	39	64	
X. cancila	Summer	61	48	0.215
	Rainy	61	46	
	Winter	70	35	

Table-15 shows the result of "chi square test". The infected host fish *P. paradiseus* was significantly associated (P>0.05) with the seasons (rainy, winter and summer) during the study period. Reverse tendency was shown in *X. cancila i. e.* the infected fish *X. cancila* was not significantly associated (P>0.05) with the seasons (rainy, winter and summer).

Infestation of parasites in relation to Sex of the fishes

Variation of parasite infestation in male and female *Xenentodon cancila* and *Polynemus paradiseus*

The present investigation was done on two species of fish, *Xenentodon cancila* and *Polynemus paradiseus.* Out of 321 *X. cancila* examined, there were 178 male (55%) and 143 female (45%). On the other, a total of 321 *P. paradiseus* dissected, there were 148 male (46%) and 173 female (54%). In *X. cancila*, the prevalence of female (75%) was higher than male (48%) and in *P. paradiseus* the prevalence was slightly more in female (51.45%) than male (46.62%) (Table-16).

Table-16: Prevalence of helminth parasites among *P. paradiseus* and *X. cancila* according to male and female

Name of the fish host	Total no. of host examined	Sex	Total no. Of host examined	% of host examined	Total no. of host infested	% of host infested	Total no. of parasites
P. paradiseus	321	Male	148	46.11	69	46.62	76
		Female	173	53.89	89	51.45	96
X. cancila	321	Male	178	55.45	85	47.75	94
		Female	143	44.54	107	74.83	124

Table-17: Association between male and female *P. paradiseus* and *X. cancila* (through chi square test)

Host fish	Sex	No. of infected fish	No. of non-Infected fish	Hypothesis	P-value (using chi Square test)
P. paradiseus	Male	69	79	H_0: There is no association between male and female to be infected in *P. paradiseus* or *X. cancila*	0.98
	Female	89	84		
X. cancila	Male	85	93		<0.001
	Female	107	36		

Table-17 shows the "chi square test" whether having association between number of infected fish and sex of the fish. There was no association between male and female *P. paradiseus* to be infected at 5% level of significance. Since P value is much smaller for *X. cancila.* The sex of *X. cancila* is statistically associated to be infected at 5% level of significance. Thus, male and female have significant contribution of being infected in *X. cancila*.

Monthly prevalence of helminth parasite in male and female *P. paradiseus* during Jan'17-Dec'18

The present investigation was done on two species of fish, *Polynemus paradiseus* and *Xenentodon cancila*. Out of 321 *P. paradiseus* examined, there were 148 male and 173 female. On the other hand, a total of 321 *X. cancila* dissected, there were 178 male and 143 female.

In the present investigation, the maximum prevalence was observed 83.33% (July'17) and 66.67% (June'18 and November'18) in male while in female, the maximum prevalence was observed 75% (July'17) and 83.33% (April'18) respectively. The lowest prevalence (28.57%) in male was observed in January, March and December of

2017 and 14.29% in January of 2018. In female, the lowest prevalence 28.57% was observed in December of 2017 and 20% was observed in December of 2018. (Tables-18, 19; Fig. 37).

Table-18: Monthly prevalence of helminth parasites in male and female *P. paradiseus* during Jan'17-Dec'17

Month	**Male**			**Female**		
	No. of Fish examined	**No. of fish infected**	**Prevalence (%)**	**No. of Fish examined**	**No. of fish infected**	**Prevalence (%)**
January	7	2	28.57	5	3	60
February	6	3	50	6	2	33.33
March	7	2	28.57	5	2	40
April	5	4	80	8	3	37.5
May	5	3	60	9	6	66.67
June	5	4	80	9	5	55.56
July	6	5	83.33	7	6	75
August	6	3	50	10	7	70
September	4	3	75	10	4	40
October	8	3	37.5	6	3	50
November	5	3	80	7	3	42.86
December	7	2	28.57	7	4	28.57
Overall	**71**	**37**	**63.17**	**90**	**48**	**52.34**

Table-19: Monthly prevalence of helminth parasites in male and female *P. paradiseus* during Jan'18-Dec'18

Month	Male			Female		
	No. of Fish examined	No. of fish infected	Prevalence (%)	No. of Fish examined	No. of fish infected	Prevalence (%)
January	7	1	14.29	6	2	33.33
February	7	2	28.57	7	2	28.57
March	8	3	37.5	6	3	50
April	6	2	33.33	6	5	83.33
May	7	3	42.86	8	5	62.5
June	6	4	66.67	8	4	50
July	5	3	60	7	5	71.43
August	6	2	33.33	7	5	71.43
September	5	3	60	8	3	37.5
October	6	2	33.33	8	4	50
November	6	4	66.67	7	2	28.57
December	8	3	37.5	5	1	20
Overall	**77**	**32**	**44.88**	**83**	**41**	**48.89**

Monthly prevalence of helminth parasite in male and female *X. cancila* during Jan'17-Dec'18

In male *X. cancila*, highest prevalence (83.33%) was recorded in December'17 and November'18. The lowest prevalence (28.57%) of male was found in April and June of 2017 and 22.22% in June of 2018. In female *X. cancila*, the highest prevalence 100% was observed in January, April, June, August of 2017; February, September and October of 2018 while the lowest prevalence 28.57% was observed in March of 2017 and 60%in August of 2018 (Table-20, 21; Fig.36).

Table-20: Monthly prevalence of helminth parasites in male and female *X. cancila* during Jan'17-Dec'17

Month	Male			Female		
	No. of Fish examined	No. of fish infected	Prevalence (%)	No. of Fish examined	No. of fish infected	Prevalence (%)
January	9	4	40	6	5	100
February	9	5	55.56	5	4	80
March	5	3	60	7	2	28.57
April	7	2	28.57	6	6	100
May	9	3	33.33	4	3	75
June	7	2	28.57	6	6	100
July	8	5	62.5	7	4	57.14
August	8	3	50	5	5	100
September	6	1	66.67	7	4	71.43
October	7	4	71.43	6	3	33.33
November	6	4	66.67	7	4	57.14
December	6	5	83.33	7	4	57.14
Overall	**87**	**41**	**53.89**	**73**	**50**	**71.65**

Table-21: Monthly prevalence of helminth parasites in male and female *X. cancila* during Jan'18-Dec'18

Month	Male			Female		
	No. of Fish examined	No. of fish infected	Prevalence (%)	No. of Fish examined	No. of fish infected	Prevalence (%)
January	7	4	57.14	6	5	83.33
February	8	4	50	5	5	100
March	8	3	37.5	5	4	80
April	8	4	50	6	5	83.33
May	7	4	57.14	8	6	75
June	9	2	22.22	7	6	85.71
July	6	3	50	7	5	71.42
August	9	4	44.44	5	3	60
September	9	4	44.44	4	4	100
October	7	3	42.86	5	5	100
November	6	5	83.33	6	4	66.67
December	7	4	57.14	6	5	83.33
Overall	**91**	**44**	**49.68**	**70**	**57**	**82.40**

Monthly Intensity of helminth parasites in male and female *X. cancila* during Jan'17-Dec'18

In the present investigation, the highest mean intensity in males was (2) observed in September'17 and 1.33 in July'18. In female, the highest mean intensity was (2) in

March'17 and (1.33) in August'18. The lowest mean intensity (1) was observed in both male and female host fish in several months of both the years (Fig-38).

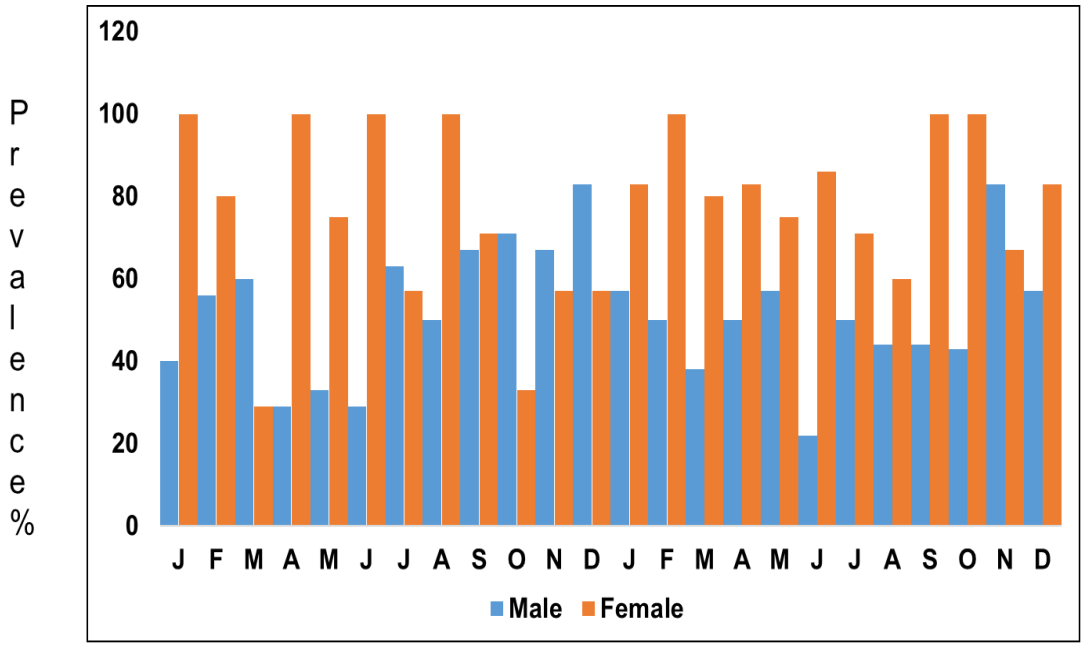


Fig. 36. Monthly prevalence of helminth parasites in male and female of *X. cancila*

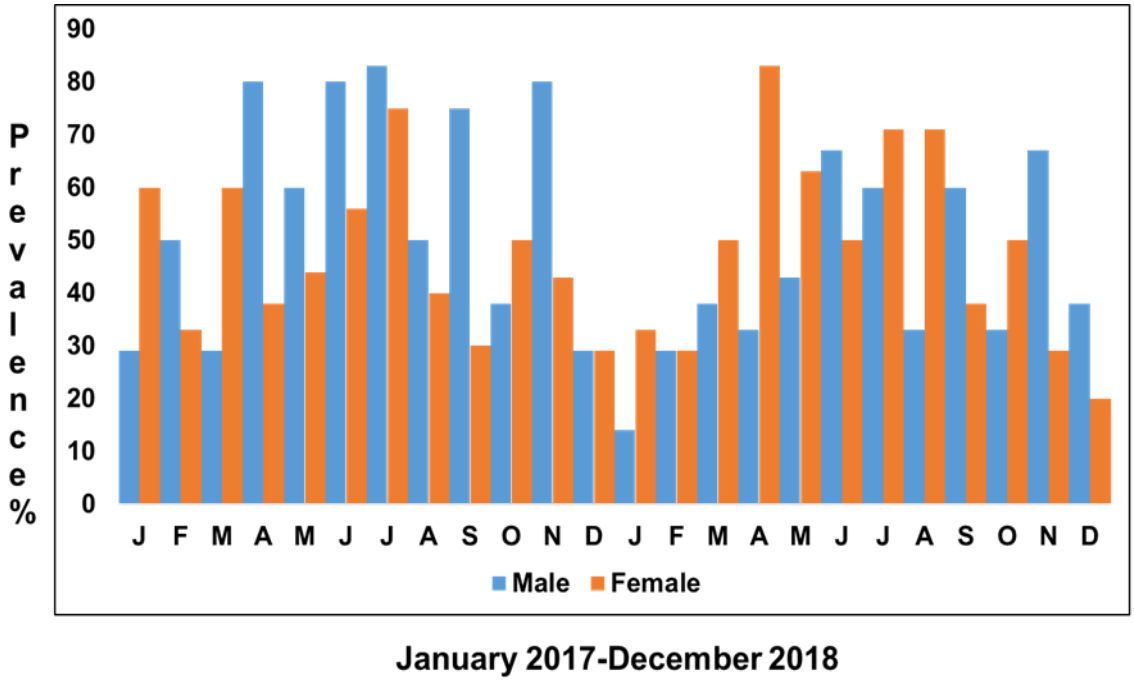
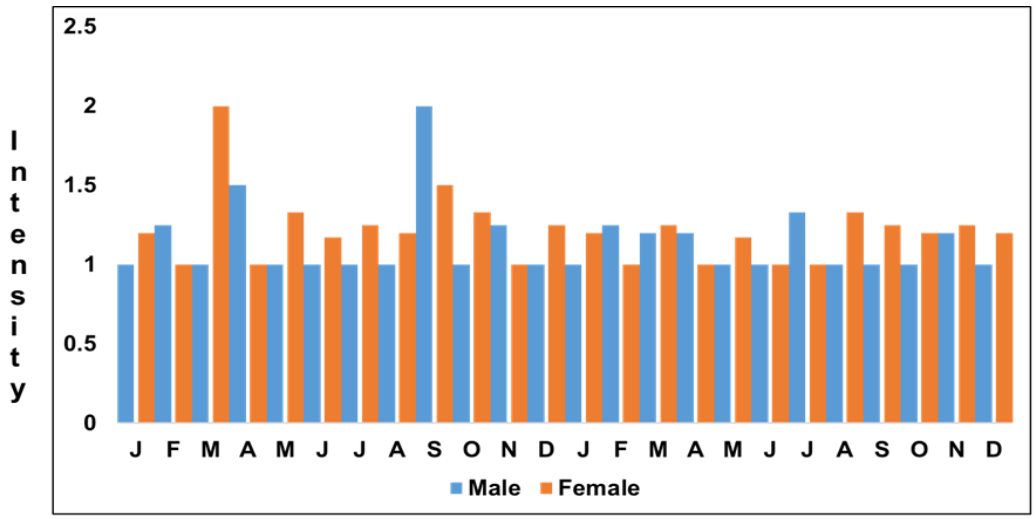


Fig. 37. Monthly prevalence of helminth parasites in male and female of *P. paradiseus*

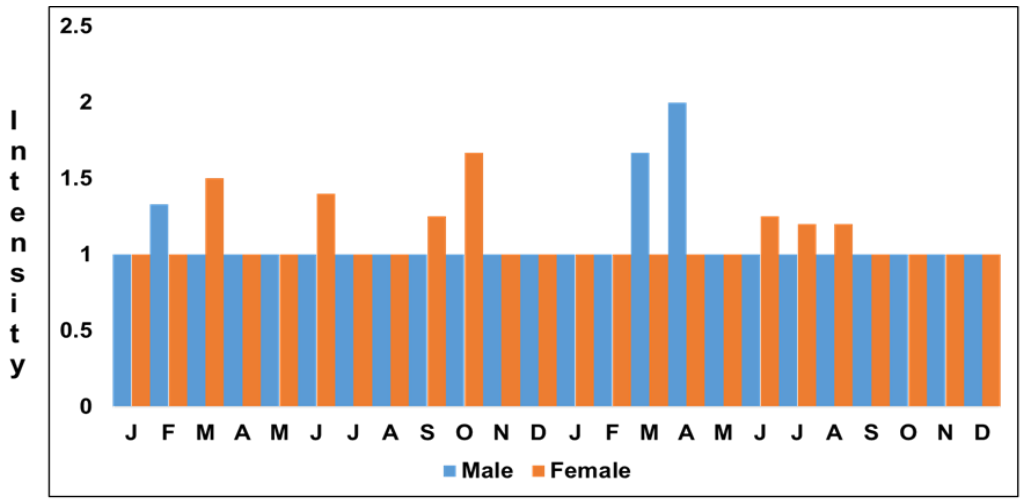


January 2017-December 2018

Fig. 38. Monthly intensity of helminth parasites in male and female of *X. cancila*

Monthly intensity of helminth parasites in male and female *P. paradiseus* during Jan'17-Dec'18

In the present investigation, the highest mean intensity in males was (1.33) in Feb'17 and 2 in April'18. In female, the highest mean intensity was (1.67) in October'17 and



January 2017-December 2018

Fig. 39. Monthly intensity of helminth parasites in male and female of *P. paradiseus*

1.25 in June'18. The lowest mean intensity in male and female was observed (1) in different months of both the years (Fig-39).

The overall proportion of infected *X. cancila* differs significantly at 5% level of significance between male and female. It reveals that the prevalence of infected male (47.75%) was significantly lower than female (74.83%) with P<0.05. However, there was no significant association found between male and female of *P. paradiseus* (P>0.05) during the study period (Table-22).

Table-22: Statistical analysis of helminth parasites in male and female *P. paradiseus* and *X. cancila* (Proportion Test)

Host fish	**Male**	**Female**	**P-value**
P. paradiseus			
Prevalence (%)	46.62	51.45	0.39
Mean Intensity of parasites	1.10	1.08	
X. cancila			
Prevalence (%)	47.75	74.83	<0.001
Mean Intensity of parasites	1.11	1.16	

Table-23: Analysis of helminth parasites between male and female over the year

Year	***P. paradiseus***					***X. cancila***				
	Male		**Female**		**P-value (using Proportion Test)**	**Male**		**Female**		**P-value (using proportion Test)**
	N	%	N	%		N	%	N	%	
2017	71	44.10	90	55.90	0.137	87	53.89	73	71.65	0.021
2018	77	48.13	83	51.88	0.6355	91	49.68	70	82.40	<0.0001
Overall	148	46.62	173	51.45	0.1935	178	47.75	143	74.83	0.052

Table-23 shows the two sample binomial proportion test result. The overall proportion of infected male and female *X. cancila* differs significantly at 5% level of significance between two periods 2017 and 2018. It reveals that the overall prevalence of infected male (47.75%) was significantly lower than female (74.83%). There was strong association (P<0.05) observed between male and female in case of *X. cancila.* However, there was no significant association found between male and female of *P. paradiseus* (P>0.05)

Monthly prevalence and intensity of trematodes in male and female *X. cancila*

In male *X. cancila*, the highest prevalence (66.67%) of trematodes was observed in August of 2017 and 50% in May of 2018. The lowest prevalence (25%) found in July, October, November, December of 2017 and February, August, September and October of 2018.

The highest prevalence (40%) of trematodes in female *X. cancila* was found in August, September of 2017 and 60% in December of 2018. Lowest prevalence 16.67% was observed in April, June of 2017 and May of 2018 (Fig. 40).

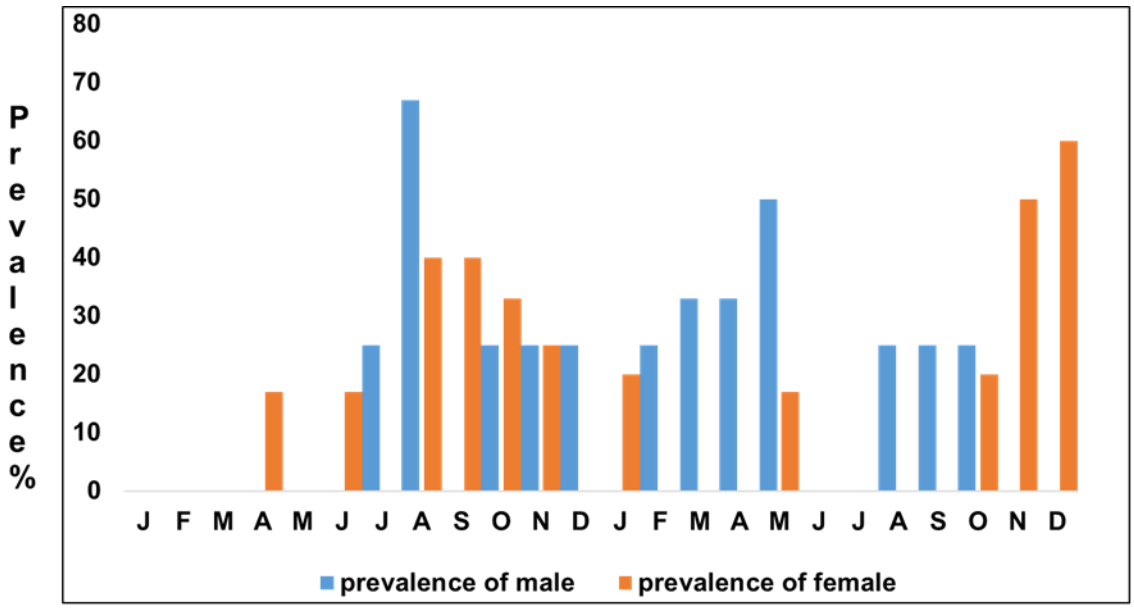


Fig. 40. Monthly prevalence of trematodes in male and female *X. cancila*

In male and female *X. cancila*, the intensity of trematodes was recorded (1) (Fig. 41).

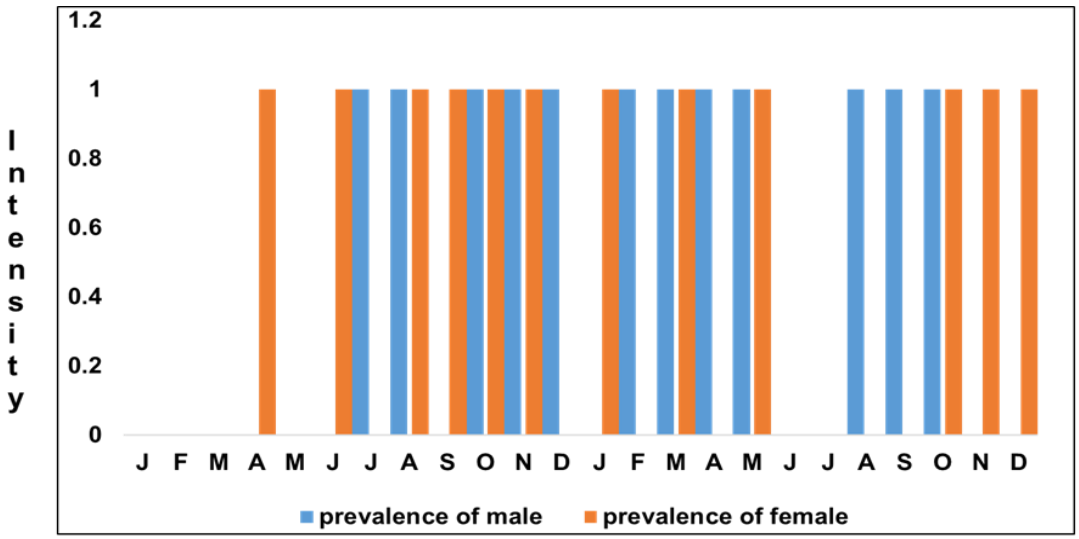


Fig. 41. Monthly intensity of trematodes in male and female *X. cancila*

Monthly prevalence and intensity of trematodes in male and female *P. paradiseus*

In male *P. paradiseus*, the highest prevalence (100%) of trematodes was observed in January, May, June of 2017 and January, February, April and July of 2018. The lowest prevalence (33.33%) was found in July of 2017 and December of 2018. No trematodes was found in August of 2018.

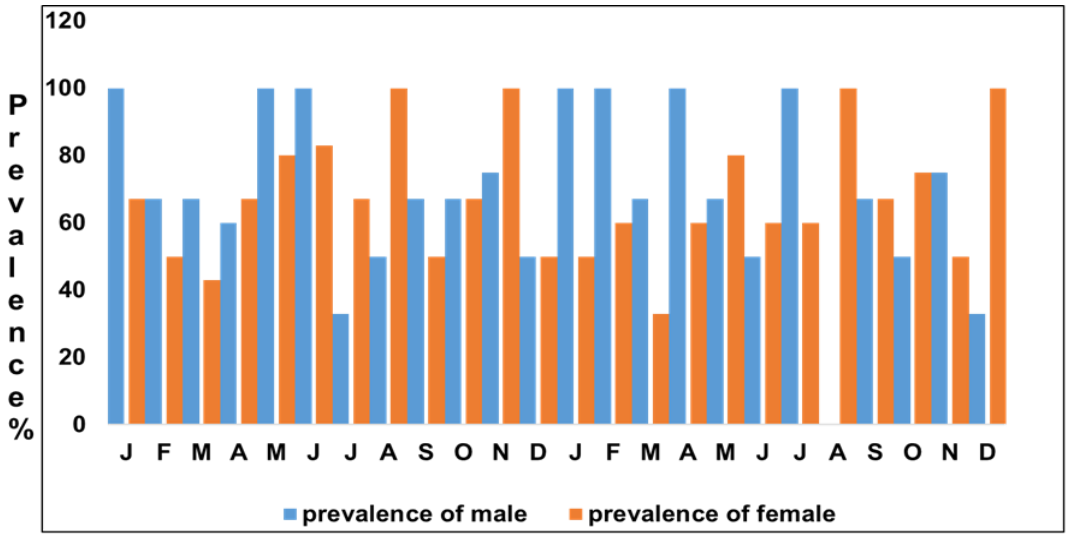


Fig. 42. Monthly Prevalence of trematodes in male and female *P. paradiseus*

The highest prevalence (100%) of trematodes in female *P. paradiseus* was found in August, November of 2017 and August, December of 2018. Lowest prevalence 42.86% was observed in March'17 and 33.33% in March of 2018 (Fig.-42).

In male *P. paradiseus*, the highest intensity of trematodes (2) was recorded in June of 2018. The intensity (1) was found to be the same for the rest of the months of both the years except for the month March'18 (1.5). The female *P. paradiseus* had the highest intensity (1.5) of trematodes in August, September, October of 2017and (1.33) in February and April of 2018 while the lowest intensity (1) was found in several months of both the years (Fig 43).

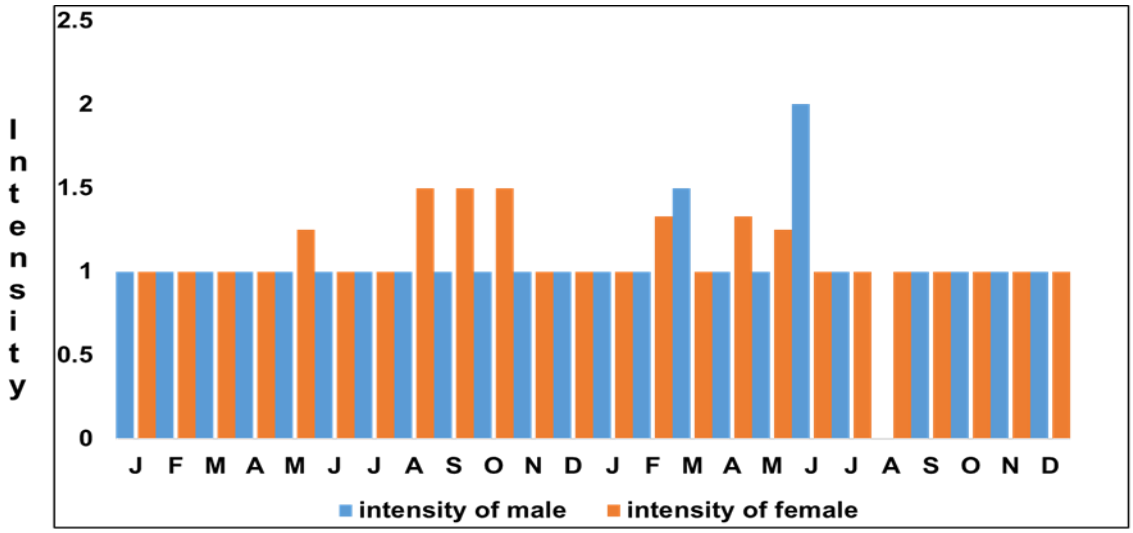
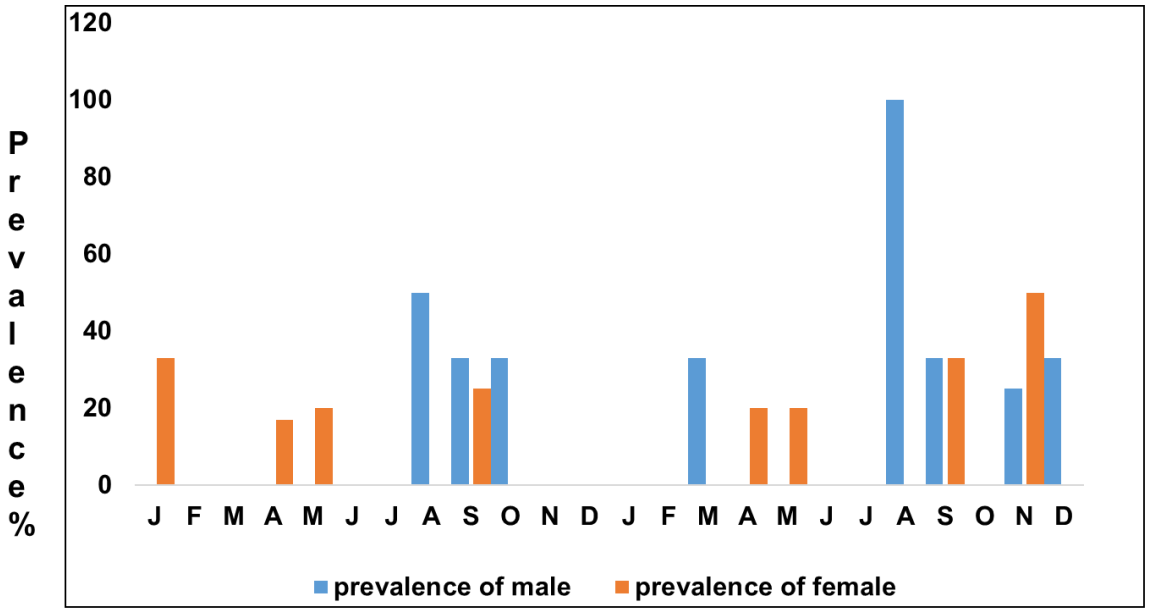


Fig. 43. Monthly intensity of trematodes in male and female *P. paradiseus*

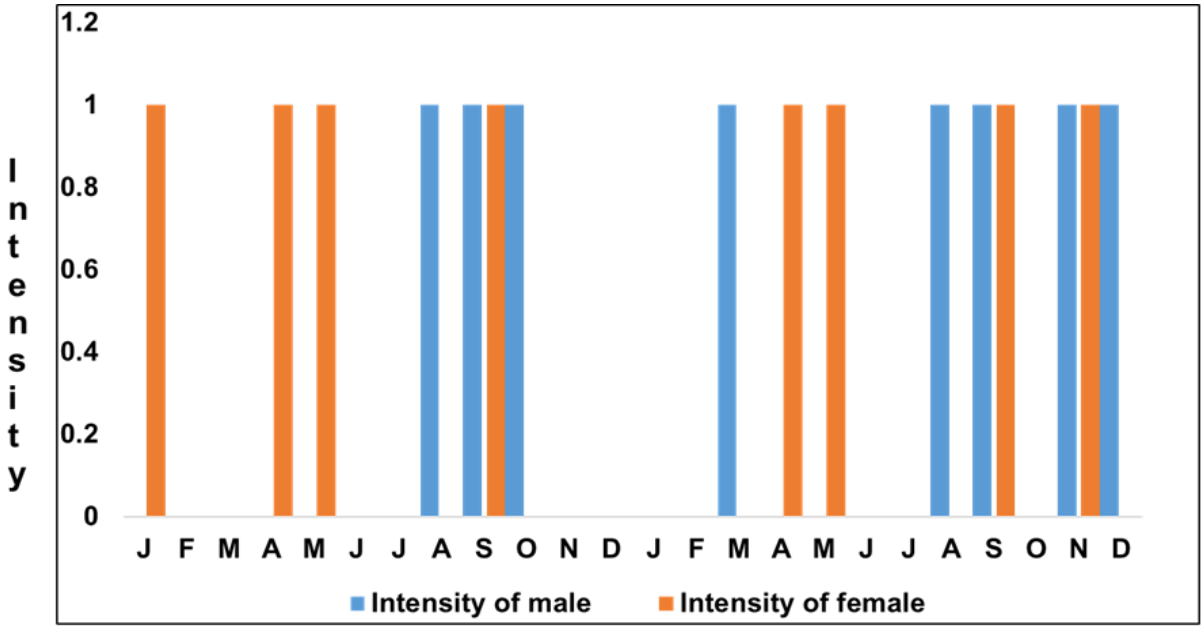
Monthly prevalence and intensity of cestodes in male and female *P. paradiseus*

In male *P. paradiseus*, the highest prevalence (50%) of cestodes was observed in August'17 and 100% in August'18; the lowest prevalence 33.33% was found in September'17, October'17 and 25% in November'18. No cestode parasite was observed in male in several months of both the years. The highest prevalence (33.33%) of cestodes in female *P. paradiseus* was found in January'17 and 50% in November'18. Lowest prevalence 16.67% was observed in April'17 and 20% in April'18 and May'18. Cestode was absent in female host fish in several months of both the years (Fig. 44).



January 2017-December 2018

Fig. 44. Monthly Prevalence of cestodes in male and female *P. paradiseus*



January 2017-December 2018

Fig. 45. Monthly intensity of cestodes in male and female *P. paradiseus*

In male and female *P. paradiseus*, the intensity of cestodes was recorded (1) in all the months of both the years (Fig. 45).

Cestodes were totally absent in *X. cancila.*

Monthly prevalence and intensity of nematodes in male and female *X. cancila*

In male *X. cancila*, the highest prevalence (100%) of nematodes was observed in month June of both the years. The lowest prevalence (25%) found in August and October of 2017 and 20% in September of 2018.

The highest prevalence (66.67%) of nematodes in female *P. paradiseus* was found in January'17 and December'18. Lowest prevalence 20% was observed in March, July of 2017 and January, April, August, October of 2018 (Fig.-46).

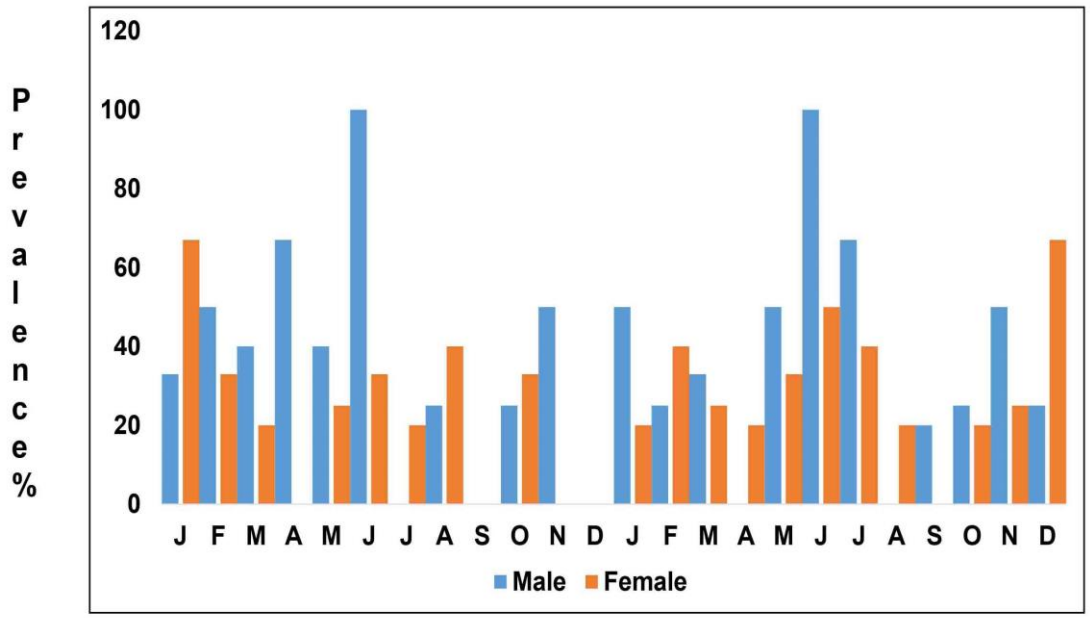
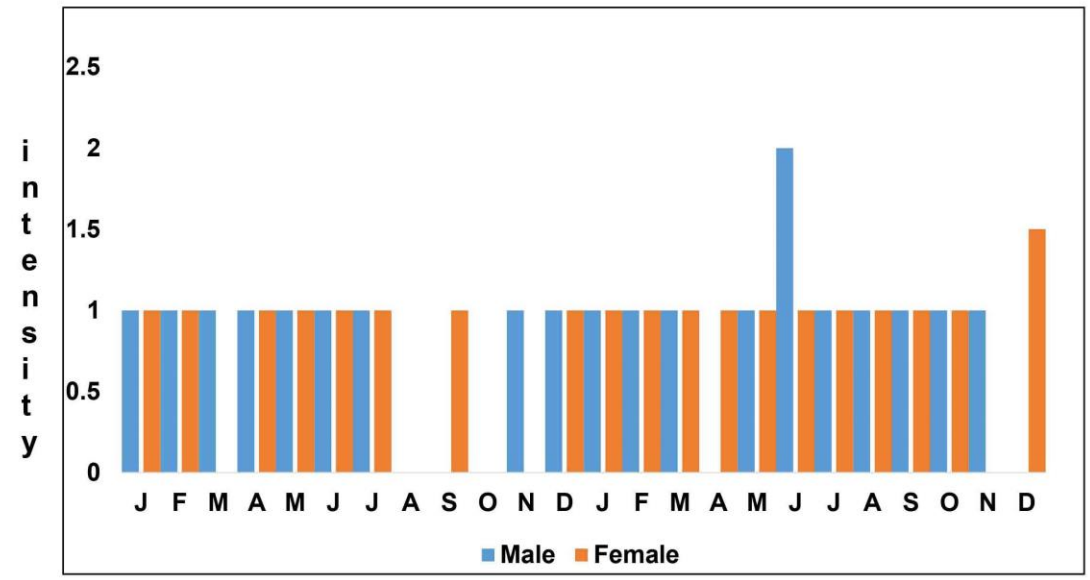


Fig. 46. Monthly prevalence of nematodes in male and female *X. cancila*

In male *x. cancila*, the highest intensity of nematodes (2) was recorded in June'18 while the lowest intensity (1) was found in rest of the months of both the years. The female *P. paradiseus* had the highest intensity (1.5) of nematodes in December'18 while the lowest intensity (1) was found in rest of the months of both the years (Fig-47).

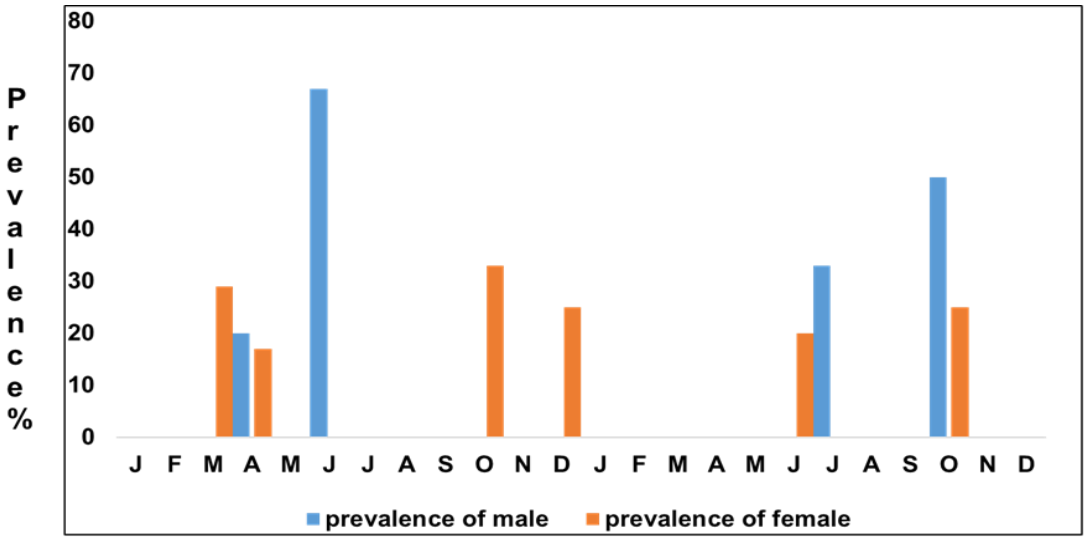


January 2017-December 2018

Fig. 47. Monthly intensity of nematodes in male and female *X. cancila*

Monthly prevalence and intensity of nematodes in male and female *P. paradiseus*

In male *P. paradiseus*, nematodes showed the highest prevalence (66.67%) in June'17 and 50% in October'18 while the lowest prevalence (20%) observed in April'17 and 33.33% in July'18. No nematodes was observed in fifteen months out of 24 months of the study periods.



January 2017-December 2018

Fig. 48. Monthly Prevalence of nematodes in male and female *P. paradiseus*

In female *P. paradiseus*, in 2017, the nematodes showed the highest prevalence (33.33%) in October of 2017 and 25% in October of 2018. The lowest prevalence (16.67%) was found in April of 2017 and 20% in June of 2018. In male and female *P. paradiseus*, the intensity of nematodes was recorded (1) (Fig.- 48, 49).

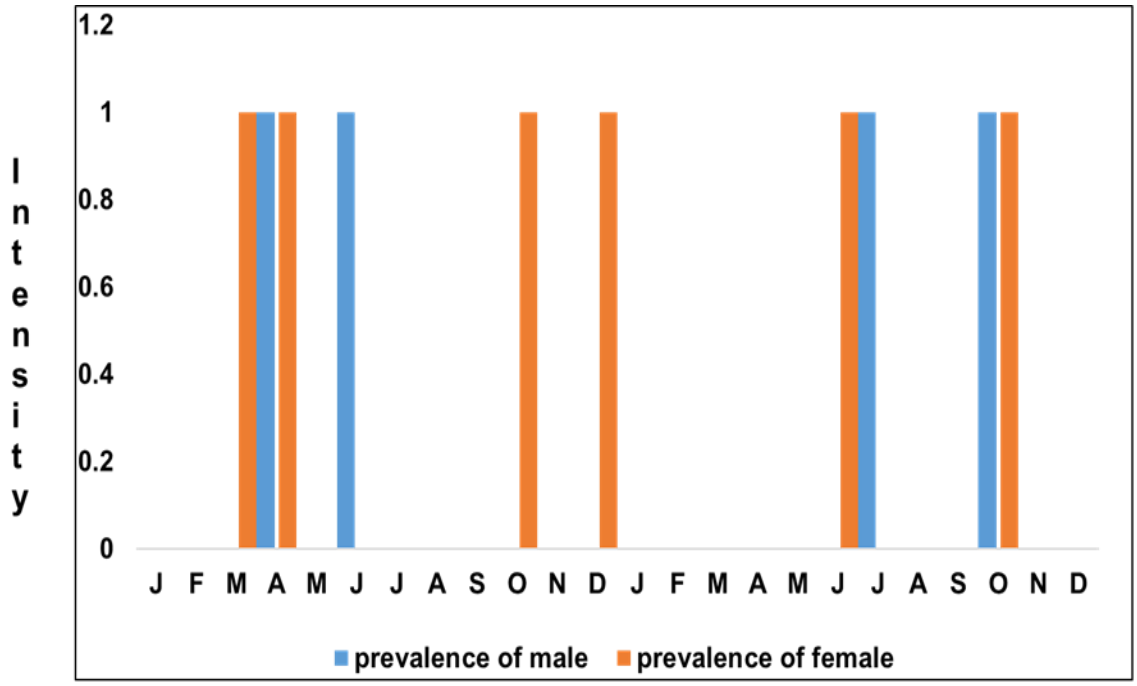
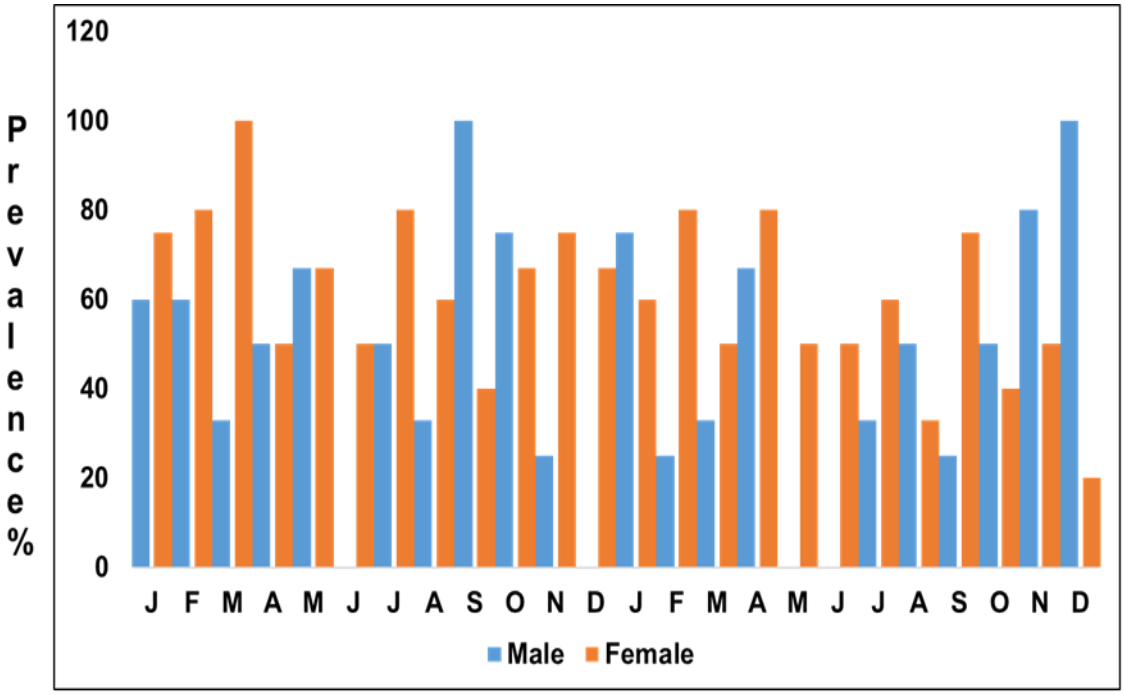


Fig. 49. Monthly intensity of nematodes in male and female *P. paradiseus*

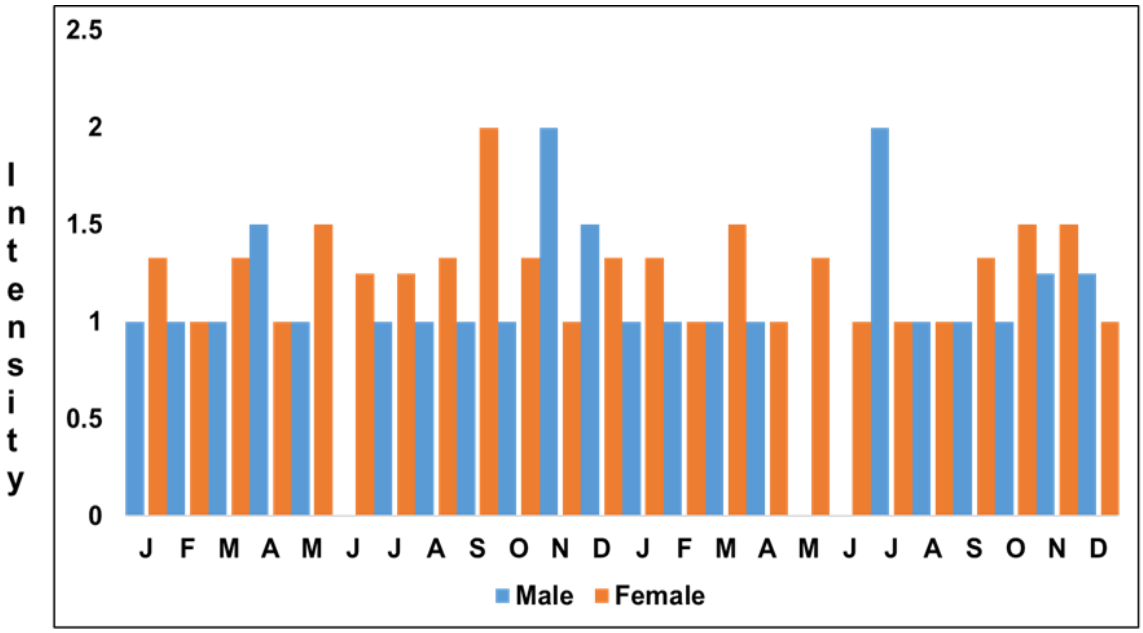
Monthly prevalence and intensity of acanthocephalan in male and female *X. cancila*

In male *X. cancila*, in 2017 the highest prevalence (100%) of acanthocephalans was observed in September of 2017 and December of 2018. The lowest prevalence (25%) was found in November of 2017 and February, September of 2018 (Fig. 50). In female *X. cancila*, in 2017, the highest prevalence (100%) was recorded in March'17 while the lowest prevalence (40%) found in September'17. On the other hand, the highest prevalence (80%) was found in February, April of 2018 and lowest prevalence (20%) was found in December of 2018 (Fig. 50).



January 2017-December 2018

Fig. 50. Monthly prevalence of acanthocephalan parasites in male and female *X. cancila*



January 2017-December 2018

Fig. 51. Monthly intensity of acanthocephalan parasites in male and female *X. cancila*

In male *X. cancila*, the highest intensity of acanthocephala was recorded 2 in November of 2017 and July of 2018. The lowest intensity (1) was found in most of the months of both the years. In female *X. cancila*, the highest intensity of acanthocephala (2) was recorded in September of 2017 and 1.5 in March, October, November of 2018. The lowest intensity (1) was found in February'17, April'17, Novembe'17, February'18, April'18, June'18, July'18, August'18 and December'18 (Fig.-51).

Monthly prevalence and intensity of acanthocephalan in male and female *P. paradiseus*

In case of male *P. paradiseus*, acanthocephala showed the highest prevalence (50%) in December'17 and June'18; the lowest prevalence (20%) in Apri'17 and 33.33% in December'18. No acanthocephalans was found in several different months of both the years (Fig. 52).

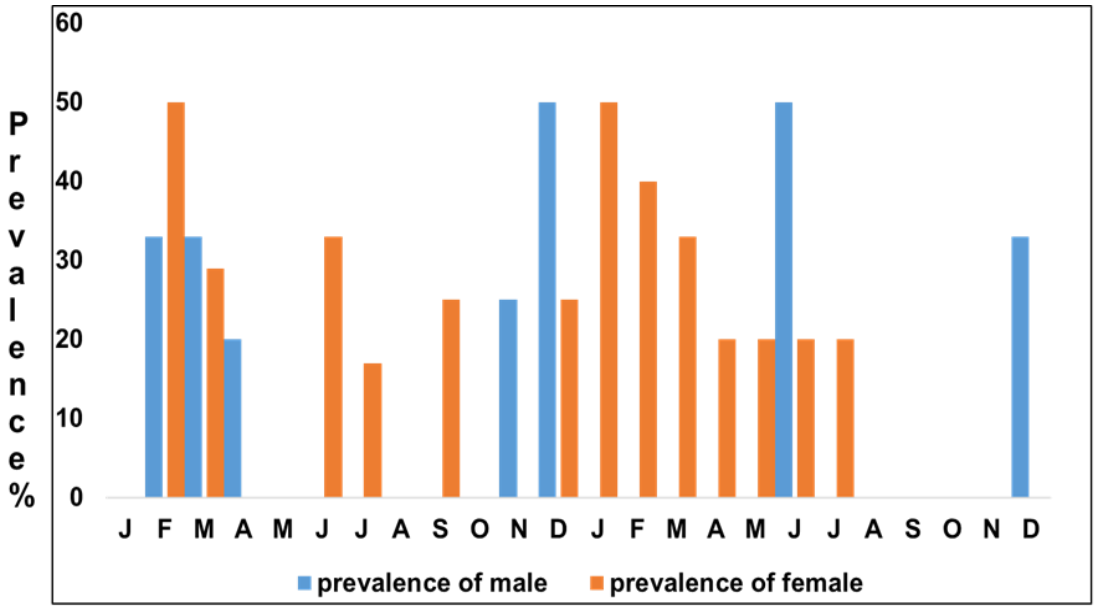


Fig. 52. Monthly Prevalence of acanthocephalans in male and female *P. paradiseus*

In case of female *P. paradiseus*, the acanthocephala showed the highest prevalence (50%) in February'17 and January'18. The lowest prevalence (16.67%) was observed in July'17 and 20% was observed from April to July of 2018 (Fig-52).

In male and female *P. paradiseus*, the intensity (1) of acanthocephala was recorded in most of the months of the study period. (Fig. 53).

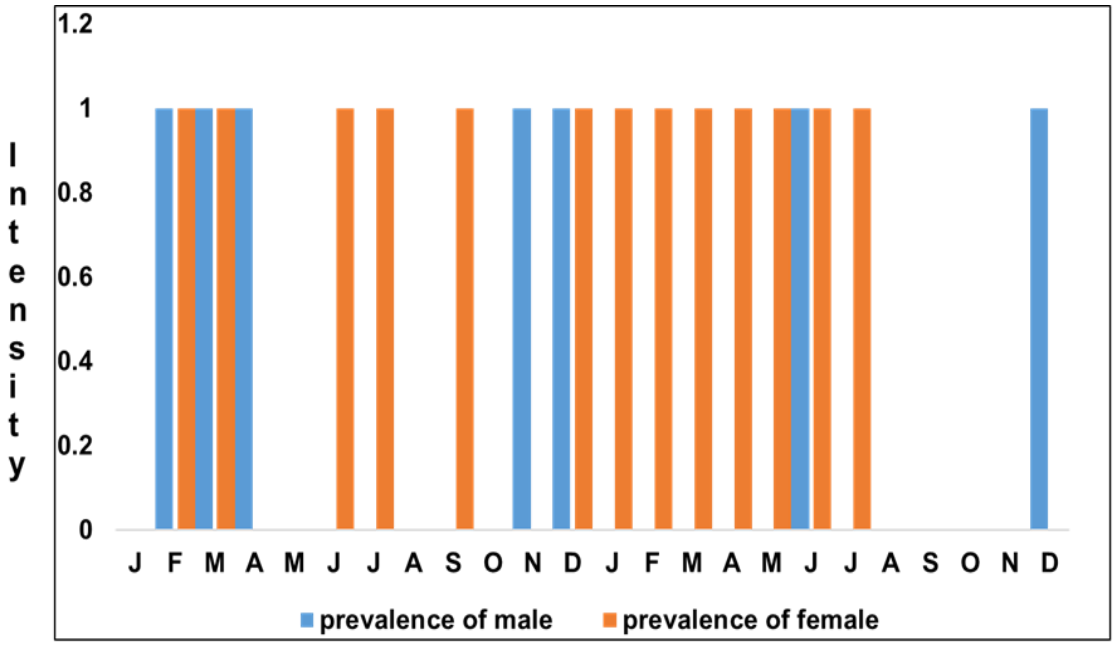


Fig. 53. Monthly intensity of acanthocephalans in male and female *P. paradiseus*

Infestation of parasites in relation to Length of the fishes

Relationship of helminth infestation with length of *Xenentodon cancila* and *Polynemus paradiseus*

The present investigation was done on two species of fish, *P. pardiseus* and *X. cancila*. A total of 321 *P. paradiseus*, 158 were infected and out of 321 *X. cancila*, 192 were infected with different helminth parasites. In the present study, *P. paradiseus* and *X. cancila* were grouped into four length groups. In *P. paradiseus*, the length groups were 7-9 cm, 9.1-11 cm, 11.1-13 cm and 13.1-15 cm. In *X. cancila*, the groups were 20-25 cm, 25.1-30 cm, 30.1-35 cm and 35.1-40 cm.

In *X. cancila*, during the study period, the maximum prevalence of helminth parasites infestation (76.2%) was observed in the largest length group 35.1-40 cm and the minimum prevalence (39.73%) was observed in the smallest (20-25 cm) length group. On the other hand, the intensity of helminth parasites in *X. cancila* was observed lowest (1.13) in the length groups 25.1-30 cm , 30.1-35cm and the highest (1.18) was observed in the smallest (20-25 cm) length group (Table-24).

Table-24: Prevalence and intensity of helminth parasites among four length groups of *X. cancila* during Jan'17-Dec'18

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
20-25	73	29	39.73	34	1.18
25.1-30	78	41	52.56	46	1.13
30.1-35	82	55	67.07	62	1.13
35.1-40	88	67	76.2	76	1.14
Total	**321**	**192**	**58.90**	**218**	**1.15**

In *X. cancila*, during Jan'17-Dec'17, the maximum prevalence of helminth parasites infestation was recorded 79.07% in the largest group (35.1-40 cm) and the minimum

prevalence (35.14%) found in the smallest (20-25 cm) length group. On the other hand, in 2018, the highest prevalence (73.33%) was recorded in the largest length group (35.1-40 cm) and the lowest prevalence (44.44%) found in 20-25 cm length group (Table-25, 26; Fig.-54, 55).

Table-25: Prevalence and intensity of helminth parasites among four length groups of *X. cancila* during Jan'17-Dec'17

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
20-25	37	13	35.14	16	1.23
25.1-30	39	18	46.15	22	1.22
30.1-35	41	26	63.41	29	1.12
35.1-40	43	34	79.07	39	1.15
Total	**160**	**91**	**55.94**	**106**	**1.18**

Table-26: Prevalence and intencity of helminth parasites among four length groups of *X. Cancila* during Jan'18-Dec'18

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
20-25	36	16	44.44	18	1.13
25.1-30	39	23	58.97	24	1.04
30.1-35	41	29	70.73	33	1.14
35.1-40	45	33	73.33	37	1.12
Total	**161**	**101**	**61.87**	**112**	**1.11**

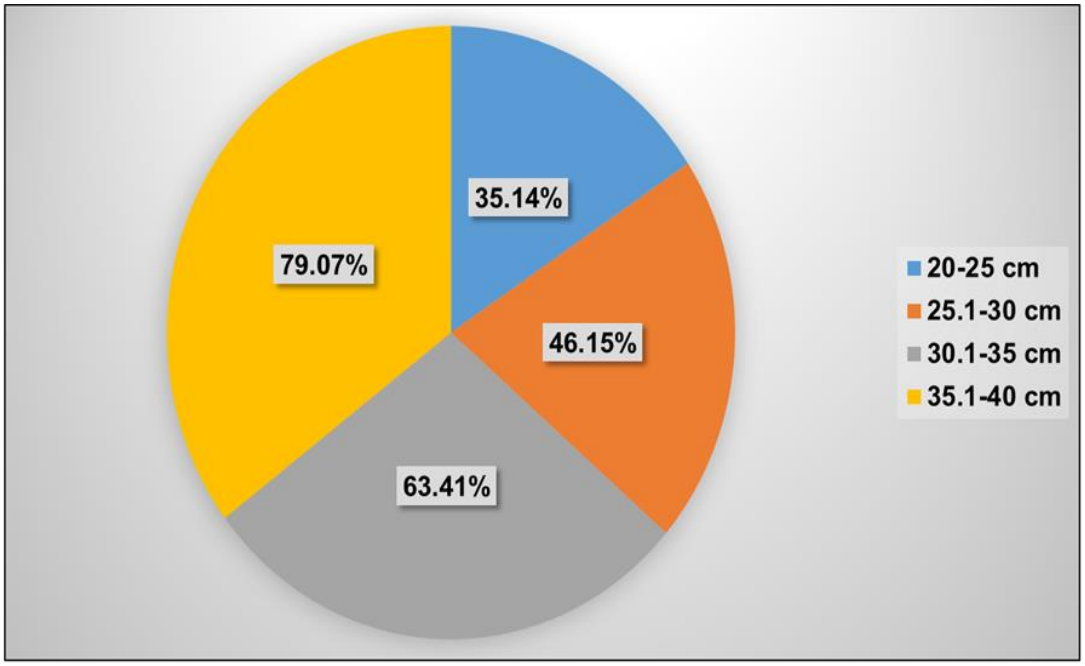


Fig. 54. Prevalence of helminth parasites in four length groups of *X. cancila* (Jan'17-Dec'17)

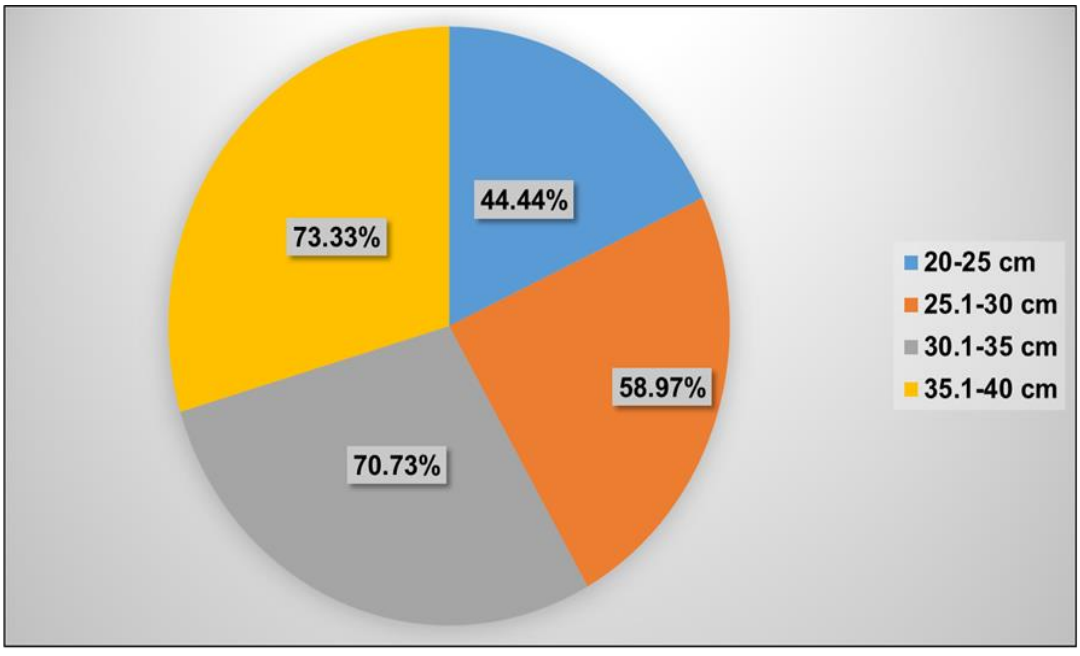


Fig. 55. Prevalence of helminth parasites in four length groups of *X. cancila* (Jan'18-Dec'18)

In 2017, the intensity of helminth parasites in *X. cancila* was found lowest (1.12) in the length group 30.1-35 cm while the highest intensity (1.23) was found in the smallest length group of 20-25 cm (Table-25, Fig-56).

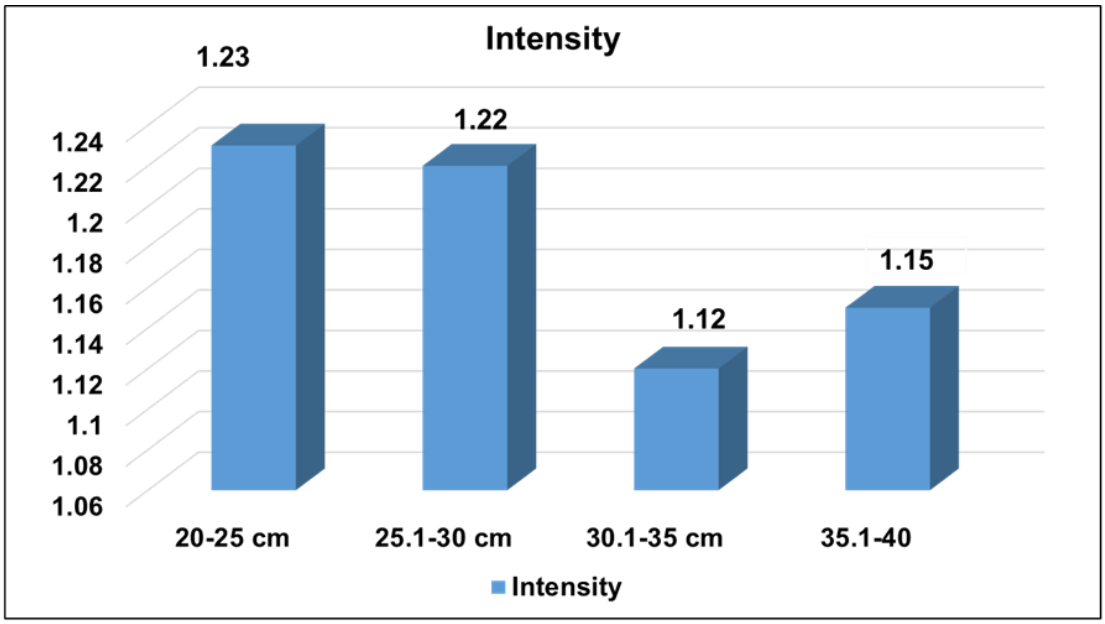


Fig. 56. Intensity of helminth parasites in four length groups of *X. cancila* (January 2017-December 2017)

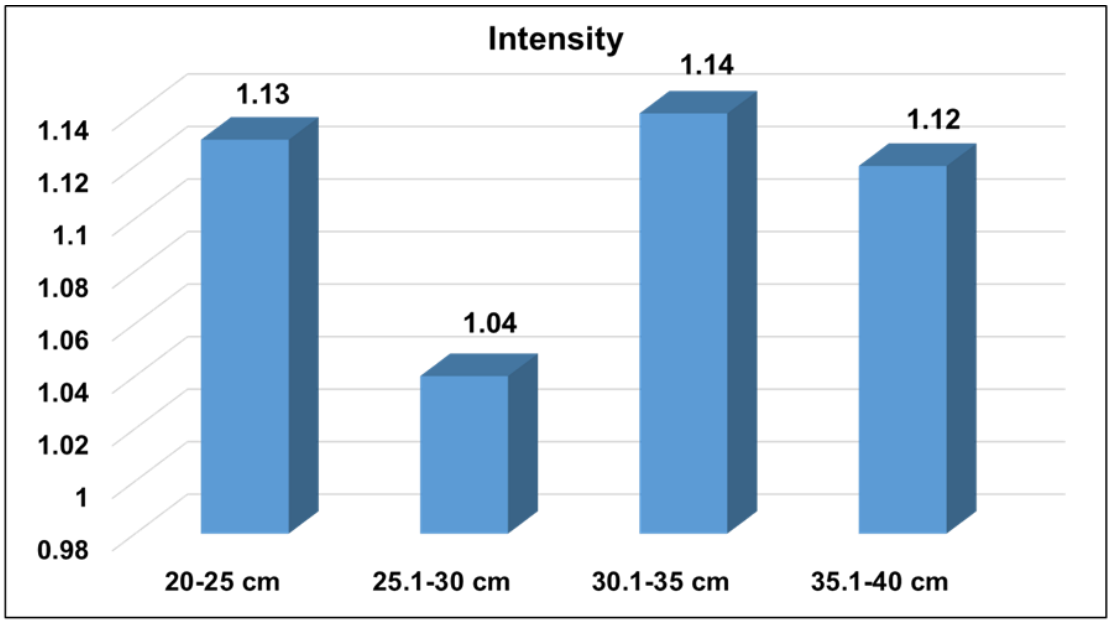


Fig. 57. Intensity of helminth parasites in four length groups of *X. cancila* (January 2018-December 2018)

On the other hand, in 2018, the highest intensity (1.14) was observed in the length group (30.1-35cm) and the lowest intensity (1.04) was found in 25.1-30 cm length group (Table-26, Fig-57).

The infection by helminth parasites was found positively correlated with length groups (r=0.99) which implied that as the length increased, infection also tends to increase.

In *P. paradiseus*, the maximum prevalence of helminth parasites infestation was 51.06% in the second smallest length group (9.1-11 cm) and the minimum prevalence (45.59%) found in the largest length group (13.1-15 cm). The highest intensity (1.16) was recorded in the largest length group 13.1-15 cm while the lowest intensity (1) found in the smallest length group (7-9 cm) (Table-27).

Table-27: Prevalence and intensity of helminth parasites among four length groups of *P. paradiseus* during Jan'17-Dec'18

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
7-9	72	36	50	36	1
9.1-11	94	48	51.06	53	1.11
11.1-13	87	43	49.42	49	1.14
13.1-15	68	31	45.59	34	1.16
Total	**321**	**158**	**49.10**	**172**	**1.10**

In *P. paradiseus*, during Jan'17-Dec'17, the maximum prevalence of helminth parasites infestation was 55.56% in the smallest length group (7-9 cm) and the minimum prevalence 51.11% found in 11.1-13 cm length group. The intensity of helminth parasites in *P. paradiseus* was lowest (1) in smallest length group (7-9 cm) and the highest intensity (1.13) was observed in the second smallest length group (11.1-13cm) (Table-28; Fig-58, 60).

On the other hand, in 2018, the highest prevalence (50%) was recorded in the 9.1-11 cm length group and the lowest prevalence (38.89%) found in 13.1-15 cm length group. The highest intensity (1.15) was observed in the length group (11.1-13 cm) and the lowest intensity (1) was found in the smallest length group (7-9cm) (Table-29; Fig.-59, 61).

The infection by helminth parasites was found negatively correlated with length groups (r=0.41 in 2017 and r=0.29 in 2018) which implied that as the length increased, infection tends to decrease.

Table-28: Prevalence and intensity of helminth parasites among four length groups of *P. paradiseus* during Jan'17-Dec'17

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
7-9	36	20	55.56	20	1±
9.1-11	48	25	52.08	28	1.12±
11.1-13	45	23	51.11	26	1.13±
13.1-15	32	17	53.13	18	1.06±
Total	**161**	**85**	**52.97**	**92**	**1.08±**

Table-29: Prevalence and intencity of helminth parasites among four length groups of *P. paradiseus* during Jan'18-Dec'18

Length Groups (cm)	No. of fish examined	No. of fish infested	Prevalence of Infestation (%)	Total no. of parasites collected	Mean Intensity of parasites
7-9	36	16	44.44	16	1±
9.1-11	46	23	50	25	1.09±
11.1-13	42	20	47.62	23	1.15±
13.1-15	36	14	38.89	16	1.14 ±
Total	**160**	**73**	**45.24**	**80**	**1.10±**

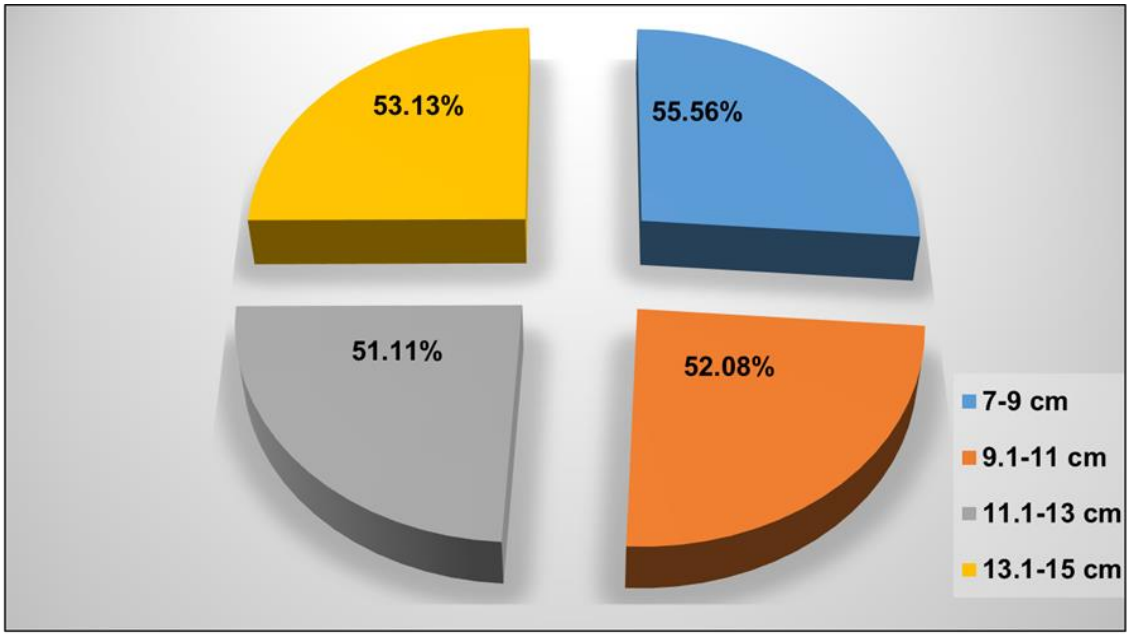


Fig. 58. Prevalence of helminth parasites in four length groups of *P. Paradiseus* (Jan'17-Dec'17)

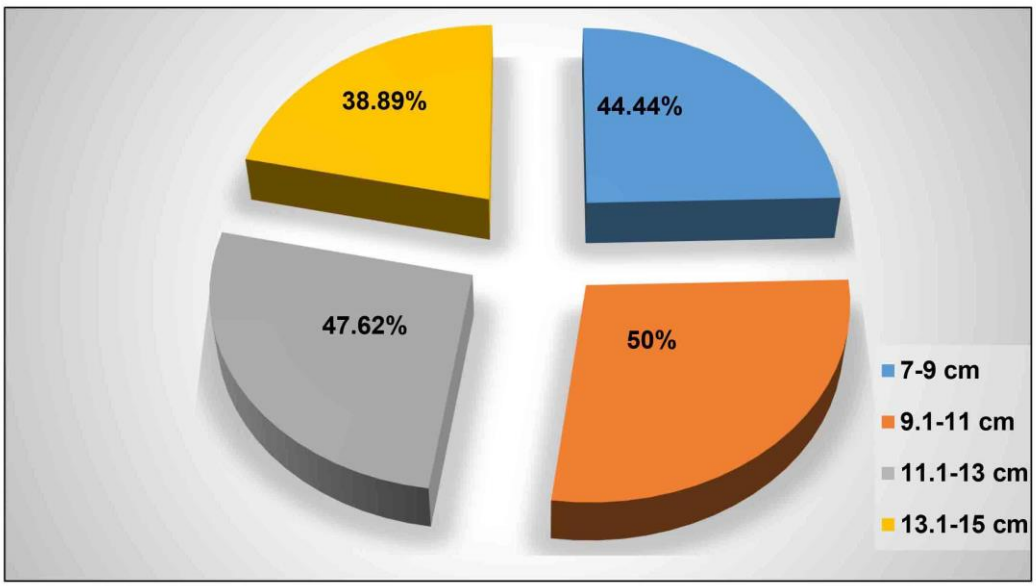


Fig. 59. Prevalence of helminth parasites in four length groups of *P. paradiseus* (Jan'18-Dec'18)

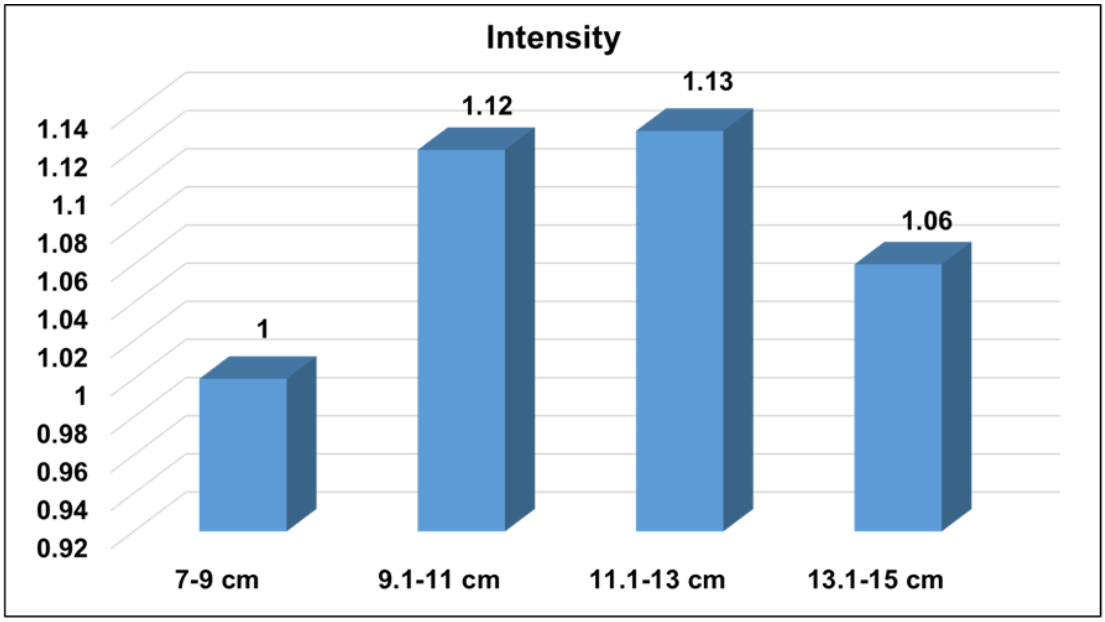


Fig. 60. Intensity of helminth parasites in four length groups of *P. paradiseus* (Jan'17-Dec'17)

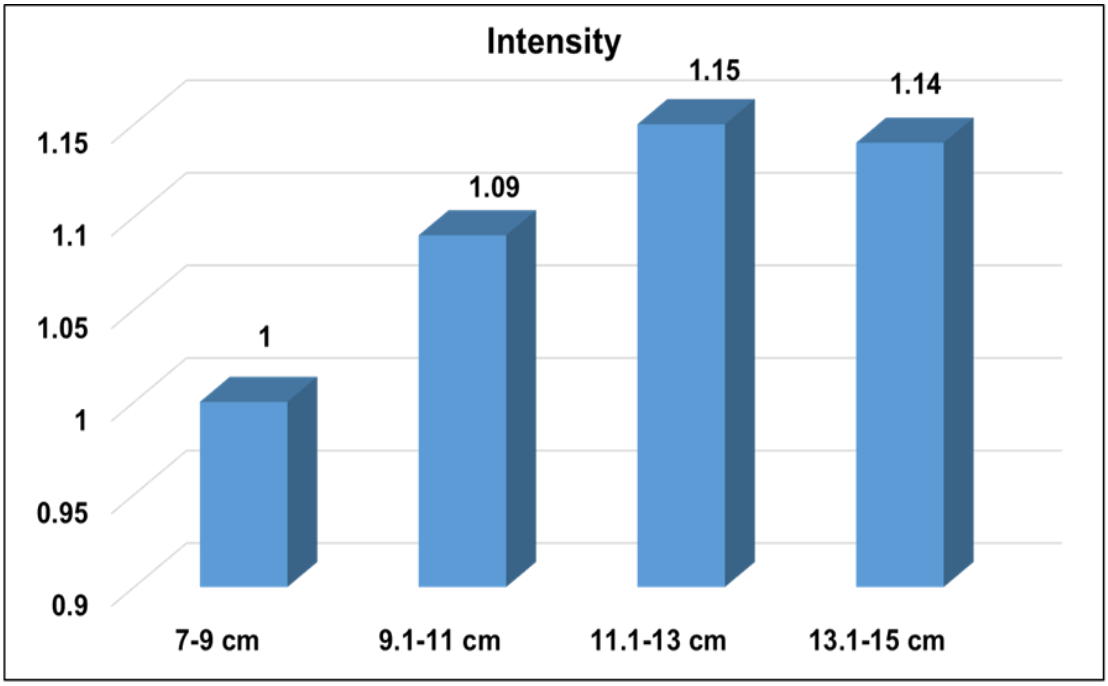


Fig. 61. Intensity of helminth parasites in four length groups of *P. paradiseus* (Jan'18-Dec'18)

Table-30: Association between length and infected fish of *P. paradiseus* and *X. cancila* by helminth parasites during 2017 and 2018 (through chi square test)

Length Groups (cm)	*P. paradiseus*			Length Groups (cm)	*X. cancila*		
	No. of infected	No. of non-infected	P-value (using chi square test)		No. of infected	No. of non-infected	P-value (using chi square test)
2017				**2017**			
7-9	20	16	0.98	20-25	13	24	<0.001
9.1-11	25	23		25.1-30	18	21	
11.1-13	23	22		30.1-35	26	15	
13.1-15	17	15		35.1-40	34	9	
2018				**2018**			
7-9	16	20	0.78	20-25	16	20	0.03
9.1-11	23	23		25.1-30	23	16	
11.1-13	20	22		30.1-35	29	12	
13.1-15	14	22		35.1-40	33	12	
Overall				**Overall**			
7-9	36	36	0.92	20-25	29	44	<0.001
9.1-11	48	46		25.1-30	41	37	
11.1-13	43	44		30.1-35	55	27	
13.1-15	31	37		35.1-40	67	21	

Table-30 shows the "chi square test" whether having association between number of infected fish of *P. paradiseus* and *X. cancila* with length groups during 2017 and 2018. The length group was significantly associated (P<0.05) with infection by helminth Parasites in case of *X. cancila*. However, there was no significant association found in *P. paradiseus.* There was no association to be infected by parasites in *P. paradiseus* with different length group during 2017.The same conclusion was drawn in 2018. In overall concept, the different length group of *P. paradiseus* were not statistically associated to be infected by parasites at 5% level of significance. Infection by parasites in *X. cancila* was found to be significantly associated (P<0.05) with length groups during 2017 and 2018. The length groups of *X. cancila* were statistically associated to be infected by parasites during 2017 at 5% level of significance. There was an

association to be infected among different length groups during 2018 at 10% level of significance. The overall infection of *X. cancila* was statistically significant with the different length groups at 5% level of significance.

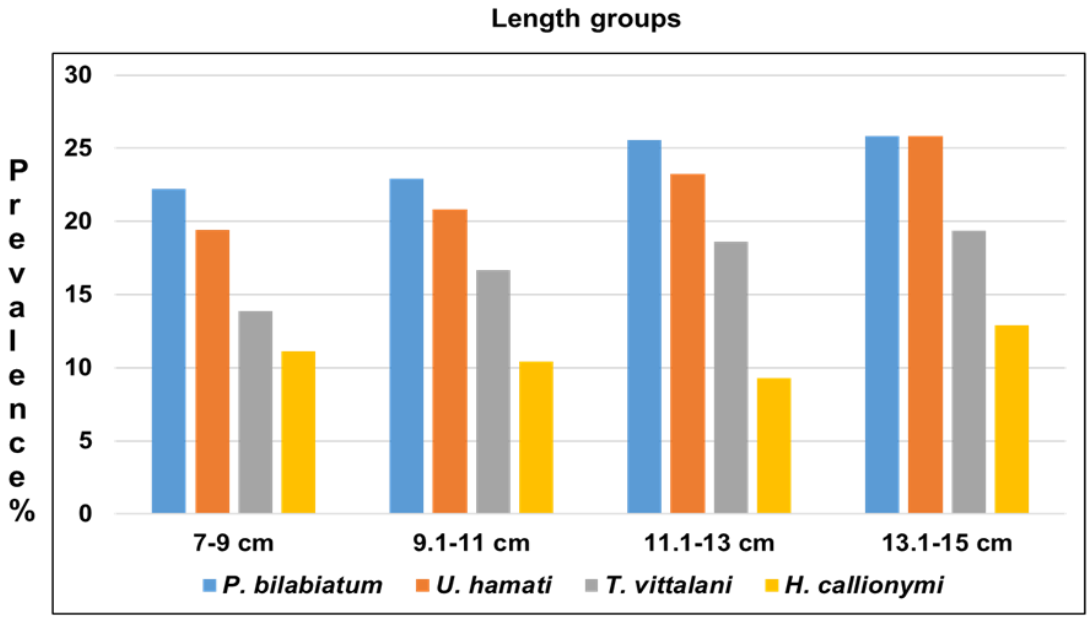


Fig. 62. Prevalence of trematodes in four length groups of *P. paradiseus*

In *P. paradiseus*, the prevalence of *P. bilabiatum* showed the highest (25.81%) in the largest length group (13.1-15cm) and the lowest (22.22 %) observed in the smallest length group (7-9cm) (Fig.-62).

The prevalence of *U. hamati* was recorded highest (25.81%) in the largest length group (13.1-15cm) and lowest (19.44%) in the length group (7-9cm) (Fig.-62).

The prevalence of *T. vittalani* was recorded highest (19.35%) in the length group (13.1-15cm) and lowest (13.89 %) in the length groups (7-9cm) (Fig.-62).

The prevalence of *H. callionymi* showed the highest (12.90%) in the length group (13.1-15cm) and the lowest (9.30 %) observed in the length group (11.1-13cm) (Fig.-62).

The prevalence of *P. trygonis* showed the highest (5.56%) in the length group (7-9 cm) and the lowest (3.23 %) observed in the length groups (13.1-15cm). The prevalence of

N. lingualis showed the highest (8.33%) in the length group (9.1-11 cm) and the lowest (3.23 %) observed in the length group (13.1-15cm) (Fig. 63).

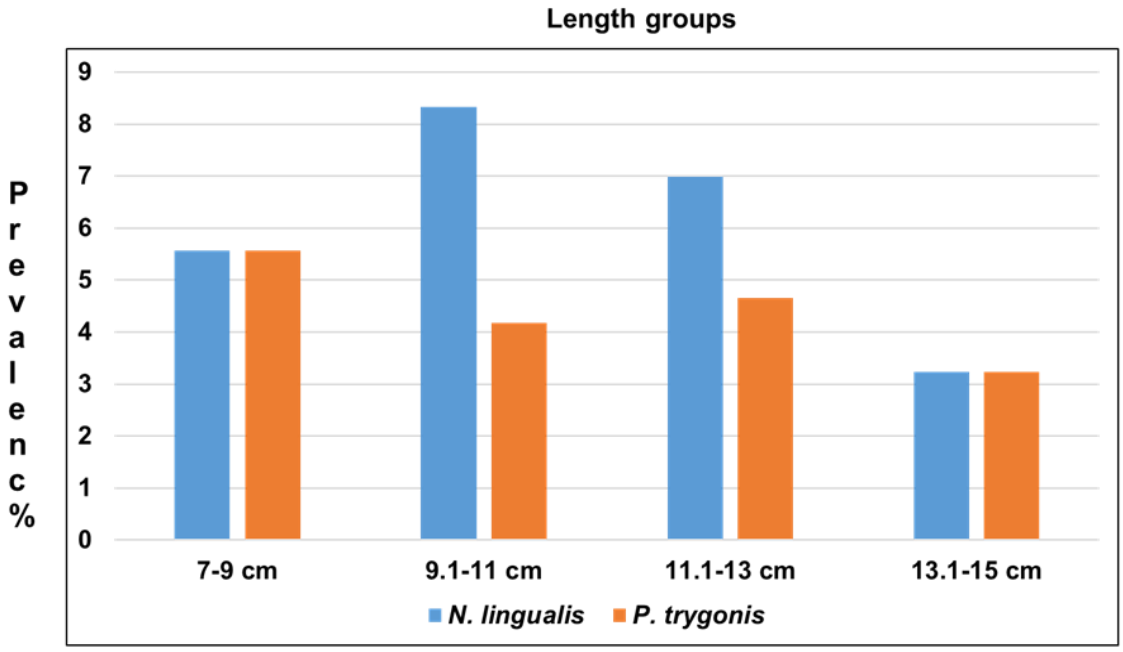


Fig. 63. Prevalence of cestodes in four length groups of *P. paradiseus*

The prevalence of *M. bagarii* showed the highest (8.33%) in the length group (9.1-11 cm) and the lowest (5.56 %) observed in the length group (7-9cm). The prevalence of *Dujardinascaris* showed the highest (8.33%) in the length group (9.1-11cm) and the lowest (2.78 %) observed in the length group (7-9cm) (Fig. 64).

The prevalence of *N. aspinosum* showed the highest (9.30%) in the length group (11.1-13 cm) and the lowest (2.08 %) observed in the length group (9.1-11cm). The prevalence of *P ophioceohali* showed the highest (6.45%) in the length group (13.1-15cm) and the lowest (2.32%) observed in the length group (11.1-13cm) (Fig-65).

In *P. paradiseus*, the intensity of *P. bilabiatum* showed highest (1.14) in the smallest length group (7-9 cm) and lowest (1) in the rest length groups (Fig.-66).

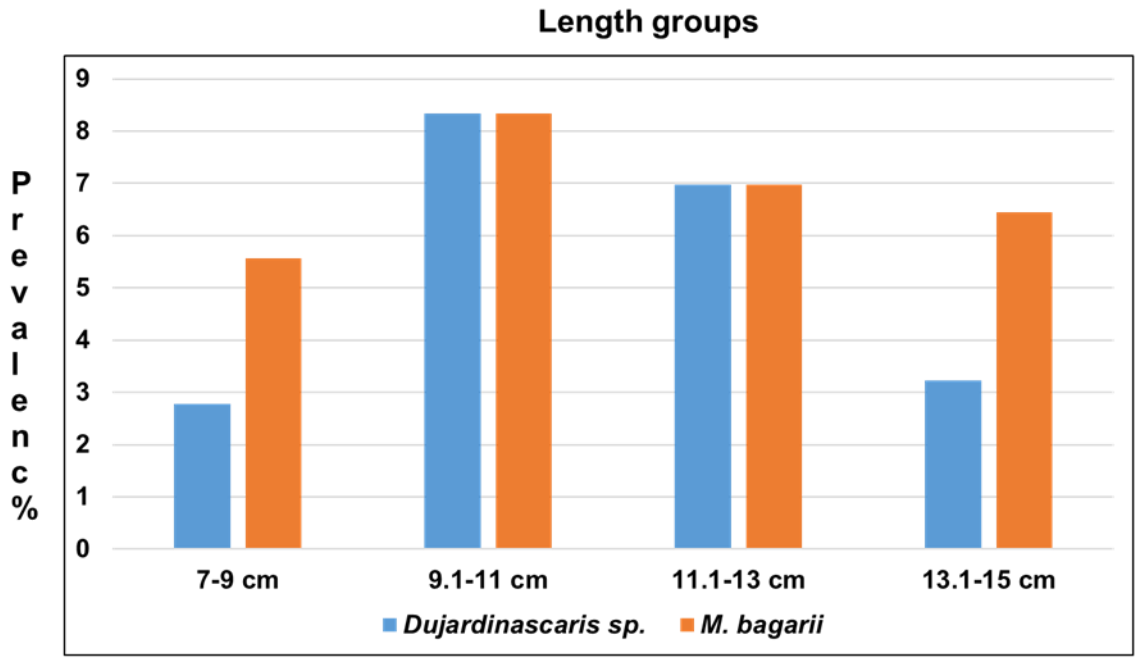


Fig. 64. Prevalence of nematodes in four length groups of *P. paradiseus*

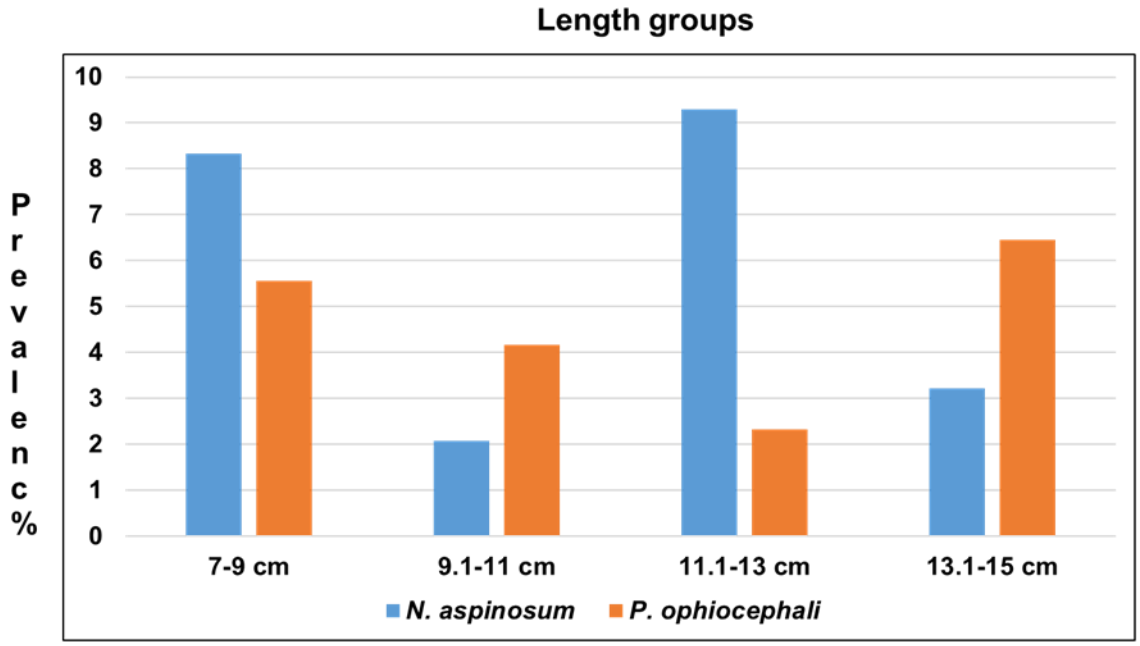


Fig. 65. Prevalence of acanthocephalans in four length groups of *P. paradiseus*

The intensity of *U. hamati* was recorded lowest (1) in the smallest length group (7-9 cm) and highest (1.14) in the rest of the length groups (Fig.-66).

The intensity of *T. vittalani* was recorded highest (1.2) in the largest length group (13.1-15 cm) and lowest (1) in the smallest length group (7-9 cm) (Fig.-66).

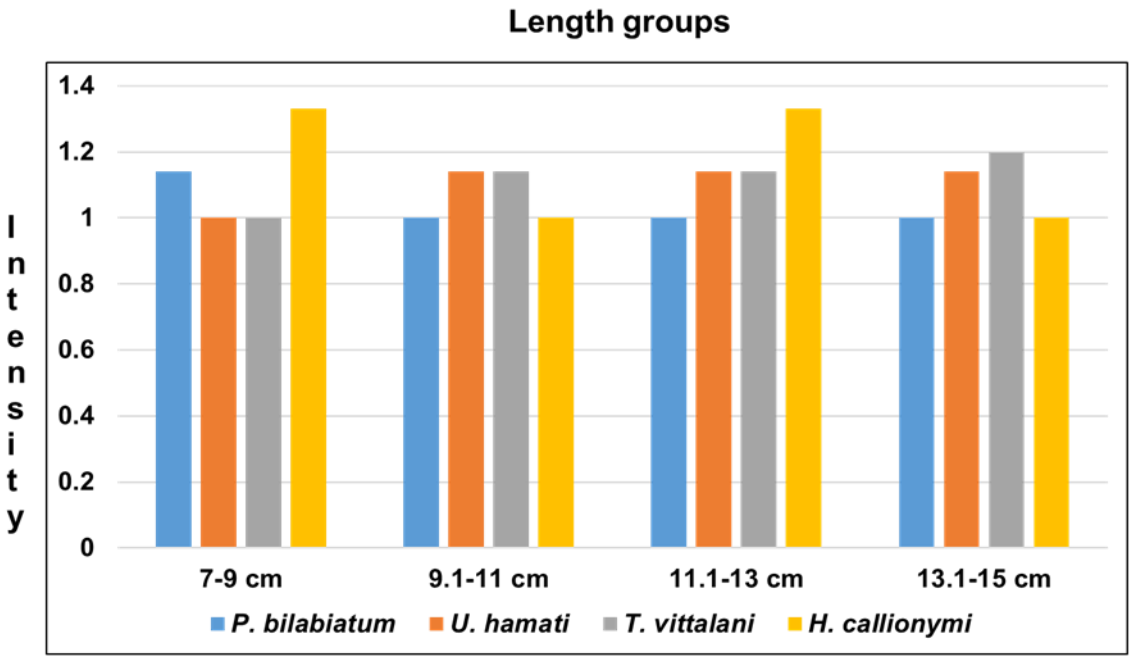


Fig. 66. Intensity of trematodes in four length groups of *P. paradiseus*

The intensity of *H. callionymi* showed the highest (1.33) in the length group of (7-9 cm), (11.1-13 cm) and lowest (1) in the length group (9.1-11cm), (13.1-15cm) (Fig-66).

The highest intensity (1.5) of *N. lingualis* was observed in the length group (11.1-13 cm) and lowest (1) was observed in the length groups (7-9 cm and 13.1-15 cm) (Fig. 67).

The maximum intensity (2) of *P. trygonis* was recorded in 9.1-11 cm length group and the minimum intensity (1) was recorded in rest of the length groups (Fig. 67).

The intensity of *Dujardinascaris* showed the highest (1.33) in the length group (9.1-11cm) and the lowest (1) was observed in other three length groups. The intensity of *M. bagarii* was observed (1) in all length groups (Fig.-68).

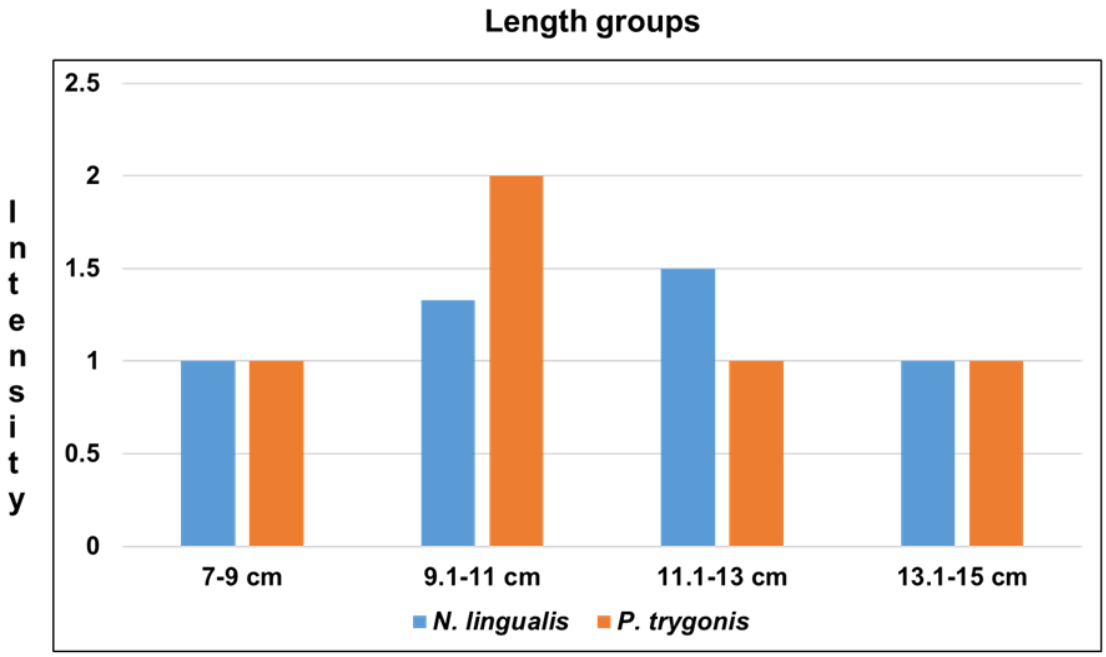


Fig. 67. Intensity of cestode parasites in four length groups of *P. paradiseus*

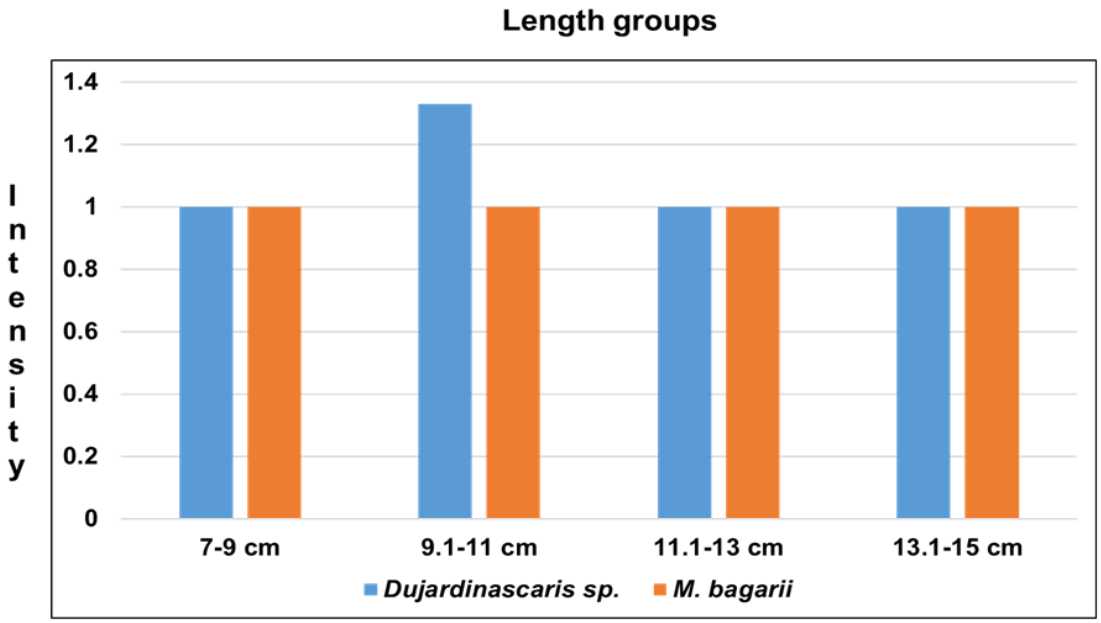


Fig. 68. Intensity of nematode parasites in four length groups of *P. paradiseus*

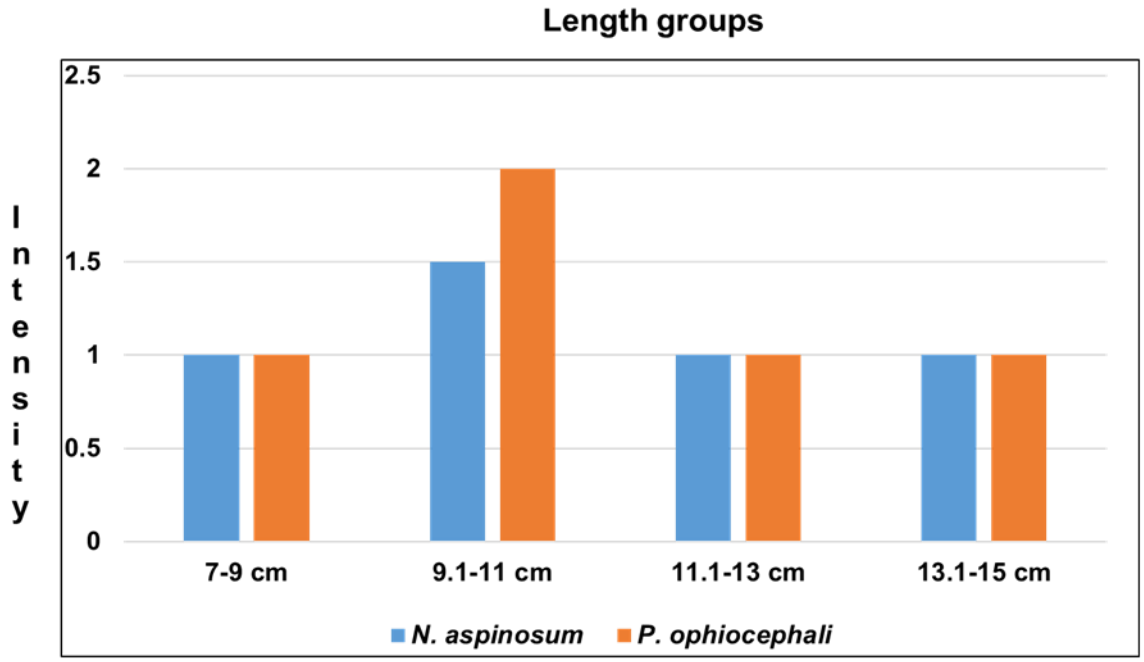


Fig. 69. Intensity of acanthocephalans in four length groups of *P. paradiseus*

The intensity of *N. aspinosum* showed the highest (1.5) in the length group (9.1-11cm) and the lowest (1) was observed in rest of the length groups (Fig.-69).

The intensity of *N. ophiocephali* showed the highest (2) in the length group (9.1-11cm) and the lowest (1) observed in rest of the length groups (Fig.-69).

In *X. cancila*, the prevalence of *Bolbocephalus sp.* showed the highest (17.24%) in the length group (20-25cm) and the lowest (9.76 %) was observed in the length group (25.1-30 cm) (Fig.-70).

The prevalence of *Isoparorchis hypselobagri* showed the highest (9.76%) in the length group (25.1-30cm) and the lowest (3.45 %) was observed in the length group (20-25 cm). *I. hypselobagri* was absent in the length group 30.1-35 cm (Fig.-70).

The prevalence of *M. bagarii* showed the highest prevalence (11.94%) in the length group (35.1-40cm) and the lowest (3.45 %) observed in the length group (20-25cm) (Fig.-71).

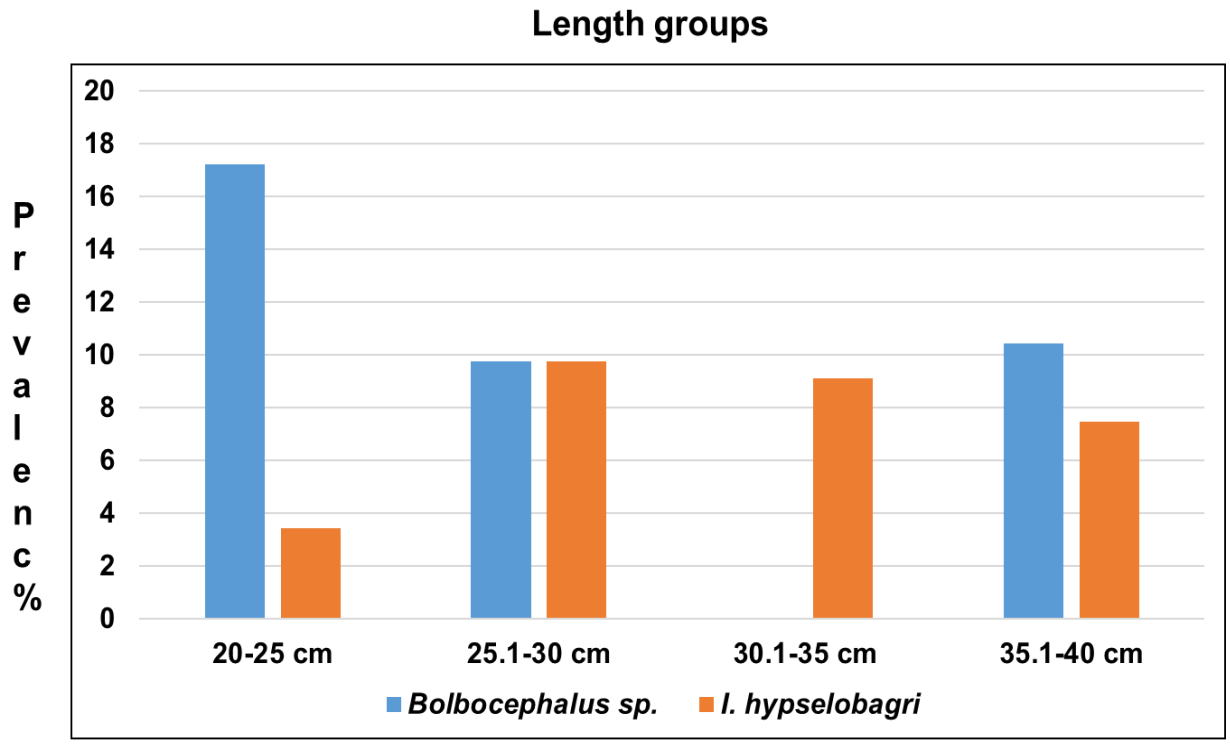


Fig. 70. Prevalence of trematodes in four length groups of *X. cancila*

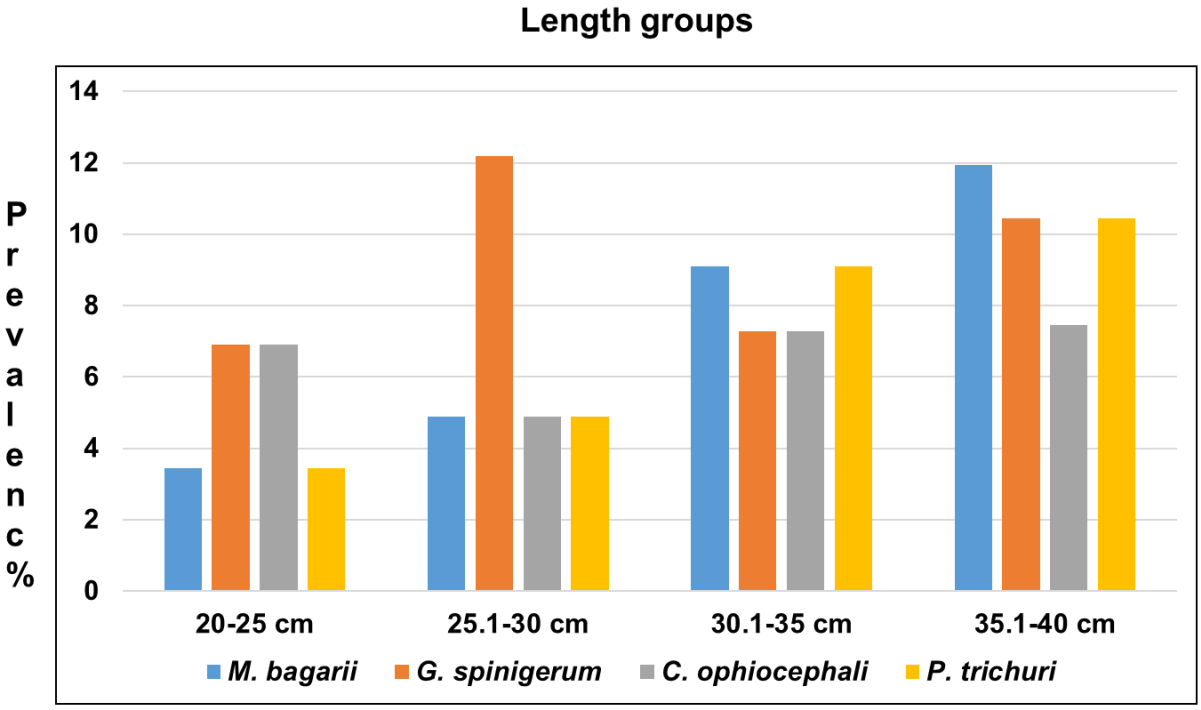


Fig. 71. Prevalence of nematodes in four length groups of *X. cancila*

The prevalence of *G. spinigerum* was recorded highest (12.20%) in the length group (25.1-30 cm) and lowest (6.90 %) in the length group (20-25cm) (Fig.-71).

The prevalence of *C. ophiocephali* was recorded highest (7.46%) in the length group (35.1-40 cm) and lowest (4.88 %) in the length group (25.1-30cm) (Fig.-71).

The prevalence of *P. trichuri* was recorded highest (10.45%) in the length group (35.1-40cm) and lowest (3.45 %) in the length group (20-25cm) (Fig.-71).

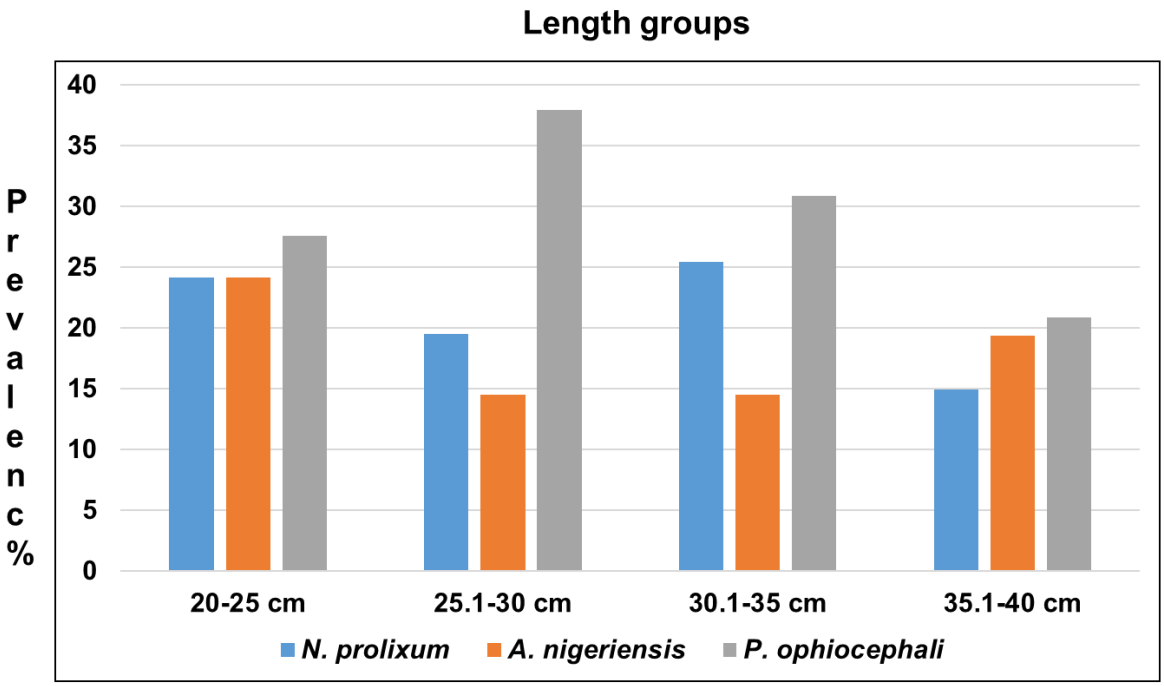


Fig. 72. Prevalence of acanthocephalans in four length groups of *X. cancila*

The prevalence of *N. prolixum* showed the highest (25.46%) in the length group (30.1-35 cm) and the lowest (14.92 %) observed in the length group (35.1-40 cm) (Fig.-72).

The prevalence of *A. nigeriensis* was recorded highest (24.14%) in the length group (20-25 cm) and lowest (14.51 %) in the length group (25.1-30 cm) (Fig.-72).

The prevalence of *P. ophiocephali* showed the highest (37.93%) in the length group (25.1-30 cm) and the lowest (20.90 %) observed in the largest length group (35.1-40 cm) (Fig.-72).

In *X. cancila*, the intensity of *Bolbocephalus sp* showed the highest (1.4) in the length group (35.1-40 cm) and the lowest (1) was observed in the remaing length groups (Fig.-73).

The intensity of *Isoparorchis hypselobagri* showed the highest (1.67) in the length group (30.1-35 cm) and the lowest (1) observed in the rest length groups (Fig.-73).

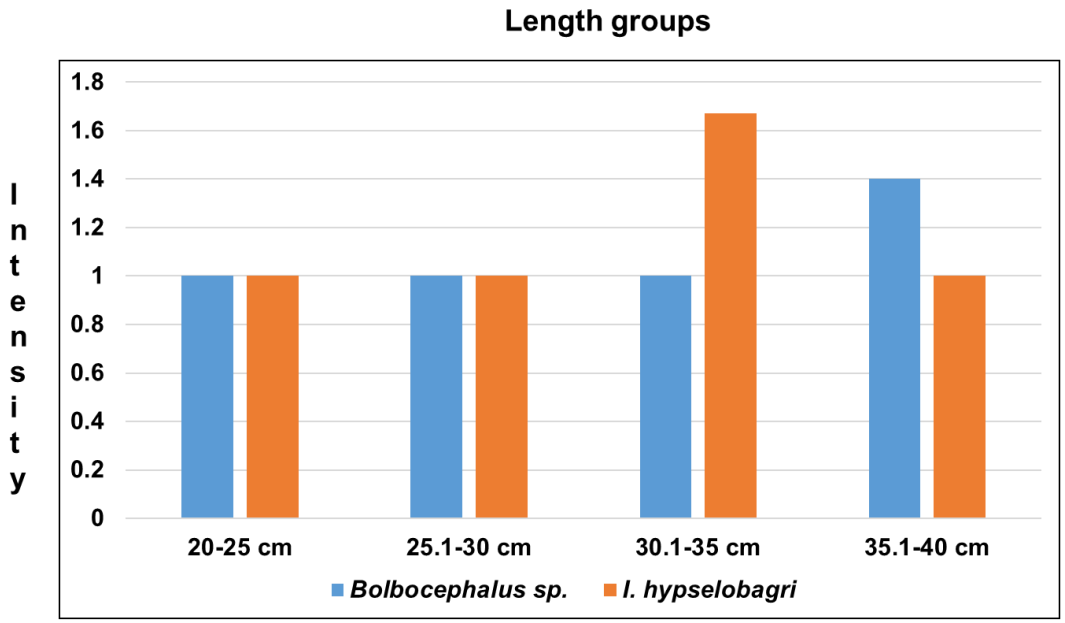


Fig. 73. Intensity of trematodes in four length groups of *X. cancila*

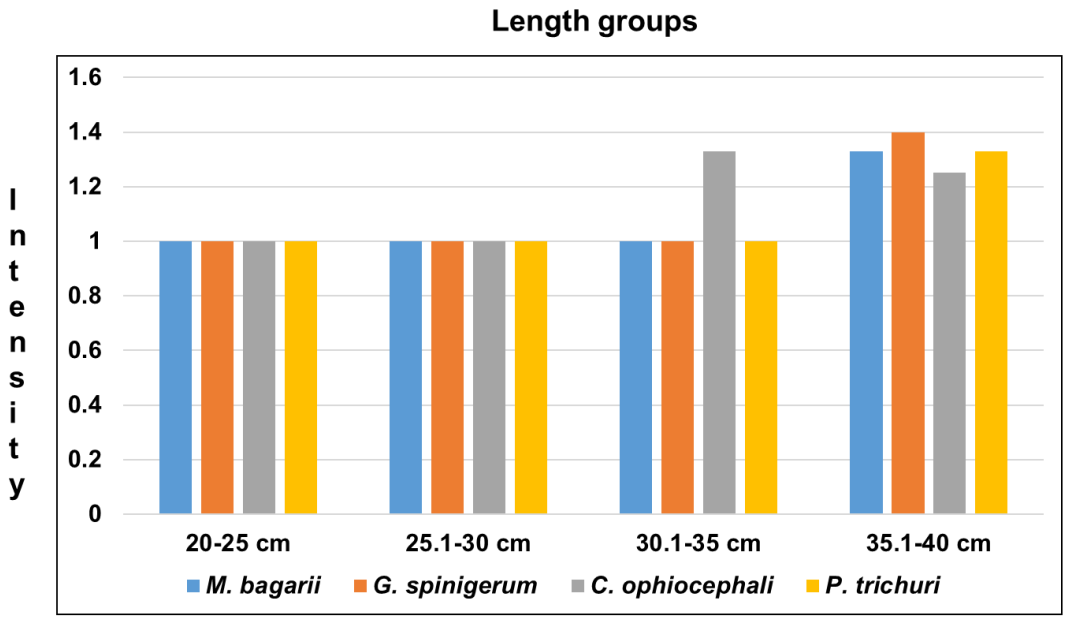


Fig. 74. Intensity of nematodes in four length groups of *X. cancila*

M. bagarii showed highest intensity (1.33) in the length group (35.1-40 cm) and lowest (1) in the rest length groups (Fig.-74).

The intensity of *G. spinigerum* was recorded highest (1.4) in the largest length group (35.1-40 cm) and lowest (1) in the rest length groups (Fig.-74).

The intensity of *C. ophiocephali* was recorded highest (1.33) in the length group (30.1-35 cm) and lowest (1) in the length group (20-25cm), (25.1-30 cm) (Fig.-74).

The maximum intensity (1.33) of *P. trichuri* found in (35.1-40 cm) length group and the minimum intensity (1) recorded in rest of the length groups (Fig.-74).

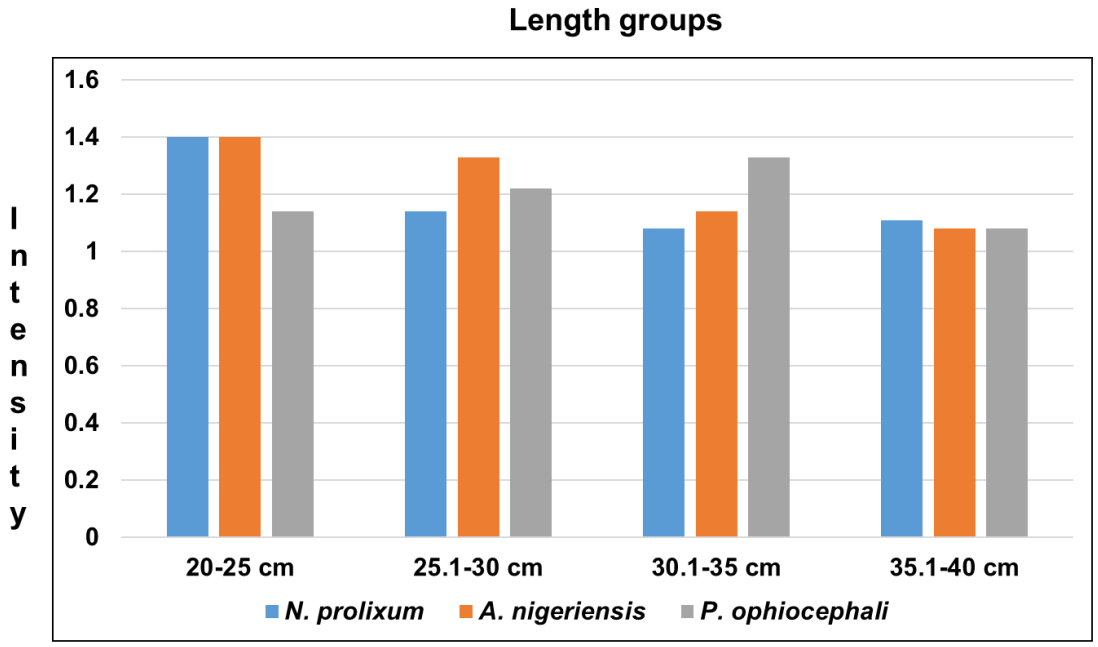


Fig. 75. Intensity of acanthocephalans in four length groups of *X. cancila*

The highest intensity (1.4) of *N. prolixum* observed in the the length group (20-25cm) and lowest (1. 11) found in the largest length group (30.1-35 cm) (Fig.75).

The intensity of *A. nigeriensis* showed the highest (1.4) in the length group (20-25 cm) and the lowest (1. 08) observed in the largest length group (35.1-40 cm) (Fig.75).

The maximum intensity (1.33) of *P. ophiocephali* found in 30.1-35cm length groups and the minimum intensity (1.08) recorded in the length group (35.1-40 cm) (Fig.- 75).

Infestation of parasites in relation to Climatic factors

Climatic factors

In the development of helminth parasites, environmental factors such as climate, rainfall and season play a very important role. Climate change may directly affect fishery production along many pathways. Fish reproduction, growth and migration patterns are all affected by temperature, rainfall and hydrology (Ficke *et al*. 2007). Knowledge of the biology of the parasite and its host(s), the host-parasite relationship and the environment can help to detect environmental change.

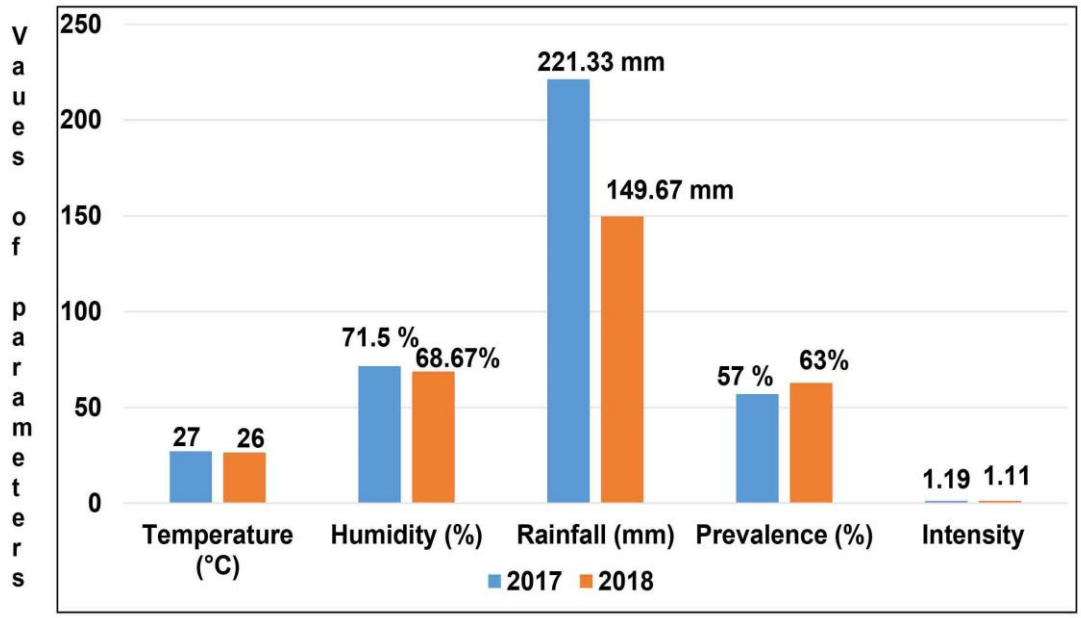


Fig. 76. Difference of temperature, humidity, rainfall, prevalence and intensity of parasites of *X. cancila* in 2017 and 2018

In *X. cancila*, the observed values of temperature, humidity, rainfall, prevalence and intensity were 27°C, 71.5%, 221mm, 56.65% and 1.19 respectively in the year of 2017. On the other hand, in 2018, the recorded values were temperature 26.33°C, humidity 68.67%, rainfall 149.67mm, prevalence 63.10% and intensity 1.11 (Fig.-76).

In *P. paradiseus*, the observed values of temperature, humidity, rainfall, prevalence and intensity were 27°C, 71.5% and 221 mm, 52.16% and 1.09 respectively in the year of 2017. On the other hand, in 2018, the recorded values were temperature 26.33°C, humidity 68.67%, rainfall 149.67mm, prevalence 45.81% and intensity 1.10 (Fig.-77).

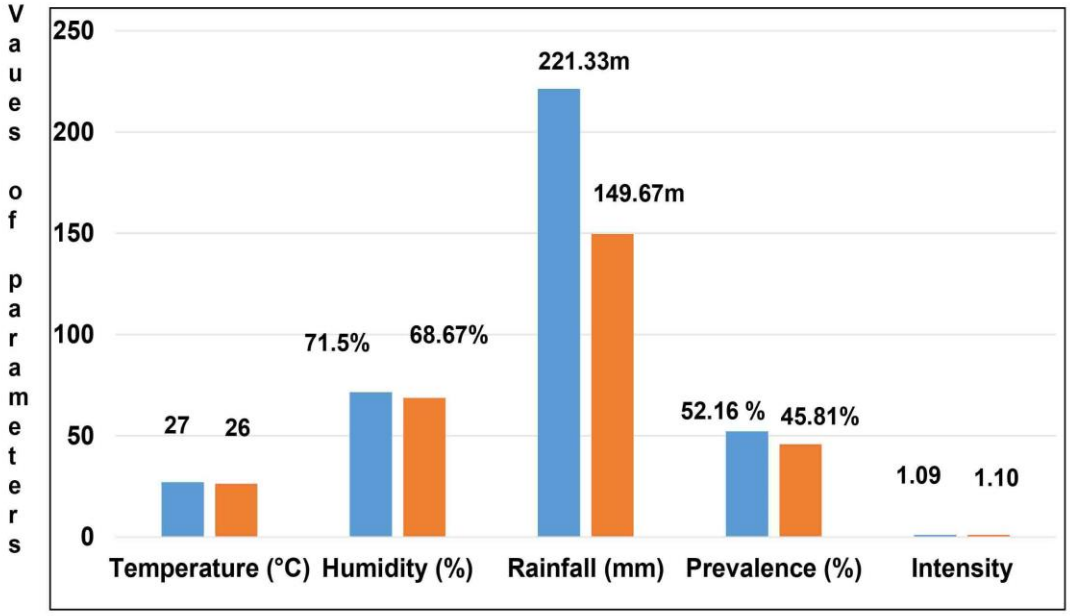


Fig. 77. Difference of temperature, humidity, rainfall, prevalence and intensity of *P. paradiseus* in 2017 and 2018

Temperature

Fish dependant parasites that have a devastating effect on fish reproduction, grow four times faster at higher temperatures providing some of the first evidence that global warming affects the interactions between parasites and their hosts. In *X. cancila*, the highest average temperature (29.25°C and 25.25°C) was recorded during summer and rainy seasons while the highest prevalence (69.23% and 75%) was recorded during winter in 2017and 2018. The lowest average temperature (23.75°C and 22.5°C) was recorded during winter in 2017 and 2018 while the lowest prevalence (38.46%, and 50%) was observed during rainy in 2017 and during summer and rainy seasons in 2018. The prevalence was found negatively correlated with temperatures (r=0.59).

In *X. cancila*, during 2017, the highest temperature (30°C) was observed in the month of May'17 and June'17 while the lowest temperature was (22°C) in January'17 and December'17 and the highest prevalence (69.23%) was observed in the month of Dec'17 and the lowest prevalence was 38.46% in Sep'17. In 2018, the highest

temperature 30°C was found in June'18, July'18, August'18, Sep'18 and lowest 18°C observed in Jan'18 while the highest prevalence 75% recorded in November'18 and lowest was 50% in June'18 and August'18 (Fig-78).

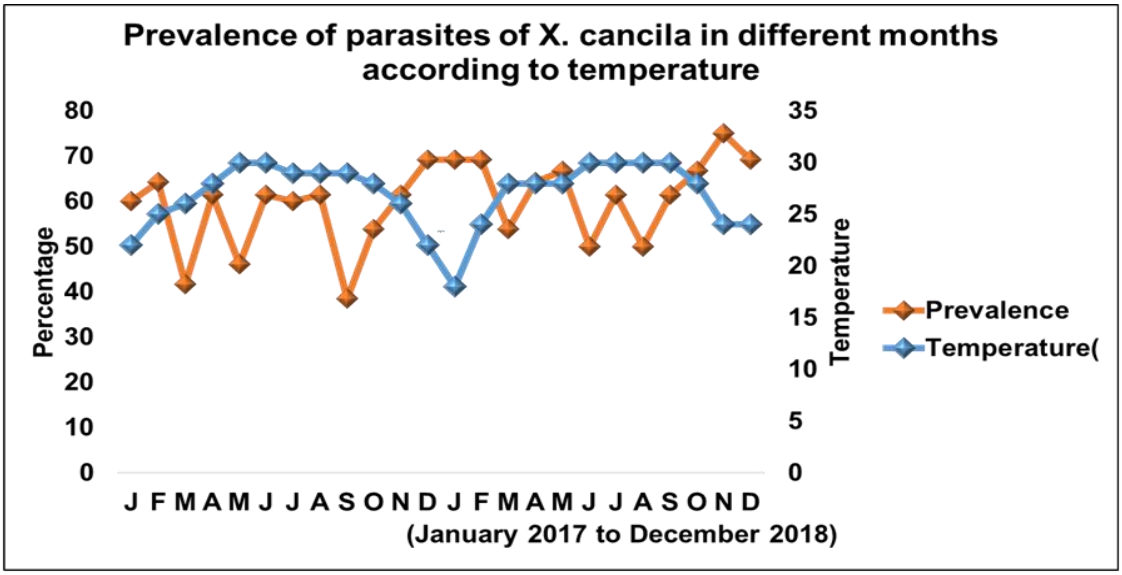


Fig. 78. Prevalence of parasites of *X. cancila* in different months according to temperature

In *P. paradiseus*, during 2017 and 2018, the highest temperature (30°C and 30°C) was recorded during summer and rainy seasons and the highest prevalence (78.57% and 66.67%) was found during rainy season. The lowest temperature (22°C and 18°C) was recorded during winter in 2017 and 2018 while the lowest prevalence (33.33% and 23.08%) was observed during summer and winter seasons in 2017 and 2018. The prevalence was weakly positively correlated with temperature in different months (r =0.37) (Fig- 79).

In *P. paradiseus*, in 2017, the highest temperature (30°C) was observed in the month of May'17 and June'17 while the lowest temperature was (22°C) in January'17 and December'17 and the highest prevalence (78.57%) was observed in the month of July'17 and the lowest prevalence was 33.33% in March'17. In 2018, the highest temperature 30°C was found in June'18, July'18, August'18, Sep'18 and lowest 18°C was observed in Jan'18 while the highest prevalence 66.67% recorded in July'18 and lowest was 23.08 % in January'18 (Fig-79).

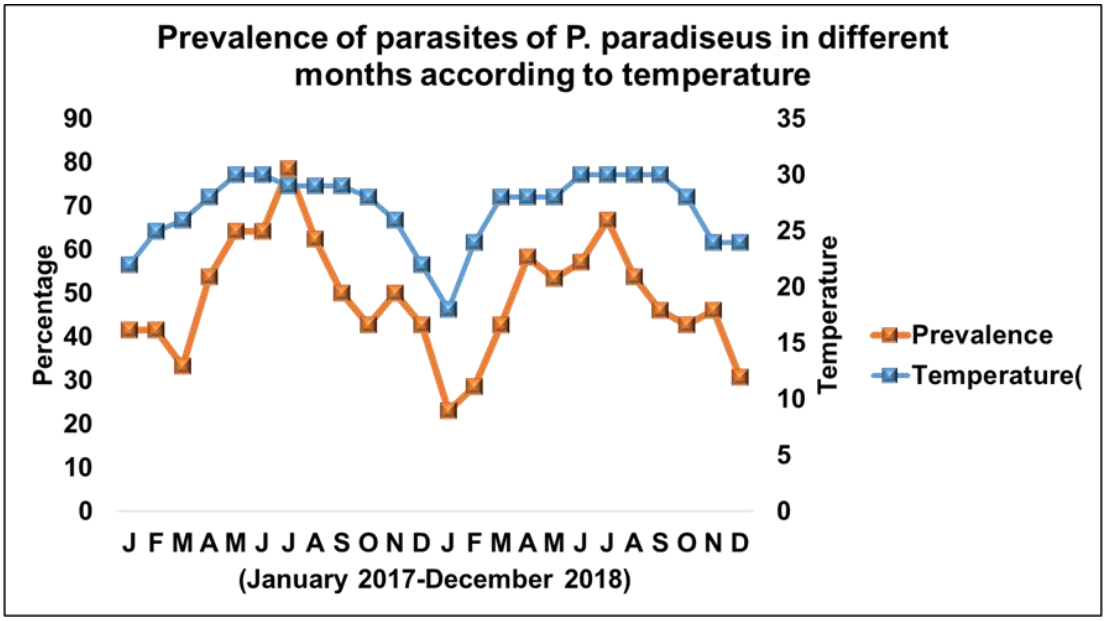


Fig. 79. Prevalence of parasites of *P. paradiseus* in different months according to temperature

Table-31: Association between temperature and infected fish of *P. paradiseus* and *X. cancila* (through chi square test)

Temperature (° C)	*P. paradiseus*			*X. cancila*		
	No. of infected	No. of Non-infected	P-value (using chi square test)	No. of infected	No. of Non-infected	P-value (using chi square test)
<25	31	52	0.07	39	39	0.17
25-27	28	28		31	22	
27--29	64	57		70	42	
>29	35	26		52	26	

Table-31 shows the "chi square test" whether having association between number of infected fish of *P. paradiseus* and *X. cancila* with temperatures. There was an association observed in *P. paradiseus* at 10% level of significance. However, there was no significant association found in *X. cancila*.

Rainfall

In animal condition and on host-parasite interactions, seasonality of rainfall can exert a strong influence. The body condition of ruminants fluctuates seasonally in response to changes in energy requirements, foraging patterns and resource availability, and seasonal variation in parasite infections may further alter ruminant body condition.

In *X. cancila*, in 2017, the highest rainfall (36763 mm) was observed in the month of July'17 and lowest rainfall was 21 mm in Jan'17 while the highest prevalence 69.23% was observed in the month of Dec'17 and lowest prevalence was 38.46% in Sep'17. In 2018, the highest rainfall (26274 mm) was observed in the month of July'18 and lowest

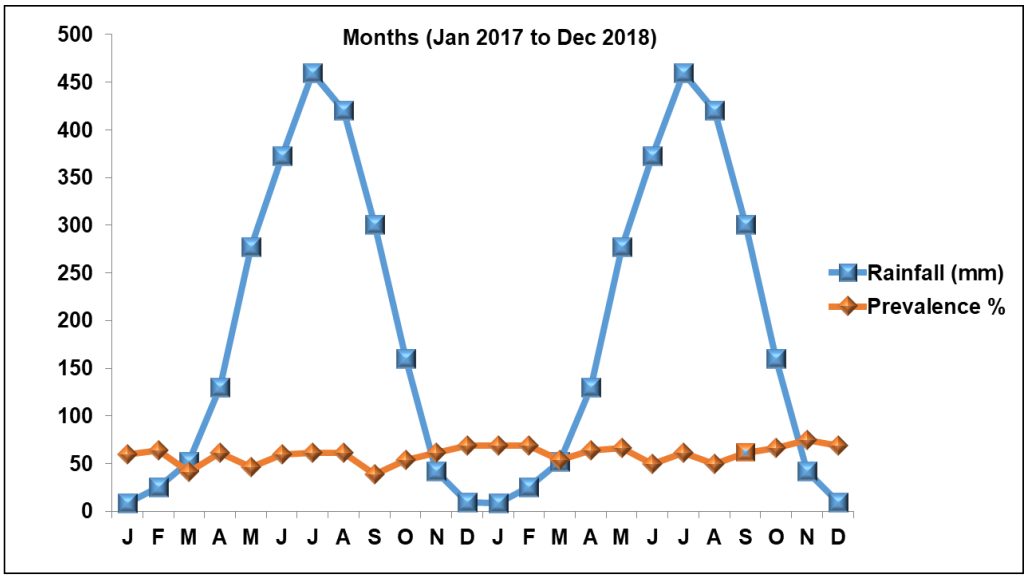


Fig. 80. Prevalence of parasites of *X. cancila* in different months according to rainfall

rainfall was 21 mm in Jan'18 while the highest prevalence 75% was observed in the month of November'18 and lowest was 50% in June'18 and August'18. The prevalence was very weakly positively correlated with rainfall (r =0.08) in different months (Fig-80).

In *X. cancila*, the highest rainfall (36763 mm and 26274 mm) was recorded during rainy season in both 2017 and 2018 while the highest prevalence (69.23% and 75%) were observed during winter season. The lowest rainfall (21mm and 21mm) was recorded

during winter in 2017 and 2018 while the lowest prevalence (38.46% and 50%) were observed during rainy and summer seasons (Fig-80).

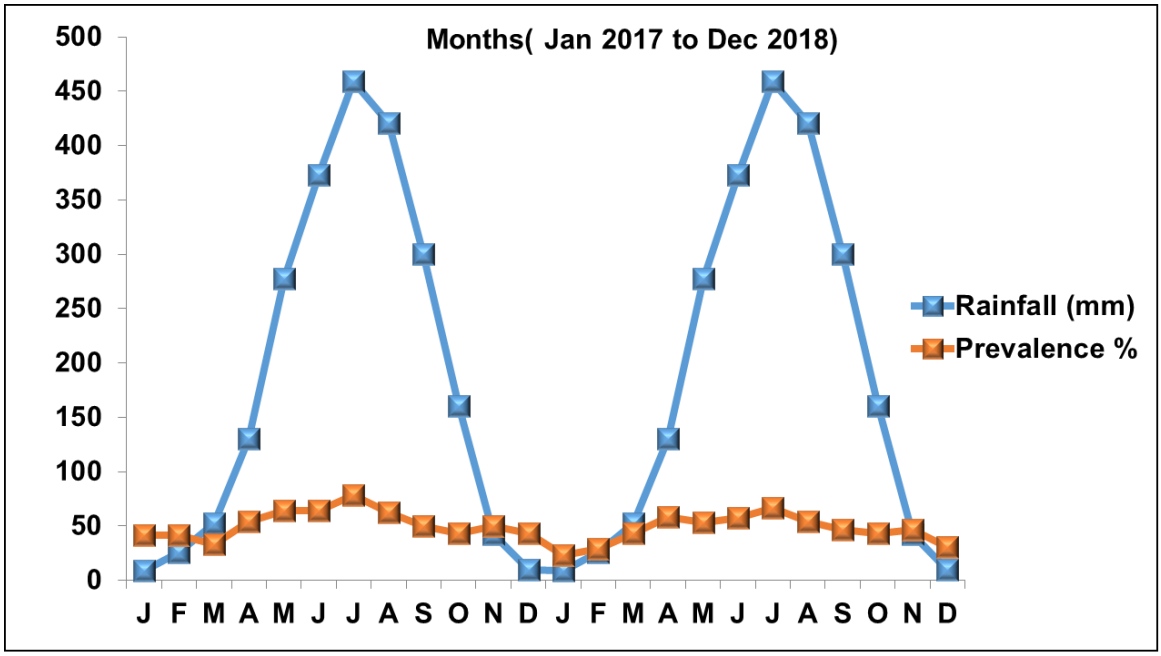


Fig. 81. Prevalence of parasites of *P. paradiseus* in different months according to rainfall

In *P. paradiseus*, during 2017, the highest rainfall (36763 mm) was observed in the month of July'17 and lowest rainfall was 21 mm in Jan'17 while the highest prevalence 78.57% was observed in the month of July'17 and lowest prevalence was 33.33% in March'17. In 2018, the highest rainfall (26274 mm) was observed in the month of July'18 and lowest rainfall was 21 mm in Jan'18 while the highest prevalence 66.67% was observed in the month of July'18 and lowest was 23.08 % in January'18. The prevalence was positively correlated with rainfall (r =0.08) in different months (Fig-81).

In *P. paradiseus*, the highest rainfall (36763 mm and 26274 mm) was recorded during rainy season and highest prevalence (78.57% and 46.67%) was also observed during rainy season in both 2017 and 2018. The lowest rainfall (21mm and 21mm) was recorded during winter in 2017 and 2018 while the lowest prevalence (33.33% and 23.08%) were observed during winter and summer (Fig-81).

Table-32: Association between rainfall and infected fish of *P. paradiseus* and *X. cancila* (through chi square test)

Rainfall (mm)	*P. paradiseus*			*X. cancila*		
	No. of infected	No. of Non-infected	P-value (using chi square test)	No. of infected	No. of Non-infected	P-value (using chi square test)
<100	38	65	0.005	56	52	0.12
100-500	51	52		66	32	
500-1000	16	15		17	14	
>1000	53	31		53	31	

Table-32 shows the "chi square test" whether having association between number of infected fish of *P. paradiseus* and *X. cancila* with rainfall. The rainfall was significantly associated (P> 0.05) with infection by helminth parasites in *P. paradiseus* and not significantly associated (P> 0.05) in *X. cancila*.

Humidity

Humidity is the amount of water vapor in the air. Water vapor is the gaseous state of water and invisible. Humidity indicates the likelihood of precipitation, dew or fog. Higher humidity reduces the effectiveness of sweating in cooling the body by reducing the rate of evaporation of moisture from the skin. This effect is calculated in a heat index table or humidity.

In *X. cancila*, during 2017, the highest humidity (82%) was observed in the month of August'17 and lowest humidity was 51 % in Feb'17 while the highest prevalence 69.23% was observed in the month of Dec'17 and lowest prevalence was 38.46% in Sep'17. In 2018, the highest humidity was (81%) in July'18, Aug'18 and the lowest (51%) observed in Dec'18 while the highest prevalence 75% was observed in the month of Novemberr'18 and lowest was 50% in June'18 and August'18. The prevalence was strongly positively correlated with humidity (r=0.98) in different months (Fig.-82).

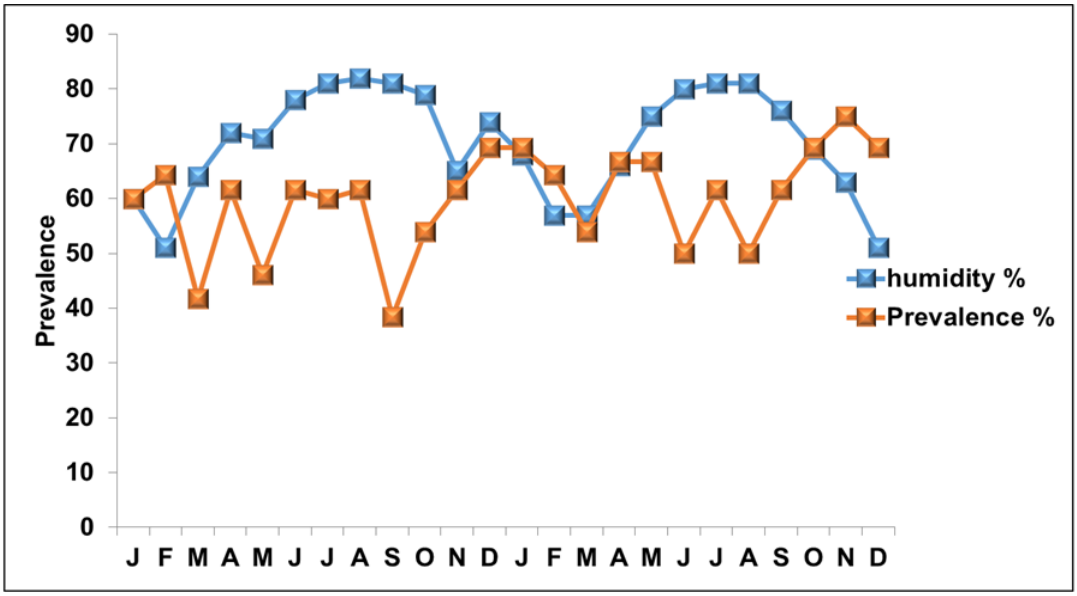


Fig. 82. Prevalence of parasites of *X. cancila* in different months according to humidity Months(Jan 2017 to Dec 2018)

In *P. paradiseus*, during 2017, the highest humidity (82%) was observed in the month of August'17 and lowest humidity was 51 % in Feb'17 while the highest prevalence 78.57% was observed in the month of July'17 and lowest prevalence was 33.33% in March'17. In 2018, the highest humidity was (81%) in July'18, Aug'18 and the lowest (51%) observed in Dec'18 while the highest prevalence 66.67% was observed in the month of July'18 and lowest was 23.08% in January'18. The prevalence was strongly positively correlated with humidity (r =0.99) in different months (Fig-83).

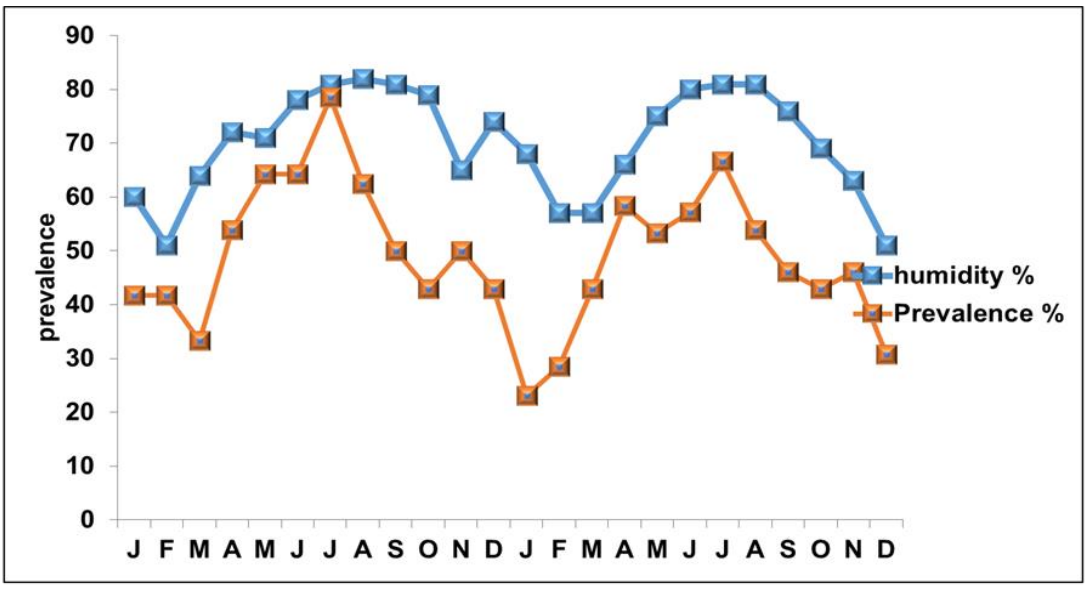


Fig. 83. Prevalence of parasites of *P. paradiseus* in different months (Jan 2017 to Dec 2018) according to humidity.

Table-33: Association between humidity and infected fish of *P. paradiseus* and *X. cancila* (through chi square test)

Humidity (%)	*P. paradiseus*			*X. cancila*		
	No. of infected	**No. of Non-infected**	**P-value (using chi square test)**	**No. of infected**	**No. of Non-infected**	**P-value (using chi square test)**
51-61	23	47	0.01	35	32	0.35
62-72	54	49		69	41	
73-83	81	67		88	56	

Table-33 shows the "chi square test" whether having association between number of infected fish of *P. paradiseus* and *X. cancila* with humidity. The humidity was found to be significantly associated ($P<0.05$) with infection by helminth parasites in *p.* paradiseus. Such association was absent in *X. cancila*.

Infestation in relation to the food and feeding habits of the host fishes

Analysis of food items in the stomach of *P. paradiseus* and *X. cancila*

To understand the functional role of the fish within its ecosystem, the study of food and feeding habit is very useful and essential. Is has importance in fishery biology and the subject has been extensively studied in the recent decades. A detail knowledge on the food and feeding habit of fishes provide keys for the selection of cultivable species and the importance of such information for successful fish farming.

The confirmation of the expected relation between fish diet and parasite occurrence in *P. paradiseus* and *X. cancila* was the main purpose of the study on food items in the fish. Little is known about the fish parasite and their correlation with the occurrence of specific type of food items.

A total of 321 stomachs of *P. paradiseus* and 321 intestines of *X. cancila* were examined from January 2017 to December 2018, to find out the food components in their diet. Attempts were made to determine the percentage of frequency of occurrence of the food items in relation to the total number of stomachs examined in each month of the study period in each species of fish. Attempts were also made to find the "most important food" or "main food" items with consideration to the views of Guziur (1976) and accordingly the food items were distinguished into three kinds (Table-34):

a) Main food- the animal components that comprise the greatest proportion of the fish diet and was been designated as "main food";
b) "the additional food"-the animal foods consumed by 5-35% of the fishes; and
c) "the incidental food"-the animal and plant components found in less than 5% of all fish examined.

The food items found in stomachs of fishes, were determined by "Occurrence Method" (Frost 1946, 1954; Hynes1950; Hunt and Carbine 1951) where the number of fish stomach in which each food item occurred was listed and expressed as a percentage of total number of stomachs examined.

The food items found in the gut of *P. paradiseus* and *X. cancila* were divided into five basic categories: fish, crustaceans, insects, molluscans and plants. The fishes also consumed macrophytes, mud and sand to a smaller extent (Fig.-84, 85). A good percentage of empty stomachs were also observed in both the fish species.

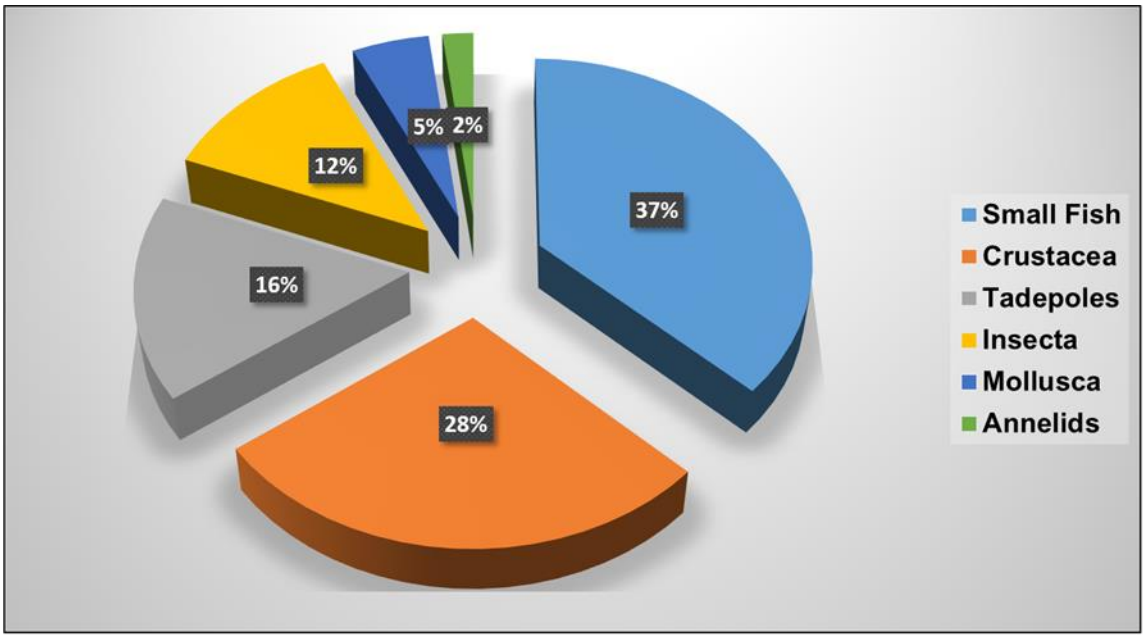


Fig. 84. Percentage of different food items found in the intestine of *Xenentodon cancila*

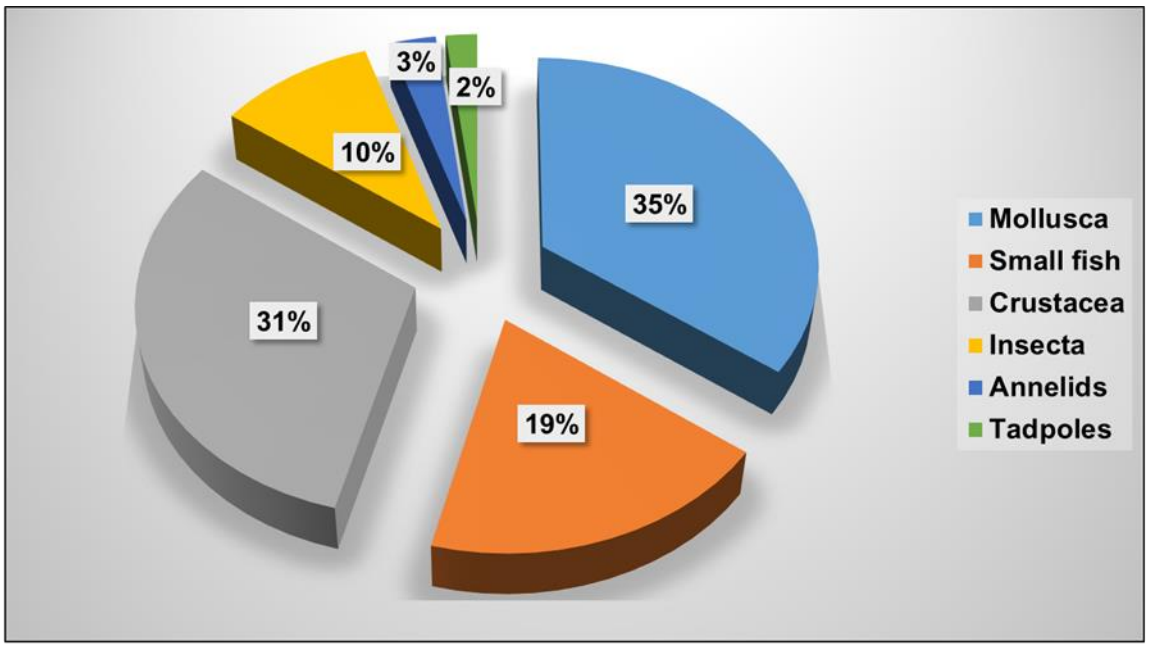


Fig. 85. Percentage of different food items found in the stomach of *Polynemus paradiseus*

Main food items

Small fishes

The most important and most frequent components of animal food were the small fishes which comprised the greatest proportion of the diet of *P. paradiseus* and *X. cancila* (Fig-84, 85). The majority of fishes in stomach were *Corica soborna*, *Amblypharyngodon mola*, *Botia dario*, *Corica* sp., *Puntius* spp. This category of food also contained considerable number of fish fry, fish scales, eggs, bones, eye balls, fins, operculum, head, and barbells. The frequency of occurrence of small fishes were 37% in *X. cancila* and 19% in *P. paradiseus* (Table- 34).

The seasonal variation of frequency of occurrence of small fish food item followed almost similar pattern in both the host fishes. In *p. paradiseus*, the highest occurrence was evident in (21% in July 2017 and 22% in June 2018) during rainy and summer season; lower consumption was observed during winter month (18%, Jan'17 and 19%, Dec'18). In *X. cancila*, the peaks were prominent (40% in April'17; 39% in April'18 and September'18) during summer and rainy seasons; lower frequency was observed during winter (33% in January'17 and 34% in December'18) (Fig-86).

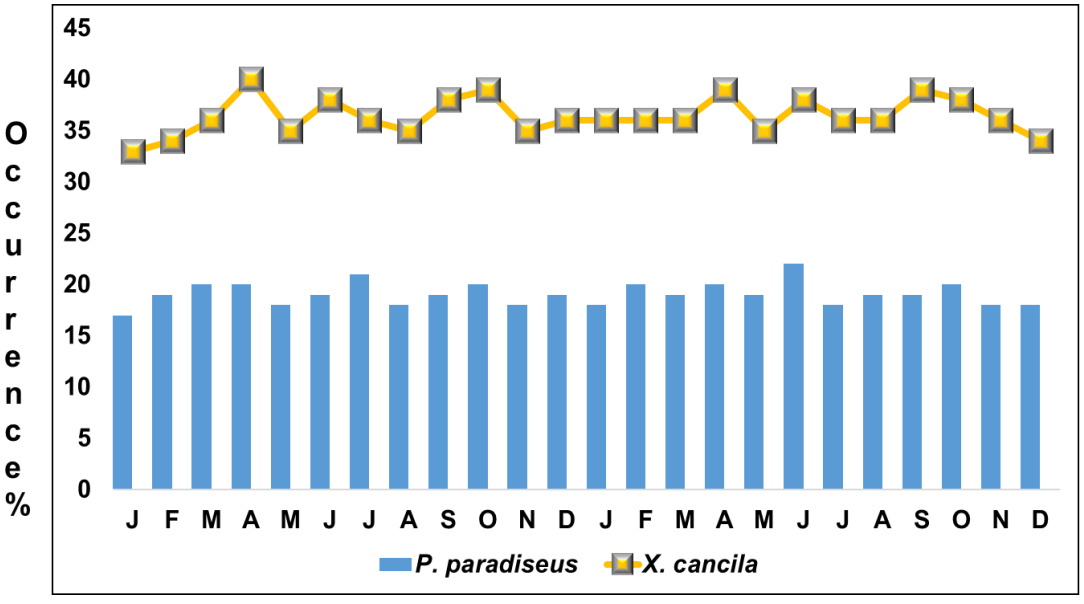


Fig. 86. Monthly Occurrence of small fish food item in stomach of *X. cancila* and *P. paradiseus*

Table-34: Frequency of occurrence and the significance of particular components in the food of *X. cancila* and *P. paradiseus*

Kind	**Significance**	**Food components**	**Occurrence in *P. paradiseus***	**Occurrence in *X. cancila***
Animal food	Main food	Fish: *Corica soborna*, *Amblypharyngodon mola*, *Puntius* spp., *Botia dario*, fish scales, eggs, eye balls, fins, operculum, head, barbell	19	37
		Crustaceans: Prawns, small crabs, copepods(*Cyclops*, Diaptomus), ostracods(*Cypris*), crustacean larva and appendages	31	28
	Additional food	Molluscans: Gastropods (*Limnaea*, Amnicola) And Bivalvia (shells and mantles of *Pila*, *Unio* etc.)	35	5
		Insects: Coleoptera, Cladocera, Diptera, Lepidoptera, nymphs of dragon flies, mosquito larvae.	10	16
	Incidental food	Tadpoles: Ragworm (Polychaetes),	02	12
Plant food		Algae, stem of aquatic plants, aquatic grass,	03	02

Crustacea

Crustaceans were the most abundantly consumed group of animal food including larvae and aduts and they filled a significant proportion of the stomachs in all the months of the study period. The item consisted of prawns, small crabs, copepods (*Cyclops*, *Diaptomus*), ostracods (*Cypris*), crustacean larva and appendages etc. Other copepods were also prevalent.

The frequency of occurrence of crustacean was recorded 31% in *P. paradiseus* and 28% in *X. cancila*. The seasonal and monthy variation of frequency in *P. paradiseus* and *X. cancila* are shown in fig -87. In *P. paradiseus*, in both the years, there was a similar sequential pattern in the peaks of the frequency curves. The peaks were evident 40% in June'17 and 41% in October'18 and lower consumption was 23% in Jan'17 and 22% in Dec'18. In *X. cancila*, the peaks were prominent in 38% in Sept'17 and 39%, in June'18 and lower frequency was observed 19% in November'17 and 20% in August'18 ((Table-34; Fig-87).

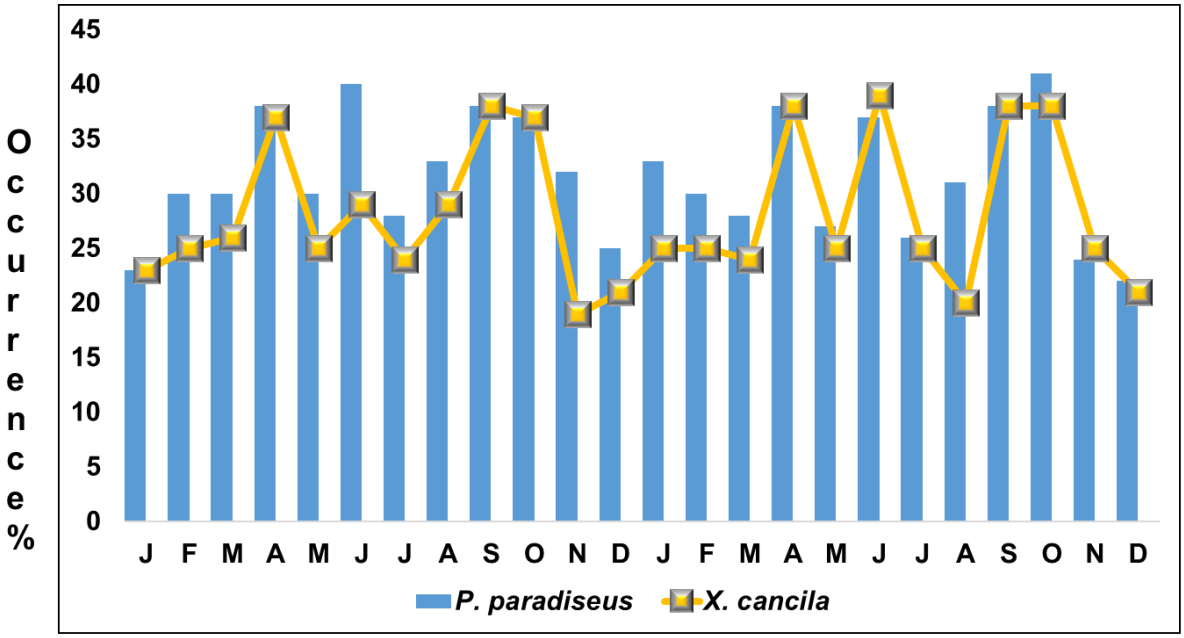


Fig. 87. Monthly Occurrence of crustacean food item in stomach of *X. cancila* and *P. paradiseus*

Additional food items

Insects

Among the additional food items cosumed by *P. paradiseus* and *X. cancila*, the aquatic insects were the representative of Coleoptera, Cladocera, Diptera, Lepidoptera etc. This group of animals were most frequent and abundant in stomachs (Table-34).

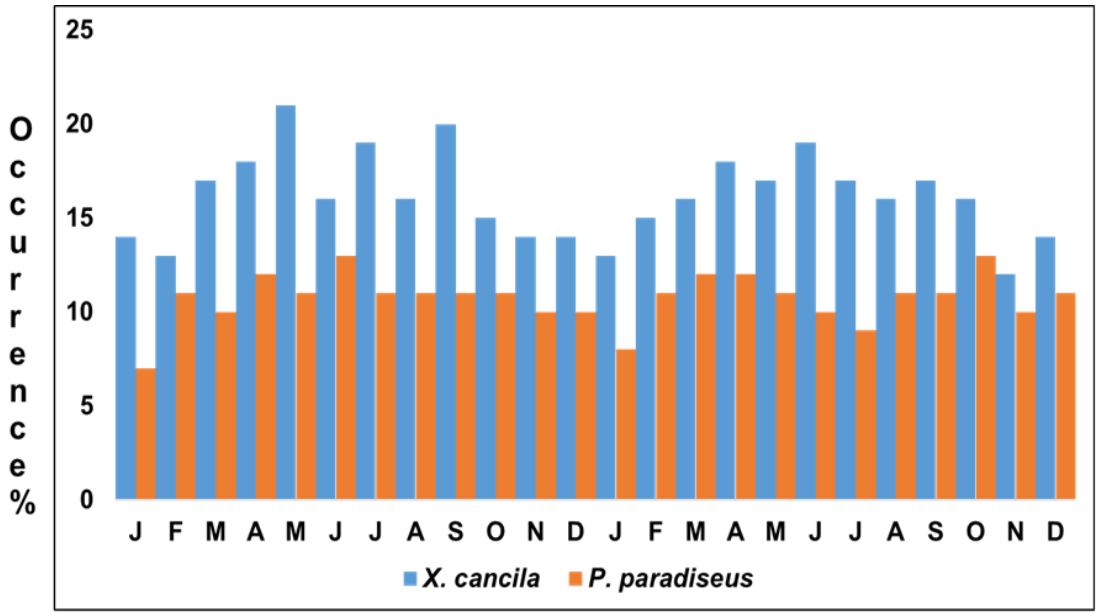


Fig. 88. Monthly Occurrence of insect food item in stomach of *X. cancila* and *P. paradiseus*

In *P. paradiseus*, the frequency of occurrence of insects was 10% and in *X. cancila*, it prevailed 16%. The occurrence did not follow any seasonal pattern in both the hosts and showed little difference in month to month during the period. The maximum frequency of occurrence was recorded 12% (June' 2017) and 13% (October' 2018) and minimum frequency was recorded 7% (January' 2017) and 8% (January' 2018) in *P. paradideus*. In *X. cancila*, the maximum frequency of occurrence was recorded 21% (May 2017) and 19% (June 2018) and minimum frequency was recorded 13% in February'17 and 12% in November'18 (Fig.-88).

Mollusca

Mollusca comprised 35% of food components consumed by *P. paradiseus* and 5% of food components consumed by *X. cancila*. The representatives of Mollusca were Gastropods (Limnaea, Amnicola) and Bivalvia (shells and mantle of *Pila, Unio*).

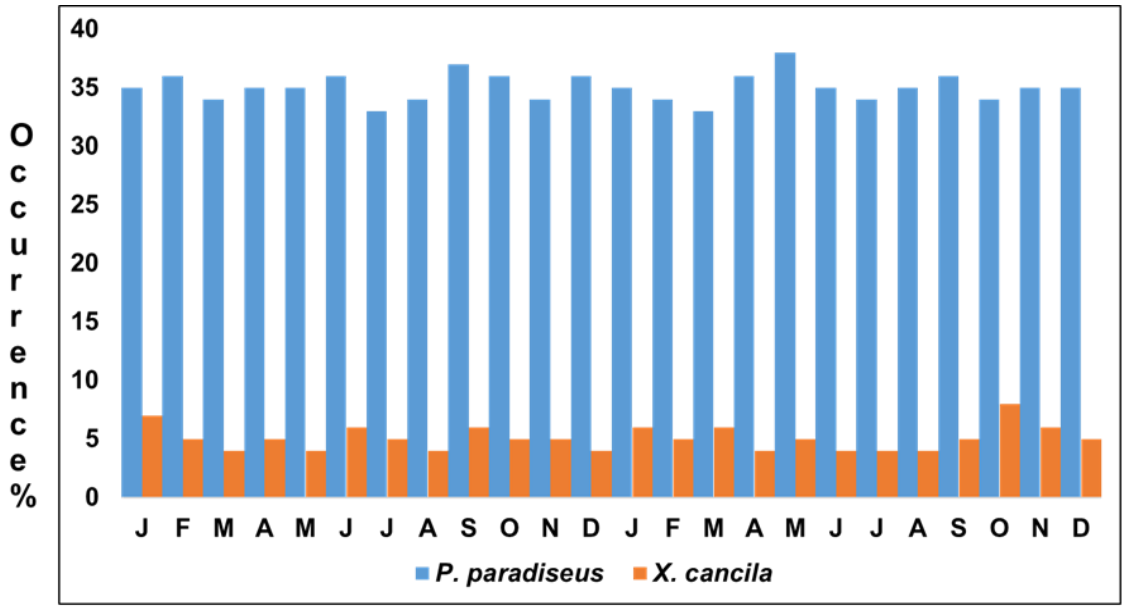


Fig. 89. Monthly Occurrence of molluscan food item in stomach of *X. cancila* and *P. paradiseus*

In *P. paradiseus*, maximum consumption, 37% occurred in September'17 and 38% in May'18. In *X. cancila*, the peaks (7% and 8%) were observed in Jan'17 and Oct'18 (Fig-89).

Incidental food items

Tadpoles

Tadpoles occupied 12%% of food items consumed by of *X. cancila* and 2% of food items consumed by *P. paradiseus* during the study period. In *P. paradiseus* and *X. cancila*, a similar sequencial pattern in the peaks of the frequency curves observed in both the years. The peaks were evident during rainy seasons (33% in Aug'17 and 28% In July'18) and lower consumption was 16% in May'17 and 21% in Sep'18 in *X. cancila*. In *P. paradiseus* the peaks were prominent (5% in July'17 and 6% in June'18) and lower frequency (2% in April'17 and 3% in Sep'18) was observed (Fig.-90).

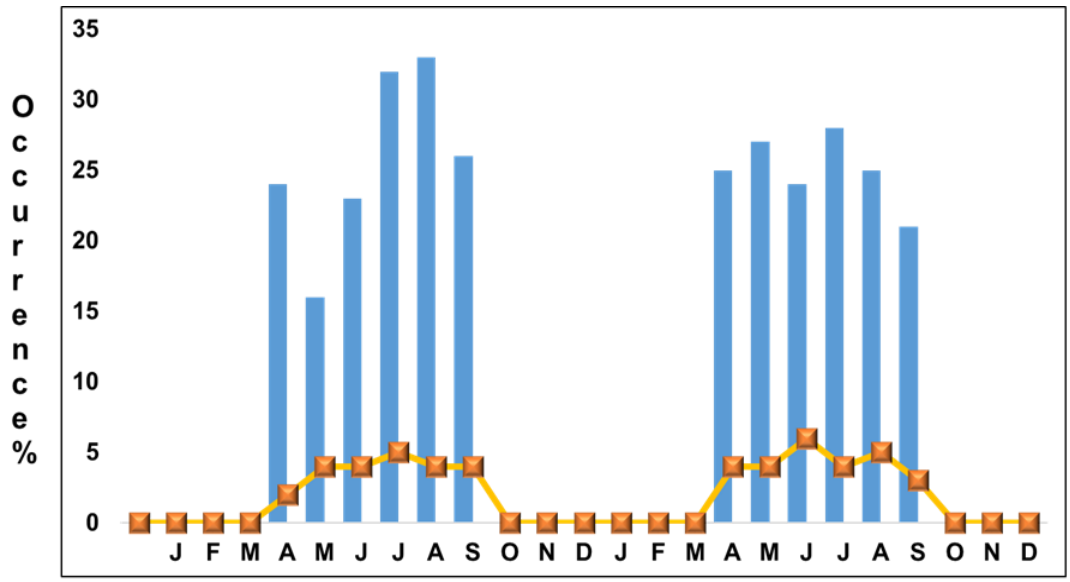


Fig. 90. Monthly Occurrence of Tadpoles food item in stomach of *X. cancila* and *P. paradiseus*

Plants

The origin of plant food included algae, stems of aquatic plants, aquatic grass etc. The occurrence of plant food item followed similar patterns in both the host fishes. In *X. cancila* and *P. paradiseus*, in both the years, there was a similar sequential patterns found in the peaks of the frequency curves. In *X. cancila*, the peaks were evident during rainy and winter seasons (3% in Aug'17, Oct'17 and Feb'17) and frequency was observed (2%) in rest of the months. In *P. paradiseus*, in both years, there were a similar sequential patterns (3%) observed except (2%) in Feb'17, Nov'17 and Sep'18 (Fig.-91).

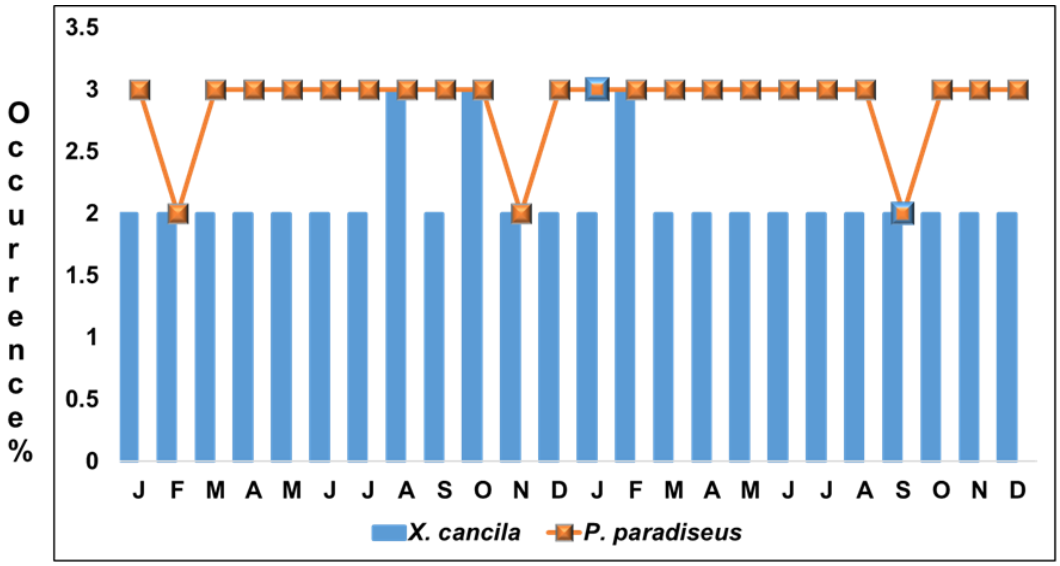


Fig. 91. Monthly Occurrence of plant food item in stomach of *X. cancila* and *P. paradiseus*

CHAPTER 6.

PATHOLOGICAL EFFECTS OF THE PARASITES

HISTO-PATHOLOGICAL EFFECTS OF THE PARASITES

Histopathological effects of helminths in different organs of *P. paradiseus* and in *X. cancila*

Parasites occupy a definite position or site in suitable environment on their hosts and damage their host. Fishes are one of the most common hosts of helminth parasites. The parasites of digestive tract feed either on the digested contents of the host's intestine or the host's own tissues (Markovy, 1946). The influence of the parasite may result in extensive change in individual organs or tissues or it can exert a general effect on host. "Like all animals, fishes have their full complement of disease and parasites and of abnormalities, both malignant and benign and there is no question that most fishes die from such disorders, natural enemies other than men" (Lagler, 1956).

The helminth parasites usually cause the damage in the surrounding of their microhabitat into the host body. This damage occurs when the parasites pierce the vicious organs of digestive system for having their food from the host's body; their migration causes disturbances to the host's multiple systems, the cluster of parasite block the channel of fluid in the host body, heavy infection causes deficiency of hosts nutrition, lesions, ulcer and finally the death of the host.

In the present study, multiple organs were observed to find out the mode of parasites present in the body of *P. paradiseus* and *X. cancila*. Some histological and pathological changes were examined on the skin, body musculature, swim bladder and visceral organs of the host fishes. Structural integrity of the skin, body musculature and visceral organs were more disrupted by the juveline trematodes *Isoparorchis hypselobagri* and larva of *Gnathostoma spinigerum* than other helminth parasites which were found in the stomach and intestine.

In the present study, *Isoparorchis hypselobagri* and third stage larva of *Gnathostoma spinigerum* were found to be the most pathogenic and damaging one [Plate-10]. As no report on the study of the pathological effects of these parasites in these host fishes are available, the name and degree of pathogenicity were chosen for this particular study.

Histopathological studies showed that skin, muscle layer, intestine, lower part of intestine, liver and kidney were damaged by the infestation of helminth parasites. Skin, muscles, intestine, liver, kidney tissues were found to be more infected and in future these infected organs causes severe problem in the growth of fish. Some parasites were deeply attached to the host muscle caused cell damage as well partly destroyed the organs function. While, some were free and only live on hosts nutrients. These free parasites present in the lumen thus the diameter of the intestine become narrower and the system faced hamper.

It was observed that, stomach and intestine of host fish were infected by trematodes, cestodes, nematode and acanthocephalan parasites. In the histopathological examination, at the site of attachment of cyst to the intestinal wall of the host, mechanical displacement and compression of tissue layer, especially muscularis were noticed. The muscularis mucosa were fully disrupted and damaged by the parasites.

Description of the damages occurred due to parasitic infestation

Due to the infestation of parasites in body cavity damages cause visceral adhesions that impair the functions of intestinal tract. However, the mechanical obstruction was caused due to the occurrence of the parasites in clusters. The pathology manifested in the form of compression of the muscular folds was due to multiple infections (Plate-12).

The internal organs of the host fish, particularly the gut generally affected by helminth parasites. They perforate the intestine heavily and inhibit host's growth. The normal growth of fish is interrupted and inhibited if they are heavily infested with endoparasites viz., trematode, nematode, cestode and acanthocephalan. The irritating activities and damage of tissues lining the walls of the oesophagus, stomach, intestine etc. cause microscopic lesions in their host's tissues which become the site for the secondary infection by bacteria (Cheng,1964). Each true fish parasite therefore uses the fish for its home and food and the total damage is related to the numbers of parasites present (Soulsby, 1968; Hoffman, 1967).

Helminths are very common in freshwater fishes. Very few lesions have been attributed to intestinal forms. Histozoic helminthes particularly migrating forms, cause greater

damage in fishes. In several cases, hyperemia, hemorrhage, cellular infiltration, lesion, nacrosist, fibrosis etc. have been observed. After encystment and fibrotic encapsulation, many larval helminthes produce no further obvious damage except pressure on adjacent host tissue. Migrating larva of trematode (cercariae), cestode (plurocercoides) and nematode produce the most serious reactions: leukocytes, fibrosis, hemorrhage and necrosis. Continual migrations, such as larvae of *Gnathostoma spinigerum* produce peritonitis which results in fibrosis and extensive adhesions. Rapid invasion by large number of cercariae produce extensive hemorrhage, hyperemia, necrosis and even death if present in sufficient numbers.

Many fishes were observed with marks of perforation, lacerations and scars on their surface and abdomen. It is assumed that many metacercarial forms might be lost through these laceration processes. Any kind of internal changes or due to the cause of host's death, metacercarial forms need to leave the host immediately. At that time they bored their way in to the external environment for their survival through laceration processes. Sometimes, it was also observed during the study period that, the trematode reaches the buccal cavity or gill and sometimes came out through the anus or genital opening of lower abdomen.

Cestodes were found to have different habits and distribution pattern to the various organs of the hosts. Some of them were attached by mean of scolex and a very few number was found to exposed freely in the gut. Most of the Caryophyllaeid cestodes shows its abundance in the first and second loop of anterior part of the intestine, immediately behind the stomach which also agreed with Mackiewcz (1972). He also predicted that the normal distribution patterns of many species may be altered in the case of heavy infections.

In the stomach and intestine, the scolex of the mature form of *Nybelinia lingualis* was embedded within the muscularis and the rest of the body hanging freely in the lumen. In some of the congested regions, there were swollen mesh works of red blood cells, fibroblast with broken blood cells. Basophilic cytoplasm and cell boundaries were poorly defined. The giant cells arranged themselves around the debris.

No significant changes being observed in the early stage of the infestation. During the later stage of the parasitic infestation, border of mucosa was irregular or disrupted, columnar cells were degenerated and goblet cells were enlarged.

The normal stomach wall consists of five layers such as sub serosa, muscularis, submucosa and mucosa. The mucosa is thrown waves like villi projecting into the lumen. The muscularis mucosa is well developed and lies below the sub-mucosa. It consists of an outer longitudinal layer and inner circular layer. The muscularis consists of a thick and prominent layer of circular muscle fibres.

The intestine consists of four layers such as serosa, muscularis, sub mucosa and mucosa. The muscularis consists of two layers: an outer thin layer of longitudinal muscle fibers and inner thick layer of circular muscle fibers. The mucosa is folded into numerous simple foods or finger like villi. These villi are numerous in the proximal part and are fused with one another distally.

The histology of liver shows sinusoids which are irregularly distributed between the polygonal hepatocytes are fewer in number and are lined by endothelial cells with very prominent nuclei. Normal liver was large and there was a compound tubular gland consisting of a large number of hepatic acini.

The globular of the pathogenic parasite posses pseudobothrial depressions and an apical sucker which penetrate the epithelium, sub mucosa and muscularis of the intestine in such a way that the head is projecting from the outside of the intestine surrounded by a capsule and causes a serious damage. This was also observed by Bovein (1926). This is however generally found only in case of heavy presence of infection of parasites which effectively blocked the tube of the gut during heavy infection. This is also supported by the findings of Mackiewcz (1972).

A number of observable histopathological changes occurred in the intestinal tissue of infected fish. The gut helminthes damaged the walls at the site of their attachment. This disruption was mainly due to the action of sucker of the parasites.

As a result, the intestinal wall was heavily destroyed. Deposited melanin was also observed inside intestinal tissue. The gut wall was perforated where the host tissue reacted vigorously. Large vacuoles were also formed. Fluid filled empty space along with debris and lymphocytes were present. The intestinal mucosa and villi tissue was disrupted, the blood vessels were ruptured and intestinal tissue showed incipient necrosis (Plate-16 C).

Apparently the intestine was seen with many external swelling other than smooth surface. It was also observed that penetration of this parasite causing proliferation of layers and protuberant curved nodules. The histopathological observation of the infected tissues revealed that this species caused serious pathological change in the gut as its scolex was deeply buried into the serosa layers. Besides this, the nodular appearance and necrosis with debris in the pit. The nodule formation which seems an inflammatory response of the host, provides sheltered habitat and firmed attachment of the worm, (Plate-16 B).

In the early stage, there are no significant histopathological changes observed in the serosa, muscularis, mucosa and sub mucosa layer of the stomach. In the layer stage, some columnar cells of mucosa layer and mucous cells were degenerated. In some severe cases, the gastric glands were ruptured (Plate-18 B).

In *P. paradiseus*, trematodes (both encysted and free), were frequently found in Stomach, intestine and more rarely in body cavity. The parasite, *P. bilabiatum* was found distributed in and around the general viscera. Some cysts of it were also observed in the liver. The main effect of the parasite was done on the skin surface, body musculature and visceral organs. *P. bilabiatum* (immature) was found attached to the body muscles causing extensive tissue damages including inflammation, necrosis and empty spaces with fragmented blood capillaries, tissue debris, lymphocytes and fluids.

Due to the structural construction and the ability of the immature *P. bilabiatum*, they are normally capable of dissolving and penetrating the skin and muscle layers when they are living in the regular habitat and corresponding micro environment. But along with the onset of decomposition of viscera or any unusual change of host body's micro environment, the trade become stimulated or compelled to utilize the dissolving and

penetrating capacity for their survival. It can be noted as a homeostatic adjustment or adaptation of the juvenile trematode *P. bilabiatum*. The juvenile parasites dissolve the skin with the help of the penetration glands, located in the oral sucker situated at the anterior region of the body and in the acetabulum (Plate-17A).

Heavy melanin deposition was observed along with accumulated melanin macrophage centers in the infected liver. Due to the presence of *P. biabiatum* as well as cyst, small vacuoles were formed in the liver. The melanin-macrophage centers should passively be considered as a component of the reticulo-endothelial system and hence, part of the defensive system of the fish against any infection. Presence of massive melanization again confirms this. Sometimes hepatic blood vessels were ruptured. The affected liver showed mild hepatic or hemopoietic degenerative changes with hemorrhages (plate-14 A).

During the migration of immature *P. bilabiatum* from visceral cavity to the swim bladder, massive disruption and dislocation of the visceral organs occurred. Irregular black pigmentation was scattered throughout the swim bladder, due to the infection by juvenile *P. paradiseus*. In severe cases, the alveolar sacs and capillary plexuses were disrupted causing necrosis (Plate-19 B).

Due to the severe infections of *Pallisentis ophiocephali*, causes damage of muscularis layer forming mass of cluster. These species obviously cause mechanical obstruction and denudation of the epithelial layer within the gut occupying a major portion of the intestinal cavity of the lumen and thus allow in a narrow space for the chime to pass through. Moreover, larval forms also cause the barrier of the chime to pass and ultimately a total blockage of the results. The chime on passing through such a narrow space of the lumen causes gradual disruption of the gut and finally disappearance of the tissue system.

All the parasites were found in the stomach and in the intestine either with their head attached to the gut or exposed eely in it. Most of the acanthocephalans were attached only superficially to the wall of the gut, but *N. aspinosum* did penetrate the epithelium, sub epithelium, sub mucosa and muscularis layer, in such way that the head penetrates out of the gut and were surrounded by a capsule.

It was revealed from the sections of a portion of the intestine that there was a severe damage in the mucous membrane with the broken villi. The parasites were observed to move forward leaving a considerable portion of the intestine only with serosal layer *Nybelinia lingualis*, the truncated cone-shaped scolex of the species was found to attach firmly to the wall of the intestine, causes lesions and inflammation; generally capable of local damage (Plate-16 C).

Lesions occur within the muscularis of the stomach and external serosa is involved in any generalized peritonitis due to tapeworms. The stomach wall of the host was infected by nematode parasites. Histopathological examination confirm that the stomach wall was severely damage due to the penetration of these larvae into the muscular layers. The serosa was destroyed and many sections of larvae were observed among the muscularis mucosa and the connective tissue. Below the encapsulated parasite, the muscularis layer was found to be damaged (Plate-18 B).

The *Dujardinascaris* larva was found in several organs namely mesenteric layer, stomach and intestine wall, liver, kidney, gonads and outer peritoneal layer etc. these larval nematodes forms cysts into the above mentioned organs of the general viscera including fat bodies (Plate-15 B, C). Each larva was enclosed with in a capsule which was a flattened spore composed primarily on the fibrous tissue of the hosts. The encystment caused retardation of the proper growth of gonads and the fibroblast tissues on the stomach and intestinal wall of the hosts.

Gnathostoma spinigerum L_3 larva found attached in the epithelial in layer of the stomach with their chitinous buccal capsules and causes local damage. The encystment of larval nematodes on the outer wall of digestive tract of *X. cancila* caused local damages and mechanical destruction which also supported by Hine and Kennedy (1974). Destruction and distention of muscularis affected due to the encystations of the nematodes associated with their presence, was a proliferation of the epithelial cells. Blood cells infiltration was being observed.

Black pigment was also found around the capsule. In severe cases of pathogenicity, muscular layer was found to become hyperplastic and at times a thin mucolid interface layer was also observed (Plate-17 C).

In some, liver infection was also extremely deep with degeneration, resisting in nodular regeneration hyperplasia, which was causing compression of hepatic parenchyma, which was severely fatty. This nodular hyperplasia caused fatty changes and mild chronic congestion of irregular distribution (Plate-14 B).

The most salient feature of the sections of the liver was damaged to the hepatic parenchyma. All gradation from degeneration to extensive necrosis of hepatic cells may be encountered depending on the duration and severity of the infection. The pathology associated with parasites was thought to be caused by proteolytic secretions of the frontal glands. However, the occurrence of necrotic debris in the present study is suggestive of the presence of proteolytic enzymes in the parasites. Histopathologically compared to that of the healthy liver cell, in the early stage of the parasite's presence there was no significant changes in the parasitic liver.

The section of cyst consisted of epithelial tissue and ultimately fibrous tissue. The hepatic cells of the infected area underwent atroy and necrosis. The lobules collapsed, circulatory disorder occurred and the degree of cell damage were mild to severe were being observed. Hemorrhagic areas were frequently seen in infected areas (Plate-14 A).

In the late stage of infestation, the liver tissue showed the inflammation of the surface layers. Some polyhedral hepatic cells near Periphery were mostly necrotized. Focal lyses was often observed in the hepatopancreas, inside the hepatic parenchyma and adjacent to the blood vessel. Blood vessel were observed as degenerated. Little dark pigmentation was also found. Histopathological sections of the infected liver tissue showed that encapsulated cystic form of nematodes were present in the liver. Sometimes the liver was destroyed by cestodes because of heavy infections. The tissue mainly caused inflammation by the cyst of cestode larvae and caused injury to the tissue, damaged liver cells, caused hemorrhage and breakage of blood cells, coagulation necrosis of parenchyma, large areas of parenchyma was replaced by the cyst of the parasites (Plate-13 C).

CHAPTER-7

PROXIMATE ANALYSIS OF THE FISHES AND VARIATION DUE TO INFESTATION

PROXIMATE ANALYSIS OF THE FISHES
AND
VARIATION DUE TO INFESTATION

Biochemical analysis of different nutritional components of *P. paradiseus* and *X. cancila*

In the present study, an attempt has been taken due to determine the percentage (g/100g) of nutrients such as moisture, ash, fat, protein, contents (mg/100g) and energy (Kcal) in *P. paradiseus* and *X. cancila.*

The results of the proximate composition of *P. paradiseus* and *X. cancila* have been analyzed to compare the values of components in the two species of infected and non-infected fishes, in different size groups and also due to determine the relationship of the nutritional components with rate of infestation.

In infected *X. cancila*, the moisture was 75.91 g/100g, ash 3.21 g/100g, fat 2.79 g/100g and protein 18.01g/100g. Besides- these, the non-infected X. *cancila* contains the nutritional components as - moisture 75.4g/100g, ash 3.37 g/100g, fat 2.92g/100g and protein was 18.31g/100g (Fig-92, 93).

In infected *P. paradiseus*, the moisture was 76.29 g/100g, ash 2.01 g/100g, fat 3.35g/100g and protein was 17.41g/100g was 6.89%. While in non-infected *P. paradiseus*, the different nutritional elements were: moisture 75.99 g/100g, ash 2.24 g/100g, fat 3.78g/100g and protein was 17.99g/100g (Fig-94, 95).

In 2017, the presence of moisture content in *X. cancila* was higher during winter (75.92 g/100g, Jan, 17) whereas the lower moisture content was recorded during summer

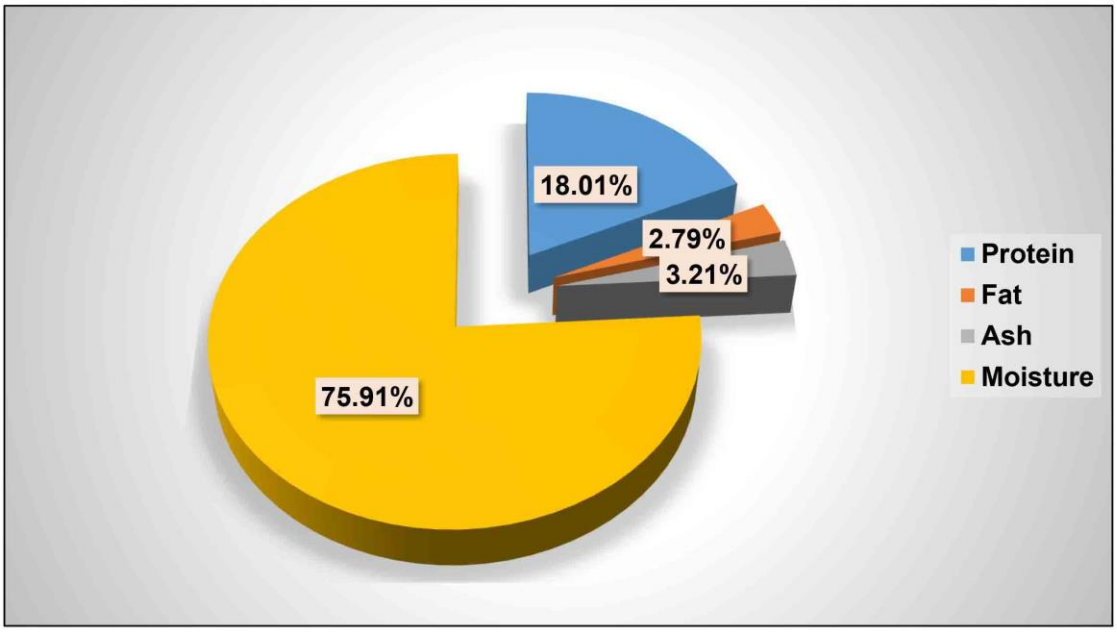


Fig. 92. Percentage of nutritional components found in infected *X. cancila*

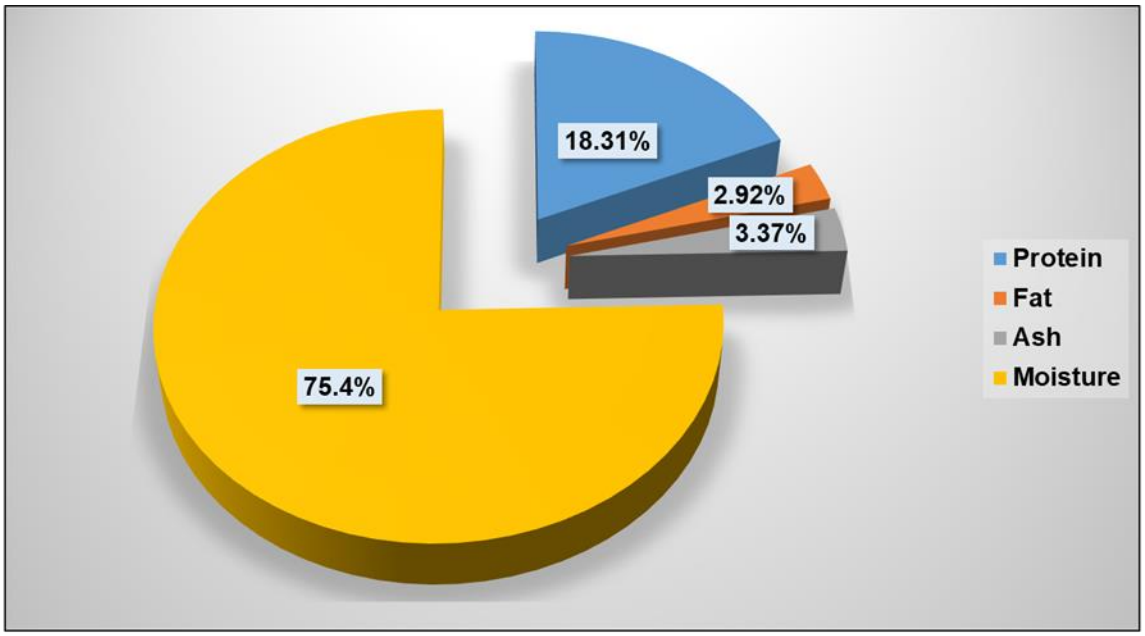


Fig. 93. Percentage of nutritional components found in non-infected *X. cancila*

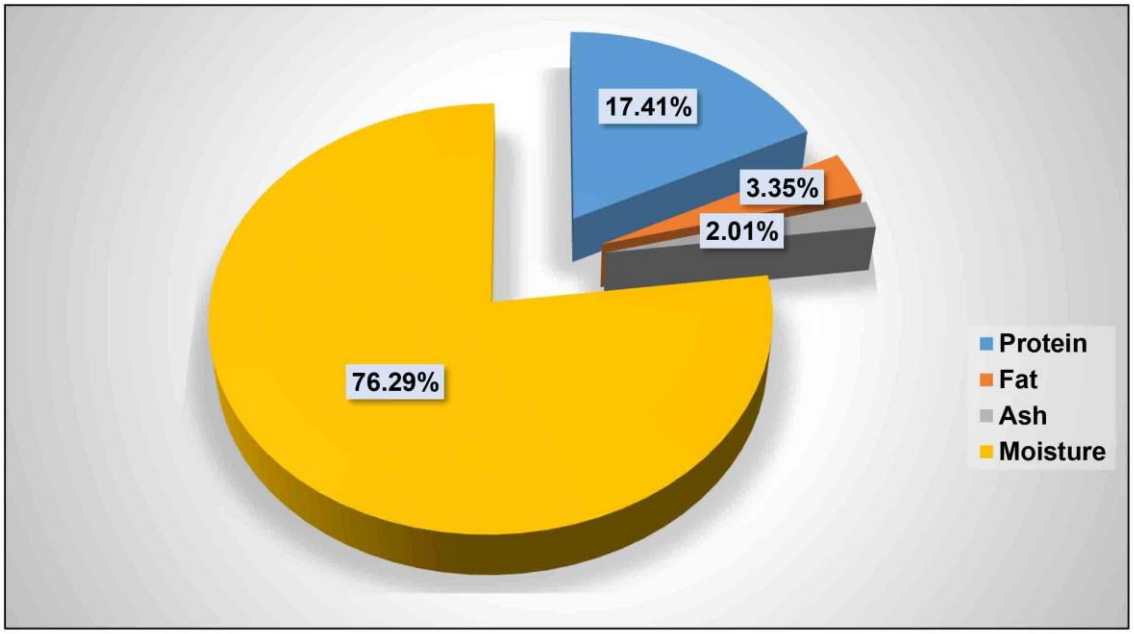


Fig. 94. Percentage of nutritional components found in infected *P. paradiseus*

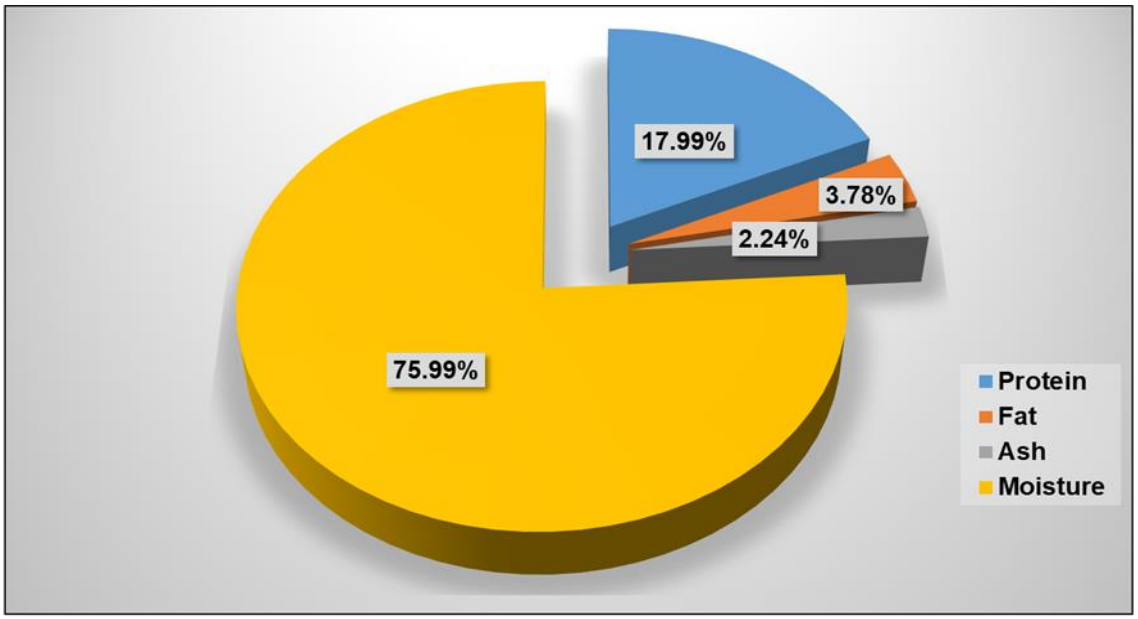


Fig. 95. Percentage of nutritional components found in non-infected *P. paradiseus*

season (75.88g/100g, April'17). In 2018, the maximum percentage of moisture was recorded during winter (75.94g/100g, Nov'18, Dec'18) while the minimum was in summer (75.88g/100g, April'18) (Fig-96).

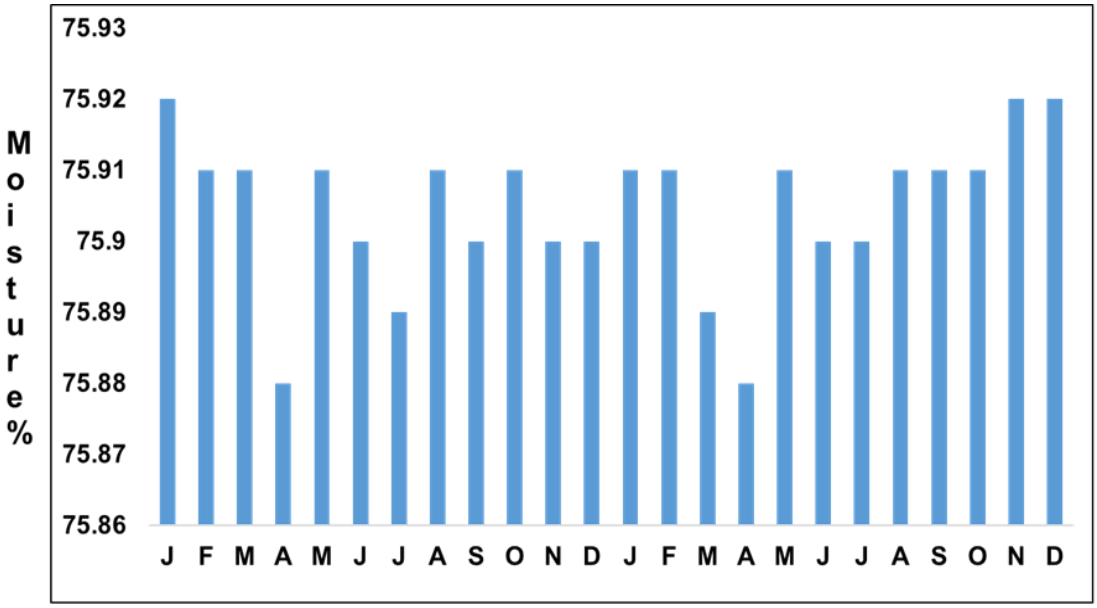


Fig. 96. Monthly presence of moisture in *Xenentodon cancila*

The pattern of seasonal variation of percentage of protein content in *X. cancila* was recorded maximum in winter (18.05 g/100g, Feb'17) and the value was lowest during summer (17.98g/100g, in April'17). In 2018, the highest percentage of protein content 18.04g/100g was observed during winter (Dec'18) while the lowest was 17.99 g/100g recorded in rainy season (Aug'18) (Fig 97).

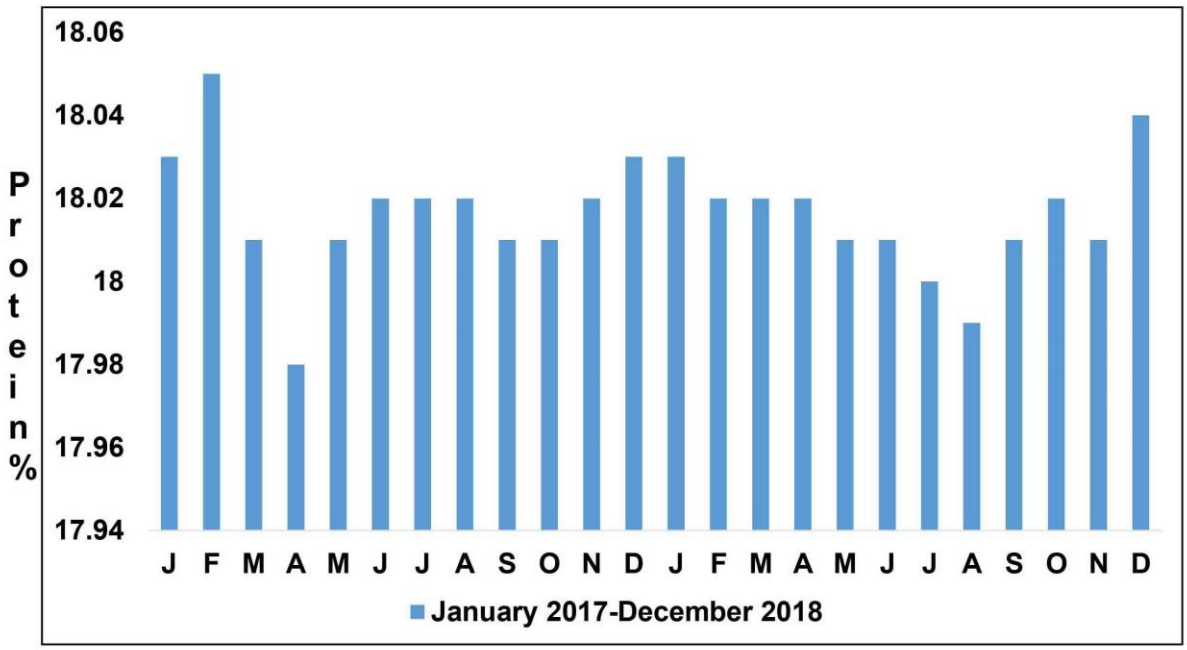


Fig. 97. Monthly presence of protein in *X. cancila*

In 2017, the highest value of fat in *X. cancila* was observed during winter (2.83g/100g, in January'17) and the lowest value was in summer (2.77g/100g, June'17). The next year, the value was also higher in winter season (2.81g/100g, Dec'18 while the lowest was found in summer and rainy seasons (2.78g/100g in May'18, July'18 and Aug'18) (Fig.-98).

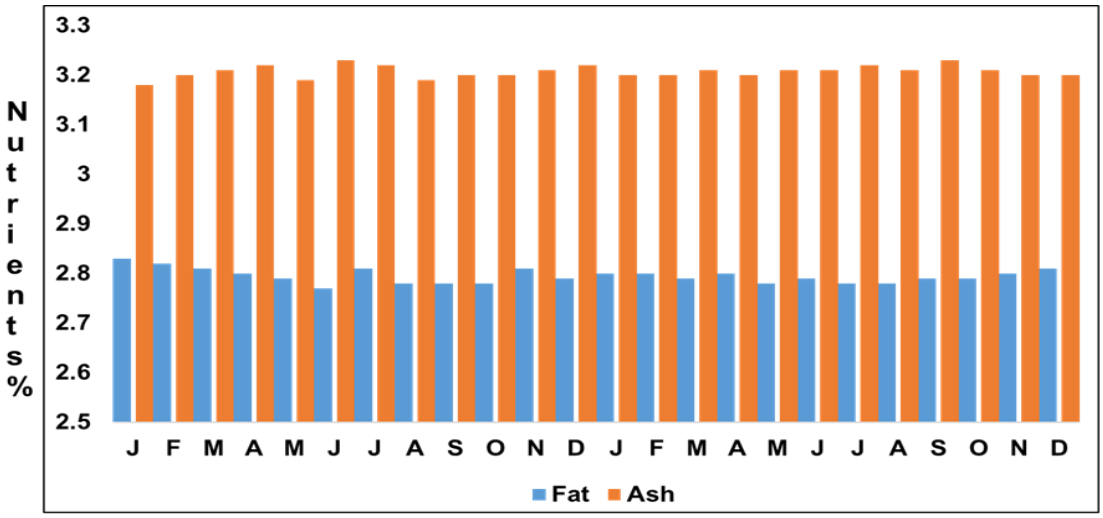


Fig. 98. Monthly presence of nutritional components (fat and ash) in *X. cancila*

In 2017, the maximum percentage of ash in *X. cancila* was recorded during rainy season (3.23 g/100g, June'17) while the minimum was in summer and rainy seasons (3.19 g/100g, May'17 and Aug'18). On the other hand, in 2018, the highest value was found

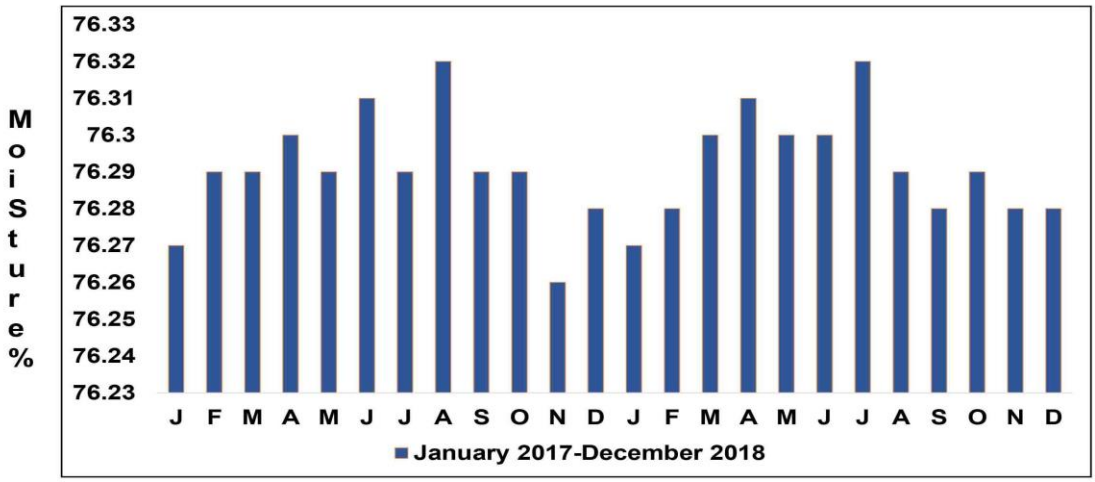


Fig. 99. Monthly presence of moisture in *P. paradiseus*

in summer season (3.23 g/100g, Sep'18) and the lowest value was found in winter season (3.2 g/100g; January'18, February'18, November'18, December'18) (Fig.-98).

In *P. paradiseus*, in both 2017 and 2018, the percentage of moisture was found highest during rainy season (76.32g/100g in August'17; 76.32 g/100g in July'18) whereas, the lower moisture content was found during winter season (76.26g/100 g in November'17; 76.27 g/100g in January'18) (Fig.-99).

The pattern of seasonal variation of percentage of protein content in *P. paradieus* was recorded maximum in summer (17.44g/100 g, May'17) and the value was lowest during rainy season (17.39 g/100 g, in Aug'17). In 2018, the highest percentage of protein content 17.43 g/100 g was observed during rainy season (Sep'18) while the lowest was 17.39 g/100g recorded also in rainy season (July'18) (Fig. - 100).

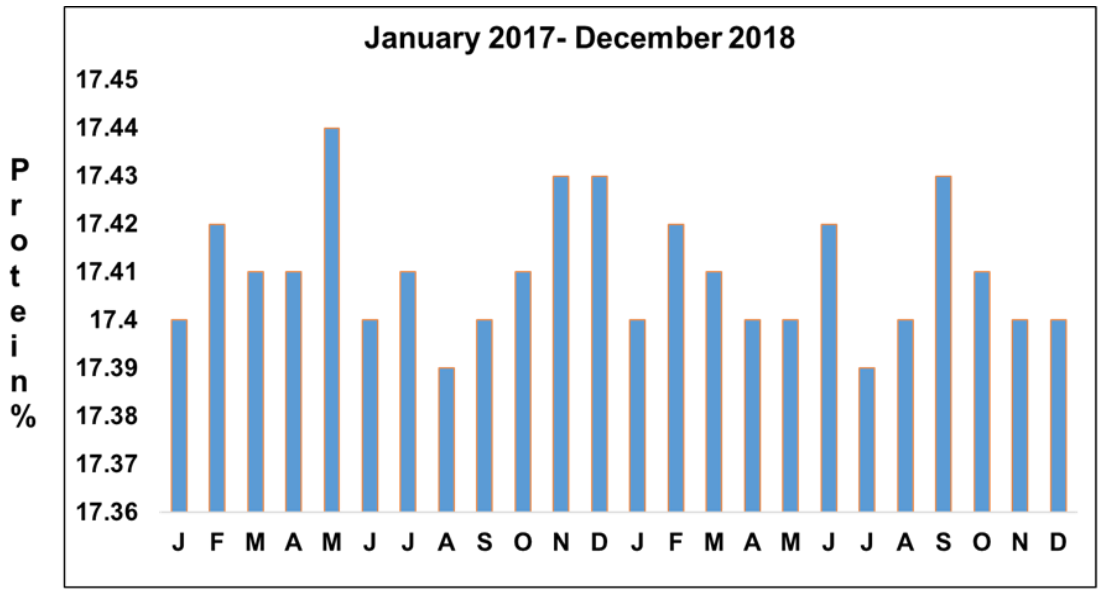


Fig. 100. Monthly presence of protein in *P. paradiseus*

In 2017, the highest value of fat in *P. paradiseus* was observed during summer (3.38 g/100g, May'17) and the lowest value was in winter (3.32g/100g, Dec'17). The next year, the value was highest in winter (3.37g/100g, Nov'18) while the lowest was found in summer (3.32g/100g, April'18) (Fig.-101).

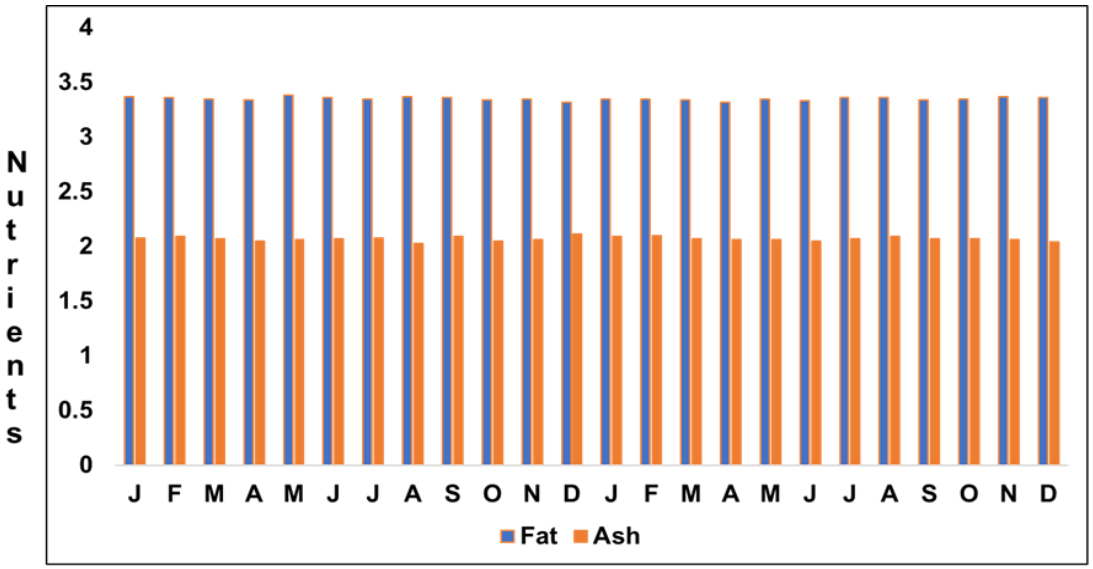


Fig. 101. Monthly presence of nutritional components (fat and ash) in *P. paradiseus*

In Jan-Dec'17, the maximum percentage of ash in *P. paradiseus* was recorded during winter (2.11 g/100g, Dec'17) while the minimum was in rainy season (2.03g/100g, Aug'17). In 2018, the highest value was found in winter (2.10 g/100g, Feb'18) and the lowest value was also found in winter (2.04g/100g, Dec'18) (Fig.-101).

CHAPTER- 8

MOLECULAR IDENTIFICATION OF ACANTHOCEPHALAN PARASITES WITH COI GENE

Molecular Identification of Acanthocephalan parasites with COI gene Morphotaxonomy

A total of 111 acanthocephalans were collected from *X. cancila* and 16 were collected from *P. paradiseus*. The detailed taxonomic study identified 4 Species as *P. ophiocephali*, *N. aspinosum*, *N. prolixum* and *A. nigeriensis*

DNA barcoding of all the parasites was performed but only the barcoding of *P. ophiocephali* was possible. In case of other parasites PCR did not take place.

Observation of PCR-amplified sequence of COI gene by gel electrophoresis

The extracted DNA from 3 acanthocephalan samples were amplified by PCR and 1% agarose gel electrophoresis was conducted to see the separated bands. Then the bands were compared with the known molecular weight ladder and observed that PCR-amplified COI regions were situated between 400bp and 500bp region. This confirmed that the PCR amplicons were the desired 432bp region of COI gene. The bands of the 432bp region of the COI sequences of *Pallisentis ophiocephali* are shown

432 bp

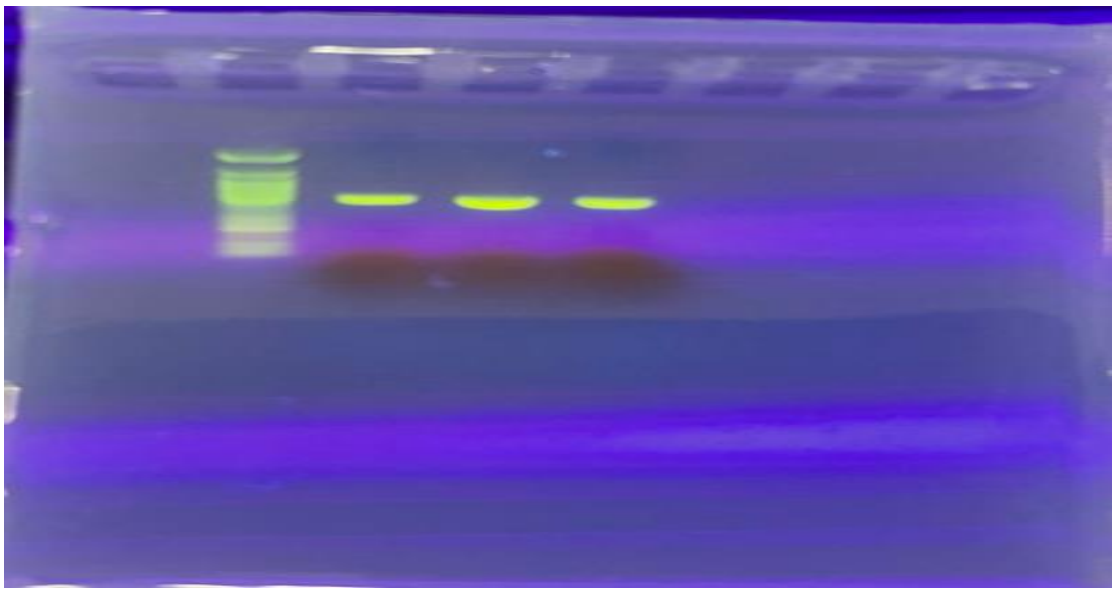


Fig. 102. Samples showing clear bands at 432 bp.

Species confirmation by BLAST search

BLAST tool was used to match the sequences with the pre-existing sequences which were already saved in NCBI. In the NCBI BLAST search, COI barcode sequence of these three specimens showed the matching (Table-36, 37) with the pre-existing sequences in generic level. However, in the experiment, accurate match of *P. ophiocephali* was not found but one related species *Pallisentis celatus* was found. This is the first report of *P. ophiocephali* in NCBI. The sequences were submitted to NCBI-GenBank and the accession numbers acquired

Sequence and phylogenetic analysis

The generated sequence, along with sequences of the other related and non-related helminth species were retrieved from GenBank for analysis

Table-35: BLAST result analysis of three specimens showing similarity with pre existing sequence from NCBI

Sl. No.	Species	Accession Number of the present study	Accession Number of the best match
1	*Pallisentis ophiocephali*	OM679999	JQ943583.1
2	*P. ophiocephali*	OM680000	JQ943583.1
3	*P. ophiocephali*	OM680001	JQ943583.1
4	*P. celatus*	-	JQ943583.1
5	*Neoechinorhynchus emyditoides*	-	*KY077095.1*
6	*N.emyditoides*	-	KY077094.1
7	*N. mexicoensis*	-	KY077093.1

Nucleotide composition analysis

The GC content is a very important parameter of a particular gene. GC content seems to be variable both in the cases of individual species and the orders they belong. Here, the GC content variation in our study is analyzed for the species in the Table-36.

Table-36: GC (%) content for seven specimens of acanthocephalans

Species	**Number of the sequenced specimen (BD)**	**Overall (%GC)**	**Overall (%AT)**
Pallisentis ophiocephali	1	44	56
P. ophiocephali	1	44	56
P. ophiocephali	1	44	56
Pallisentis celatus	1	41	59
Neoechinorhynchus emyditoides	1	34	66
Neoechinorhynchus emyditoides	1	34	66
Neoechinorhynchus mexicoensis	1	28	72

K2P (%) distance was sharply less in intra species than interspecies

Genetic divergence (K2P distance %) between intra species and interspecies were calculated. Apart from the three specimens (*Pallisentis ophiocephali*) in this study, additional COI sequence of one related species (*Pallisentis celatus*) was utilized which was downloaded from Gen Bank, NCBI. After calculation of K2P with these 4 COI sequences of 2 species, genetic divergence was observed to be increased, as expected, with higher taxonomic rank- 0% to 2% within species, whereas 22% between species. The finding of the present study, K2P (%) distance was sharply less in intra species than interspecies. Interspecies difference was greater than average. Therefore, data was retrieved from GenBank with other species (*Neoechinorhynchus emyditoides*, *Neoechinorhynchus mexicoensis*) and carried out the same procedure. After calculation of K2P with these 3 COI sequences of 2 species, genetic divergence was observed to be increased, as expected, with higher taxonomic rank- 7% within species, whereas 26% between species. And found at that they have even greater values. Thus the initial

value was deduced to be correct. The less values of intra species K2P distance than the interspecies K2P distance proved that DNA barcoding was a powerful tool to identify as well as differentiate species.

Table-37. Minimum, maximum, mean K2P (%) within and between species

	Min	Max
Intra species (*P. ophiocephali*	0	2
Interspecies (*P. celatus*)	-	22
Intra species (*N.emyditoides*)	-	7
Interspecies (*N. mexicoensis*)	-	26

Phylogenetic analysis

Phylogenetic analyses were performed to ensure the identification of species and also to ensure their taxonomic classification. For this purpose, Phylogenetic tree was constructed using Maximum Likelihood Methods. The COI sequences that were purified in this study were designated by BD (as in Bangladesh) following the accession numbers and the species names. In the study, 3 specimens belonging to one species were analyzed. A total of 7 sequences belonging to four species were analyzed (3 from the investigations and the rest four from NCBI). Relative positions of the species with respect to each other were shown in the figure 103.

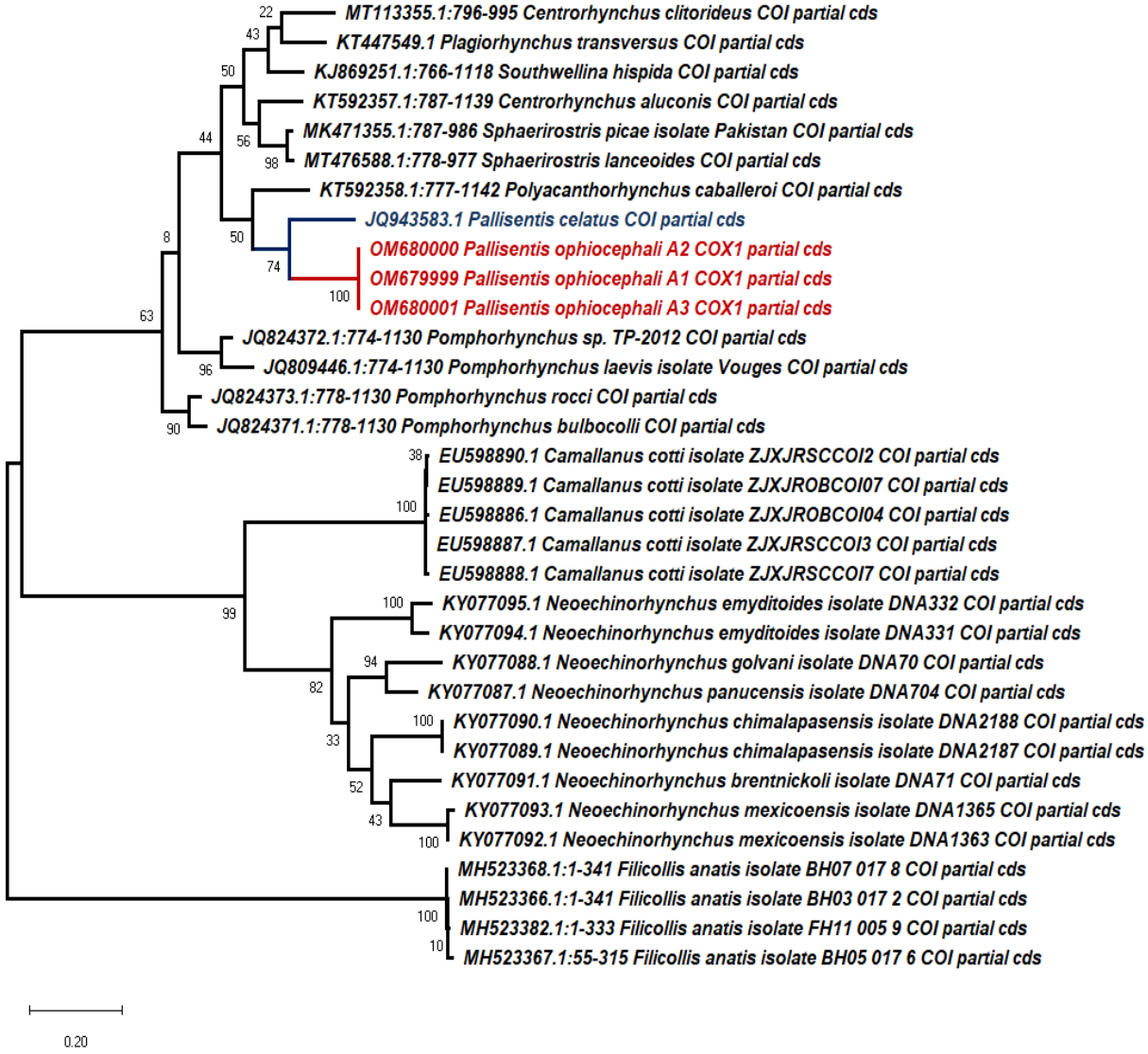


Fig. 103. Phylogenetic tree of acanthocephalans based on partial sequence of COI gene. Sequences amplified in this study were designated as BD. Neighbor-Joining (NJ) was chosen as the statistical method (500 bootstrap replicates were done and the values were included).

Findings of the molecular analysis

The detailed taxonomic study identified *Pallisentis ophiocephali.*

DNA barcoding of the species has been confirmed through sequencing, amplified mt DNA COI (432 bp) gene and it showed that the 3 sequences belong to the same species i.e. *Pallisentis ophiocephali.*

The species has been authorized GenBank accession numbers from NCBI that confirms the authentic molecular identification.

CHAPTER- 9

GENERAL DISCUSSION

GENERAL DISCUSSION

To overcome certain disease outbreaks in *X. cancila* and *P. paradiseus*, the knowledge of the metazoan parasites residing in the hosts will aid fish culturists to determine the specific type of treatment required. Performing research and obtaining the experimental results, abundant information has been collected on the parasite fauna of the Gar fish and Thread fin fish in Bangladesh. Primary information on the presence and intensities of infection of individual taxa, their pathological effects on nutritional components plus the effect of the length of the host is now available. However, the parasite may have an impact on the host in other ways as well, for example the consumption of host's food, damage of hosts tissue, abdominal growth, mechanical inferences, biological effects, various kinds of tissue reactions as well as the effects of toxins, poisons or secretion of parasites itself.

There are several diseases which affect the usual health condition, reduce growth rate, cause abnormal metabolic activities, etc. These in turn cause huge financial damage. Health of any population depends on the control of disease and maintenance of a healthy relationship between living beings and their surroundings (Snieszko, 1983). There are several limiting factors out of which diseases are most severe in aquaculture – the reason being the fact that a high density of fish live in a small amount of water. Thus, diseases can transfer from fish to fish. Huge financial loss to some extent is avoidable with good fish health management (Kabata 1985).

Loss in potential food and financial loss to culturists may result due to the infestation of parasites which can result in fish morbidity and mortality in culture fishes. The achievement of carrying out numerous fishery development programs depends on a definite level on the intensification of the fish parasitological investigation, as the enhancement of fish production can principally be attained from healthy fish stock (Srivastava 1977).

Bashirullah (1973) said that smaller fishes contained less parasites while the larger fishes contained more parasites comparatively. In a study, a total 10 species of parasites

were recovered from *P. paradiseus* whereas 9 species from *X. cancila* were recovered. The experiment showed that greater number of parasites have some special preference in their site selection to some extent. The changes occurred as the parasites become overcrowded in their niche (Mackiewicz, 1972). In the experiment, it was seen that the intestine of fish is usually infected more, probably because of the relatively abundant nutrient being present there. According to Bullock (1963) and Dogiel (1964), the intestine of fish is usually more infected than any other organ.

It is not easy to explain the relation between the infections of helminthes parasites in fishes with seasonal variation without having proper knowledge about the periodic features of the intermediate host-parasite system. Although alterations of parasite incidence are ascribed to diet and other factors like host size and development of host immunity; it is expected that immunity serves a significant role in determining incidence (Scott 1975).

In *P. paradiseus*, it was clearly seen that during rainy season (remarkably in July'17) maximum number of parasites were found. In *X. cancila*, seasonal abundances of total parasites showed distinct peak period of abundance (75% in Nov'18) during winter season. Rainy and winter months were the most vulnerable period of the year when fish parasites were found plentiful. The reason for this could be stocking density, water depth, temperature, heavy rainfall, flood, various kinds of pollutants such as industrial pollutants, pesticides, insecticides, domestic sewage etc. and decreased immunity of hosts as well. This coincides with the findings of Zaman (1985), Khanum *et al.* (1992); where they agreed that the seasonal abundance of the helminth parasites are significantly correlated with the seasonal rainfall.

A variety of factors, both destiny-dependent and destiny-independent could upset a number of features of the seasonal variation of helminthes incursion in an aquatic ecosystem (Chubb 1977, 1979, 1980, 1982). Furtado and Tan (1973) indicated increase in infection occurred with the increase in size of fish of *C. batrachus* and according to

them; it was due to a change in diet of the fish. Similarly, Pennicuick (1971) observed the huge proliferations in the incidence and intensity of *Schistocephalus* infestation in small young fish was due to the accumulation of parasites when the young fish ate more infected intermediate hosts than the larger fish. It indicates that the growth of fish promotes the tendency of parasites infestation and parasite burden. In case of male, the fish's growth promotes the prevalence and intensity also. This indicates that parasites were not strictly regulated by the host. There is a fluctuation in intensity and prevalence. But usually intensity is inversely correlated with prevalence and follows established patterns of distribution i.e. a contagious distribution (Eliott 1979).

Most of the investigations on seasonal patterns of infestation of helminthes of freshwater and estuarine fishes have been done in the temperate climate zone on earth, with limited data available on the tropical rainy climatic regions. There has been considerable speculation on the observed temperate zone seasonal patterns, with the most significant factors being thought to be water temperature variation (Aho *et al.* 1982; Camp *et al*. 1982; Esch 1983; Granath and Esch, 1983 a; Kennedy, 1977), host behavior (both dietary and social, Anderson, 1976; kennedy, 1977; Smith, 1973) and parasite population density (Esch, 1983; Granath and Esch 1983 b; Holmes *et al*. 1977).

The parasite which was dominant in a particular fish host, may or may not maintain its dominance in another host. Amin (1975) supports the view that the presence of a parasite species in significant number in a fish host, results a lower density of the other species of parasites. Dogiel (1961) discussed the dependence of the parasite fauna on the environment. He stated that the parasite fauna of all fishes depends on the geographical location, season, the characteristic of the water (temperature and chemical composition), type of the bottom and other biotic and abiotic factors. He also stated that the parasitic fauna is affected even more seriously by the physiological and biological features of the host. Food of host includes many animals which serve as the intermediate hosts for the parasites completing their life cycles in fishes. The ability of the host to develop immunity, the age of the host, health condition, spawning and migration of the fish are essential factors to determine the final configuration of parasite fauna of the fish.

The host, the pathogen plus the environment are in a constant state of instability, having the ability of changing in any step with any variation may cause new infection of parasite in *X. cancila* and *Polynemus paradiseus.* There has been considerable change in the environmental situation in Bangladesh since the 1980s. Hence, it may alter the parasite fauna of *Xenentodon cancila* and *Polynemus paradiseus* because of the excess use of inorganic fertilizer and pesticides in cultivated lands, discharge of industrial waste, insufficient waste disposal, etc. that cause changes in the aqua-environment indirectly.

Bashirullah (1972a) investigated the distribution and occurrence of *Isoparorchis hypselobagri* in different hosts and localities from Bangladesh water resources. Out of 25 hosts, he recorded seven new hosts from Dhaka, Bangladesh. He collected *Isoparorchis hypselobagri* from swim bladder of *Wallogo attu*, *Mystus aor*, *M. cavassius* and from lateral muscle of *Channa striatus*, *C. marulius*, *C. punctatus* and *Nandus nandus*. He found juvenile of *Isoparorchis* in the lateral muscle of fishes within heavily pigmented cysts. He believed that the parasite actively penetrates the gut wall and migrate into the swim bladder of siluroid fish. He also discussed the life history of the trematode parasite. In 1988, Zaman and Leong worked on two fresh water cat fishes. They described the intensity and abundance of infestation of *I. hypselobagri* from the two cat fishes *Clarias batrachus* and *C. macrocephalus*. Related consequence was also observed by Chowdhury *et al*. (1986) in genus *Mystus*. But the degree of intensity and abundance were different.

The feeding and nutrition of the two hosts followed the same pattern so the pattern of occurrences of parasites was almost similar and more than one host served as the definite host. Chauhan and Ramakhrishna (1958) worked on the distribution and abundance of cestodes of eleven species of teleosts (*Barillus sp*., *Labeo sp*., *Mastacembelus sp*., *Ophiocephalus sp*., *Schizothorax sp*. and *Tor sp*.) from Garhwal Himalayas with a note on host biology. They observed no consistent pattern in dissimilarities in the amount of food eaten by the two sexes and expected that both sexes would stand the same chance of being infested by the cestode parasites which need an

intermediate host. Differences in infestation between the sexes could be either of the two points of Wickins and MacFarlance (1973):

- It is only an expression of differential feeding either by quantity or quality of species or
- Due to changing degrees of resistance to infestation by different sexes.

The different categories of food taken by *P. paradiseus* and *X. cancila* were almost similar but the type and rate of establishment of infection were different due to susceptibility of the host, different biochemical composition and different ecological and physiological mechanism in the two host species. Besides, the diets of the two fish species were directly or indirectly related with the parasite fauna. Small fishes, insects and mollusks were found to have been consumed as major portion of the diet in both the fishes. Insects and mollusks serve as the intermediate host for many larval helminthes. Ascaroid larvae were transmitted through the small fish consumed. This view also supported by Bashirullah (1973), Wooten and Smith (1975), Khanum *et al*. (1990). Aquatic insects served as the intermediate hosts for cucullanidae, quimperiidae and other families of nematode. Insects also served as the intermediate hosts for acanthocephalan parasites where the arthropod must eat an egg voided with the feaces of a definite host. Mollusks serve as the intermediate host for many larval trematodes. The siluroid and non-siluroid fishes which feed on plankton copepod are highly susceptible to infection of *I. hypselobagri* metacercaria in the swim bladder and on feeding these infected fishes the carnivorous siluroid fishes become infected and thereafter, the metacercaria develop into juvenile or immature form, occupy the habitats like coelomic cavity, kidney, musculature and visceral organs of those fishes (Srivastava 1977 and Cribb 1988). In the similar way, the infective larvae of camallanid nematodes released into the water directly by ovoviviparous females are taken by copepods which in turn are eaten by small forage fishes and through this channels the nematodes could be passed to large predatory species when they are consumed these forage fishes (Stromberg 1973).

As it can be ascertained that, the sources of infection in both the fishes were same due to same type of food habits, different biochemical composition and manifestations of

effects of interaction of some complex and often obscure ecological and physiological mechanisms in *P. paradiseus* and *X. cancila*, the type and rate of establishment of infection were different. According to Zaman (1985), *Lytocystus lativitellarium* was dominant in *Clarias macrocephalus* but not in *C. batratchus* though they shared the same habitat and consumed same type of food.

In the present study, *Prosogonotrema bilabiatum, Uterovesiculurus hamati, Dujardinascaris sp., Metaquimperia bagarii, Neorhadinorhynchus aspinosum and Pallisentis ophiocephali* in *P. paradiseus* were restricted to specific site or organ, but other individual species of parasites were not found in specific site within the same host fish. Whereas, juvenile *P. trygonis* in *P. paradiseus* was found to very specific in site preference. In *x. cancila*, juvenile *Isoparorchis hypselobagri*, *Pallisentis ophiocephali* was found to be more specific in site preference than the others. Some species of parasites occupied narrower microhabitat whereas others may be more flexible and occupied greater areas (Awachii 1968; Mackenzie and Gibson 1970; Ulmer 1971; Holmes 1973; Hine 1980; Evans 1977) concluded that the different site preferences of the parasites may be explained by innate variances among the parasite species which indicate their reactions to stimuli thus bringing them to be confined establishment and any successive migrations, influenced by some biochemical and physiochemical gradients in the different organs of the host.

In the present study, it was evident that, prevalence and intensity of trematodes and nematodes varied greatly in both the years of the study period in both the host fishes. Few different species of parasites were also found in this investigation affecting the two host separately. It was also observed that maximum length was recorded 15 cm for *P. paradiseus*, whereas it was 39 cm for *X. cancila*.

In the present investigation, it was observed that majority of immature trematodes, cestodes, nematodes and acanthocephalans were also found to infest host fishes. According to Holmes *et al.* 1977, in catfish, the maturing of parasites might be controlled by density-dependent factor and number of population. They stated that the

greater the number of parasites in a host, fewer of them will mature. This phenomenon also was supported by Zaman 1985.

In order to determine the parasite fauna of fish (Zaman, 1985) the host age (=length) must be considered since it is an important factor. The exact mechanisms, include the following conditions (1) composition and quality of diet, (2) differences in habitat preference between juveniles and adults, (3) development of age acquired immunity to specific species of parasites of and (4) the accumulation of long-lived parasites species (Arthur *et al*.1982) and etc. The long –lived larval helminthes, such as *Isoparorchis hypselobagri* and of some nematodes occurring in the mesenteries and viscera are undoubtedly found accumulated with age as observed in the present study, but this could not be clearly demonstrated in the present analysis and an intensive, systematic investigation should be carried out for several years to obtain a clear picture of the role of host age in determining the parasite fauna of *P. paradiseus* and *X. cancila.*

Furtado and Tan (1973) showed that *Lytocestus parvulus* and *L. lativitellarium* increased with the increase of length of *Clarias batrachus* from Malaysia. Ahmed and Sanaullah (1978) showed the infection of *Djombangia penetrans* and *L. indicus* increased to maximum in the 19 cm. length groups of *Clarias batrachus* in Bangladesh and then decreased. In the present study of parasites in garfish and threadfin fish in Bangladesh, the results of the effect of age on parasite abundance varied.

Scott (1975) worked on incidence of trematode parasites of American plaice *Hippoglossoides platessoides* in relation to fish size and diet. He stated that with the length of fish and associated changes in the fish's diet, the occurrence of several trematode species changed. Associations between parasite occurence and the rate of incidence of food items showed that small crustaceans may be mediocre hosts for *Steringotrema vetustum* and *Derogenes*.

Histopathological studies showed that skin, muscle layer, stomach, intestine, swim bladder, liver were damaged by the infection of helminthes parasites. Helminthes in

fishes are also recognized as causing serious effect on their hosts (Ribelin and migaki, 1975). Numerous changes occurred in the liver for parasite infestation. One of them is formation of vacuoles, causing spongy appearance where fluid is accumulated. Therefore, the surrounding cells face an increased amount of pressure and the normal liver functions are affected. This causes the hosts' immunity to be decreased. These reasons promote the possibility of primary and secondary infection.

Mackiewiez *et al*. (1972) found cellular damage, mechanical obstruction, production of lesions and necrosis etc. caused by the helminthes in different organs in fish. Some remarkable works have been done on histopathology of catfishes by Bhattacharjees, 1986; Khanum 1994; Khanum and Farhana 2002; Soderberg 1984 etc. Due to the infestation of *I. hypselobagri* in the swim bladder, massive melanization was observed. In the body musculature, intestine and kidney fluid filled empty spaces along with debris's and lymphocytes were present. Similar observation also reported by Roberts *et al*. (1993) and Sanaullah *et al.* (1997).

In the present study, no notable pathological damage was observed due to the encysted larvae in mesenteric and viscera. Kenndy and Lie (1974) observed that capsulated larval Eustrongylids sp. found attached to the stomach wall of salmotrutta, had no local pathological effect. But Ahmed and Rahman (1979) reported pathogenicity of three encysted nematodes on the stomach wall of flat fishes where local damage and mechanical destruction were observed. The caryophyllaeid cestodes inflicts by their scolex, as they anchor to the wall of the stomach and intestine and causes shallow ulcers and lesions. Due to severe infection of these species, intestine becomes porous through the epithelial layer and ultimately become sieve-like.

In the present work, it was observed that the worms burrowing deep into the muscularis, nodule was formed, inflammation and compression of of tissue layers were noticed at the site of attachment of cysts to the intestinal wall of the host. In some cases, a thin

mucoid interface layer was seen between the host tissue and the cyst of the worm. Loose muscle fibers were also evident. Necrotic tissue surrounded the cyst of the worm and blood cells infiltration was observed. So, the present observation indicates several host

tissue reaction resulting into the formation of fibrous nodules. This also causes destruction of muscular layers of the intestine wall of the fish.

The tissue damage by the caryophyllaeid, cestodes was severe and extensive in *C. selangor*, Malaysia. It was reported from the same species of catfish from Bangladesh (Ahmed and Sanaullah 1976 and 1977). Thomas (1964) suggested that, the parasites causing the most tissue damage, might induce an impactful reaction by the host and hence causing that area of the host fish to become unsuitable for attachment by other parasites.

Fishes establish a vital source of necessary macro and micro-nutrients in which Bangladesh have been shown to be persistently lacking (Ahmed and Hassan 1982). The moisture acts an important role as a solvent, mineral nutrients and other food stuff being transferred in solution throughout the animal body and is also essential for majority of the physical reactions in animal tissue and in its absence, life does not exist (Singhvi *et al*. 1987). Ash mainly rich in minerals specially calcium, potassium, sodium, chlorine and phosphorus. Dietary fat helps in the absorption of fat-soluble vitamins, also play a good part in the regualation of the body temperature.

Protein contains nitrogen, carbon, hydrogen and oxygen. Protein got multiple physiological importance as a growth material for the organisms, a part of fuel of the organism, structure of living materials are composed of different types of protein molecules. Carbohydrate is readily available fuel of the body, constitutes the structural material of the organism plays as a main role in the metabolism of amino acids and fatty acids. The above mentioned nutritional components promote the value of *P. paradiseus* and *X. cancila* to the common people and make them more interest to take these fishes enormously as well as other fishes.

A fluctuation in the individual biochemical components of the flesh, throughout the year was observed in both infected and non-infected fises. Protein, fat contents were higher duriing winter (dry months) than the summer (hot months), analysis which agrees with Adhikari and Noor (1967) in case of *Puntius puntius*. On the other hand, it is also evident from the present results that moisture was higher during monsoon and lower during winter. Moisture content was always higher in infected female than

infected male in both the fishes because during spawning period the fishes naturally showed lower percentage of nutrient contents. Rubbi *et al*. (1987) showed that in case of some fresh water fish, moisture contents were higher, protein and fat contents were lower in matured female fish with eggs.

In this present investigation, some parasites were recovered which are basically parasites of marine fishes and piscivorous birds. This may be due to, parasites are becoming more diverged and fresh water is being contaminated with marine water in the estuaries, on the other hand these birds may transfer the parasites while taking the fishes as their food. Although considerable efforts have been given on parasites taxonomy, it is yet to be possible to acquire an exact image of the parasite fauna of Bangladeshi fishes. This is due to the fact that Bangladesh is a deltaic country and more often than not, affected by floods. It is possible for marine and estuarine fishes to move upstream, bringing with them much of their marine parasite fauna. Freshwater carnivorous fishes consume on marine or anadromous fishes which may end up in the temporary transfer of gastro intestinal parasites. In multiple occasions, this appears to have resulted in typical marine helminth fauna being testified from freshwater hosts.

In conclusion, operating measures should be considered to interfere the processes of parasitic transmission from one host body to another. A lot of attention must be paid to operate the parasites with a view to enhance the protein production together with the rapid growth of fishes. These two fishes are overburdened by a number of helminth species because the rivers and other water bodies of the country are not protected. Very frequently these are polluted by flood, climatic disaster, industrial wastes, pesticides etc. Due to these environmental degradation and continuous contamination, parasitic adaptation to the hosts are increasing day by day and gaining more diversity.

Therefore, broad range of studies should be carried out on the trematode, cestode, nematode, and acanthocephalan parasites of *Polynemous paradiseus* and *Xenentodon cancila*. Or else, damaging the tissues and decreasing the nutritional values due to the pathogenicity that occurs will lower the production of these fishes.

The present study of the identification of acanthocephalan species was based on the morphological investigation followed by DNA barcoding approach. Morphometric

study was conducted to identify acanthocephalan species morphologically. Sometimes the morphological study of the specimens lifted a few questions on the observed characteristics versus the described features and it is very difficult to discriminate morphology by their key features. The DNA barcoding method solved that identification issues and confirmed the actual species.

DNA based molecular taxonomic method has emerged as a complementary and easy alternative to the morphological identification system (Hebert *et al*. 2003). In the study, it has been demonstrated that DNA barcoding as an approach of sequencing a 432 bp region of the mitochondrial COI gene. It is an effective and most powerful tool to identify and characterize any animal and eukaryotes in general. In the study, three specimens belonging to one species of Acanthocephala were studied and compared the results to GenBank data base records by BLAST; however unfortunately the data sequences that were submitted did not match with the already existing records. But only a related species *Pallisentis celatus* was found. Therefore, the submitted data were the first reported records of the acanthocephalan parasite *Pallisentis ophiocephali*. The accession numbers have been given by NCBI that confirmed the authentic molecular identification.

In the study average nucleotide content were A 19%, T 37%, C 16%, G 28% for COI gene. The average AT and GC content were 56% and 44%. The three sequences along with the retrieved sequence of GenBank were aligned with the MUSCLE which was subjected to the K2P distance model to delineate a phylogenetic tree to trace the origin of the species and infer their evolutionary relationship. The species shown unique barcodes that could be separated and isolated from other. Genetic divergence was evaluated and found to be increased with higher taxonomic rank (0 to 2%) within species and (22%) between species for COI gene.

Study of DNA barcoding is very modern and recent in Bangladesh but till now almost no work has been done in case of fish parasites.

Very few literatures are available on fish parasites found in Bangladesh and most of them are based on morphological characteristics. Identification of fish parasite species

by COI gene is necessary to characterize the acanthocephalan with a phylogenetic relationship.

Phylogenetic tree was constructed with COI sequences using three statistical methods namely: UPGMA, Neighbor Joining and Maximum Likelihood Methods. All the sequences of *P. ophiocephali* and *P. celatus* assembled under monophyletic clade.
According to our findings it can be suggested that genetic barcoding can also be particularly useful for identifying any living being which are difficult to identify morphologically. This is accurate for acanthocephalan parasites which are difficult to detect at species level and DNA barcoding is a decent implement for species identification of such forms (Bucklin *et al*. 2010). Genetic barcoding has formerly been employed as a molecular tool to strengthen classical taxonomy in Acanthocephala.

CHAPTER-9

CONCLUSION

Conclusion

The present study revealed that a sizeable portion of the host fishes were infected with helminth parasites. Compared to parasitic fauna, *X. cancila* was more burdened and harbored with maximum number of helminth parasites than *P. paradiseus.*

It was observed that the prevalence of parasitic infestation in *X. cancila* was higher (60%) than in *P. paradiseus* (49%). The Acanthocephala showed the highest infestation (57% of the total parasites) in *X. cancila* while Trematoda showed the highest infestation (68% of the total parasites) in *P. paradiseus.*

The finding of the present study reveals that the intestine was the most infected organ both in *X. cancila* and *P. paradiseus*. In both host fishes, single parasitic infections were found highly prevalent compared to the double parasitic infections.

In case of *P. paradiseus*, helminth parasites were most prevalent in the lower medium length group (9.1-11 cm) and were least prevalent in the largest length group (13.1-15 cm). Whereas, in *X. cancia,* the highest prevalence obtained in the largest length group (35.1-40 cm) and lowest prevalence obtained in the smallest length group (20-25 cm). Possibly, that is how a parasite species evolved in nature to establish itself in different host species- the matter is believed to present an interesting problem for future study.

Among the food items found in the stomach of *P. paradiseus* and intestine of *X. cancila, the* most important and most frequent were the small fishes (*Amblypharyngodon mola, Corica* sp. etc.). The occurrence of small fish item was 37% in *X. cancila and* 19% in *P. paradiseus*. The frequency of consumption of small fishes maintained the relationship of increasing the tendency from small to larger size of fishes of both species.

In *P. paradiseus,* the prevalence of helminth parasites was shown to be the highest (78.57%) during rainy season. And in *X. cancila* the maximum prevalence (75%) was shown to be the highest during winter season

The contents of biochemical nutritional components (protein, fat and ash) of non-infected fishes were found quite higher than infected fishes (in both fish species) but the moisture contents found higher in infected fishes than the no infected fishes.

Due to the massive tissue destruction by trematodes in *P. paradiseus*, the loss of nutritional components was remarkable. The structural integrity of the visceral organs and body musculature of thread fish were disrupted by enteric parasites. With the help of penetrating glands, located in the oral sucker, the juvenile trematode dissolved the skin and muscles for making the tunnel or space in the host tissues, resulting accumulation of melanin macrophages and more moisture, necrosis, connective tissue proliferation and mixed inflammatory responses. In *X. cancila*, the acanthocephalans were the most pathogenic and damaging group of the whole. During the migration of immatures of *C. ophiocephali* and *P. trichuri* from cavity to the all internal body parts, massive disruption and dislocation of the visceral organs occurred. Irregular black pigmentation was scattered throughout the swim bladder, in severe cases, the alveolar sacs and capillary plexuses were disrupted causing necrosis.

From the results and records of the present study, it can be concluding that, though they have the similar feeding habit inspite of their different habitat, *X. cancila* was found to be infected by comparatively a large community of parasites. So it can be assumed that, *X. cancila* is more susceptible for helminth infections than *P. paradiseus* considering the parasitic problems in the fish. It is necessary to conduct different studies on aspect of biology related to nutritional values and parasitic infections of *P. paradiseus* and *X. cancila* to help the production of worms' free fishes in our country.

Execution of molecular technique can be helpful tool for animal groups that are otherwise labored to identify. It can also facilitate continuous observation of divergence of these animal groups over time. Acanthocephala comprises a highly diversified group of invertebrate that shows extreme phenotypic malleability. Despite extensive taxonomic study, identification of acanthocephalan can be uncertain and construction of phylogenetic tree based on morphological characteristics were controversial due to complex evolutionary changes. In the study, DNA barcoding has been formed an effective tool for identifying known species and new clades to be examined further,

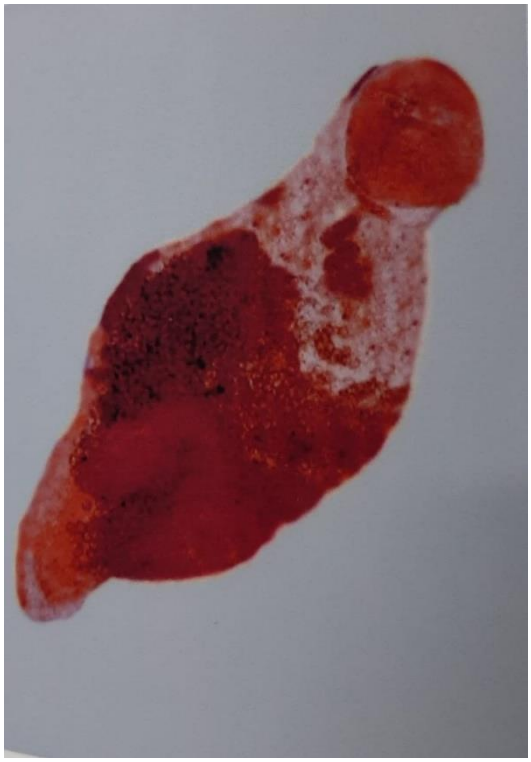
which is a first step towards one of the main motive of DNA barcoding facilitating the discovery of new species. The present study evidenced that acanthocephalan species can be efficiently identified through DNA barcoding, especially the species complex of small sized species, and present COI library can be used for subsequent applications in ecology and systematics. More extensive studies are needed involving all the available groups of acanthocephalan of our country for developing a rich database which will contain morphological as well as genetic information.

PLATE A

Xenentodon cancila

Polynemus paradiseus

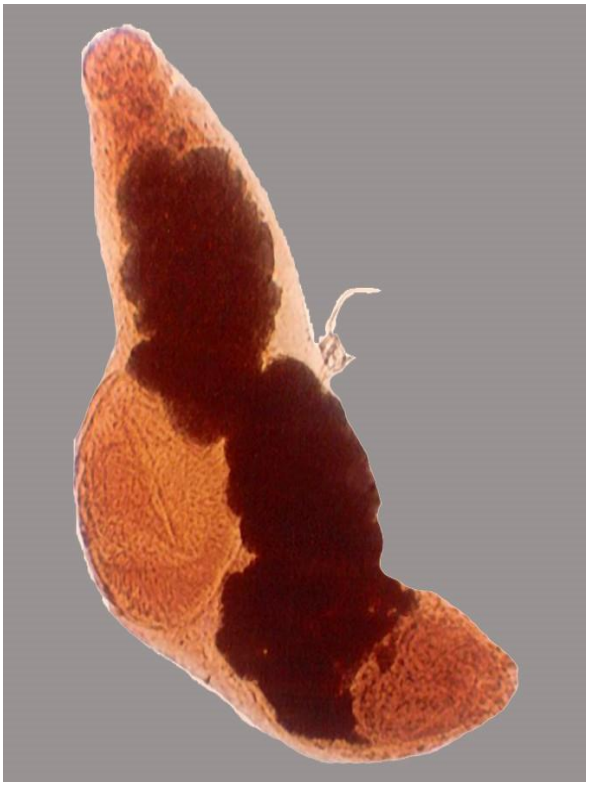
Plate-1: Trematode parasites



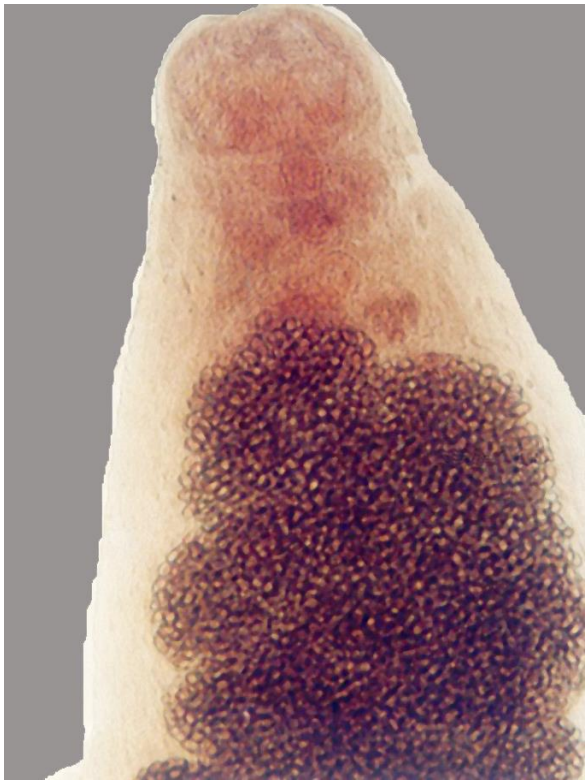
Photograph-1: ***Bolbocephalus sp.***

Photograph-2: ***Isoparorchis hypselobagri***

Plate-2: Trematode parasites

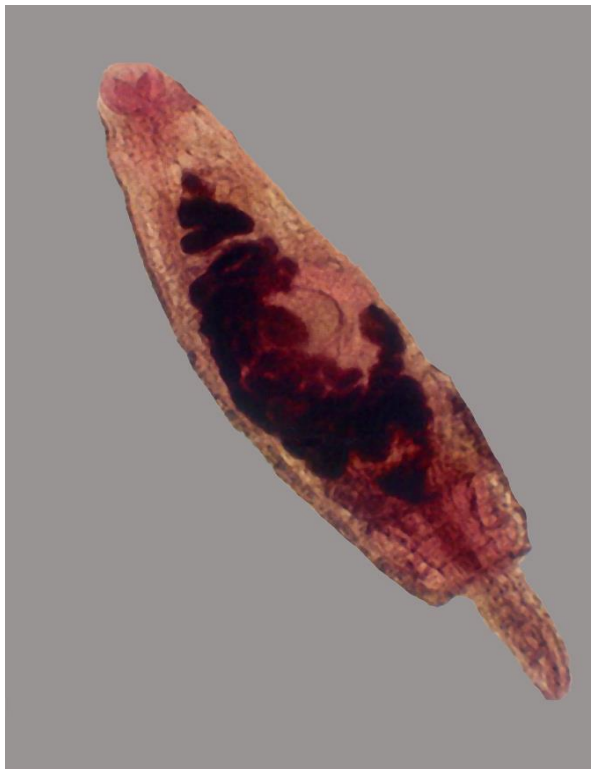


a



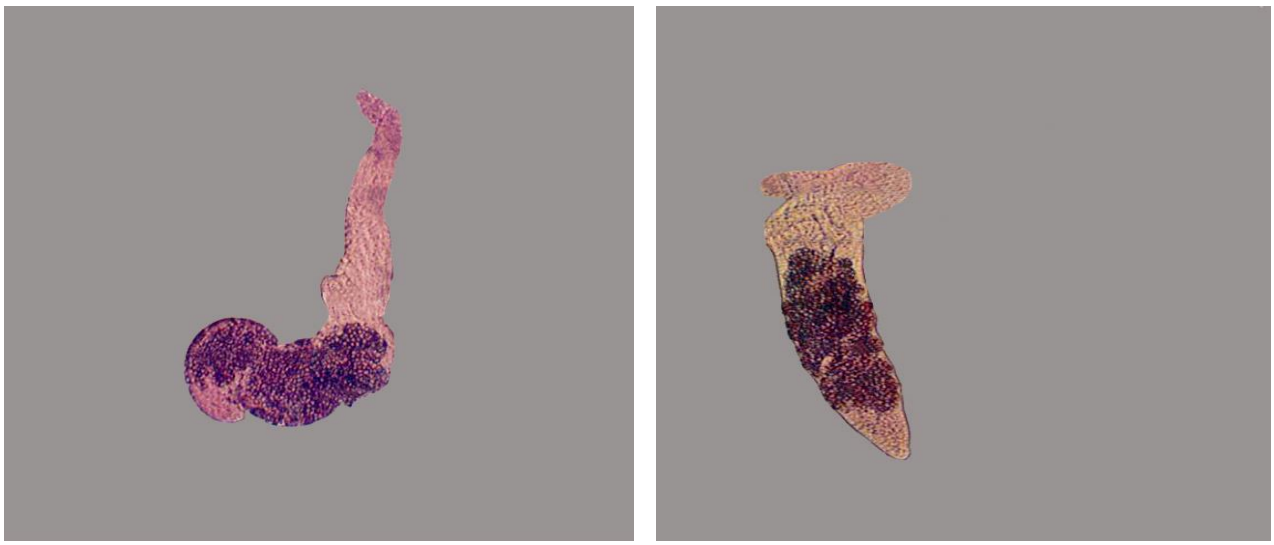
b

Photograph-3(a-b): *Prosogonotrema bilabiatum*
Photograph-(a). Whole worm; Photograph (b). Anterior Portion



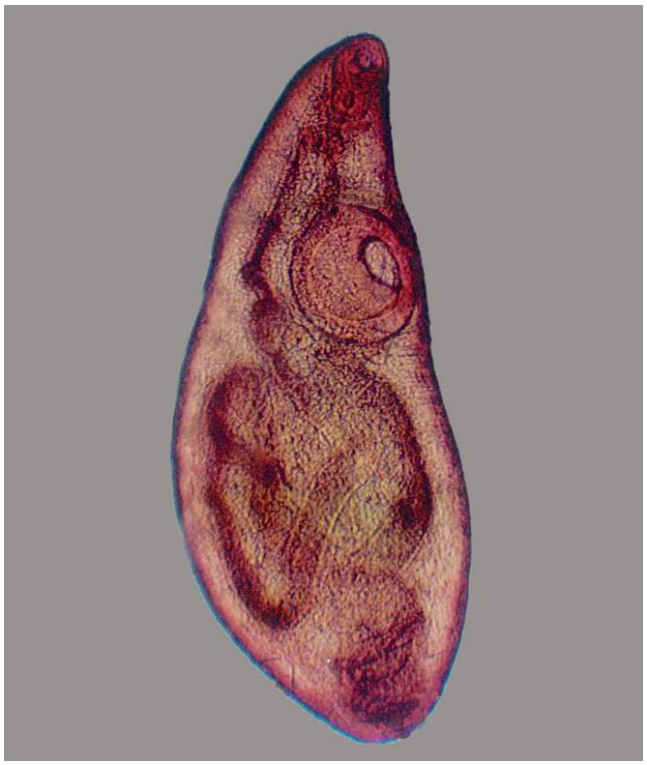
Photograph-4: *Uterovesiculurus hamati*

Plate-3: Trematode parasites



a **b**

Photograph-5(a-b): *Thaparotrema vittalani* (whole worm)

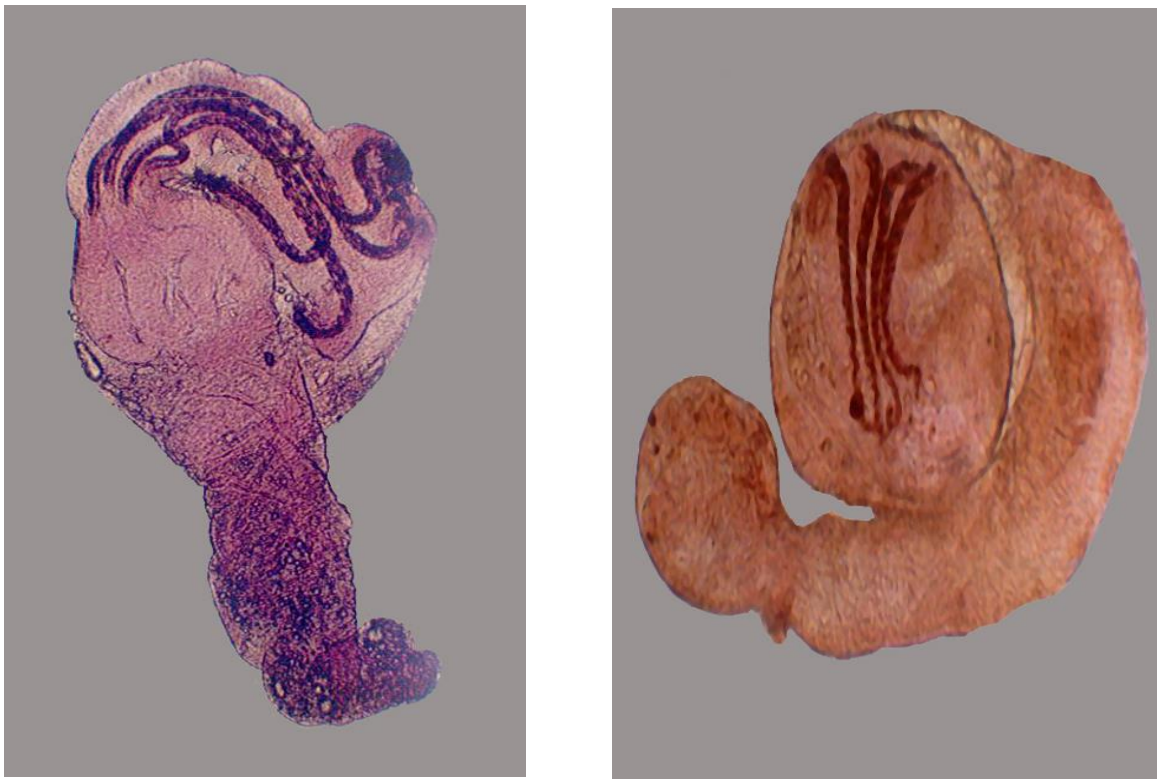


Photograph-6: *Hypohepaticola callionymi*

Plate-4: Cestode parasites

a b

Photograph-1(a-b): *Nybelinia lingualis* (Whole worm)



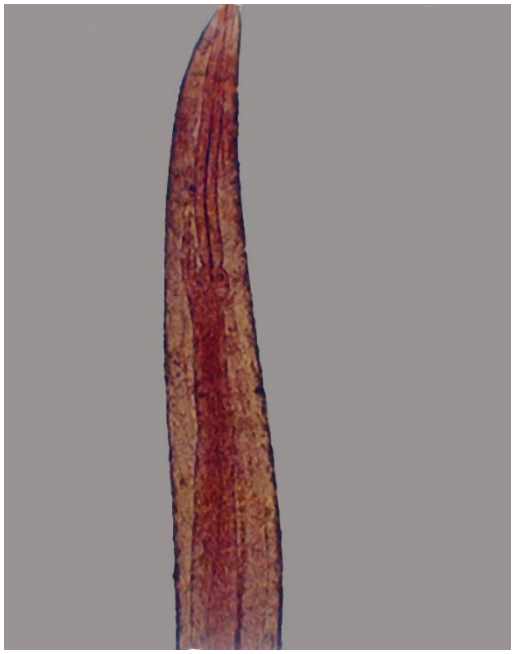
a b

Photograph-2(a and b): *Parachristianella trygonis*

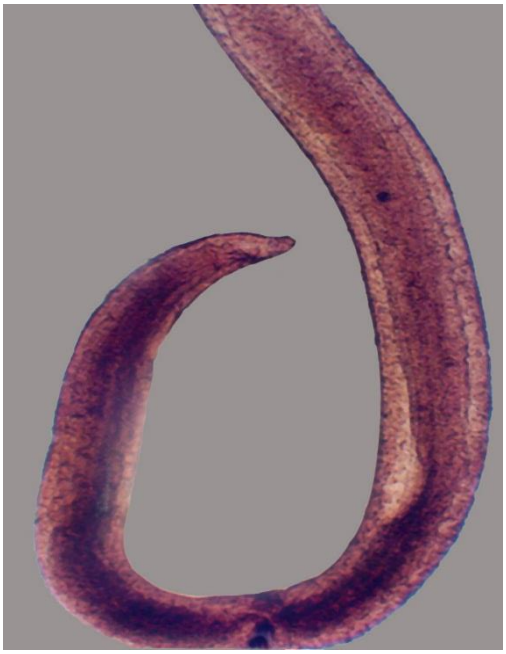
Plate-5: Nematode parasites

Photograph-1: ***Gnathostoma spinigerum*** **(L_3 larva)**

Photograph-2: ***Dujardinascaris sp.*** **(L_4 larva)**



a



b

Photograph-3 (a-b): ***Porrecaecum trichuri***

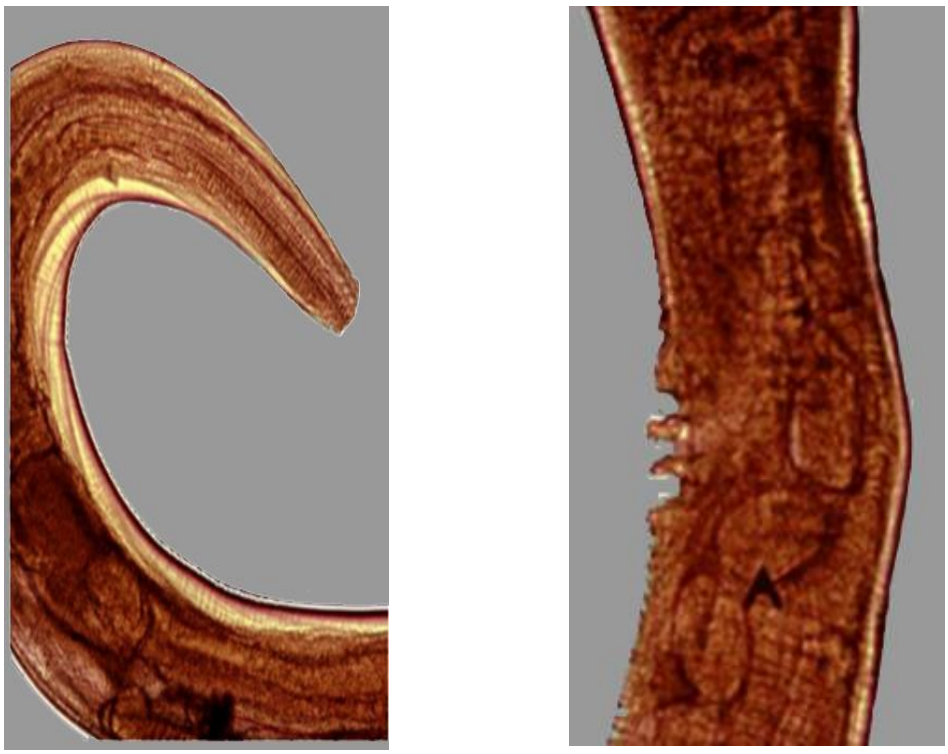
Photograph (a) Anterior portion; (b) Posterior portion

Plate-6: Nematode parasites

a b

Photograph-4(a-b): *Camallanus ophiocephali*

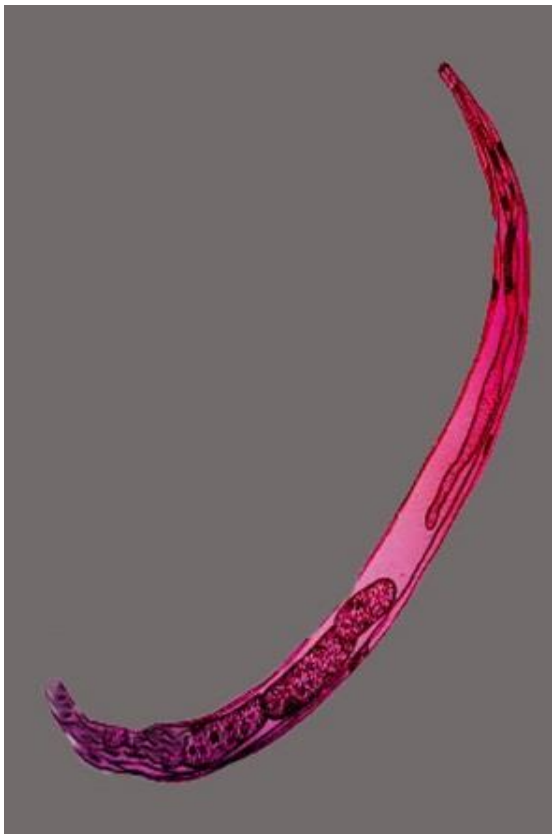
Photograph (a). Anterior portion; Photograph (b). Posterior portion



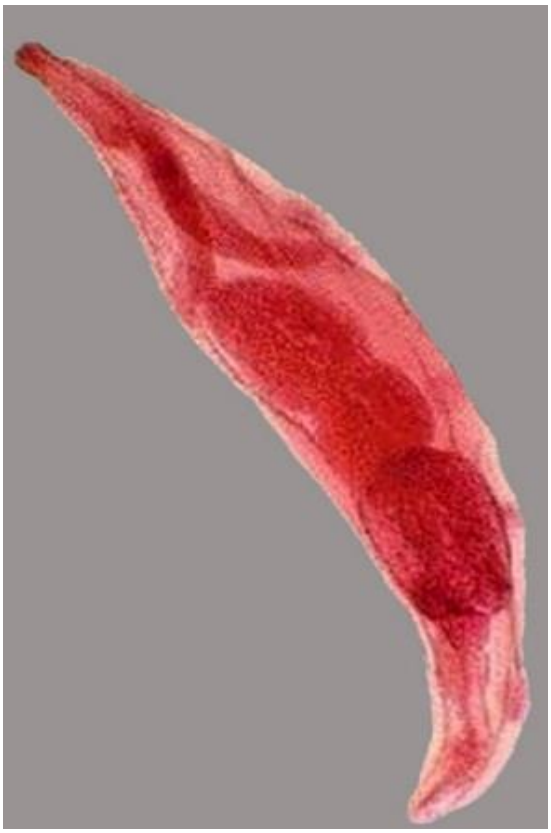
Photograph-5(a-b): *Metaquimperia bagarii*

Photograph (a). Anterior portion; Photograph (b). Posterior third of female worm

Plate-7: Acanthocephalan parasites

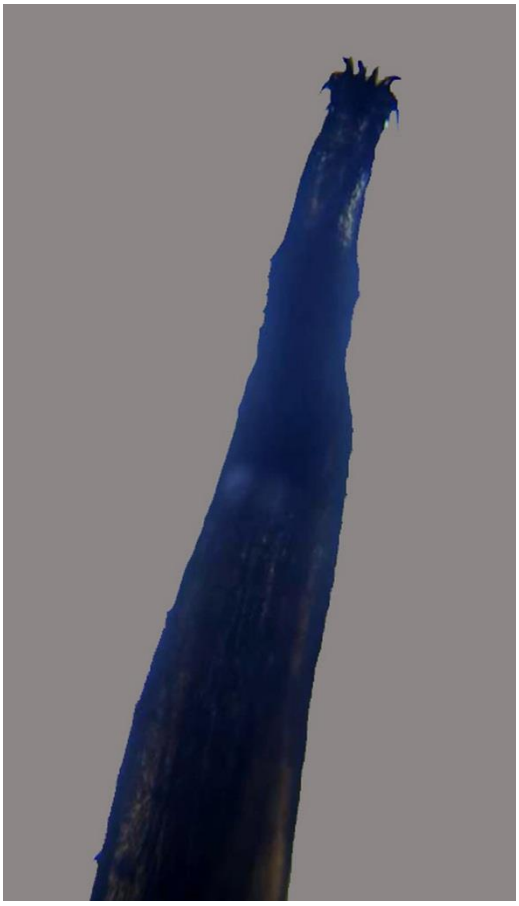


Photograph-1: ***Neoechinorhynchus prolixum***



Photograph-2: ***Acanthocentis nigeriensis***

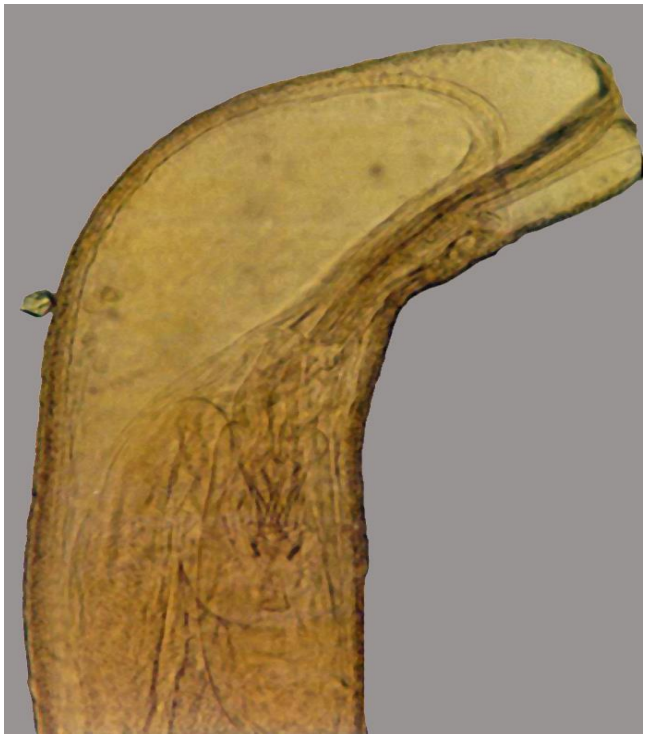
Plate-8: Acanthocephalan parasites



a

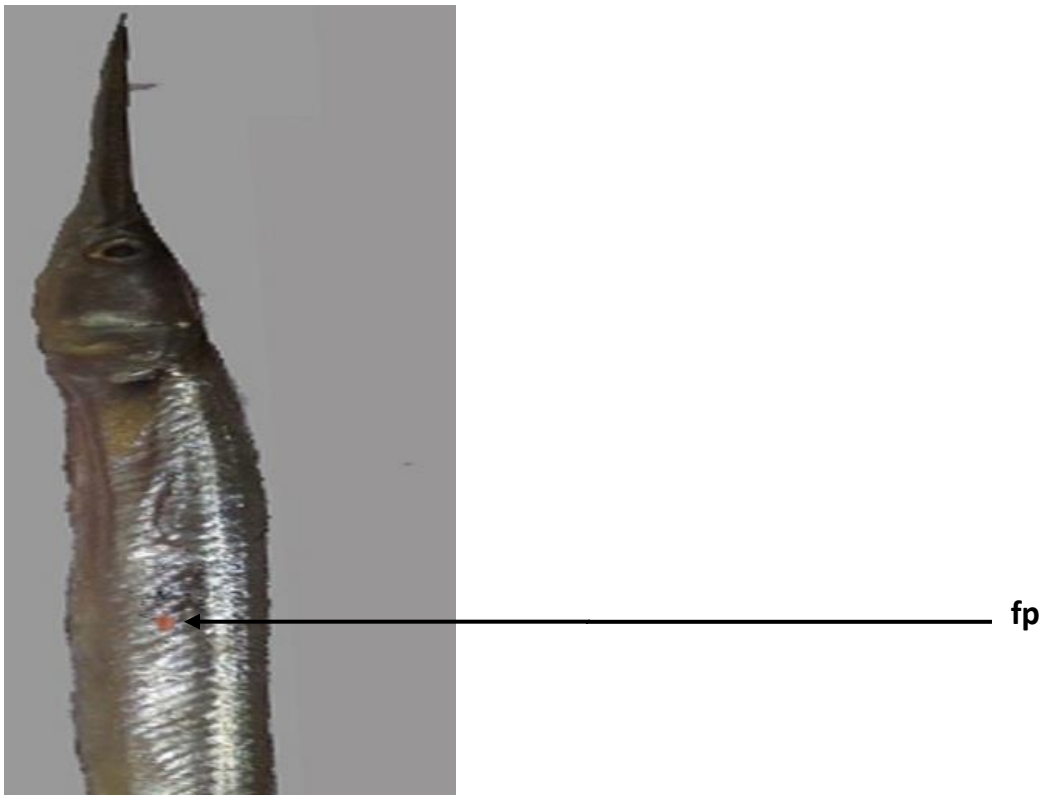
b

Photograph-3 (a - b): *Pallisentis ophiocephali* (Anterior Portion)

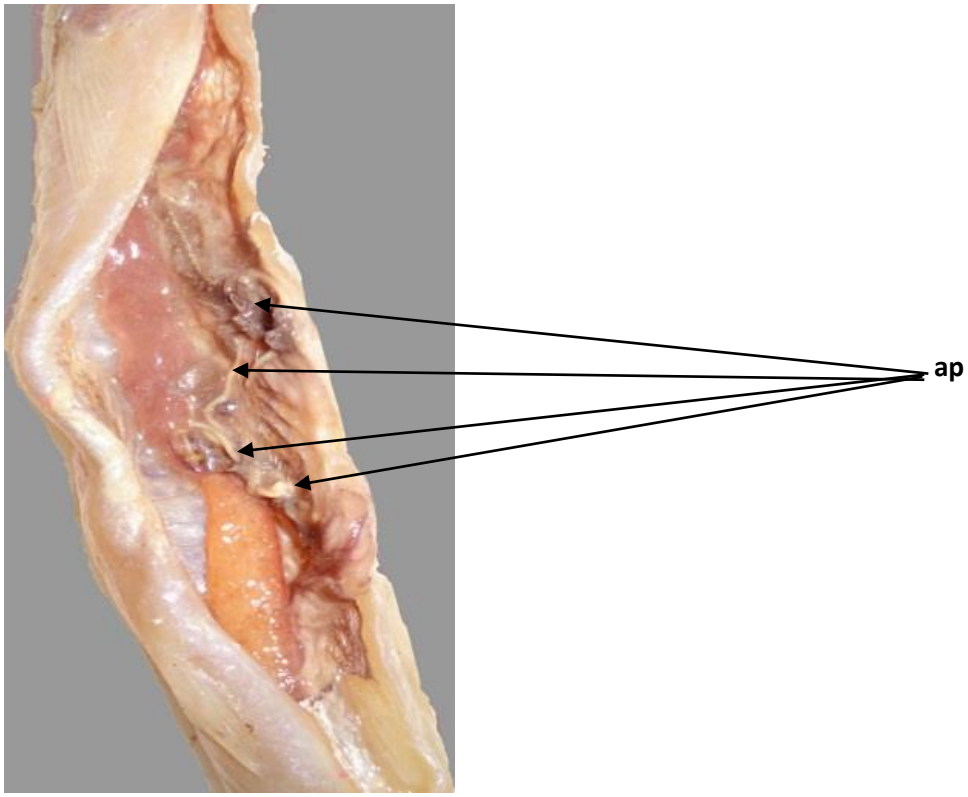


Photograph-4: *Neorhadinorhynchus aspinosum*

Plate-10

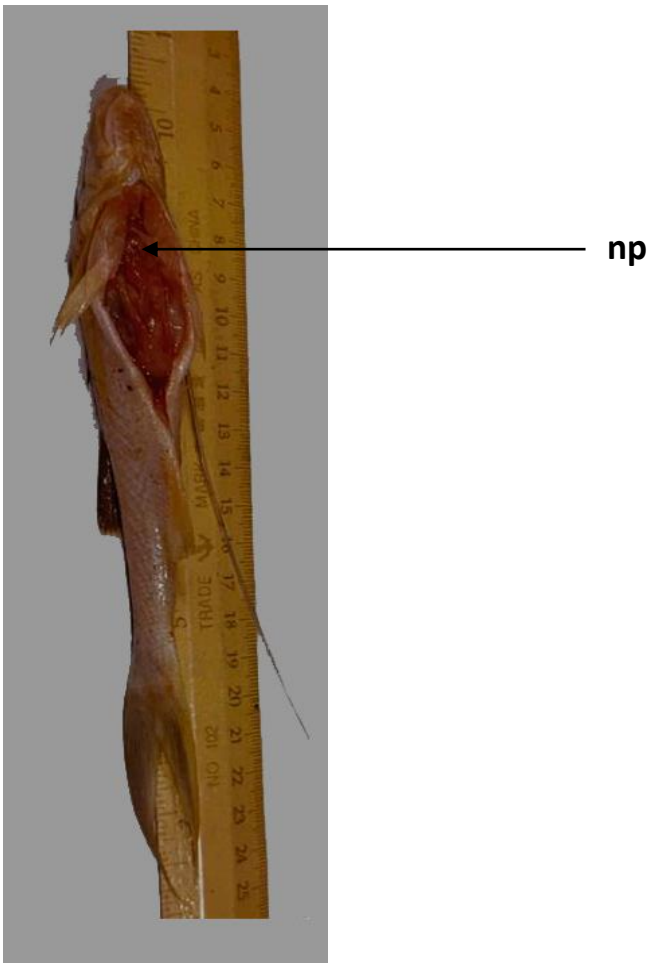


Isoparorchis hypselobagri **coming out from visceral cavity by piercing through body wall of adominal region of** ***Xenentodon cancila***

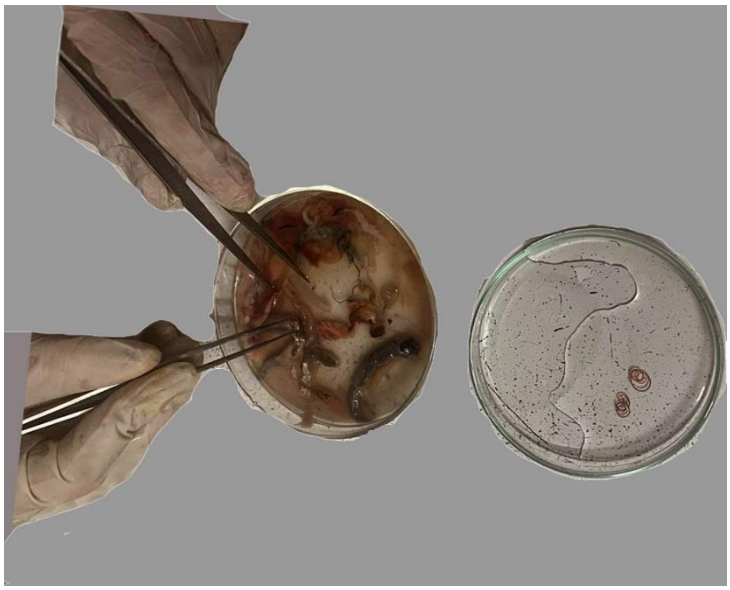


Acanthocephalan parasite found in the intestine of ***Xenentodon cancila*** **when incision was given**

Plate-11

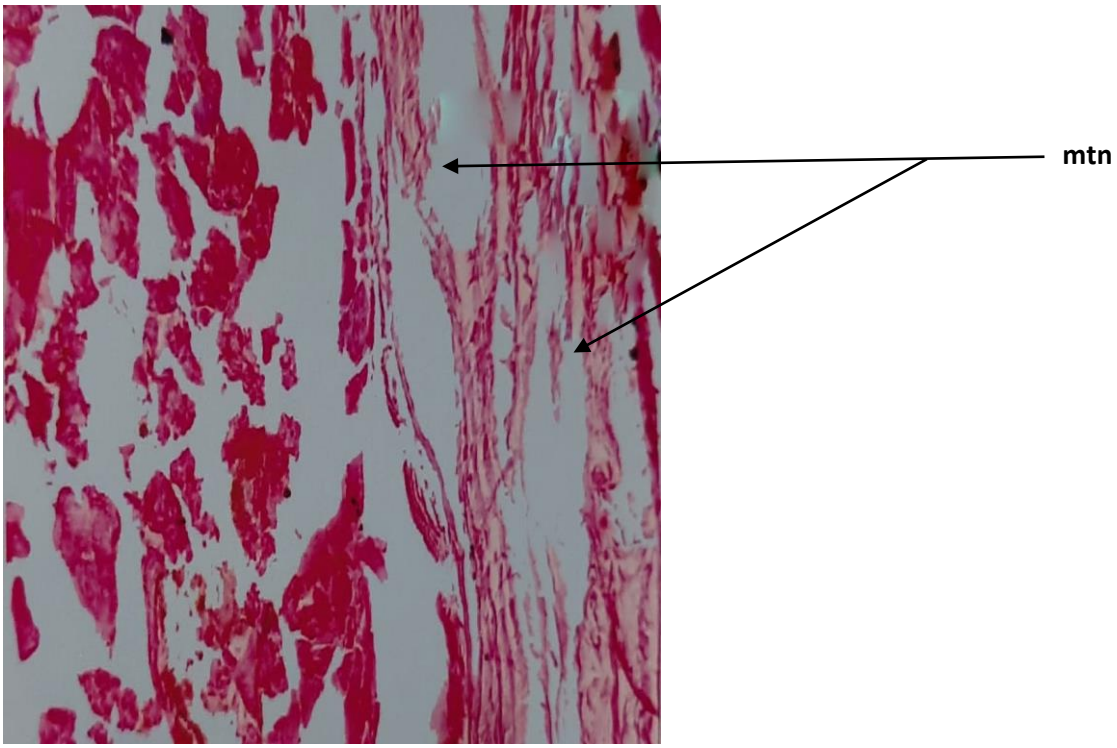


Nematode parasite found in the intestine of *Polynemus paradiseus* when incision was given



Nematode parasites are collecting from the Visceral organs of *Polynemus paradiseus*

Plate-12



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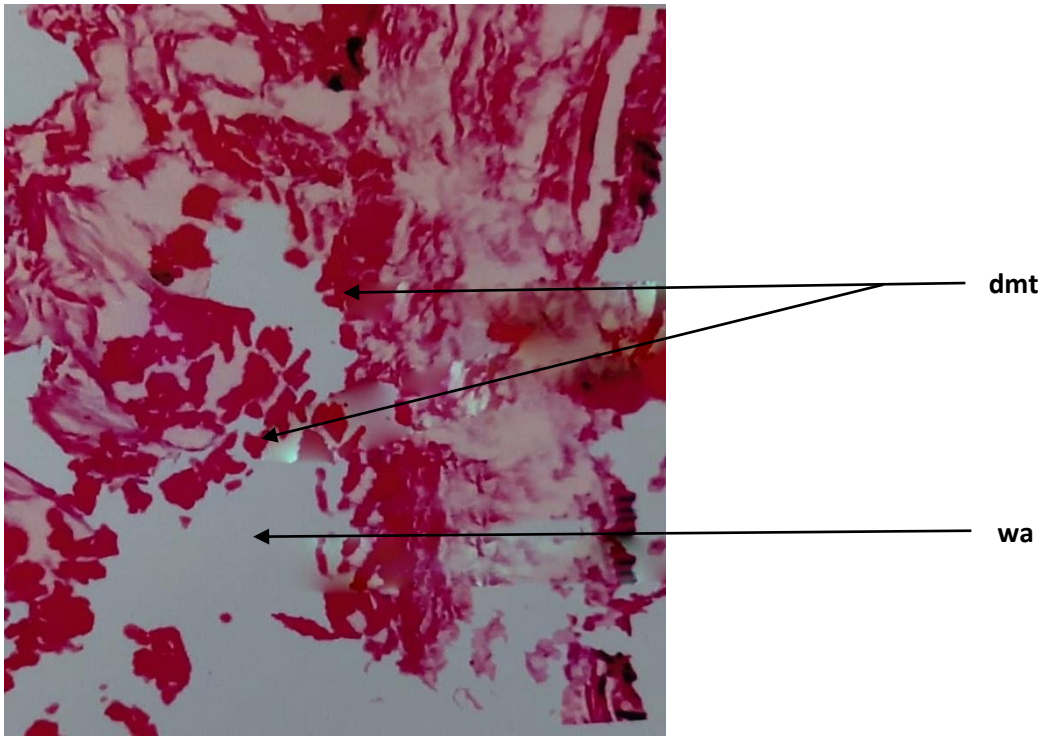

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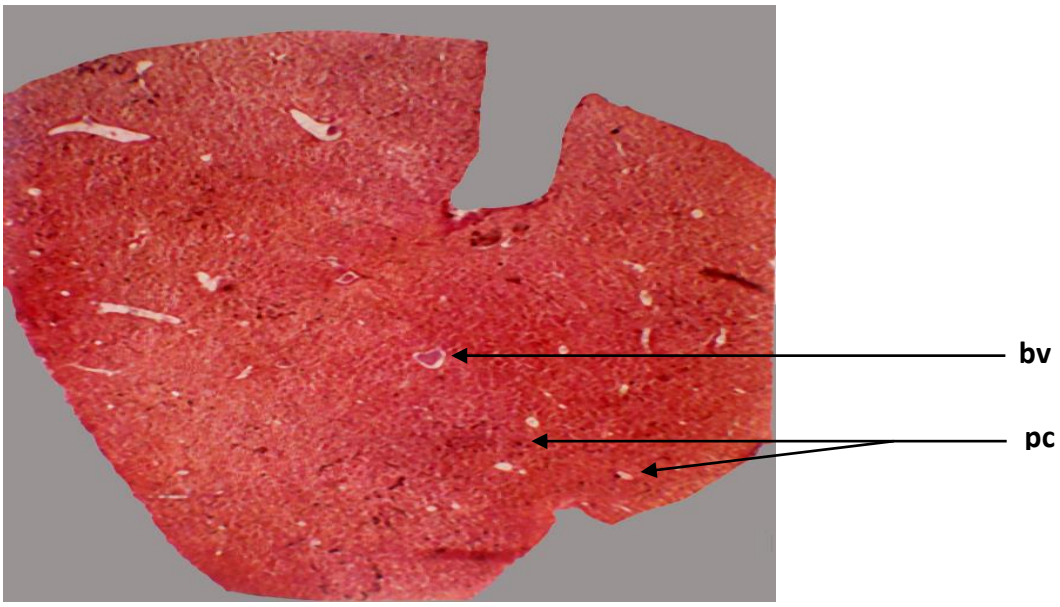

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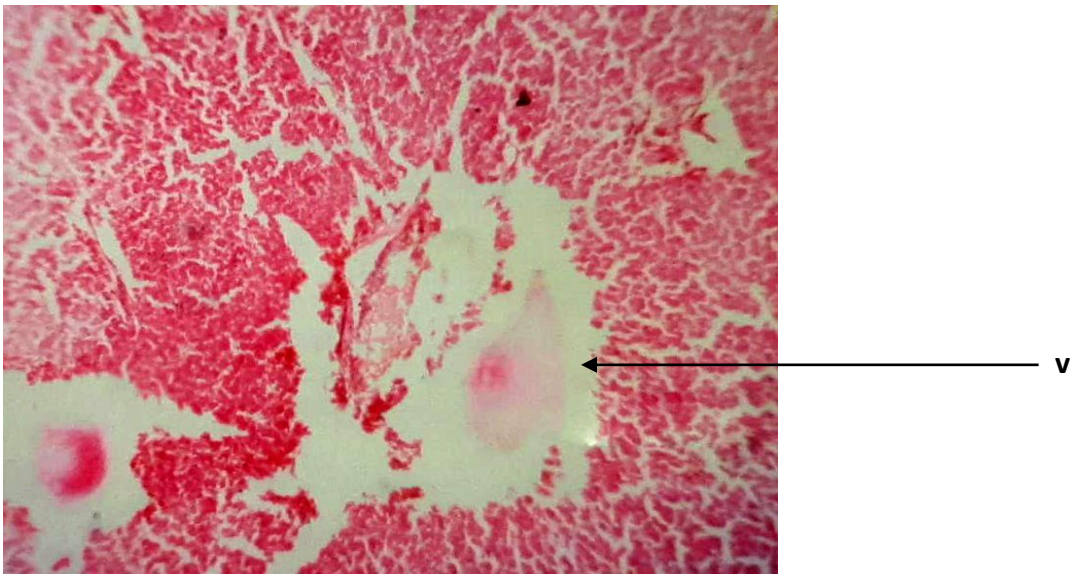

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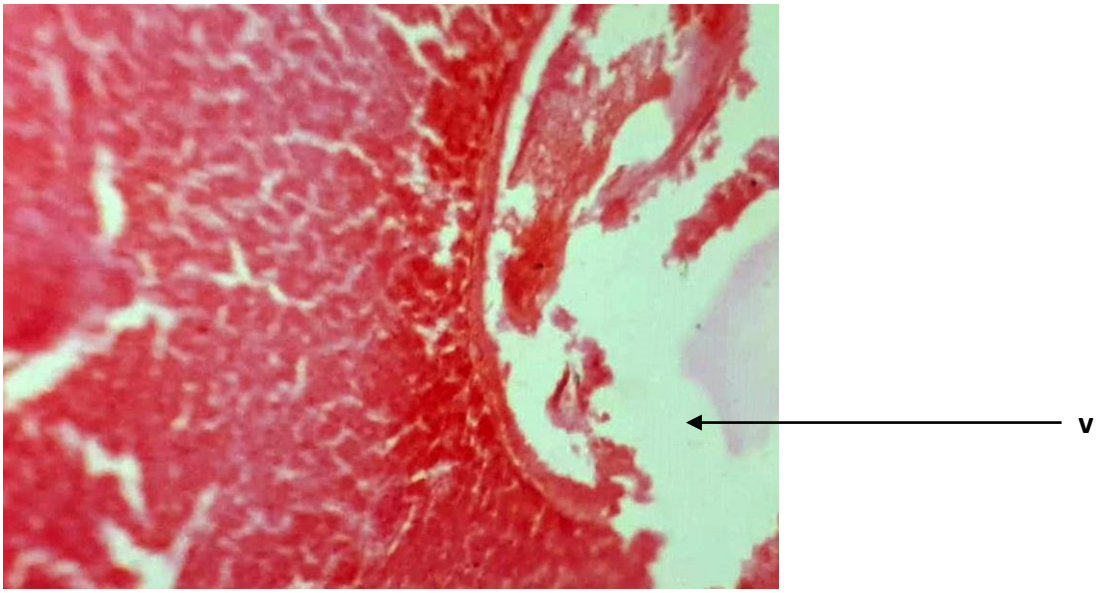

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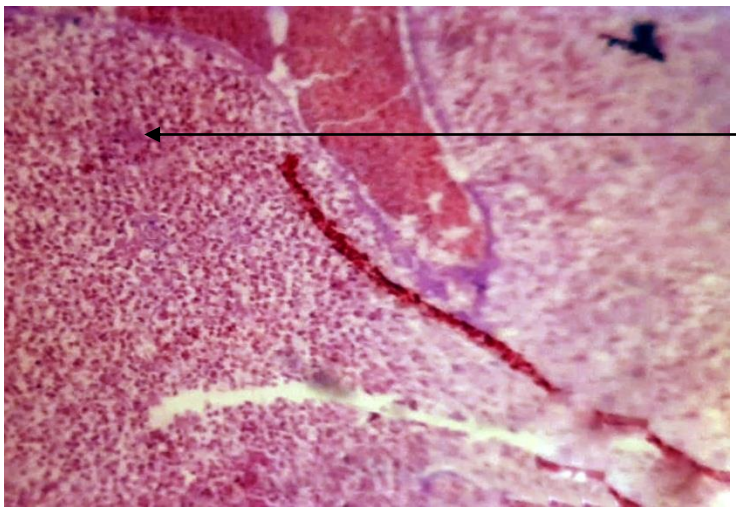

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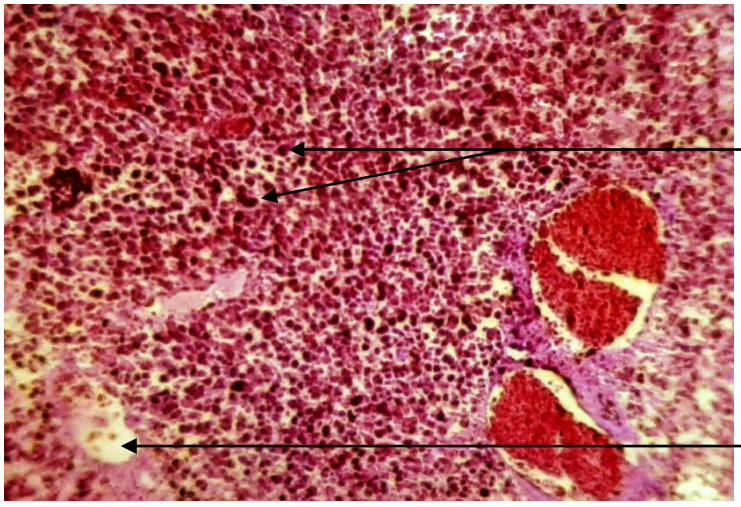

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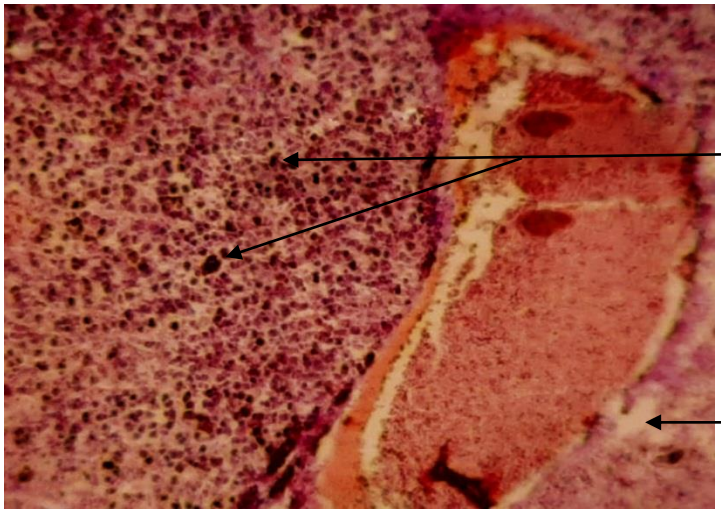

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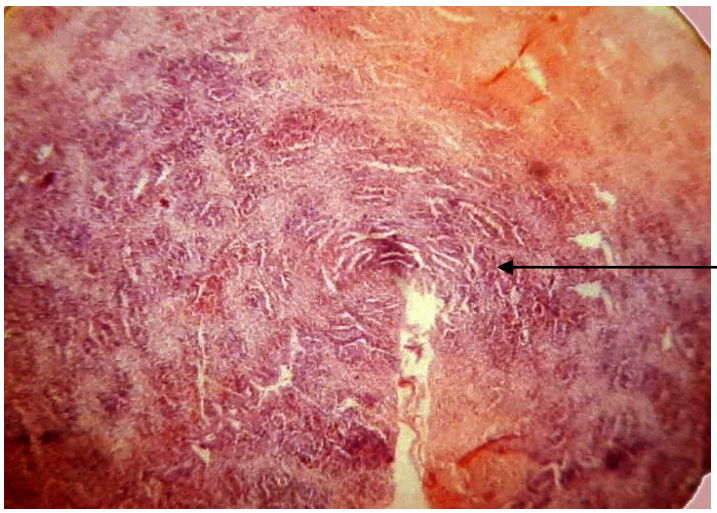

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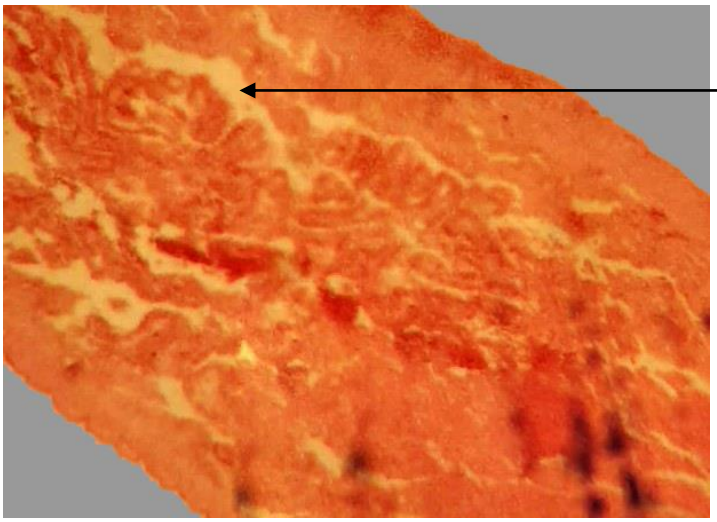

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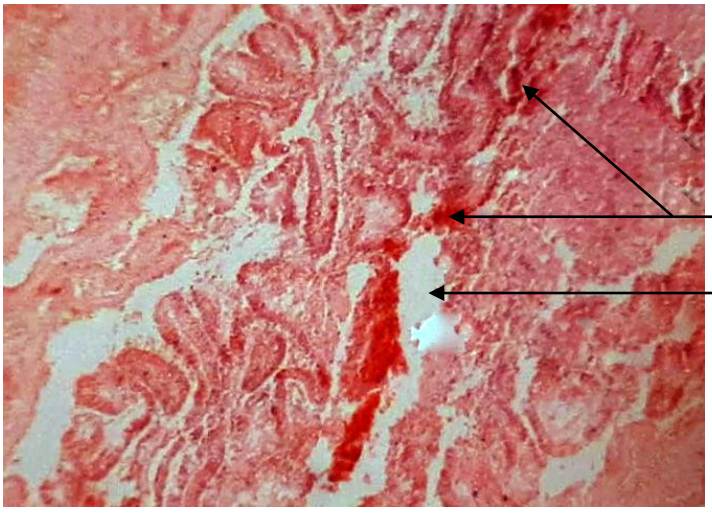

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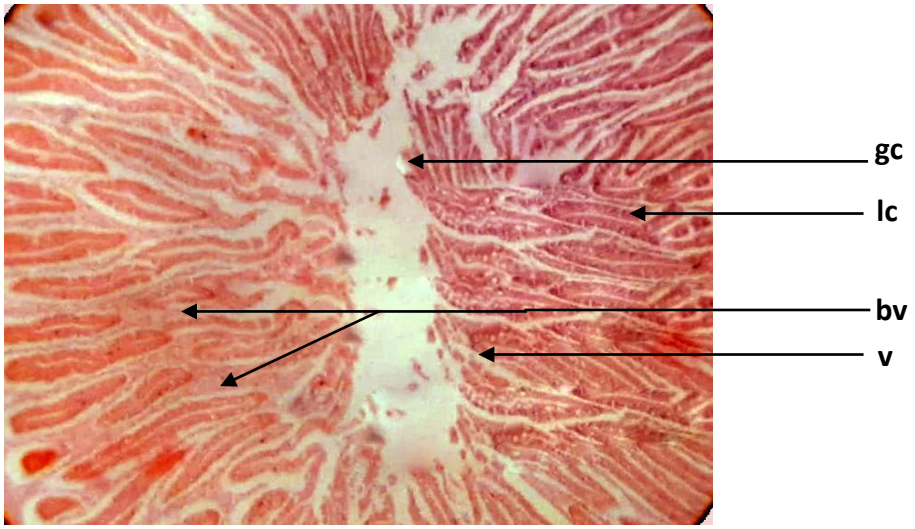

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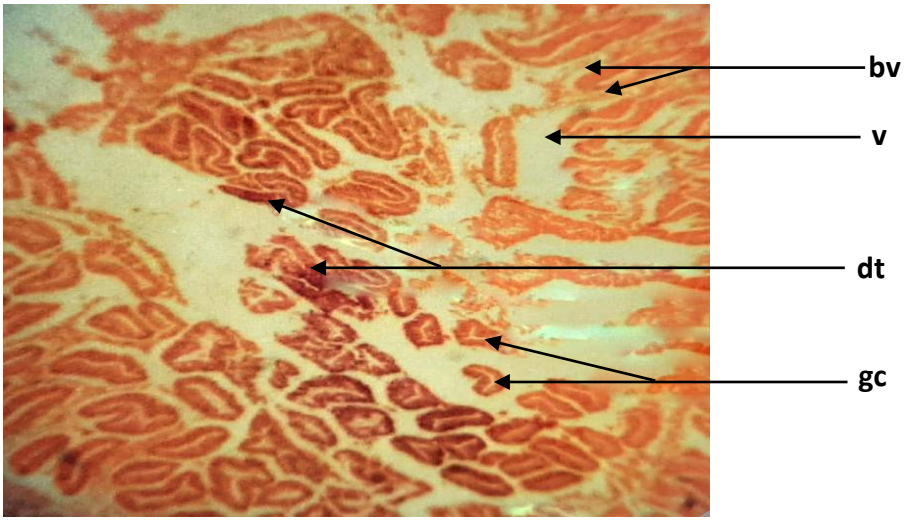

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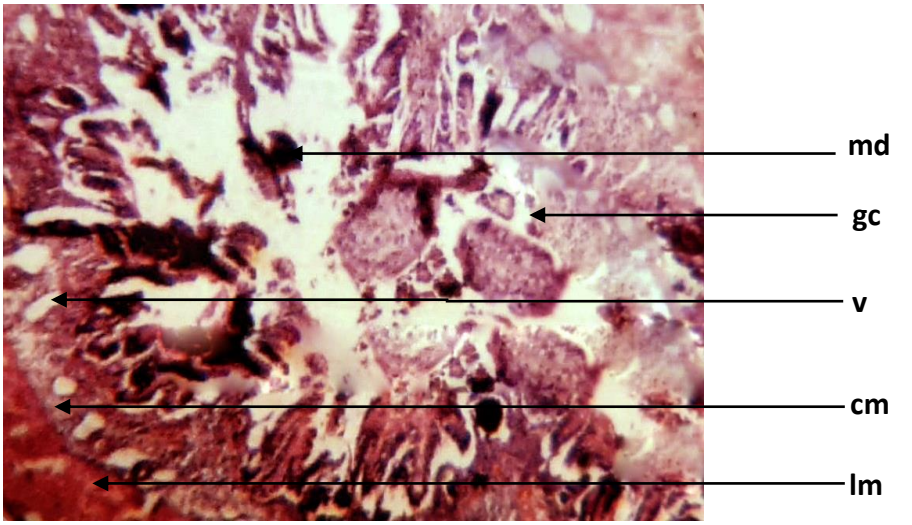

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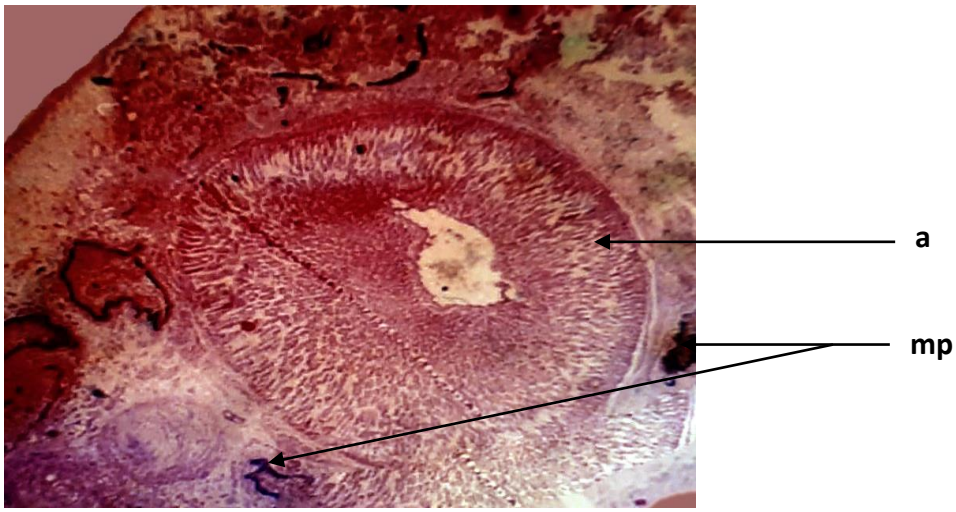

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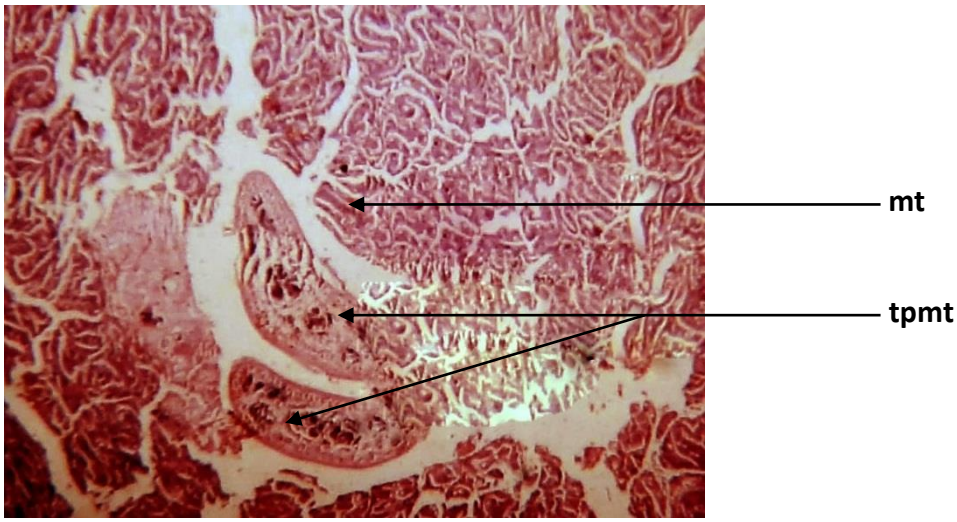

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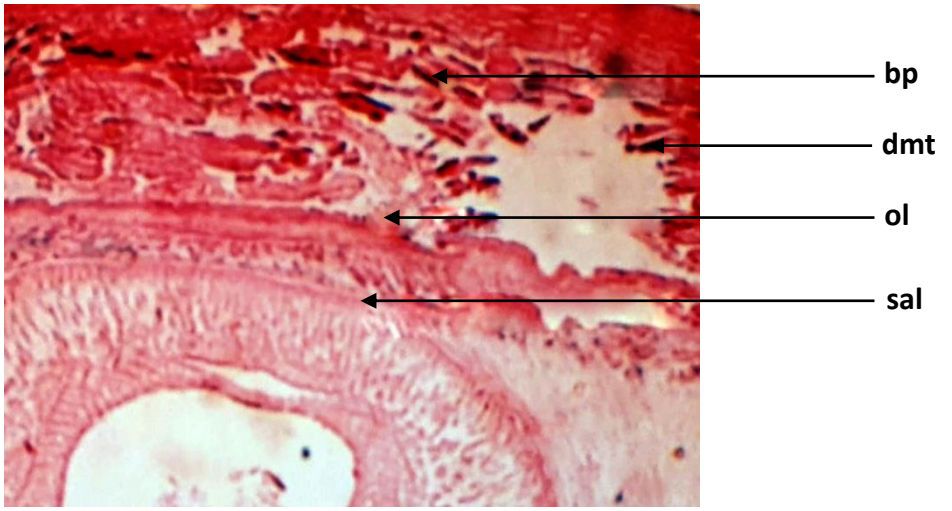

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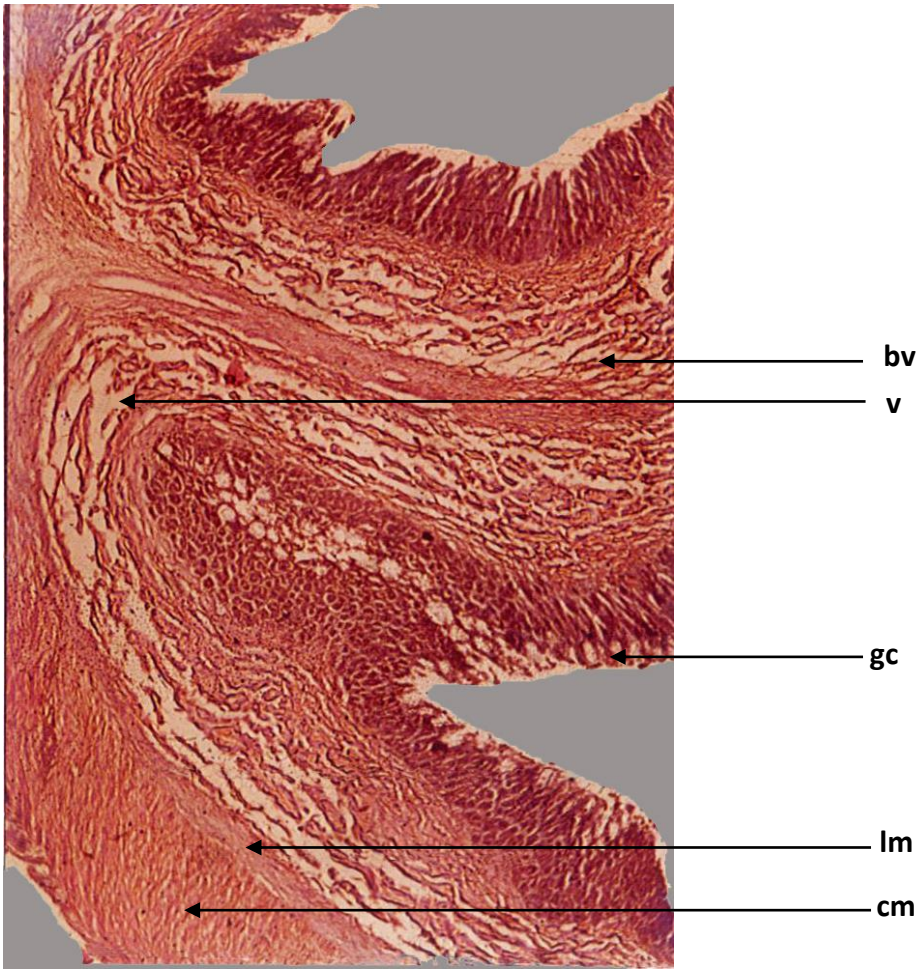
Plate-18



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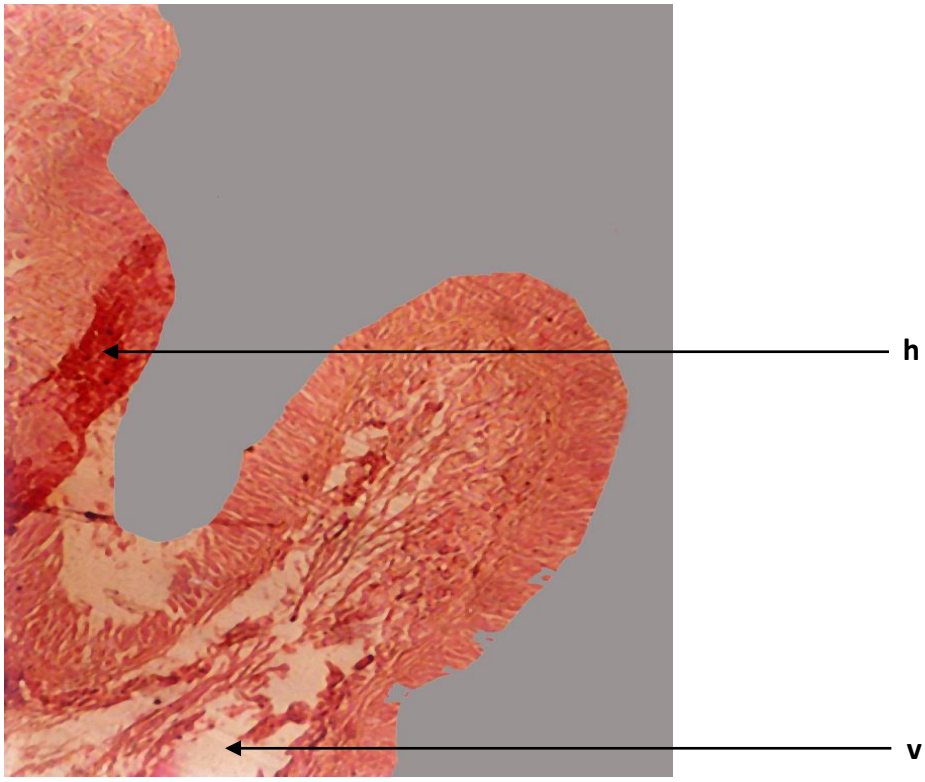

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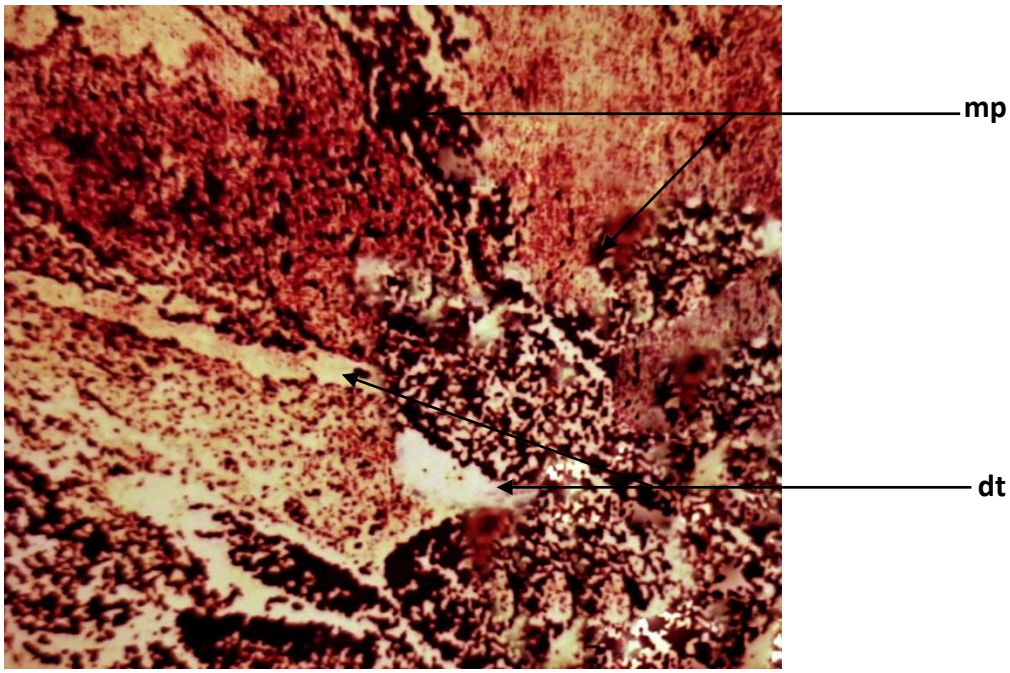

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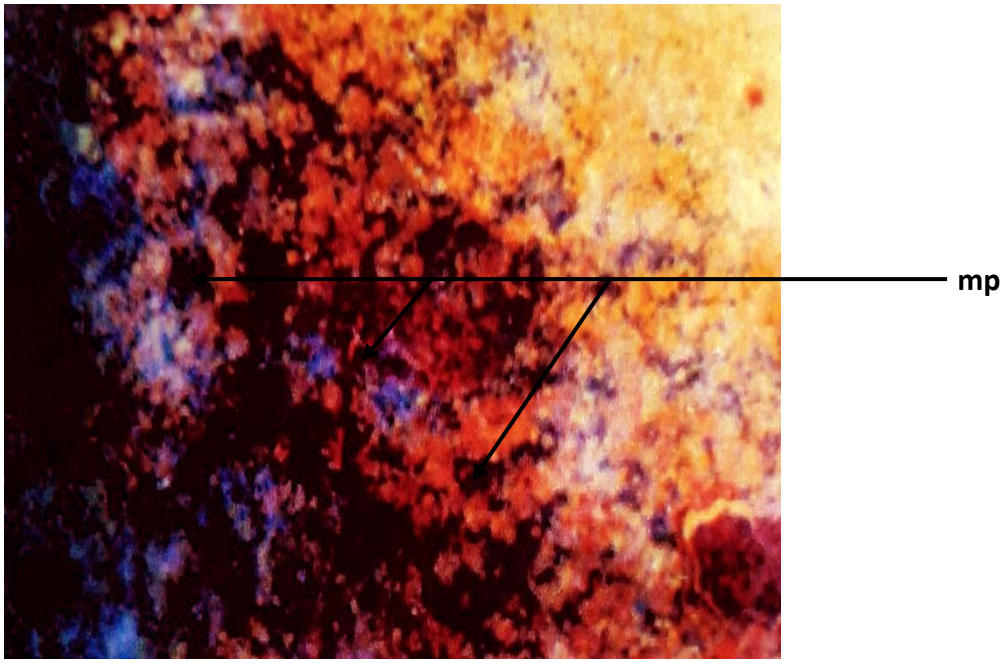

B

Plate-9

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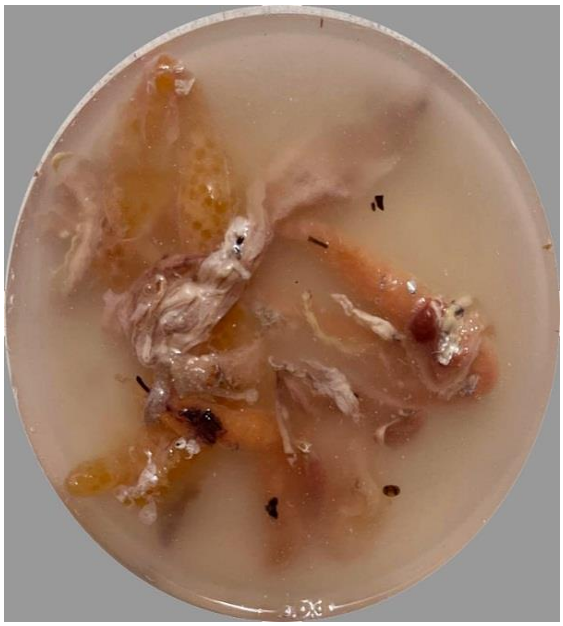

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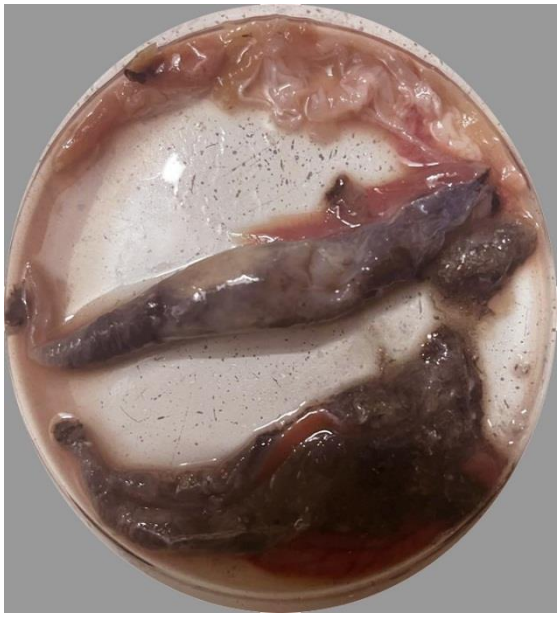

c

d

Photograph (a-d): Food items collected from the gut of *X. cancila* and *P. paradiseus*

ABBREVIATION USED IN THE PLATES

Abbreviation	Illustration
a----------	Acetabulum
b p----------	Black pigment
b v----------	Blood vessels
c m---------	Circular muscle
des----------	Debris inside empty space
dht----------	Degenerative hepatic tissue
dkt----------	Disrupted kidney tissue
dmt/m t-----	Disrupted muscle tissue/ Muscle tissue
d t----------	Disrupted tissue
ep----------	Encysted parasites
faes---------	Fluid accumulated inside empty space
fp----------	Free parasites
g c----------	Goblet cell
h----------	Hemorrhage
l c/ l m-----	Longitudinal cell/ Longitudinal muscle
m d/ m p---	Melanin deposition/ Melanin pigments
mtn---------	Muscle tissue necrosis
np----------	Nematode parasites
nckt---------	No infected compact kidney tissue
o I.----------	Outer layer of *I. hypselobagri*
p c----------	Polygonal cells
pdmt--------	Partial disruption of muscle tissue
sa I.---------	Section of acetabulum of *I. hypselobagri*
tpmt--------	Trematode parasite inside muscle tissue
v----------	Vacuole
w a----------	water accumulation

Plate-12

A. Muscle and epidermal tissues were tore due to the perforations done by *Isoparorchis hypselobagri*. These penetrations resulted into massive erotic disruption or complete destruction of the epidermis. Disrupted muscle tissues with fluid filled empty spaces are also observed. X 40.

B. Muscle fiber necrosis and little necrotic debris' suspended in the epidermis due to the infestation of trematode parasite. As a result, empty spaces are produced under dermis and dermal connective tissue proliferation with slight inflammatory responses. X 40.

Plate-13

A. Section showing no infected liver tissue with hepatic blood vessels and polygonal cells of *X. Cancila*. X 40.

B. Infected liver showing formation of vacuoles with mild hepatic degeneration. X 40.

C. Due to the presence of *I. hypselobagri* in the liver, mild hepatic degenerative tissue changes along with vacuole formation are observed. Some of the hepatic blood vessels were ruptured. X 40.

Plate-14

A. Slight to moderate hemorrhagic area due to parasitic infestation was seen in the infected liver. X 40.

B. Many pigmented macrophage were present in the liver showing early stage of melanin associated with accumulation of macrophage centers. Few lymphocytes were also observed. X 40.

C. Infected liver showing heavy melanin deposition with degenerative hepatic tissue changes. X 40.

Plate-15

A. Section of no infected compact kidney tissue of *X. cancila*. X 40.

B. Infected kidney showing degeneration of renal tissue which formed empty spaces. Inside these spaces fluid accumulated. Mild focal degenerative changes in the haemopoietic tissue were also observed. X 40.

C. Due to parasitic infestation vacuolar empty spaces with tunnels were formed, sometimes the associated haemopoietic tissue became loose and in severe cases renal tubular necrotic lesions were observed. Large macrophages containing erythrocytic debris in the hematic sinusoids were found. X 40.

Plate-16

A. Section of no infected intestinal tissue showing longitudinal cells, goblet cells, blood cells, villi etc. X 40.

B. Intestinal parasites disrupted intestinal mucosa and villi with inflammatory responses, Blood vessels were ruptured. Vacuoles were formed. Inside the vacuoles, fluid accumulated along with debris and lymphocytes. Intestinal tissue showed necrosis. X 40.

C. Structure of different cells were changed and degenerated. Sometimes intestinal wall was destroyed due to infestation. Heavy melanin deposition were also observed inside the intestine. X 40.

Plate-17

A. Deposition of melanin pigments on encysted juvenile of *I. hypselobagri*. X 40.

B. Section showing trematode parasites embedded in the muscle tissue. Tunnels were formed while migrating through the body musculature. X 40.

C. Acetabulum of *I. hypselobagri* with the musculature of *X. cancila.* The muscles tissue were disrupted and dislocated under the skin resulted into necrosis in the muscle fiber. Melanin deposition in the muscle fiber was also observed. X 40.

Plate-18

A. Section of no infected stomach tissue showing longitudinal cells, blood cells, villi etc. X 40.

B. Infected stomach of *P. paradiseus* showing hemorrhages and damages of different layers due to parasitic infestation. X 40

Plate-19

A. Irregular black pigmentation was scattered throughout the swim bladder, due to the infection by juvenile trematodes. In severe cases, the alveolar sacs and capillary plexuses were disrupted causing necrosi. X 40.

B. Massive melanin deposition inside the infected swim bladder of *P. paradiseus* during the migration of juvenile trematodes. X 40.

CHAPTER-10.
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Appendices

Appendix-I

LOCUS OM679999 432 bp DNA linear INV 15-FEB-2022
DEFINITION *Pallisentis ophiocephali* isolate A1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.
ACCESSION OM679999
VERSION OM679999
KEYWORDS .
SOURCE mitochondrion *Pallisentis ophiocephali*
ORGANISM *Pallisentis ophiocephali*
Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Acanthocephala; Eoacanthocephala; Gyracanthocephala; Quadrigyridae; *Pallisentis*.
REFERENCE 1 (bases 1 to 432)
AUTHORS Sultana,Y., Hasan,M.M., Musa,S. and Khanum,H.
TITLE Molecular Identification of *Pallisentis ophiocephali*
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 432)
AUTHORS Sultana, Y., Hasan, M.M., Musa, S. and Khanum, H.
TITLE Direct Submission
JOURNAL Submitted (15-FEB-2022) Department of Zoology, University of Dhaka, Dhaka University, Dhaka, Dhaka Dhaka-1000, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
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DEFINITION *Pallisentis ophiocephali* isolate A2 cytochrome c oxidase subunit I
(COX1) gene, partial cds; mitochondrial.
ACCESSION OM680000
VERSION OM680000

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Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Acanthocephala;
Eoacanthocephala; Gyracanthocephala; Quadrigyridae; Pallisentis.
REFERENCE 1 (bases 1 to 432)
AUTHORS Sultana,Y., Hasan,M.M., Musa,S. and Khanum,H.
TITLE Molecular Identification of *Pallisentis ophiocephali*
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 432)
AUTHORS Sultana,Y., Hasan,M.M., Musa,S. and Khanum,H.
TITLE Direct Submission
JOURNAL Submitted (15-FEB-2022) Department of Zoology, University of Dhaka,
Dhaka University, Dhaka, Dhaka Dhaka-1000, Bangladesh
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Sequencing Technology:: Sanger dideoxy sequencing
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LOCUS OM680001 432 bp DNA linear INV 15-FEB-2022

DEFINITION *Pallisentis ophiocephali* isolate A3 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.

ACCESSION OM680001

VERSION OM680001

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ORGANISM *Pallisentis ophiocephali*

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Acanthocephala; Eoacanthocephala; Gyracanthocephala; Quadrigyridae; *Pallisentis*.

REFERENCE 1 (bases 1 to 432)

AUTHORS Sultana,Y., Hasan,M.M., Musa,S. and Khanum,H.

TITLE Molecular Identification of *Pallisentis ophiocephali*

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 432)

AUTHORS Sultana,Y., Hasan,M.M., Musa,S. and Khanum,H.
TITLE Direct Submission
JOURNAL Submitted (15-FEB-2022) Department of Zoology, University of Dhaka, Dhaka University, Dhaka, Dhaka Dhaka-1000, Bangladesh
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Sequencing Technology :: Sanger dideoxy sequencing
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>KY077093.1 *Neoechinorhynchus mexicoensis* isolate DNA1365 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

TTAGTTGGTTTTGGTTTAAGTGGTGTTATTCGTCTTGAATTAATAAGAAGTGGTTC
TTGATTAGGTAGAGAAAGTTTATATAATATGGTGGTAACTAGACATGCTGTTATA
ATAGTATTTTTTCTTGTTATACCTTTATTTTTAGGGGCTTTAGGTAATATATTAACT
CCTTTAATACTTCAGATAAAAGATATGGTTTTTCCACGATTGAATAATTTAAGGAT
GCTAATAGTTTATTTATCTTTAGGTTTATTTTGTTATTCTATATGTAAAGAAGGTC
CTCAAGCAGGGTGGACATATTACCCTCCTTTGTCATCAAGAGCTTTTATGGGTGA
TGTAAGAATAGATTTAAGTATTCTTAGTTTGCATTTATTGGGTATTTCATCTATCT
TTAACTCTTTAAATATTTTAAGTACAGTAATTTTTAGGGTAACGAGAAATAGAAT
TAAGTTAGAACAACTATCTTTATTTATTTGGTCTTTATGTGTAACTAGTGTTTTAA
TTATTTTTACTGTGCCTGTTTTAGCTGCGGCTCTAGGTATGTTA

Appendix-II

Table (a). Prevalence of Parasites of *P. paradiseus* in different length groups during 2017-2018

Parasites	**Group (7-9 cm)**	**Prevalence (%)**	**Group (9.1-11cm)**	**Prevalence (%)**	**Group (11.1-13 cm)**	**Prevalence (%)**	**Group (13.1-15 cm)**	**Prevalence (%)**
P. bilabitum	8	22.22	11	22.92	11	25.58	8	25.81
U. hamali	7	19.44	10	20.83	10	23.26	8	25.81
T. vittalani	5	13.89	8	16.67	8	18.60	6	19.35
H. callionymi	4	11.11	5	10.42	4	9.30	4	12.90
N. lingualis	2	5.56	4	8.33	3	6.98	1	3.23
P. trigonis	2	5.56	2	4.17	2	4.65	1	3.23
Dujardinascaris	1	2.78	4	8.33	3	6.98	1	3.23
M. bagarii	2	5.56	4	8.33	3	6.98	2	6.45
N. aspinosum	3	8.33	3	2.08	4	9.30	1	3.23
P. ophiocephali	2	5.56	2	4.17	1	2.32	2	6.45

Table (b). Prevalence of Parasites of *X. cancila* in different length groups during 2017-2018

Parasites	**Group (20-25 cm)**	**Prevalence (%)**	**Group (25.1-30cm)**	**Prevalence (%)**	**Group (30.1-35 cm)**	**Prevalence (%)**	**Group (35.1-40 cm)**	**Prevalence (%)**
B. belonae	5	17.24	4	9.76	0	0	7	10.45
I. hypselobagri	1	3.45	4	9.76	5	9.1	5	7.46
M. bagarii	1	3.45	2	4.88	5	9.1	8	11.94
G. spinigerum	2	6.90	5	12.20	4	7.27	7	10.45
C. ophiocephali	2	6.90	2	4.88	4	7.27	5	7.46
P. trichuri	1	3.45	2	4.88	5	9.1	7	10.45
N. prolixum	7	24.14	8	19.51	14	25.46	10	14.92
A. nigeriensis	7	24.14	8	19.51	8	14.55	13	19.40
P. ophiocephali	8	27.59	11	37.93	17	30.91	14	20.90

Table (c): Monthly prevalence and intensity of trematodes in *P. paradiseus*

Month	No. of fish examined	No. of fish infected	No. of fish infected by trematodes	Prevalence of infestation (%)	No. of trematodes collected	Intensity of parasites
January	12	5	4	80	4	1
February	12	5	1	40	2	2
March	12	4	2	75	3	1. 25
April	13	7	6	14.29	6	1
May	14	9	6	77.78	7	1.29
June	14	9	8	88.89	8	1
July	14	11	7	66.63	7	1
August	16	10	5	50	5	1
September	14	7	3	57.14	4	1.25
October	14	6	1	33.33	2	2
November	12	6	3	50	3	1
December	14	6	3	50	3	1
January	13	3	2	66.67	2	1
February	14	4	2	50	2	1
March	14	6	1	33.33	2	2
April	12	7	3	57.14	4	1.5
May	15	8	6	75	6	1
June	14	8	5	75	6	1.17
July	12	8	5	75	6	1.17
August	13	7	5	71.43	7	1.4
September	13	6	4	66.67	4	1
October	14	6	4	66.67	4	1
November	13	6	4	66.67	4	1
December	13	4	1	50	2	2

Table (d): Monthly prevalence and intensity of Cestodes in *P. paradiseus*

Month	No. of fish examined	No. of fish infected	No. of fish infected by cestodes	Prevalence of infestation (%)	No. of cestodes collected	Intensity of parasites
January	12	5	1	20	1	1
February	12	5	0	0	0	0
March	12	4	1	25	1	1
April	13	7	0	0	0	0
May	14	9	1	11.11	1	1
June	14	9	0	0	0	0
July	14	11	1	9.09	1	1
August	16	10	1	10	1	1
September	14	7	2	28.57	1	1
October	14	6	2	33.33	2	1
November	12	6	0	0	0	0
December	14	6	0	0	0	0
January	13	3	1	33.33	1	1
February	14	4	0	0	0	0
March	14	6	0	0	0	0
April	12	7	0	0	0	0
May	15	8	1	12.5	1	1
June	14	8	0	0	0	0
July	12	8	1	12.5	1	1
August	13	7	0	0	0	0
September	13	6	2	33.33	2	1
October	14	6	0	0	0	0
November	13	6	2	33.33	2	1
December	13	4	1	25	1	1

Table (e): Monthly prevalence and intensity of nematodes in *P. paradiseus*

Month	No. of fish examined	No. of fish infected	No. of fish infected by nematodes	Prevalence of infestation (%)	No. of nematodes collected	Intensity of parasites
January	12	5	0	0	0	0
February	12	5	2	40	2	1
March	12	4	0	0	0	0
April	13	7	0	0	0	0
May	14	9	1	11.11	1	1
June	14	9	0	0	0	0
July	14	11	2	18.18	1	1
August	16	10	2	20	2	1
September	14	7	0	0	0	0
October	14	6	2	33.33	2	1
November	12	6	2	33.33	2	1
December	14	6	1	33.33	2	2
January	13	3	0	0	0	0
February	14	4	0	0	0	0
March	14	6	2	33.33	2	1
April	12	7	1	14.29	1	1
May	15	8	1	12.5	1	1
June	14	8	1	12.5	1	1
July	12	8	1	12.5	1	1
August	13	7	0	0	0	0
September	13	6	0	0	0	0
October	14	6	0	0	0	0
November	13	6	0	0	0	0
December	13	4	0	0	0	0

Table (f): Monthly prevalence and intensity of acanthocephalans in *P. paradiseus*

Month	No. of fish examined	No. of fish infected	No. of fish infected by cestodes	Prevalence of infestation (%)	No. of cestodes collected	Intensity of parasites
January	12	5	0	0	0	0
February	12	5	0	0	0	0
March	12	4	0	0	0	0
April	13	7	1	14.29	1	1
May	14	9	0	0	0	0
June	14	9	1	11.11	1	1
July	14	11	1	9.09	1	1
August	16	10	2	20	1	1
September	14	7	1	14.29	1	1
October	14	6	0	0	0	0
November	12	6	1	16.67	1	1
December	14	6	1	16.67	1	1
January	13	3	0	0	0	0
February	14	4	2	50	2	1
March	14	6	2	33.33	2	1
April	12	7	2	28.57	2	1
May	15	8	0	0	0	0
June	14	8	1	12.5	1	1
July	12	8	0	0	0	0
August	13	7	2	28.57	1	1
September	13	6	0	0	0	0
October	14	6	2	33.33	2	1
November	13	6	1	16.67	1	1
December	13	4	1	25	1	1

Table (g): Monthly prevalence and intensity of trematodes in *X. cancila*

Month	No. of fish examined	No. of fish infected	No. of fish infected by trematodes	Prevalence of infestation (%)	No. of trematodes collected	Intensity of parasites
January	15	9	0	0	0	0
February	14	9	0	0	0	0
March	12	5	0	0	0	0
April	13	8	1	12.5	1	1
May	13	6	2	33.33	2	1
June	13	8	1	12.5	1	1
July	15	9	1	11.11	1	1
August	13	8	2	25	2	1
September	13	5	1	20	2	2
October	13	7	2	33.33	2	1
November	13	8	2	25	2	1
December	13	9	1	11.11	1	1
January	13	9	1	11.11	1	1
February	13	9	1	11.11	1	1
March	13	7	1	14.29	1	1
April	14	9	1	11.11	1	1
May	15	10	2	20	3	1.5
June	16	8	0	0	0	0
July	13	8	0	0	0	0
August	14	7	1	14	1	1
September	13	8	1	12.5	1	1
October	12	8	1	12.5	1	1
November	12	9	2	22.22	1	1
December	13	9	1	11.11	1	1

Table (h): Monthly prevalence and intensity of nematodes in *X. cancila*

Month	No. of fish examined	No. of fish infected	No. of fish infected by nematodes	Prevalence of infestation (%)	No. of nematodes collected	Intensity of parasites
January	15	9	3	33.33	3	1
February	14	9	3	33.33	3	1
March	12	5	3	60	3	1
April	13	8	2	25	2	1
May	13	6	2	33.33	2	1
June	13	8	3	37.5	1	1
July	15	9	3	33.33	1	1
August	13	8	2	25	2	1
September	13	5	1	20	1	1
October	13	7	0	0	0	0
November	13	8	2	25	2	1
December	13	9	2	22.22	2	1
January	13	9	2	22.22	2	1
February	13	9	3	33.33	3	1
March	13	7	2	28.57	2	1
April	14	9	3	33.33	3	1
May	15	10	4	40	4	1
June	16	8	5	62.5	5	1
July	13	8	4	25	4	1
August	14	7	4	57.14	4	1
September	13	8	3	37.5	3	1
October	12	8	1	12.5	1	1
November	12	9	1	11.11	1	1
December	13	9	1	11.11	1	1

Table (i): Monthly prevalence and intensity of acanthocephalans in *X. cancila*

Month	No. of fish examined	No. of fish infected	No. of fish infected by acanthocephalans	Prevalence of infestation (%)	No. of acanthocephalans collected	Intensity of parasites
January	15	9	6	66.67	7	1.17
February	14	9	5	55.55	6	1.2
March	12	5	2	60	4	2
April	13	8	5	83.33	6	1.2
May	13	6	2	33.33	3	1.5
June	13	8	4	50	4	1
July	15	9	5	55.55	5	1
August	13	8	4	50	4	1
September	13	5	3	60	3	1
October	13	7	4	57.14	5	1.25
November	13	8	3	25	4	1.33
December	13	9	5	55.55	6	1.2
January	13	9	5	22.22	6	1.2
February	13	9	5	22.22	5	1
March	13	7	3	42.85	3	1
April	14	9	5	55.55	5	1
May	15	10	4	40	4	1
June	16	8	3	25	3	1
July	13	8	4	50	5	1.25
August	14	7	2	28.57	2	1
September	13	8	3	25	4	1
October	12	8	2	22.22	2	1
November	12	9	6	11.11	8	1.33
December	13	9	6	11.11	7	1.17

Table (j): Monthly prevalence of acanthocephalans in *X. cancila*

Month	No. of fish examined	*N. prolixum*		*A. nigeriensis*		*P. ophiocephali*	
		Host infected	prevalence	Host infected	prevalence	Host infected	prevalence
January	15	2	22.22	1	11.11	4	44.44
February	14	2	22.22	2	22.22	2	22.22
March	12	0	0	1	20	1	20
April	13	2	25	1	12.5	2	25
May	13	2	33.33	0	0	0	0
June	13	1	12.5	1	12.5	1	25
July	15	1	11.11	2	22.22	1	22.22
August	13	2	25	1	12.5	1	12.5
September	5	1	20	1	20	1	20
October	13	0	0	3	42.85	2	28.57
November	13	1	12.5	1	12.5	2	25
December	13	2	22.22	2	22.22	2	22.22
January	13	3	33.33	0	0	3	33.33
February	13	1	11.11	2	22.22	2	22.22
March	13	1	14	1	14	1	14
April	14	2	28	0	0	0	0
May	15	1	10	2	20	1	10
June	16	2	25	1	12.5	0	0
July	13	2	12.5	0	0	2	12.5
August	14	2	28	0	0	0	0
September	13	1	12.5	2	25	1	12.5
October	12	2	25	2	25	2	25
November	12	0	0	3	33.33	3	33.33
December	13	2	22.22	2	22.22	2	22.22

Table (k): Monthly prevalence of acanthocephalans in *p. paradiseus*

Month	**No. of fish examined**	**No. of host infected by acanthocephalan parasites**	***N. aspinosum***		***P. ophiocephali***	
			Host infected	**prevalence**	**Host infected**	**prevalence**
January	12	5	0	0	0	0
February	12	5	0	0	0	0
March	12	4	0	0	1	25
April	13	7	1	14	1	14
May	14	9	0	0	0	0
June	14	9	0	0	0	0
July	14	11	0	0	1	9
August	16	10	0	0	1	10
September	14	7	1	14	0	0
October	14	6	1	17	0	0
November	12	6	1	17	0	0
December	14	6	2	33.33	0	0
January	13	3	0	0	0	0
February	14	4	0	0	1	25
March	14	6	1	17	0	0
April	12	7	0	0	0	0
May	15	8	0	0	0	0
June	14	8	0	0	0	0
July	12	8	1	12.5	0	0
August	13	7	0	0	0	0
September	13	6	0	0	0	0
October	14	6	2	33.33	1	16.67
November	13	6	1	16.67	1	16.67
December	13	4	0	0	0	0

Table (l): Monthly prevalence of trematodes in *p. paradiseus*

Month	No. of fish examined	*P. bilabiatum*		*U. hamati*		*T. vittalani*		*H. callionymi*	
		Host infected	prevalence	Host infected	prevalence	Host infected	prevalence	Host infected	prevalence
January	5	1	20	0	0	0	0	1	20
February	5	1	20	1	25	1	20	0	0
March	4	1	25	0	0	0	0	1	25
April	7	1	14	0	0	1	14	1	14
May	9	2	22	2	22	3	33	0	0
June	9	2	22	2	22	2	22	1	11
July	11	4	36	3	27	2	18	1	9
August	10	3	30	2	20	1	10	1	10
September	7	1	14	2	29	1	14	1	14
October	6	1	17	1	17	1	17	1	17
November	6	1	17	2	33	1	17	0	0
December	6	1	17	1	17	0	0	0	0
January	3	0	0	1	33.33	0	0	1	33.33
February	4	1	25	0	0	0	0	1	25
March	6	1	17	1	17	1	17	1	17
April	7	2	29	2	29	2	29	0	0
May	8	3	37.5	3	37.5	1	12.5	1	12.5
June	8	3	37.5	2	25	1	12.5	0	0
July	8	2	25	2	25	2	25	0	0
August	7	1	14	2	28	2	28	0	0
September	6	0	0	1	16.67	2	33.33	1	16.67
October	6	0	0	1	16.67	1	16.67	0	0
November	6	0	0	0	0	1	16.67	1	16.67
December	4	0	0	0	0	1	25	1	25

Table (m): Monthly prevalence of nematodes in *X. cancila*

Month	No. of fish examined	*M. bagarii*		*G. spinigerum*		*C. ophiocephali*		*P. trichuri*	
		Host infected	prevalence	Host infected	prevalence	Host infected	prevalence	Host infected	prevalence
January	9	0	0	0	0	2	22.22	1	11.11
February	9	1	11.11	0	0	1	11.11	1	11.11
March	5	0	0	1	20	1	20	1	20
April	8	1	12.5	1	12.5	0	0	0	0
May	6	0	0	1	16.67	1	16.67	0	0
June	8	1	12.5	0	0	1	12.5	1	12.5
July	9	1	11.11	1	11.11	1	11.11	0	0
August	8	1	12.5	1	12.5	0	0	0	0
September	5	0	0	0	0	0	0	1	20
October	7	0	0	0	0	0	0	0	0
November	8	0	0	1	12.5	0	0	1	12.5
December	9	1	11.11	1	11.11	0	0	0	0
January	9	0	0	0	0	0	0	0	0
February	9	0	0	2	22.22	0	0	1	11.11
March	7	2	28	0	0	0	0	0	0
April	9	1	11.11	1	11.11	0	0	1	11.11
May	10	1	10	1	10	2	20	0	0
June	8	1	12.5	2	25	1	12.5	1	12.5
July	8	2	25	1	12.5	1	12.5	0	0
August	7	1	14	1	14	1	14	1	14
September	8	1	12.5	1	12.5	0	0	1	12.5
October	8	0	0	0	0	0	0	1	12.5
November	9	0	0	1	11.11	0	0	0	0
December	9	1	11.11	1	11.11	0	0	0	0

Table (n): Temperatue, Rainfall and Humidity in different months (Jan'17-Dec'18)

Month	Temperature (°C)	Rainfall (mm)	Humidity (%)
January	22°C	9.0	60
February	35°C	25.5	51
March	26°C	52.4	64
April	28°C	130.2	72
May	30°C	277.3	71
June	30°C	459.4	78
July	29°C	523.0	81
August	29°C	420.4	82
September	29°C	318	81
October	28°C	160	79
November	26°C	42.4	65
December	22°C	9.6	74
January	18°C	7.7	68
February	24°C	28.9	57
March	28°C	65.8	57
April	28°C	156.3	66
May	28°C	339	75
June	30°C	340	80
July	30°C	373	81
August	30°C	316.5	81
September	30°C	300.4	76
October	28°C	172.3	69
November	24°C	34.4	63
December	24°C	12.8	51

Table (o): Biochemical components in different months in *X. cancila*

Month	Moisture (%)	Protein (%)	Fat (%)	Ash(%)
January	75.92	18.02	2.83	3.18
February	75.91	18.04	2.82	3.20
March	75.91	18.03	2.81	3.21
April	75.88	17.98	2.80	3.22
May	75.91	18.01	2.79	3.19
June	75.90	18.02	2.77	3.23
July	75.89	18.02	2.81	3.22
August	75.91	18.02	2.78	3.19
September	75.90	18.01	2.78	3.20
October	75.91	18.02	2.81	3.21
November	75.90	18.03	2.79	3.22
December	75.90	18.03	2.80	3.20
January	75.91	18.02	2.80	3.20
February	75.91	18.02	2.79	3.21
March	75.89	18.04	2.80	3.20
April	75.88	18.01	2.78	3.21
May	75.91	18.00	2.79	3.21
June	75.90	18.02	2.78	3.22
July	75.90	18.01	2.78	3.21
August	75.91	18.00	2.77	3.23
September	75.91	17.99	2.79	3.22
October	75.91	18.02	2.79	3.21
November	75.92	18.01	2.80	3.20
December	75.92	18.01	2.81	3.20

Table (p): Biochemical components in different months in *P. paradiseus*

Month	Moisture	Protein	Fat	Ash
January	76.27	17.40	3.37	2.08
February	76.29	17.42	3.36	2.09
March	76.29	17.41	3.35	2.07
April	76.30	17.41	3.34	2.05
May	76.29	17.44	3.38	2.06
June	76.31	17.40	3.36	2.07
July	76.29	17.41	3.35	2.08
August	76.32	17.39	3.37	2.03
September	76.29	17.40	3.36	2.09
October	76.29	17.41	3.34	2.05
November	76.26	17.43	3.35	2.06
December	76.28	17.43	3.32	2.11
January	76.29	17.40	3.35	2.09
February	76.28	17.42	3.35	2.10
March	76.30	17.41	3.34	2.07
April	76.31	17.40	3.32	2.06
May	76.29	17.40	3.35	2.06
June	76.30	17.42	3.33	2.05
July	76.28	17.39	3.36	2.07
August	76.31	17.40	3.36	2.09
September	76.28	17.43	3.34	2.07
October	76.27	17.41	3.35	2.07
November	76.29	17.40	3.37	2.06
December	76.28	17.40	3.36	2.04